SPECIALIST PERIODICAL REPORTS

Amino-acids Peptides and Proteins

VOLUME 12

ROYAL SOCIETY OF CHEMISTRY

Amino-acids, Peptides, and Proteins

Volume 12

A Specialist Periodical Report

Amino-acids, Peptides, and Proteins

Volume 12

A Review of the Literature Published during 1979

Senior Reporter
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The Royal Society of Chemistry
Burlington House, London W1V0BN

British Library Cataloguing in Publication Data

Amino-acids, peptides, and proteins.-Vol. 12.
-(A Specialist periodical report)
1. Amino acids
2. Peptides
3. Proteins
I. Royal Society of Chemistry
547.7'5 QD431

ISBN 0-85186-104-0
ISSN 0306-0004

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Organic formulae composed by Wright's Symbolset method

Typeset and printed in Great Britain by John Wright & Sons (Printing) Ltd. at The Stonebridge Press, Bristol

Preface

This twelfth Report reviews papers relevant to the chemistry of amino-acids, peptides, and proteins published in the major journals during 1979. The subject matter of Chapter 4 (Peptides with structural features not typical of proteins) was omitted from Volume 11. This chapter therefore covers the two-year period 1978—1979. Chapter 5 (Chemical structure and biological activity) contains a more complete coverage of the main groups of peptide hormones than has been possible in the past. Some overlap is inevitable in a fragmented chapter of this type, and somatostatin, for example, is referred to in both the sections dealing with Hypothalamic releasing hormones (Part 1) and Pancreatic hormones (Part 4).

It is a matter of the greatest regret and concern to the Senior Reporter that recent volumes in this annual series have appeared steadily later and later. Indeed, at the time of writing (July, 1980), Volume 11 covering the literature for 1978 has not been published, and its topicality on appearance will therefore be much reduced. Readers may like to know that publication within one year of the close of literature coverage remains our objective, although it has been achieved but rarely in the immediate past. Failure to achieve it with the present volume is not to be attributed to the many contributors listed opposite, who once again deserve our warmest thanks. Thanks are also due to Barbara Rodbard, who has helped with the administrative work of the present volume and has cheerfully encouraged us all to meet tight writing schedules.

R. C. SHEPPARD

Contents

Chapter 1 Amino-acids
By G. C. Barrett

1 Introduction 1
Textbooks and Reviews 1

2 Naturally Occurring Amino-acids 1
Occurrence of Known Amino-acids 1
New Natural Amino-acids 2
New Amino-acids from Hydrolysates 3

3 Chemical synthesis and Resolution of Amino-acids 5
General Methods of Synthesis of α-Amino-acids 5
Synthesis of β-Amino-acids and Higher Homologues 6
Asymmetric Synthesis of α-Amino-acids 6
Prebiotic Synthesis; Model Reactions 7
Synthesis of Protein Amino-acids and Other Naturally Occurring Amino-acids 8
Synthesis of Aliphatic α-Amino-acids 10
Synthesis of α-Alkyl Analogues of Protein Amino-acids and of Other Natural Amino-acids 10
Synthesis of α-Heteroatom-substituted α-Amino-acids 11
Synthesis of Halogenoalkyl α-Amino-acids 11
Synthesis of Aliphatic Amino-acids Carrying Hydroxy-groups in Side Chains 11
Synthesis of α-Amino-acids with Unsaturated Side Chains 12
Synthesis of Aromatic and Heterocyclic α-Amino-acids 13
Synthesis of *N*-Substituted α-Amino-acids 15
Synthesis of Aminoxy-acids 15
Synthesis of α-Amino-acids Containing Sulphur, Selenium, or Tellurium 15
Synthesis of Phosphorus-containing α-Amino-acids 15
Amino-acids Synthesized for the First Time 15
Synthesis of Labelled Amino-acids 16
Resolution of Amino-acids 17

4 Physical and Stereochemical Studies of Amino-acids 18
Crystal Structures of Amino-acids and their Derivatives 18
N.M.R. Spectroscopy 19

O.R.D. and C.D. Spectra 20
Mass Spectrometry 21
Other Physical and Theoretical Studies 21

5 Chemical Studies of Amino-acids 23
Racemization 23
General Reactions 24
Specific Reactions of Natural Amino-acids 26
Specific Reactions and Properties of Amino-acids Related to Biochemical Processes 29
Effects of Electromagnetic Radiation on Amino-acids 30

6 Analytical Methods 31
Gas–Liquid Chromatography 31
Ion-exchange Chromatography 32
Thin-layer Chromatography 33
High Performance Liquid Chromatography 34
Fluorimetry 35
Other Separation Methods 36
Determination of Specific Amino-acids 36

Chapter 2 Structural Investigation of Peptides and Proteins
IA: Protein Isolation and Characterization
By R. Harrison

1 Introduction 37

2 Protein Isolation Methodology 37
Affinity Chromatography Methodology 38
Ligand Investigation 38
Coupling Considerations—Matrix, Spacer, Procedure, and Ligand Concentration 39
Optimum Use of Affinity Matrix—Loading and Elution 41
Affinity Chromatography *versus* Affinity Elution 42
Specific Affinity Ligands 43
Triazine Dyes 43
Hydrophobic Ligands 44
Lectin Ligands 54
Immunoaffinity Ligands 58
Other Chromatographic Procedures 60
Hydroxylapatite 60
High Performance Liquid Chromatography 60
Gel Permeation 61
Controlled Pore Glass 61
Novel Uses of Ion-exchange Resins 62

Covalent and Chelate Chromatography 62
Miscellaneous Column Chromatographic Procedures 62
Miscellaneous Non-column Procedures 63
Electrophoretic Procedures 63

3 The Isolation of Specific Classes of Protein 64
Membrane Proteins 64
Nucleic-acid Metabolism, Viral and Phage-related Proteins, and Protein Synthesis 71
Plasma Proteins 79
Proteases and Protease Inhibitors 85
Lectins and Glycosidases 85
Miscellaneous Proteins 85

4 Protein Characterization 85
Estimation of Protein Concentration 85
Determination of Molecular Weight and other Physical Parameters 98
Electrophoretic Procedures 99
Isoelectric Focusing and Isotachophoresis 99
Polyacrylamide Gel Electrophoresis in Detergents 100
Two-dimensional Procedures 101
Crossed Immunoelectrophoresis and other Affinity Electrophoretic Procedures 102
Detection of Proteins in Gels 103
Immunological Characterization of Proteins 104
Peptide Mapping 107
Thin-layer Chromatography 107
Liquid Chromatography 107
Gel Electrophoresis 107
Miscellaneous Structural Characterization 111

IB: Primary Structures
By J. Gagnon and D. L. Christie

1 Chain Cleavage 111
Chemical Cleavages 111
Enzymatic Cleavages 112

2 Separation of Peptides 113
Reversed-phase High-pressure Liquid Chromatography 113
High-pressure Gel-permeation Chromatography 115
Peptide mapping 115

3 Manual Sequencing 115

4 Liquid-phase Sequencing 116

5 Solid-phase Sequencing 117

6 Microsequencing 117

7 Phenylthiohydantoin Amino-acids 118

8 Mass Spectrometry 118

9 New Strategy 119

10 Primary Structures 120

1C: Chemical Modification of Proteins
By D. M. Moran and A. J. Garman

1 Introduction 150

2 Re-investigation of Known Reagents and Reactions 192
Cyanogen Bromide 192
Butane-2,3-dione 192
Glutaraldehyde 192
2-Nitro-5-thiocyanobenzoic acid (NTCB) 192
2-(2′-Pyridylmercapto)mercuri-4-nitrophenol 193
1-Fluoro-2,4-dinitrobenzene 193
5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) 193
Sodium Cyanoborohydride 193
4-Chloro-7-nitrobenzofurazan 193
2-Carboxy-4,6-dinitrochlorobenzene 194
Fluorescamine 194

3 New Reagents and Techniques 194

4 Chemical Cross-linking 197

5 Photocross-linking 199

6 Affinity Labelling 200

Appendix: Proteins Involved in Modifications Discussed in the Text 203

II: X-Ray Studies
By W. D. Mercer

1 Introduction 203

2 Equipment and Methods 219
General Crystallography 219
Protein Crystallography 220
Neutron Diffraction 221
Protein Dynamics by Crystallography 221
Protein Refinements 221
Structure Storage and Display 223

3 Immunoglobulins and Lectins 223
General 223
Bence-Jones protein Rhe 224
Concanavalin A 224

4 Oxygen- and Electron-carrying Proteins 224
Haem Chemistry 224
General Chemistry of Haem Proteins 225
Myoglobin 225
Haemoglobin 225
Haemoglobin C 226
Haemoglobin A 226
Haemoglobin S 227
Sickling-deer Haemoglobin 227
Haemerythrin 227
Erythrocruorin 227
Cytochrome b_5 227
Cytochrome b_{562} 228
Cytochrome c_3 228
Cytochrome c 228
Flavocytochrome b_2 229
Ferredoxin 229
Rubredoxin 229

5 Lysozyme and Ribonuclease 229
Lysozyme 229
Ribonuclease 231

6 Proteolytic Enzymes 231
Trypsin 231
Trypsin Inhibitor 232
Chymotrypsin 232
Thermolysin 232

Subtilisin 232
Streptomyces griseus Protease B 233
Streptomyces griseus Protease A 233
α-Lytic Protease 234
Acid proteases 234
General 234
Pepsin 234
Endothiapepsin 235

7 Glycolytic Enzymes 235
Phosphorylase 235
Hexokinase 236
6-Phosphogluconate Dehydrogenase 236
Phosphofructokinase 237
Phosphoglycerate Kinase 237
Pyruvate Kinase 238
Alcohol Dehydrogenase 238
Lactate Dehydrogenase 239

8 Hormones 239
Insulin 239

9 Other Globular Proteins 240
L-Arabinose Binding Protein 240
Dihydrofolate Reductase 241
Rhodanese 241
Glutathione Peroxidase 241
Bacteriochlorophyll 242
p-Hydroxybenzoate Hydroxylase 242
Gene 5 Product—Phage fd 243
Aspartate Carbamoyltransferase 243
Erabutoxin 244
Transferrin 244
Carbonic Anhydrase B 244
Citrate Synthase 244

10 Protein Conformation and Structure Prediction 244
Analysis of Protein Structures 244
Prediction of Protein Structure 246

11 Viruses 248

12 Nucleic Acids 249
tRNA 249
DNA and DNA-binding Compounds 249

13 Muscle 250

14 Membranes and Membrane-bound Proteins 251

15 Other Biological Structures 252
Bacterial Pili and Flagella 252
Ribosomes 252
Naturally Occurring Polypeptides 253
Various Other Structures 253

16 Fibrous Proteins and Polysaccharides 254
Collagen 254
Chitin 254

17 Synthetic Polypeptides 254

III: Conformation and Interaction of Peptides and Proteins in Solution
Edited by R. H. Pain

Introductory Remarks 255

1 Theoretical Aspects of Protein Conformation 255
Contributed by F. E. Cohen and M. J. E. Sternberg
Potential-energy Functions 255
Energy Calculations on Polypeptides and Proteins 256
Analysis and Prediction of Secondary Structure 257
Analysis of Tertiary and Quaternary Structure 258
Prediction of Tertiary Structure 259
Protein Dynamics 261
Conclusion 262

2 Stability and Mechanism of Folding of Globular Proteins 262
Contributed by B. Adams
Stability 262
Conformational Dynamics 263
Effect of Ligands 264
Solvent Effects on Denaturation and the Denatured State 265
Protein Fragments and Complementation 265
Synthetic Proteins which Fold 266
Identification of Folding Intermediates 266
Theories of Folding Pathways 267
Nucleation 267
Proline Isomerization 267
Cluster Model 268
Protein Folding *in vivo* 269

3 Immunological Probes of Protein Conformation 269
Contributed by A. Benson

Introduction 269
Changes and Similarities in Protein Conformation 270
Molecular Evolution 271
Antibody Binding Effects 272
Conformational Equilibria 273

4 Nuclear Magnetic Resonance 273
Contributed by H. W. E. Rattle

Methods 273
Amino-acids and Synthetic Peptides 275
Hormones and Small Natural Peptides 277
Enzymes 280
Oxidoreductases 280
Transferases 282
Hydrolases 282
Lyases 284
Isomerases and Ligases 285
Haem Proteins 285
Other Proteins 287

5 Infrared and Raman Spectroscopy 291
Contributed by R. M. Stephens

Model Compounds 291
Polypeptides 294
Proteins and Natural Peptides 295
Retina Components 295
Cytochromes 296
Alamethicin 296
Silk Fibroin 297
Flavoproteins 297
Lysozyme 297
Haemoglobin 297
Protein Interactions 298

6 Circular Dichroism 298
Contributed by T. Brittain

General 298
Reviews 298
Instrumental 298
Theory and Analysis 299
Small Molecules, Model Compounds, and Synthetic Polymers 300
Amino-acids and Derivatives 300

Dipeptides and Oligopeptides 301
Polypeptides 302
Proteins 305
Non-chromophoric Proteins 305
Chromophoric Proteins 310
Extrinsic Chromophores 312
Hormones 313
Immunoproteins 315
Membrane Proteins 315
Nuclear Proteins 315

7 Magnetic Circular Dichroism 316
Contributed by T. Brittain
Reviews 316
Theory and Analysis 316
Model Compounds 316
Proteins 317

8 Mössbauer Spectroscopy 318
Contributed by D. P. E. Dickson
Haem Proteins 319
Iron–Sulphur Proteins 322
Iron Storage and Transport Proteins 323
Dynamical Studies 324

9 Dissociation and Association of Proteins 325
Contributed by E. J. Wood
Analytical Ultracentrifugation 325
Techniques 325
Theory 327
Examples 327
Gel Chromatography 328
Examples 329
Light-scattering 329
Quasi-elastic Light-scattering 330
Transport Studies 331
Theory and Techniques 331
Examples 332
Electron Microscopy 332
Kinetic studies 334
Protein–Protein Interactions 334
Protein–Small Molecule Interactions 335
Protein–Small Molecule Equilibria 335
Subunit Structure of Proteins 338
SDS–Gel Electrophoresis 338
Examples 339

Examples of Associating–Dissociating Systems 340
Sickle-cell Haemoglobin 340
Microtubules 341

10 Fluorescence 343
Contributed by R. Devonshire
Review Articles 343
Instrumentation and Techniques 344
Data Treatment 347
Theories of Fluorescence Phenomena 348
Fluorescence Quenching 348
Singlet Energy Transfer 348
Fluorescence Polarization 349
Photophysics and Photochemistry of Aromatic Amino-acids 350
Emission Lifetimes 350
Fluorescence Quenching 351
Spectroscopy 352
Intrinsic Protein Luminescence 352
General Interest 352
Tryptophan Fluorescence 353
Tryptophan Phosphorescence 356
Tyrosine Fluorescence 356
Other Protein Chromophores 357
Covalent Probes 358
Non-covalent Probes 361

Chapter 3 Peptide Synthesis
By I. J. Galpin, with Appendices compiled by M. F. J. Galpin

1 Introduction 365

2 Methods 366
Protective Groups 366
Established Methods of Amino-group Protection 366
New Methods of Amino-group Protection 369
Protection of Terminal Carboxy-group 370
Side-chain Protection and Reactions of Amino-acids 372
Formation of the Peptide Bond 376
Racemization 380
General Deprotection 382
Repetitive Methods of Peptide Synthesis 383
Solid-phase Peptide Synthesis 383
Techniques for Monitoring Solid-phase Synthesis 388
Other Repetitive Methods 388

Synthesis of Polymeric Peptides 391
Purification Methods 391
Synthetic Operations with Peptides of Biological Origin 392

3 Syntheses Achieved 395
ACTH and MSH 395
Enkephalins and Endorphins 396
Insulin 397
Somatostatin 399
Thymic Peptides 400
Miscellaneous Peptides 400

4 Appendix 1: A list of Syntheses Reported during 1979 402
Natural peptides, Proteins, Analogues, and Partial Sequences 402
Sequential Oligo- and Poly-peptides 409
Enzyme Inhibitors 410
Miscellaneous Peptides 410

5 Appendix II: Amino-acid Derivatives Useful in Synthesis 411
Coded Amino-acids 411
Other Amino-acids 414

Chapter 4 Peptides with Structural Features not Typical of Protein
By B. W. Bycroft

1 Introduction 417

2 Cyclic Peptides (Homodetic Peptides) 417
2,5-Dioxopiperazines (Cyclic Dipeptides) 417
Naturally Occurring Cyclic Peptides 422
Viomycin, Capreomycin, Tuberactinomycin, and Related Antibiotics 423
Iron-containing Cyclic Peptides 424
Polymyxins and Related Peptides Containing Fatty Acid Residues 424
Cyclic Peptides from *Amanita phalloides* 426
Gramicidin S and Related Peptides 428
Conformational Studies on Synthetic Cyclic Peptides 430
Synthesis of Cyclic Peptides 434

3 Cyclic Depsipeptides and Cyclic Heterodetic Peptides 435
Actinomycin 436
Valinomycin, Enniatins, and Related Compounds 437
Quinoxaline Antibiotics 438
Other Naturally Occurring Cyclic Depsipeptides 439

4 Cyclopeptide Alkaloids 441

5 Penicillins, Cephalosporins, and Related Antibiotics 442

6 Linear Peptides containing Unusual Structural Features 449

7 Large Highly Modified Peptides 457

8 Peptides Linked to Carbohydrates 458
Glycopeptide Antibiotics 458
Glycopeptides from Bacterial Cell Walls 461

Chapter 5 Chemical Structure and Biological Activity of Hormones and Related Compounds

1 Hypothalamic Releasing Hormones 464
Contributed by D. H. Coy
Introduction 464
Thyrotropin-releasing Hormones 464
Luteinizing Hormone-releasing Hormone 465
Somatostatin 467

2 Anterior Pituitary Hormones 469
Contributed by J. Ramachandran
Introduction 469
Hormones of Group I 470
Corticotropin 470
Corticotropin Inhibiting Peptide (CIP) 472
Melanotropins (MSH) 472
Lipotropin (LPH) 474
Biosynthesis of ACTH-MSH-LPH-EP 475
Hormones of Group II 478
Somatotropin (GH) 478
Prolactin (PRL) 480
Biosynthesis of Somatotropin 481
Hormones of Group III 482
General 482
Lutropin (LH) 482
Follitropin (FSH) 484
Thyrotropin 484

3 Posterior Pituitary Hormones 485
Contributed by M. Manning, B. Lammek, and W. H. Sawyer
Introduction 485
Reversible Antagonists of *in vitro* and *in vivo* Oxytocic Responses to Oxytocin 485

Irreversible Antagonists of *in vitro* Oxytocic Responses to Oxytocin 487
Antagonists of the Vasopressor Response to Arginine Vasopressin (AVP) 487
Agonistic Analogues of Oxytocin 489
Agonistic Analogues of Arginine Vasopressin 492
Analogues Prepared for Conformational Studies 492
Tritiated, Deuteriated, and C-enriched Analogues of Oxytocin and AVP 496
New Synthesis of Known Analogues of Oxytocin and AVP 497

4 Pancreatic Hormones 498
Contributed By D. Brandenburg and D. Saunders
Introduction 498
Insulin 499
Isolation, Synthesis, Semisynthesis, and Chemical Modification 499
Structure, Receptor Binding, and Biological Activity 507
Biological Consequences of A-chain Modification 508
Biological Consequences of B-chain Modification 508
Correlation Between Receptor Binding and Biological Activity 509
Glucagon 510
Somatostatin 511

5 Gastrointestinal hormones 513
Contributed by D. Gillessen and R. O. Studer
Introduction 513
Gastrins 515
Cholecystokinin-Pancreozymin (CCK) 520
Secretin 525
Vasoactive Intestinal Peptide (VIP) 528
Gastric Inhibitory Peptide (GIP) 533
Motilin 535
Urogastrone 536
Possible Gastrointestinal Hormone Candidates 538

6 Calcitonin and Parathyroid Hormone 539
Contributed by S. Girgis
Introduction 539
Calcitonin 539
Isolation and Chemical Structure 539
Synthesis 539
Structure–Activity Relationships 539
Biosynthesis 542
Biological effects 542
Extra-thyroidal Calcitonin 542

Parathyroid Hormones (PTH) 543
Chemical Structure 543
Structure–Function Studies with Synthetic Peptides 545
Parathyroid Hormone Inhibitors 546
Other Synthetic Work 547
Labelled Compounds 548
Biological Effects 548
Ectopic Production 548

7 Vasoactive peptides 549
Contributed by P. D. Roy

Bradykinin 549
Angiotensin 552
Potentiating Peptides and Enzyme Inhibitors 556
Tachykinins 557

8 Enkephalins, Endorphins, and Related Peptides 558
Contributed by P. W. Schiller

Introduction 558
New Opioid Peptides, Biosynthesis and Degradation 559
Structure–Activity Relationships in Various Assay Systems 561
Isolated Tissue Assays 561
Opiate Receptor Affinities 567
•*In vivo* Analgesic Activities 570
Other Biological Activities 573
Conformation of Enkephalins and Endorphins 574
Characterization of Opiate Receptors 576
Clinical Tests with Enkephalins and Endorphins 578

Author Index 580

Abbreviations

Abbreviations for amino-acids and their use in the formulations of derivatives follow, with some exceptions, the various Recommendations of the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature which have been reprinted in Volumes 4, 5, and 8 in this series.

Other abbreviations which have been used are listed here or are defined in the text and tables.

Ac	acetyl
Acm	acetamidomethyl
Ad	adamantyl
Adoc	adamantyloxycarbonyl
Aoc	t-amyloxycarbonyl
Aox	4,5-dianisoyl-4-oxazolin-2-one amino-acid derivative
Asu	α-aminosuberic acid
Asx	aspartic acid or asparagine (not yet determined)
ATP	adenosine 5′-triphosphate
Azoc	2-(4-phenylazophenyl)isopropyloxycarbonyl
Beoc	2-bromoethyloxycarbonyl
Boc	t-butoxycarbonyl
Bpoc	2-(4-biphenylyl)isopropoxycarbonyl
BSA	bovine serum albumin
Btm	benzylthiomethyl
Bu^t	t-butyl
Bzh	benzhydryl (diphenylmethyl)
$Bzh(OMe)_2$	4,4′-dimethoxybenzhydryl
Bzl	benzyl
Bzl(4-Cl)	4-chlorobenzyl
$Bzl(2,6\text{-}Cl_2)$	2,6-dichlorobenzyl
Bzl(4-CN)	4-cyanobenzyl
Bzl(OMe)	4-methoxybenzyl
$Bzl(NO_2)$	4-nitrobenzyl
$Bzl(2\text{-}NO_2)$	2-nitrobenzyl
c.d.	circular dichroism
Cha	cyclohexylamine
Cm	carboxymethyl
Cmc	*S*-carboxymethylcysteine
Cox	4,5-di-(4-chlorophenyl-4-oxazolin-2-one amino-acid derivative

CPh_2Py	diphenyl-4-pyridylmethyl
Dcha	dicyclohexylamine
Ddz	3,5-dimthoxy(αα-dimethyl)benzyloxycarbonyl
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
Dnp	2,4-dinitrophenyl
Dns	1-dimethylaminonaphthalene-5-sulphonyl (dansyl)
2,4-Dnps	2,4-dinitrosulphenyl
Dopa	3,4-dihydroxyphenylalanine
DP	degree of polymerization
Dpp	diphenylphosphinoyl
DPtd	4,6-diphenylthieno[3,4-*d*][1,3]dioxal-2-one 5,5-dioxide
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid)
Ec	ethylcarbamoyl
edta	ethylenediaminetetra-acetate
En	ethylene diamine
e.p.r.	electron paramagnetic resonance
e.s.r.	electron spin resonance
Et	ethyl
Gal	galactose
g.c.–m.s.	gas chromatograph–mass spectrometer combination
g.l.c.	gas–liquid chromatography
Glc	glucose
Glp	pyrrolid-2-one-5-carboxylic acid
Glx	glutamic acid or glutamine (not yet determined)
GTP	guanosine 5′-triphosphate
H.p.l.c.	high performance liquid chromatography
Iboc	isobornyloxycarbonyl
i.r.	infrared
Mal=	maleoyl
Man	mannose
Mbh	4,4-dimethoxybenzhydryl
Mbs	4-methoxybenzene sulphonyl
Me	methyl
Mea	mercaptoethylamine
Mhoc	1-methylcyclohexylcarbonyl
$MePh_2Peoc$	2-methyldiphenylphosphinioethyloxycarbonyl
Msc	2-(methylsulphonyl)ethoxycarbonyl
Mtc	2-methylthioethyloxycarbonyl
NAD	nicotinamide-adenine dinucleotide (NAD^+ oxidized: NADH, reduced)
NCA	*N*-carboxyanhydride
Nma	maleimido
Nmps	4-methyl-2-nitrosulphenyl
N.m.r.	nuclear magnetic resonance
Nsu	succinimido
Nps	*o*-nitrophenylsulphenyl

Np	4-nitrophenyl
ONp	*p*-nitrophenoxy
ONp(o)	*o*-nitrophenoxy
ONSu	succinimido-oxy
OPcp	pentachlorophenoxy
OPfp	pentafluorophenoxy
OPic	4-picolyloxy
Opop	phenacyl-2-hydroxyphenyl ester
o.r.d.	optical rotatory dispersion
OTcp	2,4,5-trichlorophenoxy
Pac	phenacyl
Pcp	pentachlorophenyl
Peoc	2-triphenylphosphinioethyloxycarbonyl
Ph(SMe)	*p*-methylthiophenyl
Pic	4-picolyl
Picoc	4-picolyloxycarbonyl
Pipoc	piperidino-oxycarbonyl
Pms	*p*-tolyumethylsulphonyl
PNp	4-nitrophenyl ester
Ppoc	phenylisopropoxycarbonyl
Ppt	diphenylphosphinothioyl
Pth-Gly	the phenylthiohydantoin derived from glycine, *etc.*
Pz	*p*-phenylazobenzyloxycarbonyl
SBu^t	t-butylthio
Scm	carboxymethylsulphenyl
SCB	t-butyloxycarbonylsulphenyl
SDS	sodium dodecyl sulphate
Spr^i	isopropylthio
Sub	5-dibenzosuberyl
SZ	benzyloxycarbonylsulphenyl
Tac	toluene-*p*-sulphonylaminocarbonyl
Tcp	2,4,5-trichlorophenyl
Tfa	trifluoroacetyl
Thp	tetrahydropyranyl
T.l.c.	thin layer chromatography
Tmeda	*NNN'N'*-tetramethylethylene diamine
Tnps	2,4,6-trinitrosulphenyl
Tos	toluene-*p*-sulphonyl
Troc	2,2,2-trichloroethyloxycarbonyl
Trt	triphenylmethyl
Tse	2-toluene-*p*-sulphonyl)ethyl
U.v.	ultraviolet
Z	benzyloxycarbonyl
Z(2-Br)	2-bromobenzyloxycarbonyl
Z(OMe)	*p*-methoxybenzyloxycarbonyl
Ztf	1-benzyloxycarbonylamino-2,2,2-trifluoroethyl

1
Amino-acids

BY G. C. BARRETT

1 Introduction

The main emphasis in this Chapter is on the chemical and biochemical literature on α-amino-acids, and readers seeking references to biological aspects, such as the distribution of the common amino-acids, amino-acid metabolism, or biosynthesis, will find only scant coverage.

Textbooks and Reviews.—Distribution of non-protein amino-acids in plants[1] and amino-acids in marine organisms,[2] metabolism of aromatic amino-acids,[3] and biosynthesis of unusual amino-acids[4] are topics among areas largely excluded from this Chapter which have been reviewed recently. Fermentative[5] and chemical synthesis[6] of α-amino-acids has been reviewed, and a thorough coverage of dehydro-amino-acids and α-hydroxy- and -mercapto-α-amino-acids has appeared.[7] Specific chemical topics (non-enzymatic racemization of amino-acids,[8] N^{ω}-alkyl di- amino-acids,[9] and the conformation of γ-aminobutyric acid[10]) have been reviewed.

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—The Mighei Type II carbonaceous chondrite which fell in Russia in 1889 contains alanine, α- and β-aminobutyric acids, δ-aminoadipic acid, norvaline, and aspartic acid; since these are all racemic, it is concluded that they are indigenous to the meteorite,[11] and the same reasoning has been applied to the range of protein and non-protein amino-acids present in the Allan Hills meteorite 77306.[12] This topic has been reviewed.[13]

[1] L. Fowden, P. J. Lea, and E. A. Bell, *Adv. Enzymol.*, 1979, **50**, 117.
[2] T. Shiba, *Kagaku Sosetsu*, 1979, **25**, 246 (*Chem. Abstr.*, 1980, **92**, 71099).
[3] C.-K. Wat and G. H. N. Towers, *Recent Adv. Phytochem.*, 1979, **12**, 371.
[4] P. J. Lea, *Int. Rev. Biochem.*, 1978, **18**, 79.
[5] S. Kinoshita and K. Nadayama, *Econ. Microbiol.*, 1978, **2**, 209; O. Tajima, *Hakko Kogaku Kaishi*, 1979, **57**, 232; Y. Hirose and H. Okada, in 'Microbiological Technology', 2nd edn. 1979, Vol. 1, p. 241.
[6] K. Matsumoto and K. Harada, *Kagaku no Ryoiki*, 1979, **33**, 207.
[7] U. Schmidt, J. Hausler, E. Oehler, and H. Poisel, *Fortschr. Chem. Forsch.*, 1979, **37**, 251.
[8] J. H. McKerrow, *Mech. Ageing Dev.*, 1979, **10**, 371 (*Chem. Abstr.*, 1979, **91**, 85466).
[9] N. L. Benoiton, in 'Chemistry and Biochemistry of Amino-acids, Peptides, and Proteins', ed. B. Weinstein, Dekker, New York, 1979, Vol. 5, p. 163.
[10] I. A. Sytinskii and A. T. Soldatenkov, *Usp. Khim.*, 1979, **48**, 1256.
[11] P. H. Buhl, in 'Trace Organic Analysis: New Frontiers in Analytical Chemistry', NBS Spec. Publ., Washington, D.C., 1979, pp. 519, 771 (*Chem. Abstr.*, 1979, **91**, 104069).
[12] R. K. Kotra, A. Shimoyama, C. Ponnamperuma, and P. E. Hare, *J. Mol. Evol.*, 1979, **13**, 179.
[13] G. P. Vdovykin, *Usp. Sovrem. Biol.*, 1979, **87**, 49 (*Chem. Abstr.*, 1979, **90**, 163363).

Unusual amino-acids in fungal sources include *cis*-3-amino-L-proline in growth media and cultured mycelia of *Morchella esculanta*,[14] and L-saccharopine together with γ-L-glutamyglycine in the mushroom *Agaricus bisporus*.[15]

Plant sources and amino-acids found therein are: *Halopytis incurvus* (*N*-methyl-L-aspartic acid),[16] *Sagittaria pygmaea* (N^{ε}-carboxymethyl-L-lysine),[17] *Crotalaria juncea* (δ-hydroxynorleucine but not β-hydroxy-*N*-methyl-DL-norvaline, clearing up an earlier uncertainty),[18] seeds of *Neonotonia wightii* [3-carboxy-L-tyrosine, *alias* 3-(3′-carboxy-4′-hydroxyphenyl)-L-alanine],[19] species of fern (*Filicinae*)[20] and *Reseda luteola*[21] (4*R*-hydroxy-2*S*-aminopimelic acid and its 4*S*-diastereoisomer), *Filicinae*[20] (4*S*-hydroxy-4-methyl-*S*-glutamic acid and its 4*R*-diastereoisomer), leaves of *Acacia* (homoarginine, 4-hydroxypipecolic acid),[22] and the red seaweed *Palmaria palmata* (D-homocysteic acid).[23] The possibility of the formation of artefacts during the isolation of sensitive compounds from natural sources must always be taken into account, but an additional problem, the possibility that pesticides or herbicides are sources of unusual amino-acids found in plants, has been mentioned in connection with the isolation of N^{ε}-carboxymethyl-L-lysine.[17]

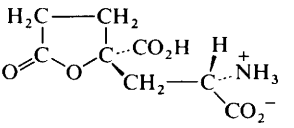
Reports describing the involvement of amino-acids as intermediates on biosynthetic pathways occasionally include information of more general importance, and, although this topic is largely excluded from this Chapter, the discussion of the natural occurrence of α-aminomalonic acid derivatives[24] (*cf.* arcamine[25]) is particularly interesting. However, at the top of the list in the 1979 literature is the splendid work[26–29] describing the establishment of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the biosynthesis of ethylene. Systems discussed in this work include *Pisum sativum* homogenates[27] and apple tissue,[28] the latter being used in a study of the pathway from methionine to ethylene *via* *S*-adenosylmethionine and 1-aminocyclopropane-1-carboxylic acid.[28]

New Natural Amino-acids.—There have been several near relatives of known amino-acids described in the recent literature. (2,3-Dihydroxyphenyl)-L-alanine, together with small amounts of *o*- and *m*-hydroxy-L-phenylalanines, has been isolated from chloridazone-degrading bacteria grown on a medium containing L-phenylalanine.[30] Aminoxyalkanoic acids have become of interest as enzyme

[14] M. Moriguchi, S. Sada, and S. Hatanaka, *Appl. Environ. Microbiol.*, 1979, **38**, 1018.
[15] Y. Oka, T. Ogawa, and K. Sasaoka, *Agric. Biol. Chem.*, 1979, **43**, 1995.
[16] S. Sciuto, M. Piatelli, and R. Chillemi, *Phytochemistry*, 1979, **18**, 1058.
[17] H. Matsutani, S. Kusumoto, R. Koizumi, and T. Shiba, *Phytochemistry*, 1979, **18**, 661.
[18] D. J. Pilbeam and E. A. Bell, *Phytochemistry*, 1979, **18**, 320, 973.
[19] M. F. Wilson, M. A. Bholah, G. S. Morris, and E. A. Bell, *Phytochemistry*, 1979, **18**, 1391.
[20] L. K. Meier and H. Sorensen, *Phytochemistry*, 1979, **18**, 1173.
[21] L. K. Meier, O. Olsen, and H. Sorensen, *Phytochemistry*, 1979, **18**, 1505.
[22] C. S. Evans and E. A. Bell, *Phytochemistry*, 1979, **18**, 1807.
[23] M. V. Laycock, A. G. McInnes, and K. C. Morgan, *Phytochemistry*, 1979, **18**, 1220.
[24] D. Schipper, J. L. van der Baan, and F. Bickelhaupt, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2017.
[25] A. W. Sangster, S. E. Thomas, and N. L. Tingling, *Tetrahedron*, 1975, **31**, 1135.
[26] K. Luerssen, K. Naumann, and R. Schroeder, *Naturwissenschaften*, 1979, **66**, 264; K. Luerssen, K. Naumann, and R. Schroeder, *Z. Pflanzenphysiol.*, 1979, **92**, 285.
[27] J. R. Konze and H. Kende, *Planta*, 1979, **146**, 293; T. Boller, R. C. Herner, and H. Kende, *Planta*, **145**, 293.
[28] D. O. Adams and S. F. Yang, *Proc. Natl. Acad. Sci. USA.*, 1979, **76**, 170.
[29] M. C. C. Lizada and S. F. Yang, *Anal. Biochem.*, 1979, **100**, 140.
[30] R. Buck, J. Eberspaecher, and F. Lingens, *Liebigs Ann. Chem.* 1979, 564.

inhibitors (*e.g.* L-2-aminoxy-3-phenylpropionic acid against phenylalanine ammonia-lyase [31]), and the co-occurrence of β-aminoxy-D-alanine and cycloserine, both possessing antimicrobial properties, has been established in *Streptomyces.*[32]

Lycoperdic acid [1; 3-(5*S*-carboxy-2-oxotetrahydrofuran-5-yl)-*S*-alanine] has been isolated from the mushroom *Lycoperdon perlatum,*[33] tetrahydrolathyrine [2; 3-(2-amino-3,4,5,6-tetrahydropyrimidin-4-yl)-*S*-alanine] from seeds of *Lanchocarpus costaricensis* (configuration at C-4 of tetrahydropyrimidinyl moiety not established),[34] mugeneic acid (3) from root washings of water-cultured barley (*Hordeum vulgare*),[35] and 2*S*-acetylamino-4*R*-carboxypiperidine (4) from leaves of *Calliandra haematocephala.*[36] Higher sources are the mussel *Mytilus galloprovincialis* (mytilins A and B, 5 and 6 respectively)[37] and the toad *Bufo americanus,* from whose skin 3-suberoyl-L-glutamine esters have been isolated.[38]



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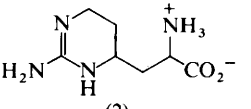

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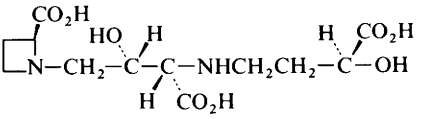

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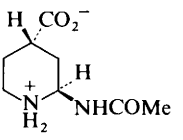

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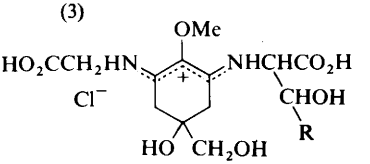


(5) R = H
(6) R = Me

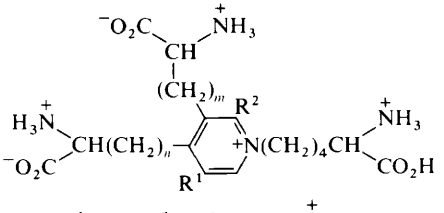
An area of viral chemistry whose importance has now been realized is represented this year (see also Vol. 10, p. 3, Vol. 11, p. 3) by the report of the existence of large amounts of a new metabolite, agropine, in crown gall tumours of tobacco. The compound ($C_{11}H_{17}NO_7$) is considered to be a condensation product of a sugar with an amino-acid.[39]

New Amino-acids from Hydrolysates.—A topic which is not covered as a rule in this Chapter in more than a brief fashion is the area of amino-acid–nucleoside

[31] N. Amrhein and J. Gerhardt, *Biochim. Biophys. Acta*, 1979, **583**, 434.
[32] K. Yagishita, J. Hideaki, Y. Haron, and M. Kiruko, *Nippon Daigaku Nojuigakubu Gakujutsu Kenkyu* Hokoku 1979, **36**, 1 (*Chem. Abstr.*, 1979, **90**, 199 159).
[33] N. Rhugenda-Banga, A. Welter, J. Jadot, and J. Casimir, *Phytochemistry*, 1979, **18**, 482.
[34] L. Fellows, E. A. Bell, T. S. Lee, and D. H. Janzen, *Phytochemistry*, 1979, **18**, 1333.
[35] T. Takemoto, K. Nomoto, S. Fushiya, R. Ouchi, G. Kusano, H. Hikino, S. Takagi, Y. Matsuura, and M. Kakudo, *Proc. Jpn. Acad., Ser. B*, 1978, **54**, 469.
[36] M. Marlier, G. Dardenne, and J. Casimir, *Phytochemistry*, 1979, **18**, 479.
[37] F. Chioccara, G. Misuraca, E. Novellino, and G. Prota, *Tetrahedron Lett.*, 1979, 3181.
[38] K. Shimada and T. Nambara, *Tetrahedron Lett.*, 1979, 163.
[39] J. L. Firmin and G. R. Fenwick, *Nature* (*London*), 1978, **276**, 842.

derivatives. *N*-[(9-β-D-ribofuranosyl-2-methylthiopurin-6-yl)carbamoyl] threonine is not only a new nucleoside (rabbit liver transfer RNA) but also represents the first example of a mammalian tRNA carrying a methylthio-substituent.[40]

Recurring topics in this section in previous volumes have been novel cross-links in proteins and novel amino-acids found in peptide antibiotics. In the former category, the structure determination and synthesis of anabilysine, previously reported in preliminary communication form (Vol. 10, p. 5), have been fully described,[41] and the existence of dityrosine cross-links in elastin and structural glycoproteins from young chicks [42-44] has been reported. A simple synthesis of this bis(amino-acid) involves the oxidation of L-tyrosine with hydrogen peroxide in the presence of horseradish peroxidase.[44] Desmosine cross-linked elastin carries a second cross-link, possibly lysinonorleucine, about 35 amino-acid residues removed from the desmosine cross-link;[45] a new desmosine isomer, photodesmosine (7) is formed (*via* pyridinium ring-opening) through irradiation of desmosine at



(7) $R^1 = H$, $R^2 = (CH_2)_2CH(\overset{+}{N}H_3)CO_2^-$
$n = 2$, $m = 3$

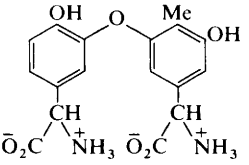
254 nm.[46] Ovamucoid proteins treated with alkali and by cyanolysis contain lysinoalanine cross-links, demonstrated through the isolation of N^{ε}-(DL-2-amino-2-carboxyethyl)-L-lysine from hydrolysates.[47] Lanthionine is also present.[47]

The *C*-terminal residue of the lipophilic undecapeptide rhodotorucine A is *S-trans, trans*-farnesyl-L-cysteine.[48]

Further reports from research groups who have found themselves studying the same antibiotic (ristocetin is identical with ristomycin A) have settled the structure of the bis(phenylglycine) moiety (8) (see Vol. 11, p. 5) of this condensed peptide antibiotic[49, 50] and of the biphenyl homologue actinoidinoic acid (2',4,6-trihydroxybiphenyl-2,5'-diyl)diglycinate,[50, 51] which is also present. This latter component has the *S*-configuration and is at the *N*-terminus of the antibiotic.[52] A

[40] Z. Yamaizumi, S. Nishimura, K. Limburg, M. Raba, H. J. Gross, P. F. Crain, and J. A. McCloskey, *J. Am. Chem. Soc.*, 1979, **101**, 2224.
[41] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2282.
[42] V. Malanik and M. Ledvina, *Connect. Tissue Res.*, 1979, **6**, 235 (*Chem. Abstr.*, 1979, **91**, 118996).
[43] V. Malanik and M. Ledvina, *J. Chromatogr.*, 1979, **170**, 254.
[44] V. Malanik and M. Ledvina, *Prep. Biochem.*, 1979, **9**, 273.
[45] R. P. Mecham and J. A. Foster, *Biochem. J.*, 1978, **173**, 617.
[46] J. F. Larochelle and F. Lamy, *Biochem. Biophys. Acta*, 1979, **584**, 327.
[47] R. G. Walsh, A. S. Nashef, and R. E. Feeney, *Int. J. Pept. Protein Res.*, 1979, **14**, 290.
[48] Y. Kamiya, A. Sakurai, S. Tamura, and N. Takahashi, *Agric. Biol. Chem.*, 1979, **43**, 1049.
[49] T. M. Harris, C. M. Harris, J. R. Fehlner, R. Bognar, and F. Sztaricskai, *J. Org. Chem.*, 1979, **44**, 1009.
[50] C. M. Harris, J. J. Kibly, J. R. Fehlner, A. B. Raabe, T. A. Barber, and T. M. Harris, *J. Am. Chem. Soc.*, 1979, **101**, 437.
[51] D. H. Williams, V. Rajananda, and J. R. Kalman, *J. Chem. Soc., Perkin Trans. 1*, 1979, 737.
[52] D. H. Williams, V. Rajananda, G. Bojesen, and M. P. Williamson, *J. Chem. Soc., Chem. Commun.*, 1979, 906.

triaminotricarboxylic acid (9; R′ = H) from the same family of antibiotics is built up from β-hydroxychlorotyrosine units,[53, 54] and actinoidin differs from vancomycin in carrying one fewer chlorine atom in this portion of the molecule.[53]



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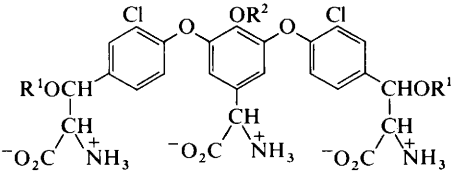


(9)

3 Chemical Synthesis and Resolution of Amino-acids

General Methods of Synthesis of α-Amino-acids.—Established methods continue to provide reliable preparative routes to a wide variety of α-amino-acids; the acetamidomalonate route ($RX + AcNHCH(CO_2Et)_2 \rightarrow AcNHCR(CO_2Et)_2 \rightarrow H_3\overset{+}{N}CHRCO_2^-$) has been used perhaps more than any other general route, as represented in the 1979 literature. The synthesis of amino-acids by this route is mentioned elsewhere in this Chapter.[21, 55, 89, 125, 147, 162] The use of the analogue $R^1CONHCR(OAc)CO_2Et$ in the synthesis of 2-substituted α-amino-acids and dehydro-amino-acids has been described.[56] A further example of the introduction of a variation of a standard method is the conversion of an α-bromoalkanoic acid into the α-isocyanato analogue with a metal isocyanate, followed by conversion into the corresponding α-amino-acid (in yields of 91—99%) *via* the carbamates.[57]

Aldehydes offer a variety of entries to amino-acids, and as well as standard Strecker procedures[90] (*cf.* earlier volumes) the conversion of aldoximes into aminomalononitriles and thence into amino-acids by hydrolysis[58] and Zelinsky–Stadnikoff synthesis illustrated for the synthesis of DL-serine from glycollaldehyde and masked equivalents[59] have been described. Alkylation of a variety of substrates by ethyl 3-bromo-2-hydroxyiminopropanoate in the presence

[53] F. Sztaricskai, C. M. Harris, and T. M. Harris, *Tetrahedron Lett.*, 1979, 2861.
[54] G. S. Katrukha, B. Diarra, A. B. Silaev, Z. P. Trifonova, B. V. Rozynov, and O. S. Rashetova, *Antibiotiki*, 1979, **24**, 179.
[55] G. R. Pettit and T. S. Krupa, *J. Org. Chem.*, 1979, **44**, 396.
[56] Y. Ozaki, T. Iwasaki, H. Horikawa, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, 1979, **44**, 391.
[57] F. Effenberger and K. Drauz, *Angew. Chem.*, 1979, **91**, 504.
[58] S. Y. Sizov, L. V. Semenova, and N. P. Utrobin, *Prikl. Biokhim. Mikrobiol.*, 1978, **14**, 915.
[59] L. Bassagnani, B. Biancini, A. Brandt, V. Caciagli, G. E. Bianchi, L. Re, A. Rossodivita, and P. Zappelli, *Chem. Ber.*, 1979, **112**, 148.

of sodium carbonate, followed by aluminium amalgam reduction, provides a general route to α-amino-acid esters of the β-substituted alanine family:[60]

$$BrCH_2C(=NOH)CO_2Et \longrightarrow RCH_2C(=NOH)CO_2Et \longrightarrow RCH_2CH(NH_2)CO_2Et$$

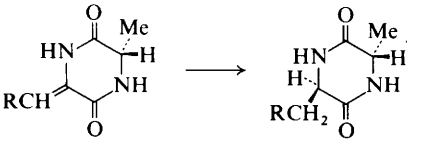
Synthesis of β-Amino-acids and Higher Homologues.—General routes to higher homologous amino-acids are not easily classified, and, as in earlier years, a crop of papers has been collected from the recent literature, each describing a specific class of ω-aminoalkanoic acid.

A convenient general synthesis of β-amino-acids[61] employs conjugate addition of hydroxylamine to αβ-unsaturated alkanoic acids, followed by hydrogenation over a palladium catalyst. A new synthesis of L-4-amino-2-hydroxybutanoic acid[62] starts from L-asparagine, and 4-aminobut-2-enoic acids have been obtained for evaluation as analogues of γ-aminobutyric acid, using αβ-unsaturated alkanoic acids as starting materials.[63]

Asymmetric Synthesis of α-Amino-acids.—Further development of methods fully described in previous volumes has been reported. A general review[64] and a review of catalytic asymmetric hydrogenation of dehydro-amino-acids[65] have appeared.

Homogeneous asymmetric catalytic hydrogenation of 2-acylaminoalken-2-oic acids using rhodium–chiral phosphine catalysts continues to be studied in several laboratories.[65–69] High optical yields (*e.g.* 90 ± 3%[66]) are being secured. The hydrogenation of unsaturated azlactones (4-alkylidene- and 4-arylidene-oxazolin-5-ones) over palladium in the presence of *S*-1-phenylethylamine gives modest asymmetric induction,[70] L-tyrosine and its *O*-methyl derivative being obtained with enantiomeric excesses up to 60%. An alternative approach, in which a chiral derivative of the acylaminoalkenoic acid is subjected to palladium-catalysed hydrogenation, leads to extraordinarily high (99%) asymmetric induction for cyclodipeptides (10) → (11).[71] A remaining variation of the asymmetric hydrogenation route, in which palladium-catalysed hydrogenation of a Schiff base formed between an α-keto-ester and a chiral benzylamine leads to an α-amino-acid with optical purity reaching *ca.* 60%, has been illustrated further for the asymmetric synthesis of L-alanine using *R*-phenylglycine.[72] An alternative use of Schiff-base intermediates, obtained from a chiral benzylamine and an aldehyde, has been illustrated for the asymmetric synthesis of *S*-(+)-2-amino-3-(1-adamantyl) pro-

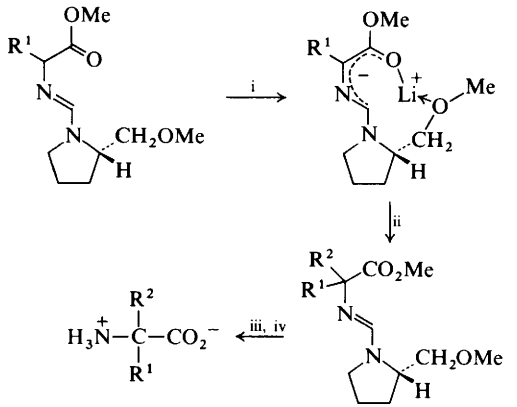
[60] T. L. Gilchrist, D. A. Lingham, and T. G. Roberts, *J. Chem. Soc., Chem. Commun.*, 1979, 1089.
[61] K. Basheeruddin, A. A. Siddiqui, N. H. Khan, and S. Saleha, *Synth. Commun.*, 1979, **9**, 705.
[62] T. Yoneta, S. Shibahara, S. Fukatsu, and S. Seki, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 3296.
[63] R. D. Allan and B. Twitchin, *Aust. J. Chem.*, 1978, **31**, 2283.
[64] N. Ikota and T. Shioiri, *Kogaku no Ryoiki*, 1979, **33**, 507.
[65] Y. Sugi, *Yuki Gosei Kagaku Kyokaishi*, 1979, **37**, 71.
[66] M. D. Fryzuk and B. Bosnich, *J. Am. Chem. Soc.*, 1978, **100**, 5491.
[67] H. Brunner and W. Pieronczyk, *Angew. Chem.*, 1979, **91**, 655.
[68] K. Onuma, T. Ito, and A. Nakamura, *Tetrahedron Lett.*, 1979, 3163.
[69] D. Lafont, D. Sinou, and G. Descotes, *J. Organomet. Chem.*, 1979, **169**, 87.
[70] E. S. Neupokoeva, E. I. Karpeiskaya, L. F. Godunova, and E. I. Klabunovskii, *Izv. Akad. Nauk SSSR.*, 1979, 139; E. S. Neupokoeva, E. I. Karpeiskaya, L. F. Godunova, and E. I. Klabunovskii, *Izv. Akad. Nauk SSSR*, 146.
[71] T. Kanmera, S. Lee, H. Aoyagi, and N. Izumiya, *Tetrahedron Lett.*, 1979, 4483; S. Lee, T. Kanmera, H. Aoyagi, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1979, **13**, 207.
[72] K. Harada and M. Tamura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1227.



(10) (11)

pionic acid.[73] Asymmetric addition of HCN to the Schiff base and following stages have been established earlier (see Vol. 10, p. 5).[73]

Asymmetric alkylation of (+)-bornyl or (−)-menthyl isocyanoacetates under phase-transfer catalysis conditions, followed by conventional reactions, has been shown to give products of moderate optical purity (*e.g.* L-alanine, 48% optical purity).[74] Alkylation of Schiff bases formed between an α-amino-acid ester and *S*-(−)-1-dimethoxymethyl-2-methoxymethylpyrrolidine (Scheme 1) occurs with a moderate degree of asymmetric induction (highest enantiomeric excess 50%),[75] dependent on the size of the electrophile R^2X (enantiomeric excess only 5% with R^2 = 3,4-$(OMe)_2C_6H_3CH_2$).[75] The stereochemistry of the diastereoisomer formed predominantly implies preferential *re*-face approach of the electrophile towards the anion. An advantage of the route displayed in Scheme 1 is the recovery of the chiral reagent.[75]



Reagents: i, $LiNPr^i_2$; ii, R^2X; iii, $MeOH–H_2O$, 25 °C; iv, 3M-HCl, reflux 60 h, neutralize

Scheme 1

Prebiotic Synthesis; Model Reactions.—Nearly 20 years after the experiment performed by Urey and Miller, and with many papers of a similar theme appearing in the intervening period, the formation of glycine, alanine, serine, aspartic acid, and glutamic acid in xenon lamp-irradiated mixtures of titanium dioxide, aqueous ammonia, and NH_4Cl has been described, a novel variation on the original system.[76] Many of the current studies of the same type, while employing ammonia

[73] K. Q. Do, P. Thanei, M. Caviezel, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 956.
[74] B. Laangstroem, B. Stridsberg, and G. Bergson, *Chem. Scripta*, 1979, **13**, 49.
[75] M. Kolb and J. Barth, *Tetrahedron Lett.*, 1979, 2999.
[76] H. Reiche and A. J. Bard, *J. Am. Chem Soc.*, 1979, **101**, 3127.

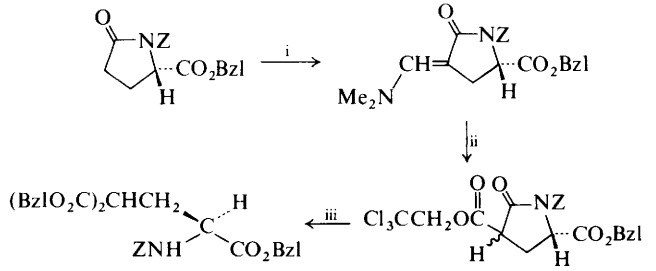
as the nitrogen source, choose some other simple organic compound and alternative energy source (ethanol subjected to neutron flux,[77] two-carbon hydrocarbons under electric discharge,[78] formaldehyde in sunlight,[79] glycollaldehyde, glyceraldehyde, or higher sugars,[80] metal salts of acetic acid [81] or citraconic, citraconamic, or itaconamic acids [82]). Further studies of the use of hydroxylamine (see Vol. 10, p. 8) and formaldehyde in the synthesis of glycine, serine, aspartic acid, and β-alanine have been reported.[83]

The other major area of chemistry, within which model reactions relevant to the prebiotic synthesis of amino-acids are being explored, is based on condensation and oligomerization of inorganic and organic cyanides. A complex, probably $H_2[(CN)_2$-$Cu_2NC \cdot CN(CN)_2] \cdot 2H_2O$, which is formed in solutions containing NaCN and $CuSO_4$, gives glycine on boiling in dilute hydrochloric acid.[84] Cyanamide–KNO_2 reaction products yield amino-acids, nucleosides, and other organic compounds on hydrolysis.[85] A polemical exchange against [86] and for [87] the oligomerization of HCN as the prebiotic origin of amino-acids and peptides highlights the main experimental facts and the possible interpretations. Earlier work on the formation of insoluble microspheres from aldehyde–cyanide reaction mixtures has been extended,[88] and the amino-acids released from this material have been shown to be formed in proportions dependent on the structures of the aldehydes.

Synthesis of Protein Amino-acids and Other Naturally Occurring Amino-acids.— General methods of synthesis of α-amino-acids, discussed in an earlier section, have been used routinely for the synthesis of natural amino-acids, further examples being a new synthesis of DL-δ-aminoadipic acid from diethyl acetamidomalonate and $I(CH_2)_3CN$[89] and Strecker synthesis of lysine and ornithine starting from 2-methoxy-*N*-acylpiperidine as a masked aldehyde.[90] The generation of an appropriate aldehyde for the synthesis of *erythro*-β-L-histidine, a component of the peptide antibiotic bleomycin, has been achieved starting from D-glucosamine.[91]

A simple synthesis of γ-carboxy-L-glutamic acid from L-pyroglutamic acid [92] (Scheme 2) is a substantial achievement. An alternative approach to this protein amino-acid starts from *N*-benzyloxycarbonyl-*O*-toluene-*p*-sulphonyl-DL-serine methyl ester, nucleophilic displacement of the toluene-*p*-sulphonyloxy group by di-

[77] K. Kawamoto, K. Kawai, H. Maki, and M. Akaboshi, *Ann. Repts. Res. React. Inst., Kyoto Univ.*, 1978, **11**, 147 (*Chem. Abstr.*, 1979, **91**, 5444).
[78] H. Ebisawa, E. Miyoshi, T. Shirai, and S. Yanagisawa, *Nippon Kagaku Kaishi*, 1979, 331 (*Chem. Abstr.*, 1979, **90**, 181958); H. Ebisawa, E. Miyoshi, T. Shirai, and S. Yanagisawa, *Nippon Kagaku Kaishi*, 1979, 1304.
[79] S. Ranganayaki and M. Srivastava, *Zh. Org. Khim.*, 1979, **15**, 1124.
[80] H. Yamagawa, Y. Kobayashi, and F. Egami, *J. Biochem. (Tokyo)*, 1980, **87**, 359.
[81] S. Ranganayaki, B. Srivastava, and U. Jyotishmati, *Anusandnan Patrika*, 1978, **21**, 185 (*Chem. Abstr.*, 1979, **90**, 181839).
[82] K. Harada and M. Matsuyama, *Biosystems*, 1979, **11**, 47.
[83] Kamaluddin, H. Yamagawa, and F. Egami, *J. Biochem. (Tokyo)*, 1979, **85**, 1503.
[84] M. Beck, V. Gaspar, and J. Ling, *Magy. Kem. Foly.*, 1979, **85**, 147.
[85] G. Wollin and W. B. F. Ryan, *Biochim. Biophys. Acta*, 1979, **584**, 493.
[86] J. P. Ferris, *Science*, 1979, **203**, 1135.
[87] C. N. Matthews, *Science*, 1979, **203**, 1136.
[88] G. E. Pollock and R. Heiderer, *J. Mol. Evol.*, 1979, **13**, 253.
[89] S. Wolfe and M. G. Jokinen, *Can. J. Chem.*, 1979, **57**, 1388.
[90] K. Warning, M. Mitzlaff, and H. Jensen, *Annalen*, 1978, 1707.
[91] S. M. Hecht, K. M. Rupprecht, and P. M. Jacobs, *J. Am. Chem. Soc.*, 1979, **101**, 3982.
[92] S. Danishefsky, E. Berman, L. A. Clizbe, and M. Hirama, *J. Am. Chem. Soc.*, 1979, **101**, 4385.



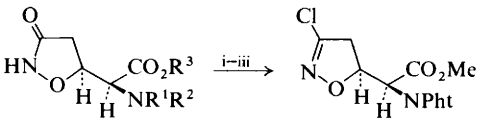
Reagents: i, $(Me_2N)_2CHOBu^t$; ii, $ClCO_2CH_2CCl_3$; iii, $PhCH_2OH$

Scheme 2

t-butyl malonate anion yielding the protected γ-carboxyglutamic acid.[93] A related side-chain modification approach has been used for the synthesis of L-(+)-discadenine and its desamino- and descarboxy-derivatives, from *N*-phthaloyl-4-bromo-2-aminobutanoic acid ethyl ester and adenines.[94]

2*S*-Amino-3*S*-phenylbutyric acid, synthesized from diethyl *R*-(1-phenylethyl) malonate *via* conventional bromination, decarboxylation, and amination stages, is identical with the amino-acid present in bottromycin, thus establishing its absolute configuration.[95]

A total synthesis of the antitumour antibiotic AT-125 (2-amino-3′-chloro-4′,5′-dihydroisoxazol-5′-ylacetic acid) has been reported (Scheme 3),[96] starting from tricholomic acid.



Tricholomic acid
$R^1 = R^2 = R^3 = H$

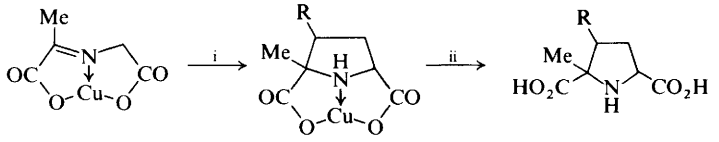
Pht = phthalimido

Reagents: i, *N*-carbethoxyphthalimide; ii, CH_2N_2; iii, $(Me_2N)_3PCl_2$–THF

Scheme 3

Further development of recently introduced synthetic routes has been described for the synthesis of L-aspartic acid, from *meso*-dibromosuccinic esters and *R*-1-phenylethylamine followed by hydrolysis and hydrogenolysis of the resulting chiral aziridinedicarboxylic esters (*cf.* Vol. 11, p. 8)[97], and a use of *N*-pyruvylideneglycinate copper(II) complexes (Scheme 4) in a new route to proline derivatives has been reported.[98]

[93] N. T. Boggs, B. Goldsmith, R. E. Gawley, K. A. Koehler, and R. G. Hiskey, *J. Org. Chem.*, 1979, **44**, 2262.
[94] F. Seela and D. Hasselmann, *Chem. Ber.*, 1979, **112**, 3072.
[95] G. Tsuchihashi, S. Mitamura, and H. Ogura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2167.
[96] R. C. Kelly, I. Schletter, S. J. Stern, and W. Wierenga, *J. Am. Chem. Soc.*, 1979, **101**, 1054.
[97] K. Harada and I. Nakamura, *Chem. Lett.*, 1978, 1171.
[98] L. Casella, M. Gullotti, A. Pasini, and R. Psaro, *Synthesis*, 1979, 150.



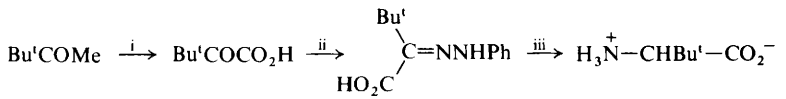
Reagents: i, $RCH{=}CH_2$, pyridine, NEt_3; ii, H_3O^+

Scheme 4

Conversion of glycine into L-serine through the use of microbial L-serine hydroxymethyltransferase[99] illustrates the possibilities for enzymic synthesis of protein amino-acids.

Synthesis of Aliphatic α-Amino-acids.—Amination of $CH_2{=}C(CO_2Et)CH(CO_2Et)_2$ with chloramine and NaH, followed by hydrolysis, yields β-methyleneaspartic acid[100] through a procedure which is only rarely brought into use in amino-acid synthesis.

A variation on a standard route to t-leucine is conveniently operated on a large scale (Scheme 5).[101]



Reagents: i, alkaline $KMnO_4$; ii, $PhNHNH_2$; iii, H_2–Pd

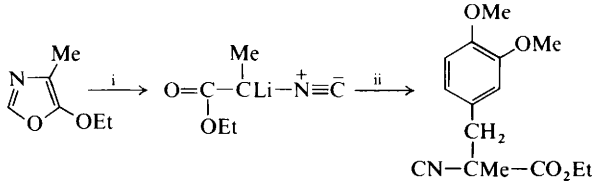
Scheme 5

Synthesis of α-Alkyl Analogues of Protein Amino-acids and of Other Natural Amino-acids.—Alkylation of 4-substituted oxazolin-5-ones, followed by hydrolysis, provides a general route to the title compounds.[102] A specific version of this route providing α-hydroxymethyl analogues through alkylation by formaldehyde has been used for the synthesis of *N*-benzoyl-*S*-benzyl-α-hydroxymethyl-L-cysteine (racemization ensues in this route if an optically active amino-acid is used as starting material, and the optically active product was obtained after resolution by quinine of the hydrolysed oxazolin-5-one).[103]

The dimethoxy analogue of α-methyldopa (α-methyl-3,4-dihydroxyphenylalanine) has been obtained from 4-methyl-5-ethoxyoxazole through alkylation of the lithiated α-isocyanopropionate formed on ring opening (Scheme 6).[104]

The carbon–carbon bond-forming potential of organocuprate reagents has now been applied to the synthesis of optically active α-alkyl- or α-aryl-amino-acids from *N*-toluene-*p*-sulphonyl-L-amino-acids.[105] Inversion of configuration accompanies the introduction of the substituent by a lithium dialkyl- or lithium diaryl-cuprate.

[99] M. Ema, T. Kakimoto, and I. Chibata, *Appl. Environ. Microbiol.*, 1979, **37**, 1053.
[100] P. Dowd and C. Kaufman, *J. Org. Chem.*, 1979, **44**, 3956.
[101] T. Miyazawa, K. Takashima, Y. Mitsuda, T. Yamada, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1539.
[102] B. Kuebel, P. Gruber, R. Hurnaus, and W. Steglich, *Chem. Ber.*, 1979, **112**, 128.
[103] M. T. Leplawy and A. Olma, *Pol. J. Chem.*, 1979, **53**, 353 (*Chem. Abstr.*, 1979, **91**, 39815); Z. Kaminski, M. Leplawy, and J. Zobrocki, Pol. Patent 86403 (*Chem. Abstr.*, 1979, **91**, 21107).
[104] P. A. Jacobi, S.-N. Ueng, and D. Carr, *J. Org. Chem.*, 1979, **44**, 2042.
[105] S. Terashima, C. C. Tseng, and K. Koga, *Chem. Pharm. Bull.*, 1979, **27**, 747.



Reagents: i, BuLi; ii, 3,4-$(MeO)_2C_6H_3CH_2Br$

Scheme 6

Synthesis of α-Heteroatom-substituted α-Amino-acids.—Less new material has been reported recently in this area, perhaps because one major research group has invested its efforts into compiling an extensive review.[7] α-Alkoxy-α-amino-acid esters, formed by electrochemical alkoxylation of 2-acetamido-2-alkylmalonic half-esters (see Vol. 10, p. 11), are useful precursors of dehydro-amino-acids and of α-benzylthio-α-amino-acids,[106] and *N*-pyrimidinyl-amino-acids.[131]

Synthesis of Halogenoalkyl Amino-acids.—With the exception of a full account [107] of results published last year in preliminary form (Vol. 11, p. 11), describing the preparation of α-halogenomethyl-α-amino-acids from Schiff bases, all the work currently published concerns fluorine analogues of protein amino-acids. These have acquired special interest as potential enzyme inhibitors. A new synthesis of β-fluoroaspartic acid involves the diazotization of *meso*-diaminosuccinic acid by $NaNO_2$ in liquid HF.[108] A hydroxy-amino-acid (serine, threonine, or *threo*-β-hydroxyphenylalanine) yields the corresponding fluoro-amino-acid on treatment with SF_4 in liquid HF at −78 °C.[109] A novel route to β-fluoro-α-amino-acids [110] and ββ-difluoro-analogues [111] is based on the opening by HF of 3-carbethoxy-2-phenylazirine [110] or its 2-methyl analogue.[111] The azirines are conveniently prepared from β-azido-αβ-unsaturated esters.

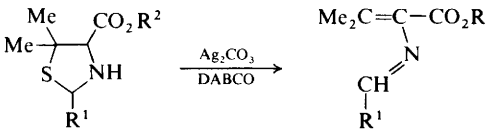
Synthesis of Aliphatic Amino-acids Carrying Hydroxy-groups in Side Chains.—A crop of papers has appeared dealing with the synthesis of β-hydroxy-α-amino-acids. *erythro*-Diastereoisomers are obtained through the reaction of a ketone with *NN*-bis(trimethylsilyl)glycine trimethylsilylester carbanion,[112] while *threo*-diastereoisomers are formed from *N*-benzyloxycarbonylglycine ethyl ester on treatment with $LiNPr^i_2$ and a ketone *via* the derived *trans*-oxazolidin-2-one [112] or from the *trans*-oxazoline formed between an aldehyde and isocyanoacetamide.[113] An asymmetric synthesis of L-threonine uses *erythro*-2,3-dibromobutanoic acid and *S*-1-phenylethylamine, cleavage of the resulting *N*-1-phenylethylaziridine with aqueous acid, and hydrogenolysis (H_2–Pd) of the *N*-substituent (see also Vol. 11, p. 8);[114] the required stereoisomer is accompanied by *ca.* 15% of the *erythro*-

[106] T. Iwasaki, H. Horikawa, K. Matsumoto, and M. Miyoshi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 826.
[107] P. Bey, J. P. Vevert, V. Van Dorsselaer, and M. Kolb, *J. Org. Chem.*, 1979, **44**, 2732.
[108] K. Matsumoto, Y. Ozaki, T. Iwasaki, H. Horikawa, and M. Miyoshi, *Experientia*, 1979, **35**, 850.
[109] J. Kollonitsch, S. Marburg, and L. M. Perkins, *J. Org. Chem.*, 1979, **44**, 771.
[110] T. N. Wade, F. Gaymard, and R. Guedj, *Tetrahedron Lett.*, 1979, 2681.
[111] T. N. Wade and R. Guedj, *Tetrahedron Lett.*, 1979, 3953.
[112] A. Shanzer, L. Somekh, and D. Butina, *J. Org. Chem.*, 1979, **44**, 3967.
[113] Y. Ozaki, S. Maeda, M. Miyoshi, and K. Matsumoto, *Synthesis*, 1979, 216.
[114] I. Nakamura and K. Harada, *Chem. Lett.*, 1979, 313.

diastereoisomer (L-isothreonine).[114] A synthesis of D-isothreonine from L-threonine follows conventional steps (replacement of NH_2 by Br with retention of configuration using NOBr, and amination with NH_4OH) but the stereospecificity is ascribed to the intermediacy of the 2*R*,3*R*-oxiran.[115] Diastereoisomers of β-hydroxy-L-aspartic acid formed from cysteine sulphinate and dihydroxyfumaric acid mediated by aspartate aminotransferase can be separated without difficulty by ion-exchange chromatography.[116]

Borane reduction of *N*-phthaloyl-γ-carboxy-DL-glutamic acid α-methyl ester, followed by de-protection, gives 5,5′-dihydroxy-DL-leucine.[117]

Synthesis of α-Amino-acids with Unsaturated Side Chains.—The so-called 'dehydro-amino-acids', *alias* 2-amino alken-2-oic acids, continue to receive more attention than their alkenyl isomers, or alkynyl analogues, even though the latter classes are important as potential enzyme inhibitors. Some particularly interesting synthetic work has been described in the recent literature, concerning dehydro-amino-acids, notably the first synthesis of an *N*-arylidene derivative (12) from a thiazolidine-5-carboxylic-ester[118] and the condensation of ethyl cyanoformate with an active methylene compound in the presence of $ZnCl_2$ and triethylamine [$EtO_2CCN + XCH_2Y \rightarrow H_2NC(=CXY)CO_2Et$].[119]



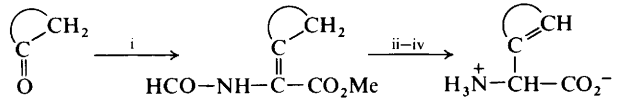
(12)

A variation of the synthesis of dehydro-amino-acids *via* 4-alkylidene- or 4-arylidene-oxazolin-5-ones has been described,[120] in which the intermediates are obtained from 2-phenyl-5-trimethylsilyloxyoxazole by condensation with an aldehyde or ketone in CH_2Cl_2 in the presence of $SnCl_4$, or with the corresponding acetal under similarly mild conditions. This procedure will be useful with base-sensitive substrates for which the classical route *via* 2-phenyloxazolin-5-one would be unusable.

A route to dehydro-amino-acids from 2-azidoalken-2-oate esters,[121] and another from α-keto-esters involving condensation with benzyl carbamate[122] ($R^1CH_2COCO_2Et + PhCH_2OCONH_2 \rightarrow ZNHC(=CHR^1)CO_2Et$), have been explored; the latter appears particularly promising but will depend on suitable methods for selective removal of protecting groups. Double-bond migration also occurs in a condensation of cyclic ketones with methyl isocyanoacetate (Scheme 7),[123] possibly at the saponification stage, where proton abstraction by the

[115] Y. Shimohigashi, M. Waki, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 949.
[116] W. T. Jenkins, *Anal. Biochem.*, 1979, **93**, 134.
[117] S. Bory, M. Gaudry, A. Marquet, and R. Azerad, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 85.
[118] E. Oehler and U. Schmidt, *Chem. Ber.*, 1979, **112**, 107.
[119] T. Iimori, Y. Nii, T. Izawa, S. Kobayashi, and M. Ohno, *Tetrahedron Lett.*, 1979, 2525.
[120] H. Takagaki, S. Tanabe, M. Asaoka, and H. Takei, *Chem. Lett.*, 1979, 347.
[121] C. G. Shin, Y. Yonezawa, K. Unoki, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1657.
[122] C. Shin, Y. Yonezawa, K. Unoki, and J. Yoshimura, *Tetrahedron Lett.*, 1979, 1049.
[123] K. Nunami, M. Suzuki, and N. Yoneda, *J. Chem. Soc. Perkin Trans. 1*, 1979, 2224.

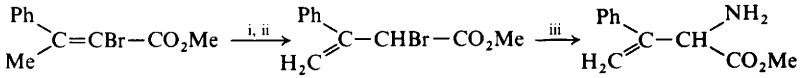
carboxylate anion could explain the production of $\beta\gamma$-unsaturated α-amino-acids.[123]



Reagents: i, $CNCH_2CO_2Me$; ii, 2M-KOH in MeOH, 50 °C; iii, HCl (aq) in THF; iv, neutralization

Scheme 7

Another new approach to this class of amino-acid depends on bond migration in 2-bromoalk-2-enoate esters (Scheme 8).[124]



Reagents: i, $LiNPr^i_2$–THF, −78 °C; ii, 2.5% HCl (aq); iii, NH_3–DMSO, 24 °C

Scheme 8

Alkylation of diethylacetamidomalonate with a non-conjugated diene, using palladium(II) acetate with PPh_3 and sodium phenoxide as catalyst system, gives alka-4,ω-dienyl-amino-acids;[125] alkylation by allylic alcohols or chlorides can also be achieved with this system.[125]

'α-Acetylenic' α-amino-acids can be obtained by carboxylation of the di-anion of t-butyl *N*-trimethylsilyl prop-2-ynylcarbamate, followed by methylation (CH_2N_2), anion formation, and alkylation with an alkyl halide.[126] L-Propargylglycine has been prepared from diethyl acetamidomalonate.[127]

Synthesis of Aromatic and Heterocyclic α-Amino-acids.—This section excludes compounds in which amino- and carboxy-groups are substituted directly on aromatic or heteroaromatic systems, but covers arylglycines ($H_3N^+CHArCO_2^-$) and relatives of the naturally occurring aromatic and heterocyclic amino-acids.

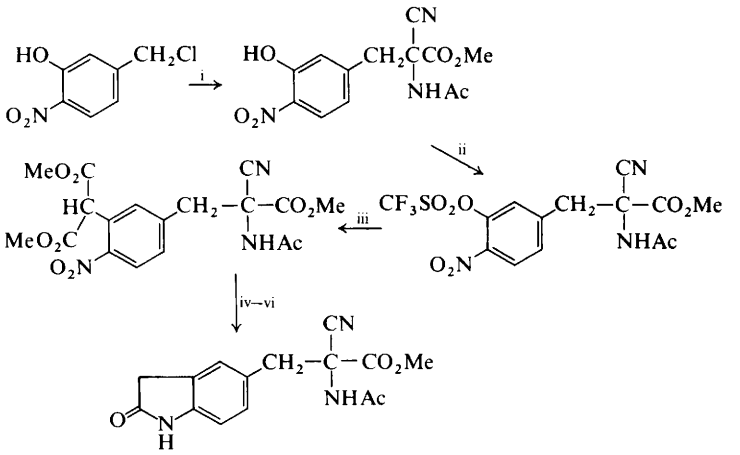
Phase-transfer catalysed conversion of benzaldehydes RC_6H_4CHO into arylglycines (R = H, 4-Cl, 4-F, 3-F, 4-MeO, 4-Me) by reaction with NH_3 and $CHCl_3$[128] competes with well established routes to these compounds.

An example of microbial synthesis relevant to this section is the conversion of L-tyrosine into L-dopa by *Actinomycetes*.[129]

The simplest heterocyclic amino-acid for which new work has been published in 1979 is pyrrol-1-ine-5-carboxylic acid, the form in which L-glutamic acid γ-semialdehyde is isolated after its formation from L-ornithine by enzyme-catalysed oxidation.[130] A range of α-pyrimidinyl-amino-acids has been obtained through

[124] R. V. J. Chari and J. Wemple, *Tetrahedron Lett.*, 1979, 111.
[125] J. P. Haudegoud, Y. Chauvin, and D. Commerenc, *J. Org. Chem.*, 1979, **44**, 3063.
[126] B. W. Metcalf and E. Bonilavri, *J. Chem. Soc., Chem. Commun.*, 1978, 915 (α-vinyl-α-amino-acids); B. W. Metcalf and P. Sasara, *J. Chem. Soc., Chem. Commun.*, 1979, 119.
[127] J. L. Fauchere, O. Leukart, A. Eberle, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 1385.
[128] D. Landini, F. Montanari, and F. Rolla, *Synthesis*, 1979, 26.
[129] C. P. Sukumaran, D. V. Singh, P. D. Khedkar, and P. R. Mahadevan, *J. Bioscience*, 1979, **1**, 235.
[130] D. J. Hayzer, R. V. Kirshna, and R. Margraff, *Anal. Biochem.*, 1979, **96**, 94.

reaction of *N*-chloropyrimidines with *N*-acyl-α-acetoxy-α-amino-acid esters,[131] and several β-heteroaryl-α-methylalanines have been prepared through alkylation of the potassium salt of *N*-benzylidenealanine methyl ester with chloromethyl-heterocycles.[132] A different approach has been used for the synthesis of 2-selenienylalanine [2-amino-3-(selenophen-2-yl)propanoic acid], in which the heterocyclic moiety is built on to *N*-acetylpropargylglycine ethyl ester;[133] the same starting material is used in an improved synthesis of L-*o*-carboranylalanine.[127] The heterocyclic side chain is constructed through novel transformation of the *o*-nitrophenol group of 3′-hydroxy-4′-nitrophenylalanine, in a synthesis of 5-(oxindolyl)alanine in improved (25%) yield.[134] The route (Scheme 9) has also been used for the synthesis of the α-methyl analogues of this amino-



Reagents: i, $MeCONHCH(CN)CO_2Me$; ii, $(CF_3SO_2)_2O$; iii, dimethyl malonate; iv, NaOH (aq); v, H_3O^+; vi, $SnCl_2$–HCl

Scheme 9

acid,[134] using methyl 2-isocyanopropionate as reagent (i) in the synthesis in place of acetamidocyano-acetate. A superficially similar approach is used in the synthesis of tryptophans from nitrotoluenes, through Michael addition of diethyl formamido-malonate to the α-nitroarylacrolein followed by reduction of the nitro-group and indolization.[135] A modified procedure using methyl nitroacetate gives *R*-6-methyltryptophan in 82% enantiomeric excess *via* the heteroarylidene-glycine ester and hydrogenation catalysed by a Rh-chiral phosphine catalyst.[135]

Full details have been published of the synthesis of anabilysine [1-(5-amino-5-carboxypentyl)pyridinium chloride][136] (see Vol. 10, p. 5).

[131] T. Nishitani, T. Iwasaki, Y. Mushika, and M. Miyoshi, *J. Org. Chem.*, 1979, **44**, 2019.
[132] J. W. Tilley, P. Levitan, and R. W. Kierstead, *J. Heterocycl. Chem.*, 1979, **16**, 333.
[133] P. M. Jacobs and M. A. Davis, *J. Org. Chem.*, 1979, **44**, 178.
[134] J. G. Atkinson, B. K. Watson, J. J. Fuenles, Y. Girard, and C. S. Rooney, *Tetrahedron Lett.*, 1979, 2857.
[135] U. Hengartner, A. D. Batcho, J. F. Blount, W. Leimgruber, M. E. Larscheid, and J. W. Scott, *J. Org. Chem.*, 1979, **44**, 3748.
[136] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2282.

Synthesis of *N*-Substituted α-Amino-acids.—As in previous volumes, this section deals with compounds of potential biological interest, but excludes *N*-protected amino-acids prepared for peptide synthesis. *N*-Nitroso-amines can be converted into *N*-nitroso-amino-acids through successive anion formation ($LiNPr^i_2$) and reaction with methyl chloroformate.[137]

Synthesis of Aminoxy-acids.—These compounds are being used as enzyme inhibitors[138] and for the synthesis of analogues of physiologically active peptides,[139] and can be obtained in optically active form from D- or L-amino-acids *via* the corresponding α-bromo-acids, using an *N*-alkoxycarbonylhydroxylamine and NaH.[139]

Synthesis of α-Amino-acids Containing Sulphur, Selenium, or Tellurium.—Whereas the lighter chalcogens have been represented in earlier volumes, and examples are included elsewhere in this Chapter, this section is reserved this year for the reports on synthesis of DL-telluromethionine, $MeTe(CH_2)_2CH(NH_3^+)CO_2^-$,[140] and its phenyl analogue,[141] formed from 2-bromoethylhydantoin and methaneteullurol and tellurophenol, respectively.

Synthesis of Phosphorus-containing α-Amino-acids.—DL-Phosphinothricin, $MeP(O)(OH)(CH_2)_2CH(NH_3^+)CO_2^-$, has been prepared through Strecker synthesis using $MeP(O)(OEt)CH_2CHO$.[142]

Amino-acids Synthesized for the First Time.—New amino-acids not mentioned elsewhere in this Chapter are collected here:

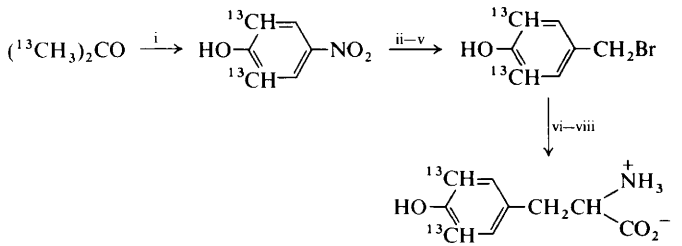
Compound	*Ref.*
DL-2-Amino-3-carboxymethylbutyric acid	143
DL- and *meso*-β-Aminoaspartic acid	144
DL-β-(Diazoacetyl)alanine	145
DL-β-(*O*-DL-Serylacetyl)alanine	145
DL-β-(*O*-DL-Threonylacetyl)alanine	145
DL-*S*-(Trialkylstannyl)cysteines	146
DL-*S*-(Trialkylstannyl)homocysteines	146
DL-*p*-Benzylphenylalanine	147
L-Tryptophan analogue with O in place of indole NH	148
L-Tryptophan analogue with S in place of indole NH	148
6-Fluoro-L-tryptophan	148
5-Fluoro-L-tryptophan	148
Tropolone analogue of DL-mimosine [DL-α-amino-β-(2-hydroxy-5-methyl-3-oxo-cyclohepta-1,4,6-trienyl)propanoic acid]	149
Tropolone analogue of DL-mimosine [DL-α-amino-β-(3,5-disubstituted 4-oxo-cyclohepta-1,4,6-trienyl propanoic acid]	150

[137] K. Piotrowska, *Synthetic Commun.*, 1979, **9**, 765.
[138] N. Amrhein and H. Hollaender, *Planta*, 1979, **144**, 385.
[139] M. T. Briggs and J. S. Morley, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2138.
[140] F. F. Knapp, *J. Org. Chem.*, 1979, **44**, 1007.
[141] F. F. Knapp, K. R. Ambrose, and A. P. Callahan, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 157.
[142] E. Gruszecka, M. Soroka, and P. Mastalerz, *Pol. J. Chem.*, 1979, **53**, 937.
[143] S. Mishima and K. Sakai, *Nippon Kagaku Kaishi*, 1978, 1675 (*Chem. Abstr.*, 1979, **90**, 120958).
[144] Y. Ozaki, T. Iwasaki, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, 1979, **44**, 1714.
[145] P. K. Chang, L. B. Lachman, and R. E. Handschumacher, *Int. J. Pept. Protein Res.*, 1979, **14**, 27.
[146] P. J. Smith, R. L. Hyams, J. S. Brooks, and R. W. Clarkson, *J. Organomet. Chem.*, 1979, **171**, C29.
[147] W. Podkoscielny, M. Podgorski, and E. Smulkowska, *Pol. J. Chem.*, 1978, **52**, 2455.
[148] H. M. Rajh, J. H. Uitzetter, L. W. Westerhuis, C. L. van den Dries, and G. I. Tesser, *Int. J. Pept. Protein Res.*, 1979, **14**, 68.
[149] T. Teitei, *Aust. J. Chem.*, 1979, **32**, 1631.
[150] T. Teitei and R. L. N. Harris, *Aust. J. Chem.*, 1979, **32**, 1329.

Synthesis of Labelled Amino-acids.—A larger number of papers than usual have been sieved from the literature on which this Chapter is based, partly because the proceedings of two major conferences have been published in 1979.

Raney nickel-catalysed deuteriation of phenylalanine and tyrosine brings about total exchange of aromatic protons after extended reaction periods, except the protons *ortho* to CH_2; while the α-CH proton exchanges slowly, the β-CH_2 protons exchange rapidly.[151] Specific labelling leading to *R*-4-[4-^{2}H]aminobutyric acid through decarboxylation of L-glutamic acid in ^2H_2O implies retention of configuration.[152] The ^{3}H-analogue is obtained similarly, from L-glutamic acid in ^3H_2O in the presence of L-glutamate decarboxylase,[153] whereas *S*-[^{3}H]-γ-aminobutyric acid is prepared from L-alanine with L-alanine transaminase in ^3H_2O.[154] The lability of protons α to sulphimide sulphur has been exploited in a conversion of L-methionine into its C^2H_3 analogue *via* 'dehydro-L-methionine' in MeO^2H–MeONa.[155]

The whole range of carbon isotopic labelling has been represented. ^{11}C-Carboxy-labelled hippuric acid,[156] L-alanine,[157] DL-alanine,[158] DL-tryptophan,[159] and a series of amino-acids[160] have been prepared from $^{11}CO_2$ through various strategies. $^{13}CH_3{}^{13}CO_2H$ and $(^{13}CH_3)_2CO$ have been employed in syntheses of [2,3-$^{13}C_2$]-D- and L-alanines,[161] and [3,5-$^{13}C_2$]-DL-tyrosine (Scheme 10),[162]



Reagents: i, $NaC(CHO)_2NO_2$; ii, $SnCl_2$, HCl; iii, diazotization, $Cu(CN)_2$; iv, H_3O^+; v, PBr_3; vi, $MeCONHCNa(CO_2Et)_2$; vii, NaOH (aq); viii, H_3O^+

Scheme 10

respectively. Alkylation of diethylphthalimidomalonate with $PhtNCH_2CH_2O$-$^{14}CH_2CH_2Cl$ and subsequent conventional steps leads to ^{14}C-labelled DL-2-amino-4-(2-aminoethoxy)butanoic acid.[163]

[151] R. C. Woodworth and C. M. Dobson, *FEBS Lett.*, 1979, **101**, 329.
[152] M. Kauska and S. Drabarek, *Radiochem. Radioanal. Lett.*, 1979, **38**, 155.
[153] E. Santaniello, M. G. Kienle, A. Manzocchi, and E. Bosisio, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1677.
[154] G. Burnett, C. Walsh, K. Yonaha, S. Toyama, and K. Soda, *J. Chem. Soc., Chem. Commun.*, 1979, 826.
[155] D. C. Billington and B. T. Golding, *Tetrahedron Lett.*, 1979, 2937.
[156] S. J. Gatley, J. S. Crawford, J. R. Halama, R. D. Hichwa, M. T. Madsen, J. L. Martin, R. J. Nickles, and D. J. Simpkin, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 182.
[157] M. B. Cohen, L. Spolter, C. C. Chang, and N. S. MacDonald, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 63.
[158] B. Langstrom and B. Stridsberg, *Int. J. Appl. Radiat. Isotopes*, 1979, **30**, 151.
[159] L. C. Washburn, T. T. Sun, B. L. Byrd, R. L. Hayes, and T. A. Butler, *J. Nuclear Med.*, 1979, **20**, 857.
[160] L. C. Washburn, T. T. Sun, B. L. Byrd, R. L. Hayes, T. A. Butler, and A. P. Callahan, *Report 1979* abstracted in *Chem. Abstr.*, 1979, **91**, 170853.
[161] V. N. Kerr and D. G. Ott, *J. Labelled Comp. Radiopharm.*, 1978, **15**, 503.
[162] V. Viswanatha and V. J. Hruby, *J. Org. Chem.*, 1979, **44**, 2892.
[163] Y. Y. Liu, E. Thom, and A. A. Liebman, *Can. J. Chem.*, 1978, **56**, 2853.

Double labelling achieved through relatively intricate synthetic routes, to provide amino-acids for use in biosynthetic investigations, has featured less in the 1979 literature than in preceding years; the examples which have been reported, 4*S*-[5-^{3}H, 5-^{14}C]-L-leucine and its [2-^{14}C]-isomer,[164] were prepared by a modification of a previously described route,[165] starting from 2*R*-[1-^{3}H]-2-methyl-4-phenylbutan-1-ol.

[β-^{13}N]-L-Asparagine[166] and [β-^{15}N]-L-leucine[167] have been prepared by conventional methods, and one standard procedure is illustrated[168] in the formation of [^{15}N]-L-glutamic acid from 2-ketoglutaric acid, $^{15}NH_4Cl$, and glutamate dehydrogenase–alcohol dehydrogenase.

Both oxygen atoms of the carboxy-group of an α-amino-acid can be exchanged, to an extent >90%, with $H_2{}^{18}O$ at elevated temperatures.[169]

^{76}Br- and ^{77}Br-labelled bromo-L-dopa and bromo-5-hydroxytryptophan have been prepared from the amino-acids and metal bromides, using chloroperoxidase.[170]

Resolution of Amino-acids.—Diastereoisomeric 2,5-dioxomorpholines formed between D-$HOCH_2CMe_2CH(OH)CO_2H$ and DL-proline can be separated and hydrolysed to yield the two enantiomers, with return of chiral reagent.[171] A similar example of this familiar general approach is the acylation of a DL-amino-acid with (+)-*trans*-1-cyclohexanedicarboxylic anhydride, followed by fractional crystallization and hydrolysis.[172] Further examples of techniques which have been represented regularly in this section in previous volumes are preferential crystallization [the D-isomer from aqueous solutions of DL-*p*-$HOC_6H_4CH(NH_3{}^+)$-$CO_2HArSO_3{}^-$][173] and ligand-exchange chromatography (in which chloromethylated polystyrene is treated with a chiral amine, complexed with Cu^{2+} or Ni^{2+} ions, and used in the conventional column chromatography mode).[174,175] A novel variation of the ligand-exchange technique[176] employs an aqueous eluant containing a chiral copper(II)–proline complex for resolving DL-amino-acids by ion exchange and depends on the different stabilities of the diastereoisomeric amino-acid–copper(II) complexes. Partial asymmetric transformations of DL-α-amino-acids can be effected through reversible Schiff-base complex formation with cobalt(III) [1-(−)-menthyl β-(2-hydroxybenzoyl)propionate].[177]

[164] C. Fuganti, P. Grasselli, and G. Pedrocchi-Fantoni, *Tetrahedron Lett.*, 1979, 2453.
[165] R. Cardillo, C. Fuganti, D. Ghiringhelli, P. Graselli, and G. Gatti, *J. Chem. Soc., Chem. Commun.*, 1977, 474.
[166] D. R. Elmaleh, D. H. Hnatowich, and S. Kulprathipanja, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 92.
[167] R. A. Asaturyan, *Biol. Zh. Arm.*, 1978, **31**, 720 (*Chem. Abstr.*, 1979, **90**, 99 297).
[168] O. Bojan, M. Bologa, G. Niac, N. Palibroda, E. Vargha, and O. Barzu, *Anal. Biochem.*, 1979, **101**, 23.
[169] R. C. Murphy, F. A. Anderson, and K. L. Clay, in 'Stable Isotopes: Proceedings of the Third International Conference', ed. E. R. Klein and P. D. Klein, Academic Press, New York, 1979.
[170] A. M. Friedman, J. Cheronis, M. Zalutsky, M. Cooper, P. Hoffman, A. Heller, D. Israelstam, P. Herper, and T. Hill, *J. Labelled Comp. Radiopharm.*, 1979, **16**, 66.
[171] T. D. Marieva, V. M. Kopelovich, and V. I. Gunar, *Khim. Prir. Soedin.*, 1979, 106.
[172] K. Murakami, N. Katsuka, K. Takano, Y. Yamamoto, T. Kakegawa, K. Saigo, and H. Nohira, *Nippon Kagaku Kaishi*, 1979, 765.
[173] S. Yamada, C. Hongo, and I. Chibata, *Agric. Biol. Chem.*, 1978, **42**, 1521.
[174] I. A. Yamskov, B. B. Berezin, V. E. Tikhonov, and V. A. Davankov, *Biorg. Khim.*, 1979, **5**, 492.
[175] A. A. Kurganov, L. Y. Zhuchkova, and V. A. Davankov, *Makromol. Chem.*, 1979, **180**, 2101.
[176] P. E. Hare and E. Gil-Av, *Science*, 1979, **204**, 1226.
[177] Y. Numata, H. Okawa, and S. Kida, *Chem. Lett.*, 1979, 293.

Further results from Cram's group on the differential complexation of D-and L-amino-acid ester salts by macrocyclic chiral polyethers have been reported,[178] and the demonstration of preferential complexation of an amino-acid ester in the presence of simple amines by an acyclic phosphine oxide ligand[179] opens up the possibility of resolution by chiral ligands of this family.

Explanations for the enantioselection which favoured the L-enantiomers of amino-acids in prebiotic times continue to be sought. The rare occurrence of a constructive outcome to a polemical debate can be reported; stereospecific binding of L-leucine, L-aspartic acid, and D-glucose to colloidal clay (bentonite)[180] has been noted earlier,[181] and this correction of a claim for a first finding has also led to the reasoning that now the implicit chirality of clay should be verified by other means.[182,183] An important observation,[184] that left circularly polarized light (284 nm) is absorbed and re-emitted by D-tryptophan in MeOH, with appreciably higher fluorescence efficiency than is shown by the L-enantiomer, may imply that enhanced stereoselectivity should be observed in photoreactions of these enantiomers, where intramolecular energy transfer is involved. A role for chiral electromagnetic radiation in enantioselection has long been sought (see previous volumes) and there should be scope for further deductions and associated experimentation based on this result.

A theoretical discourse on those weak neutral current effects that are capable of ensuring that only one enantiomer of an amino-acid is selected in the formation of self-replicating molecules has been published.[185]

4 Physical and Stereochemical Studies of Amino-acids

Crystal Structures of Amino-acids and Their Derivatives.—Routine *X*-ray crystal-structure determinations, on which no further description is offered here, have been reported for γ-carboxy-L-glutamic acid,[186] α-methyl-DL-glutamic acid hemihydrate,[187] two crystal modifications of 3,5-dinitro-L-tyrosine,[188] *S*-carboxymethyl-L-cysteine,[189] sarcosine hydrochloride,[190] thienyl-DL-serine hydrate,[191] *N*-acetyl-L-4-hydroxyproline hydrate,[192] *N*-acetyl-DL-methionine diethyl- and *N*-acetyl-DL-ethylmethyl-amide,[193] *N*-acetyldehydroalanine,[194] *N*-t-butoxycarbonyldehydro-

[178] S. C. Peacock, L. A. Domeier, F. C. A. Gaeta, R. C. Helgeson, J. M. Timko, and D. J. Cram, *J. Am. Chem. Soc.*, 1978, **100**, 8190.
[179] A. H. Alberts, K. Timmer, J. G. Noltes, and A. L. Spek, *J. Am. Chem. Soc.*, 1979, **101**, 3375.
[180] S. C. Bondy and M. E. Harrington, *Science*, 1979, **203**, 1243.
[181] T. A. Jackson, *Science*, 1979, **206**, 483; *Science, Chem. Geol.*, 1971, **7**, 295.
[182] S. C. Bondy, *Science*, 1979, **206**, 484.
[183] D. Wellner, *Science*; 1979, **206**, 484.
[184] C. D. Tran and J. H. Fendler, *J. Am. Chem. Soc.*, 1979, **101**, 1285.
[185] K. Tennakone, *Prog. Theor. Phys.*, 1979, **62**, 841.
[186] K. A. Satyshur and S. T. Rao, *Acta Crystallogr.*, 1979, **B35**, 2260.
[187] C. Derricott and J. Trotter, *Acta Crystallogr.*, 1979, **B35**, 2230.
[188] V. Cody, D. A. Langs, and J. P. Hazel, *Acta Crystallogr.*, 1979, **B35**, 1829.
[189] A. D. Mighell, C. R. Hubbard, J. Harris, J. A. Staffa, and C. Zervis, *Acta Crystallogr.*, 1979, **B35**, 1258.
[190] S. C. Bhattacharyya and N. N. Saha, *J. Cryst. Mol. Struct.*, 1979, **8**, 105.
[191] S. Swaminathan and K. K. Chacko, *Acta Crystallogr.*, 1979, **B35**, 208.
[192] M. Hospital, C. Courseille, F. Leroy, and B. P. Roques, *Biopolymers*, 1979, **18**, 1141.
[193] A. Aubry, J. Protas, M. T. Cung, and M. Marraud, *Acta Crystallogr.*, 1979, **B35**, 2634.
[194] D. Ajo, G. Granozzi, E. Tondello, A. Del Pra, and G. Zanotti, *J. Chem. Soc., Perkin Trans. 2*, 1979, 927.

leucine (the *Z*-configuration is adopted),[195] creatine monohydrate,[196] and L-pyroglutamyl-*NN'*-dicyclohexylurea.[197]

The absolute configuration of (+)-coronamic acid is incorporated in its systematic name (+)-(1*S*,2*S*)-1-amino-2-ethylcyclopropanecarboxylic acid, and this result from *X*-ray crystal analysis corrects an earlier assignment (Vol. 10, p. 10) based on circular dichroism (c.d.) data.[198] A combined *X*-ray–i.r.–c.d. study of *N*-t-amyloxycarbonyl-L-proline,[199] and an *X*-ray–c.d. study of the four stereoisomers of *N*-acetyl-S-(2-nitro-1-phenylethyl)cysteine,[200] have been reported.

X-Ray analysis of the inclusion complex which separates from supersaturated solutions of *NN*-diethyl-β-alanine in benzene reveals the conformational features of the amino-acid derivative which facilitate complex formation.[201]

Recently reported *X*-ray and neutron-diffraction crystal structures of amino-acids have been studied [202] to determine the range of hydrogen-bonding distances involved.

N.M.R. Spectroscopy.—The continuing development of instrumental techniques is represented in the recent literature, which also contains reports of new results obtained with routine laboratory spectrometers.

Conformational studies dominate this section, with studies of the amino-acids themselves being outnumbered by studies of *N*-acyl amino-acid esters or amides to simulate the behaviour of side chains in peptides. ^{1}H-N.m.r. data of deuteriated DL-histidine at $p^2H = 8.2$ in ^2H_2O compared with data for the L-enantiomer permit the assignment of the lower and higher field resonances associated with the β-protons to the pro-*R*- and pro-*S*-protons, respectively.[203] DL-Carnitine studied under the same conditions has been shown [204] to adopt the *gauche* conformation about the C-3—C-4 bond and to undergo rapid rotation about the C-2—C-3 bond. *cis*-D-*allo*-Hydroxyproline [205] and *cis*-4-fluoro-L-proline,[206] the latter as its diketopiperazine derivative with D- or L-phenylalanine, have been studied; this project amounts to a re-investigation of ring conformations of the pyrrolidine amino-acids and reveals a γ-C-*endo*–δ-C-*exo*-envelope conformation for the former amino-acid.[205] ^{13}C-N.m.r. studies of glycine, alanine, and lysine in H_2O and ^2H_2O as a function of pH reveal ^{2}H isotope effects of up to 0.9 p.p.m.[207]

A comparison of vicinal proton coupling constants between α- and β-protons in amino-acids, with vicinal carboxylate ^{13}C–β-H coupling constants, has been made,

[195] V. S. Chauhan, C. H. Stammer, L. Norskov-Lauritzen, and M. G. Newton, *J. Chem. Soc., Chem. Commun.*, 1979, 412.
[196] Y. Kato, Y. Haimoto, and K. Sakurai, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 233.
[197] F. Bechtel, J. P. Bideau, and M. Cotrait, *Cryst. Struct. Commun.*, 1979, **8**, 815.
[198] A. Ichihara, K. Shiraishi, S. Sakamura, A. Furusaki, N. Hashiba, and T. Matsumoto, *Tetrahedron Lett.*, 1979, 365.
[199] E. Benedetti, A. Ciajolo, B. Di Blasio, V. Pavone, C. Pedone, C. Toniolo, and C. M. Bonora, *Int. J. Pept. Protein Res.*, 1979, **14**, 130.
[200] W. Winter, G. Heusel, H. Fouad, and G. Jung, *Chem. Ber.*, 1979, **112**, 3171.
[201] M. A. Peterson, H. Hope, and C. P. Nash, *J. Am. Chem. Soc.*, 1979, **101**, 946.
[202] S. N. Vinogradov, *Biopolymers*, 1979, **18**, 1559.
[203] M. Kainosho, K. Ajisaka, S. Sawada, M. Tanokura, and T. Miyazawa, *Chem. Lett.*, 1979, 395.
[204] G. Agostini, F. Coletta, A. Gambaro, and S. Castellano, *Spectrochim. Acta, Part A*, 1979, **35**, 733.
[205] M. J. O. Anteunis, P. Cuypers, and M. Budesinsky, *Bull. Soc. Chim. Belg.*, 1979, **88**, 147.
[206] M. J. O. Anteunis, P. Cuypers, B. Liberek, and A. Kolodziejczyk, *Bull. Soc. Chim. Belg.*, 1978, **87**, 877.
[207] J. J. Led and S. B. Petersen, *J. Magn. Reson.*, 1979, **33**, 603.

with the objective of improved conformational diagnosis in the amino-acid series.[208] Wide-line n.m.r. of amino-acids has received further attention with particular reference to their dynamic properties in solution.[209]

Conformational studies for *N*-acetyl-L-phenylalanine methyl ester[210] and *N*-acetyl- or *N*-benzoyl-L-proline methyl ester[211] have sought similar information in terms of side-chain structure and mobility. The use of lanthanide shift reagents shows[211] that the pyrrolidine ring in the proline derivatives in C^2HCl_3 is mainly (60%) in a half-chair conformation (C-γ up, C-β down, CO_2R up) and 40% in an envelope conformation (C-γ down). Self-association of *N*-acetyl-L-alanine methyl ester or methylamide[212] in solution takes the form of dimerization, with the N–H proton bonded intermolecularly to the carboxyl oxygen atom in the case of the ester, and the amide N–H proton bonded to the acetyl carbonyl oxygen atom in the case of the amide.[212] Self-association is also observed with *N*-acetyl-amino-acid diethylamides in CCl_4.[213]

The chemical shift of the amide proton in an α-acyl-amino-acid is in a linear relationship with the electronegativity of the acyl group [δ (p.p.m.) = $-0.22\,pK_a + 9.18$].[214] The comparable relationship between C=O stretching frequency and electron release by the acyl group was established several years ago.[215]

Significant deductions for other structural features from n.m.r. data have been reported for histidine,[216] where the small red shift in the visible absorption spectrum and associated ^{1}H-n.m.r. changes in the presence of the dye Rose Bengal suggest an interaction in solution between the imidazole ring and the dye, and for N^G-nitroarginine methyl ester hydrochloride, where ^{15}N- and ^{1}H-n.m.r. data reveal the adoption of the nitrimine tautomeric structure $H_2NC(=NNO_2)$-$NH(CH_2)_3$ $CH(NH_3^+)CO_2Me\ Cl^-$.[217]

O.R.D. and C.D. Spectra.—Routine uses of c.d. are represented in studies combining several spectroscopic techniques, *viz.* *X*-ray, i.r., and c.d. studies of *N*-t-amyloxycarbonyl-L-proline revealing solvation effects and solute–solute association,[199] and in the assignment of absolute configuration to (+)-coronamic acid.[198] There are lessons to be learned from these studies; c.d. data taken alone can lead to incorrect information on solution conformations of amino-acid derivatives,[199] and use of an empirical sector rule led to the wrong absolute configuration in the latter case.[198] Although a chirality rule has been proposed[218] to relate the sign of the Cotton effect near 330 nm with absolute configuration for the 1-pyrrolinones obtained from α-amino-acid esters with fluorescamine, a major

[208] R. B. Martin, *J. Phys. Chem.*, 1979, **33**, 2404.
[209] S. Ganapathy and R. Srinivasan, *Ind. J. Biochem. Biophys.*, 1979, **16**, 310.
[210] N. Nicolai and E. Tiezzi, *J. Phys. Chem.*, 1979, **83**, 3249.
[211] D. F. DeTar and N. P. Luthra, *J. Org. Chem.*, 1979, **44**, 3299.
[212] T. Asukura, M. Kamio, and A. Nishioka, *Biopolymers*, 1979, **18**, 467.
[213] K. Mizuno, S. Nishio, and Y. Shindo, *Biopolymers*, 1979, **18**, 693.
[214] Y. Shimohigashi, T. Kato, S. Kang, Y. Minematsu, M. Waki, and N. Izumiya, *Tetrahedron Lett.*, 1979, 1327.
[215] H. Determann, J. Heuer, P. Pfaender, and M.-L. Reinartz, *Liebigs Ann. Chem.*, 1966, **694**, 190.
[216] A. Sidorowicz, *Stud. Biophys.*, 1978, **73**, 185.
[217] D. Gust, G. Dirks, and G. R. Pettit, *J. Org. Chem.*, 1979, **44**, 314.
[218] V. Toome and B. Wegrzynski, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1496.

revision of this rule is needed since examples of false conclusions have been uncovered.[219]

Further examples of chromophoric derivatives of amino-acids have been reported, namely *N*-(2-pyrazinoyl)-α-amino-acid esters,[220] stereoisomers of *N*-acetyl-*S*-(2-nitro-1-phenylethyl)cysteine,[200] and the 2,4-dinitrophenyl *p*-methoxyanilides of the iturinic acids, $MeCHR(CH_2)_8CH(NH_2)CH_2CO_2H$ (R = Me, Et), released from iturin A on hydrolysis (c.d. data permit the assignment of the *R*-configuration at C-3).[221]

An analysis of the c.d. and magnetic c.d. of phenylalanine and its derivatives has been reported,[222] and in a different region of the electromagnetic spectrum an extension of work reported last year (Vol. 11, p. 20) on vibrational c.d. involves studies of L-alanine and L-serine in both solid and solution states.[223]

Mass Spectrometry.—The non-routine literature has moved entirely across to the applications of sophisticated instrumentation for gathering structural and analytical information. In the former category, developments of earlier studies have been reported on the determination of proton affinities of amino-acids [224] and of gas-phase acidity and basicity of glycine by ion cyclotron resonance.[225] In the latter category, all the papers cited here are based on chemical ionization mass spectrometry data for *N*-trifluoroacetyl amino-acid carboxybutyl esters,[226] protonated di-amino-acids,[227] trimethylsilylated amino-acids,[228] and thiohydantoins.[229] Further references to mass spectrometric analysis of amino-acids can be found in the later section covering gas–liquid chromatography.

Other Physical and Theoretical Studies.—Infrared and Raman spectrometric studies seem to have returned to greater favour in the amino-acid field, with studies of alanine in solid and solution states,[230] of *NN*-dibutylglycine in solution and vapour states,[231] of serine in solid [232] and solution states,[233] and *N*-acetyl methylamides of glycine, alanine, and leucine in $CHCl_3$ or CCl_4.[234] Points of interest from these studies include evidence from Raman spectra that L-serine exists in at least two different conformations in aqueous solutions,[233] and clear evidence for strong NH hydrogen bonding was not obtained for the C-7 (eq) conformation of an *N*-acetyl-amino-acid methylamide.[234]

[219] K. L. Kovacs, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 995.
[220] M. M. El-Abadelah, S. S. Sabri, A. A. Jarrar, and M. H. Abu Zarga, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2881.
[221] U. Nagai, F. Besson, and F. Peypoux, *Tetrahedron Lett.*, 1979, 2359.
[222] T. Komiyama and M. Miwa, *Chem. Phys. Lett.*, 1979, **65**, 136.
[223] M. Diem, E. Photos, H. Khouri, and L. A. Nafie, *J. Am. Chem. Soc.*, 1979, **101**, 6829.
[224] M. Meot-Ner, E. P. Hunter, and F. H. Field, *J. Am. Chem. Soc.*, 1979, **101**, 686.
[225] M. J. Locke, R. L. Hunter, and R. T. McIver, *J. Am. Chem. Soc.*, 1979, **101**, 272.
[226] E. E. Kingston and A. M. Duffield, *Biomed. Mass Spectrom.*, 1978, **5**, 621.
[227] R. J. Weinkam, *J. Org. Chem.*, 1978, **43**, 2531.
[228] H. Budzikiewicz and G. Meissner, *Org. Mass Spectrom.*, 1978, **13**, 608.
[229] K. Okada and A. Sakuno, *Org. Mass Spectrom.*, 1978, **13**, 535.
[230] M. Castineira and J. Herranz, *An. Quim.*, 1979, **75**, 40, 250.
[231] G. S. Denisov, I. G. Rumynskaya, and V. M. Shraiber, *Zh. Prikl. Spektrosk.*, 1979, **31**, 275.
[232] C. Madec, J. Lauransan, and C. Garrigou-Lagrange, *Can. J. Spectrosc.*, 1978, **23**, 166.
[233] C. Madec, J. Lauransan, and C. Garrigou-Lagrange, *C. R. Hebd. Seances Acad. Sci., ser. B*, 1979, **288**, 69.
[234] F. R. Maxfield, S. J. Leach, E. R. Stimson, S. P. Powers, and H. A. Scheraga, *Biopolymers*, 1979, **18**, 2507.

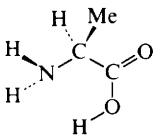
Polarized Raman spectra of a single crystal of α-glycine-C-$^2H_2$ and DL-alanine-α,β-$^2H_4$ have been determined.[235]

Photoelectron spectra of amino-acids have been surveyed,[236] and a new application of this technique to gaseous L-tryptophan has been reported.[237] *X*-Ray electron and Auger spectroscopic studies of glycine, alanine, and serine deposited on silver sheets from aqueous solutions have been described.[238]

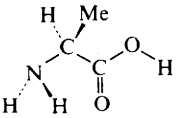
Solute–solvent interactions are open to study by ultrasonic absorption methods, and the pH dependence of the interaction of amino-acids with water has been studied.[239]

A range of titration studies have been reported, covering first and second dissociation constants of glycine in THF–H_2O mixtures,[240] p*K*–structure relationships for *N*-substituted α,ω-di-amino-acids,[241] self-association constants of L-amino-acids in aqueous solutions,[242] and demonstration of the effect of a 4-nitro-group on enhancing the acidity of histidine.[243]

Molecular orbital calculations include a topic related to the experimental studies mentioned in the preceding paragraph, a ranking of side-chain proton affinities Arg > His > Ser ≈ Tyr > Lys.[244] Apart from a study in which experimental data on geminal carboxyl-^{13}C–H coupling constants $^3J(C^1H)$ are compared with calculations for α-amino-acids,[245] the other theoretical papers collected here are concerned with conformational assignments. *N*-Acetyl-amino-acid methylamides have available to them a number of minimum-energy conformations when varying hydration patterns are considered, and molecular orbital calculations have been employed to define the different forms.[246] Minimum-energy conformations of L-glutamine and of its metabolic antagonist *S*-carbamoyl-L-cysteine show that these two amino-acids have complementary conformational features, which may be a major factor in accounting for their functions.[247] The conformations (13) and (14) emerge from molecular orbital calculations for alanine as the most stable forms of



(13)



(14)

[235] K. Machida, A. Kagayama, and Y. Saito, *J. Raman Spectrosc.*, 1979, **8**, 133.
[236] P. H. Carrington and N. S. Ham, *J. Electron Spectrosc. Rel. Phenom.*, 1979, **15**, 79.
[237] K. Seki and H. Inokuchi, *Chem. Phys. Lett.*, 1979, **65**, 158.
[238] R. J. Cotton, J. S. Munday, J. R. Wyatt, and J. J. DeCorpo, *Surface Sci.*, 1979, **84**, 235.
[239] A. P. Sarvazyan, D. P. Kharakoz, and P. Hemmes, *J. Phys. Chem.*, 1979, **83**, 1796.
[240] R. N. Roy, J. J. Gibbons, J. L. Padron, K. Buechter, and S. Faszholz, in 'Thermodynamic Behaviour of Electrolytes in Mixed Solvents', Advances in Chemistry Series, American Chemical Society, 1979, No. 177, p. 277.
[241] J. T. Edward, P. G. Farrell, J. C. Halle, J. Kirchnerova, R. Schaal, and F. Terrier, *J. Org. Chem.*, 1979, **44**, 615.
[242] J. Vliegen and L. C. Van Poucke, *Bull. Soc. Chim. Belg.*, 1978, **87**, 837.
[243] E. Gitalt, M. D. Ludevid, F. Albericio, and M. Bassedas, *Bioorg. Chem.*, 1979, **8**, 59.
[244] P. G. Mezey, J. J. Ladik, and S. Suhai, *Theor. Chim. Acta*, 1979, **51**, 323.
[245] P. Mohanakrishnan and K. R. K. Easwaran, *Org. Magn. Reson.*, 1979, **12**, 196.
[246] Z. I. Hodes, G. Nernethy, and H. A. Scheraga, *Biopolymers*, 1979, **18**, 1565.
[247] A. U. De and A. K. Ghose, *Ind. J. Chem.*, 1978, **16B**, 717.

the amino-acid;[248] these are closely similar to the preferred conformations established by various methods for glycine.

5 Chemical Studies of Amino-acids

Racemization.—The use of racemization kinetic data of amino-acids to provide estimates of the age of recent fossils, average temperatures at sites where these fossils are found, and other geochronological information has been a feature of this section in each of the preceding volumes, and Bada's pioneering work has been augmented by studies by other research groups. There is once again no shortfall in the chemical and intellectual interest to be found in the work reported this year, a spectacular example [249] being the use of the degree of conversion of isoleucine to alloisoleucine in fossil bones of 'Peking man' to provide a date 3.7—4.6 $\times$ 10^5 years for this specimen. Data for 'Lantian man' and 'Yuanmo man' are also included in this study.[249] The dangers of false conclusions arising from such factors as catalysis of the racemization process, temperature variations at the site over the years, and differential leaching of amino-acid enantiomers from the specimen have all been well appreciated; one cautionary result [250] is the finding that the rate of epimerization of isoleucine in peptides depends on the structure of the peptide, and another is the fact that the racemization of L-aspartic acid in aqueous solution is accelerated when D-glucose is added, as a result of Schiff-base formation.[251] The analytical method based on g.l.c. separation of diastereoisomeric derivatives (see later section) must also be reliable, and comparisons of data obtained in different laboratories for fossil bones show high reproducibility for the D : L ratio for aspartic acid (the amino-acid best suited for dating purposes since it racemizes more rapidly than other protein amino-acids).[252] However, it is not clear why comparable reproducibility for the D : L ratios for glutamic acid and for alanine from the same sources in different laboratories could not be secured.[252] Further work (see Vol. 11, p. 23) on the use of the γ-carboxyglutamic acid content of a fossil bone as a quantitative index of leaching suggests that there should be no significant error introduced in this way.[253]

Dating a femur from a Yuha skeleton at 23 600 years, using a measured D : L ratio for aspartic acid of 0.52—0.56 and taking into account the known temperature variation of racemization for this amino-acid, gives a result which is consistent with both ^{14}C and ^{230}Th dates for the fossil, based on sampling of the calcrete coating on the bone.[254]

Ongoing studies of amino-acid racemization, which are aimed at the implications of the findings that amino-acids present in meteorites are invariably racemic, involve 'radioracemization'.[255, 256] Optically pure protein amino-acids suffer

[248] H. L. Sellers and L. Schaefer, *Chem. Phys. Lett.*, 1979, **63**, 609.
[249] R.-W. Li and D.-X. Lin, *Ti Chih K'o Hsueh*, 1979, 56 (*Chem. Abstr.*, 1979, **90**, 167 295).
[250] N. Kriausakal and R. M. Mitterer, *Science*, 1978, **201**, 1011.
[251] J. E. Zumberge, *Geochim. Cosmochim. Acta*, 1979, **43**, 1443.
[252] J. L. Bada, E. Hoopes, D. Darling, G. Dangworth, H. J. Kessels, K. A. Kvenvolden, and D. J. Blunt, *Earth Planet. Sci. Lett.*, 1979, **43**, 265.
[253] K. King and J. L. Bada, *Nature (London)*, 1979, **281**, 135.
[254] J. L. Bischoff and W. M. Childers, *Earth Planet. Sci. Lett.*, 1979, **45**, 172.
[255] W. A. Bonner, N. E. Blair, and R. M. Lemmon, *Origins Life*, 1979, **9**, 279.
[256] W. A. Bonner, N. E. Blair, and R. M. Lemmon, *J. Am. Chem. Soc.*, 1979, **101**, 1049.

racemization in neutral (but not in acid) solutions, as well as decomposition under ^{60}Co γ-ray irradiation, and even isovaline (present, like certain protein amino-acids, in the Murchison meteorite) undergoes 4.8% racemization as well as *ca.* 79% destruction under these conditions.[256]

Racemization of amino-acids *via* Schiff-base formation in the presence of metal ions is a well known phenomenon. The role of Zn^{2+} ions in the racemization of L-alanine in media containing pyruvic acid is to stabilize the Schiff base since Zn^{2+} ions alone do not promote racemization of this amino-acid at pH $\geqslant$10 and at $\leqslant$80 °C.[257] Poly(acylamidosalicylaldehyde)s have been employed in the racemization of L-amino-acids.[258] α-Amino-ε-caprolactam is rapidly racemized as its nickel(II) chloride complex $(DL\text{-aa})_3NiCl_2$ in refluxing EtOH containing catalytic amounts of EtONa.[259]

The mechanism of the direct exchange racemization of cysteine derivatives, involving the β-heteroatom, has been investigated further,[260] supporting the view that electronic factors determine the rate of base-catalysed racemization through a series of cysteine analogues.

General Reactions.—Sensitized photo-oxidation [261] and ultrasonic degradation [262] are not responded to equally by the common protein amino-acids. Glycine is unaffected by the former treatment,[261] while serine, threonine, proline, and valine are not degraded during ultrasonication.[262] Slight losses of aspartic acid, alanine, and alloisoleucine must be expected during ultrasonic treatment of geological samples in preparation for amino-acid analysis, and this must be borne in mind as a source of error in amino-acid dating studies. The slight *increases* in the amounts of glycine, glutamic acid, leucine, and especially isoleucine which result from ultrasonic treatment of amino-acid mixtures [262] must mean that these amino-acids are formed from the amino-acids which are 'lost' from the mixtures.

More drastic degradation is brought about by pyrolysis; crystalline anhydrous L-leucine-^{14}C has been subjected to treatment at 451—578 K in order to check the likely survival of amino-acids in ancient (*i.e.* pre-Cambrian) geological samples.[263] A result with frightening dietary implications has been described; amino-acids heated with benzaldehyde or carbohydrates at 40—80 °C in a buffer at pH = 5.4 generate HCN as long as oxygen is not excluded.[264] The kinetics of $KMnO_4$ oxidation of amino-acids in moderately concentrated H_2SO_4 in the presence of a silver(I) salt represents another area of study in which a wide range of variations of the available parameters of this well studied reaction continues to be investigated.[265] Complex formation rather than oxidation is the result of the interaction of amino-acids with osmium(IV) oxide [266] (see also ref. 319).

Reduction of amino-acids to aldehydes is not a well established preparative procedure, and the problem has been tackled by two groups in different

[257] A. Dempsey and S. A. Phipps, *Inorg. Chim. Acta,* 1979, **36**, L425.
[258] I. A. Yamskov, V. E. Tikhonov, and V. A. Davankov, *Vysokomol. Soedin.*, 1979, **21A**, 1838.
[259] W. J. Boyle, S. Sifniades, and J. F. Van Peppen, *J. Org. Chem.*, 1979, **44**, 4841.
[260] M. Barber, J. H. Jones, and M. J. Witty, *J. Chem. Soc., Perkin Trans. 1,* 1979, 2425.
[261] R. Straight and J. D. Spikes, *Photochem. Photobiol.*, 1978, **27**, 565.
[262] B. J. Katz and E. H. Man, *Geochim. Cosmochim. Acta,* 1979, **43**, 1567.
[263] G. A. Lavrentev, T. F. Strigunkova, and I. A. Egorov, *Dokl. Akad. Nauk. SSSR,* 1979, **248**, 1474.
[264] G. Lehmann and H. D. Zinsmeister, *Z. Lebensm.-Unters. -Forsch.*, 1979, **169**, 357.
[265] V. S. Rao, B. Sethuram, and T. N. Rao, *Int. J. Chem. Kinet.*, 1979, **11**, 165.
[266] A. J. Nielson and W. P. Griffith, *J. Chem. Soc., Dalton Trans.*, 1979, 1084.

ways;[267, 268] a reliable, though long, procedure[267] involves conversion of the amino-acid into the corresponding diazomethylketone (*via* the mixed anhydride), thence to the trifluoroacetoxymethyl ketone and the derived hemiacetal ($RCO_2H \rightarrow RCOCHN_2 \rightarrow RCOCH_2OCOCF_3 \rightarrow RCH(OH)CH_2OH \rightarrow RCHO$). Alternatively,[268] the amino-acid may be converted into an *N*-protected amino-acid imidazolide, which is then subjected to reduction by di-isobutyl aluminium hydride.

A mechanistic study of the decomposition of *N*-chloro-amino-acids into aldehydes, NH_3, CO_2, and α-keto-acid has been undertaken, starting with *N*-chloroalanine.[269]

Dehydroascorbic acid reacts with an α-amino-acid to give several products, one of which is the tri(2-deoxy-2-L-ascorbyl)amine, the source of the relatively stable blue radical formed by air oxidation.[270] The converse process, the reactions of α-amino-acids with radicals, is represented by reactions with chlorine radical anions involving H-abstraction,[271] and with azide radicals.[272] Kinetic studies of reactions of α-amino-acids with 4-dimethylamino-1-methoxycarbonylpyridinium chloride,[273] with 1,2-diketo-compounds,[274] and with *o*-phthalaldehyde and mercaptoethanol[275] have been described. Points of interest arising from these studies are the wide variations in reaction rates with 1,2-diketones, the highest rates being for the basic and the hydroxy-amino-acids,[274] the fact that the *o*-phthalaldehyde system is now widely used for fluorimetric analysis of amino-acids, and rate differences between the different amines can be exploited in novel ways (*e.g.* the assay of α-amino-acid esters in the presence of amino-acids).[275]

Amino-acid derivatives have a number of uses in synthesis, not only in areas of heterocyclic chemistry; but their reactions are also of interest as models of the behaviour of amino-acid residues in peptides and proteins. Hydrolysis of amino-acid esters catalysed by copper or nickel complexes of polystyrene-bound L-cysteine proceeds at different rates for D- and L-enantiomers.[276] Similar rate differences are found in the hydrolysis of alkoxycarbonyl-L-amino-acid *p*-nitrophenyl esters catalysed by *N*-lauroyl-D- or *N*-lauroyl-L-histidine in the presence of a cationic micellar compound.[277] Conversion of α-amino-acid esters into α-keto-esters through reaction with toluene-*p*-sulphenyl chloride followed by treatment with PPh_3 and silica also constitutes a synthesis of α-(toluene-*p*-sulphenylimino)esters.[278] Further intramolecular thermal cycloaddition reactions of Schiff bases derived from α-amino-acid esters have been described (see also Vol. 11, p. 25).[279]

[267] R. P. Sharma, M. G. Gore, and M. Akhtar, *J. Chem. Soc., Chem. Commun.*, 1979, 875.
[268] H. Khatri and C. H. Stammer, *J. Chem. Soc., Chem. Commun.*, 1979, 79.
[269] W. D. Stanbro and W. D. Smith, *Environ. Sci. Technol.*, 1979, **13**, 446.
[270] T. Hayashi and M. Namiki, *Tetrahedron Lett.*, 1979, 4467.
[271] T. Masuda, S. Shimada, and M. Kondo, *J. Radiat. Res.*, 1979, **20**, 209.
[272] E. J. Land and W. A. Pruetz, *Int. J. Radiat. Biol. Relat. Stud. Phys., Chem., Med.*, 1979, **36**, 75.
[273] G. Guillot-Edelheit, M. Laloi-Diard, E. Guibe-Jampel, and M. Wakselman, *J. Chem. Soc., Perkin Trans. 2*, 1979, 1123.
[274] M. Piloty and W. Baltes, *Z. Lebensm.-Unters. -Forsch.*, 1979, **168**, 368, 374.
[275] V. J. K. Svedas, I. J. Galaev, I. L. Borisov, and I. V. Berezin, *Anal. Biochem.*, 1979, **101**, 188.
[276] I. A. Yamskov, B. B. Berezin, L. A. Belchich, and V. A. Davankov, *Makromol. Chem.*, 1979, **180**, 799.
[277] K. Yamada, H. Shosenji, and H. Ihara, *Chem. Lett.*, 1979, 491; K. Yamada, H. Shosenji, H. Ihara, and Y. Otsubo, *Tetrahedron Lett.*, 1979, 2529.
[278] E. M. Gordon and J. Pluscec, *J. Org. Chem.*, 1979, **44**, 1218.
[279] R. Grigg, M. Jordan, and J. F. Malone, *Tetrahedron Lett.*, 1979, 3877.

Reactions of *N*-substituted α-amino-acids described in the recent literature include Kolbe electrolysis of *p*-nitrobenzoylglycine in MeOH to give the acetal *p*-$NO_2C_6H_4CONHCH_2OMe$[280] and the synthesis of symmetrical anhydrides from *N*-alkoxycarbonyl-α-amino-acids using 0.5 equiv. *N*-ethyl-*N'*-3-dimethylaminopropylcarbodi-imide hydrochloride;[281] a full account has been published [282] of the alternative course for this reaction, the cyclization of *N*-benzyloxycarbonyl-L-amino-acids into 2-benzyloxy-4-substituted L-oxazolin-5-ones (see Vol. 10, p. 23).[282] An interesting result, established by the isolation of $HO_2CNHCH_2CONH_2$ from the hydrogenolysis of *N*-benzyloxycarbonylglycinamide, confirms that carbamic acids are intermediates in this widely used *N*-protection–deprotection technique.[283]

A useful paper defining the preparation of L-amino-acid 2,2,2-trichloroethyl esters has been published.[284] A mild methyl esterification procedure for amino-acids has been uncovered[285] during studies of the four-component condensation reaction; an *N*-substituted amino-acid and the enamine $Me_2C{=}CNR^1R^2$ [$R^1R^2 = (CH_2)_2O(CH_2)_2$] yield the methyl ester with Bu^tNC and MeOH.

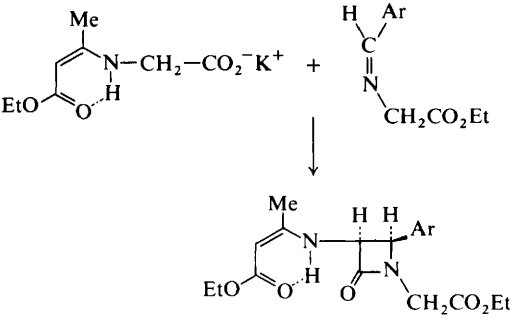
A study of the nitrosation of functional groups in the protein amino-acids, other than the α-amino-group, has been fully described;[286] aqueous acetic acid solutions of *N*-acetylcysteine methyl ester to which $NaNO_2$ is added yield the corresponding cystine derivative *via* the thionitrite, while the tyrosine analogue gives a mixture of 3-nitro- and 3-nitroso-derivatives. *N*-Acetyl-histidine methyl ester and the methionine analogue are not nitrosated under these conditions, but only in more highly acidic media. Methionine gives a mixture of *N*-nitroso-amide and *N*-nitrosamine-*S*-oxide, while nitrosation of tryptophan derivatives at the indole nitrogen atom gives a derivative easily solvolysed in aqueous MeOH.[286] Two reports have appeared on the decarboxylation of *N*-nitroso-α-amino-acids, one dealing with the reaction of the sodium salts in refluxing diglyme,[287] the other employing lead tetra-acetate as reagent.[288]

Specific Reactions of Natural Amino-acids.—Papers dealing with reactions of side-chain functional groups are collected in this section, together with some uses in synthesis of natural amino-acids (other than use for the synthesis of peptides).

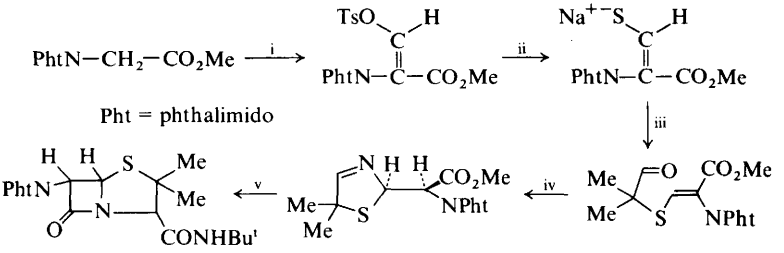
Formaldehyde reacts with L-lysine to give N^{ε}-formyl-lysine as well as N^{ε}-methyl-lysine already reported as reaction product,[289] but mono- and di-methyl-lysines are the only derivatives so far isolated from formaldehyde-treated proteins, *e.g.* casein.[290] Reversible reductive alkylation of N^{α}-acetyl-L-lysine with an α-hydroxyaldehyde or ketone in the presence of $NaBH_4$ has been demonstrated.[291]

[280] U. Hess, T. Gross, and R. Thiele, *Z. Chem.*, 1979, **19**, 195.
[281] F. M. F. Chen and N. L. Benoiton, *Synthesis*, 1978, 928.
[282] J. H. Jones and M. J. Witty, *J. Chem. Soc., Perkin Trans. 1*, 1979, 3203.
[283] I. Schon and L. Kisfaludy, *Z. Naturforsch., Teil B*, 1978, **33**, 1196.
[284] J. F. Carson, *Synthesis*, 1979, 24.
[285] G. Skorna and I. Ugi, *Chem. Ber.*, 1979, **112**, 776.
[286] R. Bonnett and P. Nicolaidou, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1969.
[287] M. Nakajima and J.-P. Anselme, *Tetrahedron Lett.*, 1979, 4037.
[288] J. E. Saavedra, *J. Org. Chem.*, 1979, **44**, 4511.
[289] L. Treszd, I. Rusznak, E. Tyihak, T. Szarvas, I. Markus, and T. Muller, *Proc. Hung. Ann. Meetings Biochem.*, 1979, 33 (*Chem. Abstr.*, 1980, **92**, 36 263).
[290] T. Nakai, T. Ohta, and N. Hayase, *Agric. Biol. Chem.*, 1979, **43**, 1779.
[291] K. F. Geoghegan, D. M. Ybarra, and R. E. Feeney, *Biochemistry*, 1979, **18**, 5392.

Glycine provides the starting material for both components in a synthesis of β-lactams (Scheme 11),[292] and is used in a synthesis of penicillins employing the four-component condensation (Scheme 12).[293]



Scheme 11



Reagents: i, HCO_2Me + Na, then TsCl; ii, NaSH; iii, $BrCMe_2CHO$; iv, NH_4OAc; v, LiI, dmf–py, heat, then Bu^tNC

Scheme 12

Serine and threonine are degraded in aqueous HCO_2H at 10 °C during 16 h, to alanine and α-aminobutyric acid respectively.[294] Reactions at the hydroxy-group in these amino-acids include a convenient methylation procedure using MeI and NaOMe,[295] and condensation of a Schiff base obtained by reaction with formaldehyde to give the corresponding oxazolidinecarboxylic acids.[296] The analogous reaction with cysteine has been known for many years; a recent report[297] that unequal amounts of diastereoisomers are formed in the condensation of *p*-tolualdehyde with L-cysteine has been shown to be incorrect, since epimerization occurs readily.[298] On a preparative scale, epimerization of thiazolidin-4-carboxylic acids (at the C-4 chiral centre) is effected without ring opening in Ac_2O at 100 °C.[298] The SH group reacts more readily than the NH_2

[292] S. D. Sharma, M. Sunita, and P. K. Gupta, *Tetrahedron Lett.*, 1979, 1265.
[293] A. Schutz and I. Ugi, *J. Chem. Res.* (*S*), 1979, 157.
[294] A. S. Subbaraman, Z. A. Kazi, and A. S. U. Choughuley, *Ind. J. Biochem. Biophys.*, 1979, **16**, 253.
[295] F. M. F. Chen and N. L. Benoiton, *J. Org. Chem.*, 1979, **44**, 2299.
[296] S. Wolfe, G. Militello, C. Ferrari, S. K. Hasan, and S. L. Lee, *Tetrahedron Lett.*, 1979, 3913.
[297] R. Parthasarathy, B. Paul, and W. Korytuyk, *J. Am. Chem. Soc.*, 1976, **98**, 6634.
[298] L. Szilagyi and Z. Gyorgydeak, *J. Am. Chem. Soc.*, 1979, **101**, 427.

group in the reaction of cysteine with isothiocyanates.[299] *S*-Alkylation of cysteine can be achieved by reaction with thiols and sulphides under the conditions normally used for protein hydrolysis,[300] revealing a source of potential artefacts in this process. *S*-Alkylation has, however, not been observed in the reaction of alanine-3-sulphinic acid with 2-mercaptoethanol at 110 °C at pH = 15, (cysteine, cystine, and L-2-amino-7-hydroxy-4,5-dithiaheptanoic acid are formed) or at pH = 4, when the mixed cysteine–mercaptoethanol disulphide is formed.[301]

A reaction which is well known as a general procedure, the conversion of α-amino-acids into hydroxy analogues, involves retention of configuration when used with D-methionine.[302]

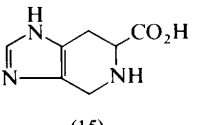
Optically pure (*N*-tetrafluoropropionyl-L-prolyl)anhydride can be obtained from the optically pure acid by reaction with the corresponding acid chloride, even though the latter is partially racemized whatever method is used for its preparation.[303]

The chemistry of the phenyl and heteroaromatic moieties of phenylalanine, tyrosine, histidine, and tryptophan is discussed in several recent papers. These are largely unrepresentative of current trends in aromatic and heteroaromatic chemistry since the objectives of work with these amino-acids are frequently in response to observations of unusual reactions occurring during peptide synthesis, or in response to problems arising from side-chain protection. Indoles are particularly susceptible to substitution by t-butyl carbonium ions, and tryptophan yields the 2,5,7-tri-t-butyl derivative with Bu^{t}OH–TFA;[304] its *N*-benzyloxycarbonyl benzyl ester undergoes substitution at positions 1, 3, and 5 of the indole moiety through reaction with t-butyl acetate and TFA,[305] while its *N*-(*p*-methoxybenzyl)oxycarbonyl derivative suffers *p*-methoxybenzylation at positions 1, 2, and 5 on acidolysis in TFA.[306] Tyrosine gives 3′-t-butyl tyrosine with t-butyl trifluoroacetate.[307]

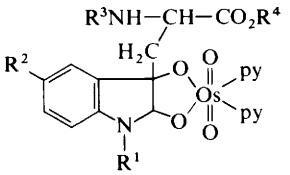
Protection of the imidazole moiety of histidine, and reactions of this grouping, are represented in several papers; *N*-acetyl histidine methyl ester gives equal amounts of N^τ- and N^π-hydroxyethyl-L-histidines after reaction with ethylene oxide followed by hydrolysis,[308] N^τ-phenacyl histidine derivatives predominate in the reaction product from the silver(I) salt of *N*-benzyloxycarbonyl histidine methyl ester and phenacyl bromide,[309] while the N^π-phenacyl isomers can best be made *via* the N^τ-trityl analogues.[309] N^τ-Methyl- or ethyl-L-histidines are prepared by cyclization of histidine methyl ester to the imidazo[1,5-*c*]pyrimidine derivative

[299] D. Podhradsky, L. Drobnica, and P. Kristian, *Experientia*, 1979, **35**, 154.
[300] C. J. Calleman, J. Ehrenberg, G. Osterman-Golkar, and D. Segerbaeck, *Acta Chem. Scand.*, 1979, **33B**, 488.
[301] A. J. Finlayson, S. L. Mackenzie, and J. W. Finlay, *Can. J. Chem.*, 1979, **57**, 2073.
[302] A. Kleemann, B. Lehmann, and J. Martens, *Angew. Chem.*, 1979, **91**, 858.
[303] I. Tomida and M. Matsuzaki, *Agric. Biol. Chem.*, 1979, **43**, 925.
[304] M. Low, L. Kisfaludy, E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *Z. Physiol. Chem.*, 1978, **359**, 1637.
[305] E. Jaeger, P. Thamm, S. Knof, and E. Wünsch, *Z. Physiol. Chem.*, 1978, **359**, 1629.
[306] H. Ogawa, T. Sasaki, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, 1978, **26**, 3144.
[307] B. F. Lundt, N. L. Johansen, and J. Markussen, *Int. J. Pept. Protein Res.*, 1979, **14**, 344.
[308] C. J. Calleman and C. A. Wachtmeister, *Acta Chem. Scand.*, 1979, **33B**, 277.
[309] A. R. Fletcher, J. H. Jones, W. I. Ramage, and A. V. Stachulski, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2261.

using *NN'*-carbonyldi-imidazole, followed by alkylation and hydrolysis with 6M hydrochloric acid.[310] Attack at the side chain is not significant when N^{im}-*p*-methoxybenzenesulphonylhistidine is deprotected with TFA at room temperature during 1 h, in the presence of Me_2S,[311] and the toluene-*p*-sulphonyl analogue is also cleaved in this way.[311] Observations of less direct relevance to peptide work are the demonstration that some racemization accompanies nitration of L-histidine,[312] a study of attack by aryldiazonium cations (also capable of substituting tyrosine and lysine, the latter through the ε-amino-group),[313] the demonstration by low-temperature n.m.r. that dye-sensitized photo-oxidation of histidine involves an unstable 2,5-*endo*-peroxide,[314] and the astonishing conclusion that the imidazo-tetrahydropyridine (15) discovered as an impurity during the preparation of ^{14}C-labelled histidine is formed through reaction of the amino-acid with formaldehyde present in the atmosphere of the laboratory.[315]



(15)



(16)

Mercuration of C-4 of the imidazole moiety of histidine, and one of the imidazole nitrogen atoms, also C-3 and C-5 of the tyrosine phenolate moiety, occurs by contact with mercuric acetate.[316] Catalysis by a copper(II) salt facilitates hydroxylation of phenylalanine by H_2O_2;[317] hydroxylated phenylalanines have been subjected to ^1H–^2H exchange, a rapid process studied kinetically by stopped-flow fluorimetry.[318]

Adduct formation involving osmium(IV) tetroxide, pyridine, and tryptophan [*e.g.* (16); $R^1 = R^2 = R^3 = R^4 = H$] has been fully explored;[319] this behaviour is in contrast with the complex formation observed in this system with other amino-acids.[266]

Specific Reactions and Properties of Amino-acids Related to Biochemical Processes.—A number of processes referred to in preceding and later sections might have been located here, and the small scope of this section is therefore an artificial indication of the amount of work which has been carried out under this category. Binding studies (tryptophan to reptilian plasma proteins,[320] *N*-acetyl-L-

[310] A. Noordam, L. Maat, and H. C. Beyerman, *Recl. Trav. Chim. Pays-Bas*, 1978, **97**, 293.
[311] K. Kitagawa, K. Kitade, Y. Kito, T. Akita, S. Fumakoshi, N. Fujii, and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1979, 955.
[312] E. Giralt and M. D. Ludevid, *An. Quim.*, 1979, **75**, 331.
[313] C. H. Paik, W. C. Eckelman, and R. C. Reba, *Bioorg. Chem.*, 1979, **8**, 25.
[314] H. S. Ryang and C. S. Foote, *J. Am. Chem. Soc.*, 1979, **101**, 6683.
[315] S. Hrncir, J. Kopoldova, K. Veres, V. Dedkova, V. Hanus, and P. Sedmera, *J. Labelled Comp. Radiopharm.*, 1978, **15**, 47.
[316] A. P. Korn, F. P. Ottensmeyer, and T. R. Jack, *J. Inorg. Biochem.*, 1979, **10**, 235.
[317] S. Ishimitsu, S. Fujimoto, and A. Ohara, *Chem. Pharm. Bull.*, 1979, **27**, 2286.
[318] M. Nakanishi and M. Tsuboi, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1337.
[319] J. S. Deetz and E. J. Behrman, *J. Org. Chem.*, 1980, **45**, 135.
[320] J. Hattingh and D. C. Klein, *S. Afr. J. Zool.*, 1979, **14**, 109.

tryptophan to monomeric and dimeric forms of chymotrypsin,[321] branched side-chain amino-acids to *E. coli* periplasmic protein,[322] and phenylalanine or tryptophan to β-nicotinamide adenine dinucleoside[323]) have been reported. The last two results derive from n.m.r. data, T_2-relaxation times for different protons being used to show the existence of two types of bond, one from the amino-group and the other from the hydrocarbon moiety, in interactions of branched side-chain amino-acids with proteins.[322]

Effects of Electromagnetic Radiation on Amino-acids.—Well defined categories have been established for this section by a continuing series of papers over recent years, with more laboratories entering the field.

Fluorescence studies of tryptophan and its N^{α}-acetyl amide[324–327] deal with quenching by H_2O_2[324] and *N*-bromosuccinimide;[325,326] the effects of KH_2PO_4 on tyrosine fluorescence have been described.[328] Salts can often greatly enhance the phosphorescence[329] and delayed luminescence[330] of tryptophan[329] and tyrosine;[330] light emission from photo-excited tryptophan[331,332] and tyrosine[333] is influenced by several parameters.

Photochemical studies supporting the intermediacy of the tricyclic hydroperoxide in the photodegradation of tryptophan to formylkynurenine in aqueous solutions have been reported.[334,335] Nakagawa's earlier formulation of this reaction pathway has been verified in the most direct way, the isolation of the hydroperoxide.[334,335] Flash photolysis of *N*-acetyl-L-tryptophanamide[336] and photoionization of tryptophan[337] have been described. Reaction products resulting from hydrated electrons liberated from irradiated aqueous solutions of tyrosine have been analysed,[338] and degradation rates for amino-acids subjected to laser-generated singlet oxygenation in ^2H_2O have been reported.[339]

Higher-energy radiation generates radicals in samples of amino-acids, both in solution and in the solid state. Short-lived radicals formed by γ-irradiation of aqueous DL-methionine[340] have been spin-trapped and studied. Dityrosine is formed in γ- or *X*-irradiated aqueous solutions of tyrosine even after careful deoxygenation;[341] the same process is readily brought about by H_2O_2 oxidation.[44]

[321] R. Tellam, J. De Jersey, and D. J. Winzor, *Biochemistry*, 1979, **18**, 5316.
[322] A. M. Chavret-Monges, J. P. Monti, A. Crevat, C. Gaudin, J. C. Sari, and J. P. Belaich, *J. Chim. Phys. Phys.-Chim., Biol.* 1979, **76**, 714.
[323] K. J. Neurohr and H. H. Mantsch, *Can. J. Chem.*, 1979, **57**, 2297.
[324] P. Cavatorta, R. Favilla, and A. Mazzini, *Biochim. Biophys. Acta*, 1979, **578**, 541.
[325] B. F. Peterman and K. J. Laidler, *Can. J. Chem.*, 1979, **57**, 1471.
[326] B. F. Peterman and K. J. Laidler, *Biochim. Biophys. Acta*, 1979, **577**, 314.
[327] J. P. Privat, P. Wahl, and J. C. Auchet, *Biophys. Chem.*, 1979, **9**, 223.
[328] O. Shimizu, J. Watanabe, and K. Imakule, *Photochem. Photobiol.*, 1979, **29**, 915.
[329] M. L. Meyers and P. G. Seybold, *Anal. Chem.*, 1979, **51**, 1609.
[330] S. Yameshita and G. Tomita, *Z. Naturforsch., Teil B*, 1979, **34**, 344.
[331] B. T. Thu, *J. Chem. Phys.*, 1979, **70**, 3544.
[332] B. T. Thu, *J. Chem. Phys.*, 1979, **70**, 3536.
[333] B. T. Thu and A. Petit, *J. Phys. Chem.*, 1979, **83**, 1300.
[334] M. Sun and S. Zigman, *Photochem. Photobiol.*, 1979, **29**, 893.
[335] M. Nakagawa, S. Kato, S. Kataoka, and T. Hino, *J. Am. Chem. Soc.*, 1979, **101**, 3136.
[336] R. F. Evans, R. R. Kuntz, W. A. Volkert, and C. A. Ghiron, *Photochem. Photobiol.*, 1978, **27**, 511.
[337] E. Amouyal, A. Bernas, and D. Grand, *Photochem. Photobiol.*, 1979, **29**, 1071.
[338] J. F. Baugher and L. I. Grossweiner, *Photochem. Photobiol.*, 1978, **28**, 175.
[339] I. B. C. Matheson and J. Lee, *Photochem. Photobiol.*, 1979, **29**, 879.
[340] K. Makino and H. Hatano, *Chem. Lett.*, 1979, 119; K. Makino, *J. Phys. Chem.*, 1979, **83**, 2520.
[341] G. Boguta and A. M. Dancewicz, *Stud. Biophys.*, 1978, **73**, 149.

Similar studies have been reported for crystalline L-arginine hydrochloride, L-asparagine hydrate, and L-histidine hydrochloride,[342] and for *N*-acetyl-alanine and glutamic acid at 77 K;[343] e.s.r. monitoring is both routine and mandatory in all these projects.

Pulse radiolysis of ^{2}H-exchanged glycine yields a mixture of the radical cation $^2H_2NCH_2CO_2^-$ and radical anion $^2H_3N^+CH_2CO_2{}^2H$.[344]

An important extension of earlier results dealing with the interaction of chiral radiation with racemic amino-acids is the report[345] that 13.9—21–5% higher concentrations of radicals are formed in ^{90}Y-β-irradiated crystalline D-alanine than in the L-enantiomer.

6 Analytical Methods

Gas–Liquid Chromatography.—The quantitative estimation of amino-acids by g.l.c. methods is an undisputed rival to the ion-exchange amino-acid analyser technique, and g.l.c. is more compatible with mass spectrometric instrumentation and with optical purity determination. All these aspects are represented in the recent literature.

A review of the g.l.c. and h.p.l.c. analysis of amino-acids and peptides has appeared.[346] The conversion of amino-acids into volatile derivatives, a prerequisite for g.l.c. analysis, continues to provide a topic for study, and *N*-trifluoroacetyl amino-acid n-butyl esters,[347–351] pentafluoropropionyl analogues[352–354] (in one case as hexafluoroisopropyl esters),[354] and *N*-heptafluorobutyryl amino-acid isobutyl esters[355–358] are widely used. *N*-Acetyl amino-acid n-propyl esters[359, 360] and *N*-trifluoroacetyl analogues,[360] *N*-trimethylsilyl amino-acid trimethylsilyl esters,[361, 362] in one case[362] a particularly extensive study (58 compounds, including assessment of *N*-trimethylsilyl amino-acid n-butyl or (−)-methyl esters), and also condensation products of amino-acids with 1,3-dichlorotetrafluoroacetone[363, 364] provide suitable samples for g.l.c. analysis. As is so often the

[342] J. J. Tria, D. Hoel, and R. H. Johnsen, *J. Phys. Chem.*, 1979, **83**, 3174.
[343] M. D. Sevilla, J. B. D'Arcy, and K. M. Morehouse, *J. Phys. Chem.*, 1979, **83**, 2893.
[344] P. O. Samskog, T. Gillbro, and G. Nilsson, *Chem. Phys. Lett.*, 1979, **64**, 162.
[345] M. Akaboshi, M. Noda, K. Kawai, H. Maki, and K. Kawamoto, *Origins Life*, 1979, **9**, 181 (*Chem. Abstr.*, 1979, **91**, 170929).
[346] R. F. Adams, *Chromatogr. Sci.*, 1979, **9**, 1273.
[347] M. Matucha and E. Smolkova, *J. Chromatogr.*, 1979, **168**, 255.
[348] J. J. Rafter, M. Ingelman-Sundberg, and J. A. Gustafsson, *Acta Biol. Med. Ger.*, 1979, **38**, 321.
[349] S. Nagy and N. T. Hall, *J. Chromatogr.*, 1979, **177**, 141.
[350] N. T. Hall and S. Nagy, *J. Chromatogr.*, 1979, **171**, 392.
[351] C. W. Gehrke, D. R. Younker, K. O. Gerhardt, and K. C. Kuo, *J. Chromatogr. Sci.*, 1979, **17**, 301.
[352] N. Mahy, J. Tussell, and E. Gelpi, *Agents Actions*, 1978, **8**, 399 (*Chem. Abstr.*, 1979, **90**, 83 118).
[353] F. Artigas and E. Gelpi, *Analyt. Biochem.*, 1979, **92**, 233.
[354] M. Wolfensberger and H. C. Curtius, *J. Chromatogr.*, 1979, **172**, 471.
[355] S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 1979, **171**, 195.
[356] S. L. MacKenzie and D. Tenaschuk, *J. Chromatogr.*, 1979, **173**, 53.
[357] G. Bengtsson and G. Odham, *Anal. Biochem.*, 1979, **92**, 426.
[358] J. Desgres, D. Boisson, and P. Padieu, *J. Chromatogr.*, 1979, **162**, 133.
[359] W. P. Leighton, S. Rosenblatt, and J. D. Chanley, *J. Chromatogr.*, 1979, **164**, 427.
[360] D. Lecavalier and J. C. Crawhall, *Union Med. Can.*, 1979, **108**, 566.
[361] K. L. Clay and R. C. Murphy, *J. Chromatogr.*, 1979, **164**, 417.
[362] H. Iwase, Y. Takeuchi, and A. Murai, *Chem. Pharm. Bull.*, 1979, **27**, 1307.
[363] P. Husek, V. Felt, and M. Matucha, *J. Chromatogr.*, 1979, **180**, 53.
[364] P. Husek, *J. Chromatogr.*, 1979, **172**, 468.

case when competing with well established alternatives, a promising one-step derivatization procedure (the use of 1,3-dichlorotetrafluoroacetone) is used only in the laboratory of its inventor (see Vol. 7, p. 26). Extensions of these techniques to the assay of steric purity of amino-acid samples are also well established, and are represented in derivatization of amino-acids with *N*-trifluoroacetyl-L-alanine or its chloride,[365] or with *N*-trifluoroacetyl-L-prolyl chloride;[366] the latter study includes an interesting extension of the diastereoisomer quantification technique, since if derivatization is performed using a mixture of *N*-trifluoroacetyl-L-prolyl chloride and *N*-trifluoroacetyl-D-prolyl-1-^{2}H chloride, and the relative amounts of the resulting four diastereoisomers are determined by c.i.m.s., then a source of error, which can be introduced in the simpler method if diastereoisomer formation does not go to completion, is avoided.[366] G.c.–m.s. studies are implied in many of the references cited here [348, 352–354, 357, 361, 362, 366] and in the earlier section on mass spectrometry.[226–229] Quantitative analysis of certain unusual amino-acids in physiological samples can be achieved by methylation followed by g.c.–m.s., illustrated this year for N^4-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-asparagine[367] and *N*-(phosphonoacetyl)-L-aspartic acid.[368]

Established chiral stationary phases have been used in the determination of steric purity of amino-acids as their perfluoroalkanoyl ester derivatives,[369–372] including g.c.–m.s. isotope-labelling (hydrolysis of peptides with ^{2}HCl–^2H_2O followed by exchange of labile ^{2}H with ^{1}H [369]) and use of the 'non-natural' enantiomer of an amino-acid as internal standard, avoiding errors arising through incomplete recovery, hydrolysis, and derivatization,[370] as discussed in the preceding paragraph.[366] A novel chiral stationary phase, formed by grafting L-valine-t-butylamide on to a silicone, has been advocated for this work.[373]

Ion-exchange Chromatography.—As in the preceding section, discussion of the papers appearing under this heading is very brief where development of existing techniques is concerned. This applies to nearly all published work in this area. Instrumental innovations are excluded.

Ion-exchange chromatographic identification of proline,[374] 3-methylhistidine,[375] and *S*-adenosyl-L-methionine,[376] the last-mentioned amino-acid on sulphopropyl-Sephadex, has been reported and the simultaneous determinations of amino-acids, amino-sugars, and carbohydrates have been described.[377] γ-Carboxy-L-glutamic acid content of proteins has been determined by alkaline

[365] K. Kruse, W. Francke, and W. A. König, *J. Chromatogr.*, 1979, **170**, 423.
[366] C. Wiecek, B. Halpern, A. M. Sargeson, and A. M. Duffield, *Org. Mass Spectrom.*, 1979, **14**, 281.
[367] P. Maury, *J. Lab. Clin. Med.*, 1979, **93**, 718.
[368] J. M. Strong, Y. E. Kinney, A. R. Branfman, and K. L. Cysyk, *Cancer Treat. Rep.*, 1979, **63**, 775 (*Chem. Abstr.*, 1979, **91**, 71 142).
[369] H. Frank, W. Woiwode, G. J. Nicholson, and E. Bayer, in 'Stable Isotopes: Proceedings of the Third International Conference', ed. E. R. Klein and P. D. Klein, Academic Press, New York, 1979, p. 165.
[370] H. Frank, G. J. Nicholson, and E. Bayer, *J. Chromatogr.*, 1978, **167**, 187.
[371] G. J. Nicholson, H. Frank, and E. Bayer, *J. High Resolution Chromatogr.*, 1979, **2**, 411.
[372] W. Woiwode, D. List, and H. Weichardt, *J. Clin. Chem. Clin. Biochem.*, 1979, **17**, 251.
[373] T. Saeed, P. Sandra, and M. Verzele, *Adv. Chromatogr.*, 1979, **14**, 699.
[374] R. Paquin and P. Lechasseur, *Can. J. Bot.*, 1979, **57**, 1851.
[375] M. Neuhauser and P. Furst, *Anal. Biochem.*, 1979, **92**, 294.
[376] R. I. Glazer and A. L. Peale, *Anal. Biochem.*, 1978, **91**, 516.
[377] M. M. Tikhomirov, A. Y. Khorlin, W. Voelter, and H. Bauer, *J. Chromatogr.*, 1978, **167**, 197.

hydrolysis followed by ion exchange,[378] and by using the anion-exchange auto-analyser;[379] a problem in the confluence of this amino-acid with taurine has been noted.[379] Other amino-acid analyser studies (selected from a larger field) concern the use of 3-nitro-L-arginine as internal standard,[380] studies of sulphur-containing amino-acids with particular reference to variable colour yields and proposals for ninhydrin colour factors,[381] dithiasuccinoyl-amino-acids,[382] and 3-hydroxy-L-proline assays.[383] Reports have been published on the use of a micro-column amino-acid analyser [384] and microbore analyser,[385] the latter study being one of a crop of papers on *o*-phthalaldehyde [385–387] or fluorescamine detection.[386] There is good agreement between two groups [385, 387] that high precision is possible with *o*-phthalaldehyde–2-mercaptoethanol fluorimetric detection (see also the later section on fluorimetry) at the 10 picomole level.

Thin-layer Chromatography.—Studies range from a claim that leucine, isoleucine, and phenylalanine, whose separation is a test piece for t.l.c. methodology, can be resolved by one-dimensional t.l.c.,[388] separation of (2*S*,3*R*,4*R*)-4-hydroxyisoleucine (the major free amino-acid of fenugreek seed), whose separation by ion-exchange is complicated by lactonization,[389] and ion-exchange t.l.c. of methionine, cysteine, cystine, and cysteic acid in plant extracts.[390] 'Three-dimensional t.l.c.' turns out to be a variation of two-dimensional t.l.c. in which a square plate is used in the normal way, with one side for sample, the other half for standards, then successively developed in the second dimension from one side and from the other;[391] this gives a better basis for visual comparison.

Dansyl derivatives of more than 90 'uncommon' amino-acids have been studied by two-dimensional polyamide t.l.c.,[392] and the dansylation–fluorimetry technique has been used for the assay of amino-acids in blood samples.[393] The relatively rarely used combination of t.l.c. with m.s. has been applied for the estimation of γ-aminobutyric acid, as dansyl-γ-butyrolactam.[394] Dabsyl chloride (4-dimethylaminoazobenzene-4′-sulphonyl chloride) gives photostable coloured derivatives which, although detectable on t.l.c. plates at some 60-fold lower levels than 2,4-dinitrophenyl analogues, are less satisfactory for trace analysis of amino-acids than dansyl derivatives.[395]

[378] D. Madar, R. A. Willis, K. A. Koehler, and R. G. Hiskey, *Anal. Biochem.*, 1979, **92**, 466.
[379] L. B. James, *J. Chromatogr.*, 1979, **175**, 211.
[380] R. C. White and T. E. Nelson, *J. Chromatogr.*, 1979, **176**, 430.
[381] M. Friedman, A. T. Noma, and J. R. Wagner, *Anal. Biochem.*, 1979, **98**, 293.
[382] G. Baremy and R. B. Merrifield, *Anal. Biochem.*, 1979, **95**, 160.
[383] A. Szymanovicz, G. Poulin, A. Randoux, and J. P. Borel, *Clin. Chim. Acta*, 1979, **91**, 141.
[384] J. P. Vergues and I. L. Freeman, *Anal. Biochem.*, 1979, **99**, 427.
[385] H.-W. Lee, M. D. Forde, M. C. Lee, and D. J. Bucher, *Anal. Biochem.*, 1979, **96**, 298.
[386] H.-W. Lee, D. J. Bucher, and R. C. Seid, *Ind. Eng. Chem. Prod. Res. Dev.*, 1979, **18**, 122.
[387] H. Nakamura, C. L. Zimmerman, and J. J. Pisano, *Anal. Biochem.*, 1979, **93**, 423.
[388] R. W. McBride, D. W. Jolly, B. M. Kadis, and T. E. Nelson, *J. Chromatogr.*, 1979, **168**, 290.
[389] R. Hardman and I. M. Abu-Al-Futuh, *Planta Med.*, 1979, **36**, 79.
[390] A. Varadi and S. Pongor, *J. Chromatogr.*, 1979, **173**, 419.
[391] M. Messripour, S. Naderi, and A. Wise, *Anal. Biochem.*, 1979, **97**, 328.
[392] H. Laatsch, *J. Chromatogr.*, 1979, **173**, 398.
[393] B. M. Fetterroll and J. Sander, *Monatsschr. Kinderheilkd.*, 1979, **127**, 411.
[394] P. H. Wu, D. A. Durden, and L. Hertz, *J. Neurochem.*, 1979, **32**, 379.
[395] J.-K. Lin, C.-A. Chen, and C.-H. Wang, *Proc. Nat. Sci. Council, Republic of China*, 1979, **3**, 158 (*Chem. Abstr.*, 1979, **91**, 136402).

N-Acetyl-amino-acids can be detected on t.l.c. plates by successively spraying with 12M hydrochloric acid, heating at 120 °C during 10—15 min, and then by ninhydrin colour formation.[396]

Routine t.l.c. studies with 3-phenylthiohydantoins[397] and 4-[4-(dimethylaminophenyl)azophenyl] analogues[398] have been described; in one of these,[397] detection at the 5 nanomole level was established.

High-performance Liquid Chromatography.—The volume of published work in this area continues to increase, and a number of reviews have appeared, covering h.p.l.c. of amino-acids and peptides,[346, 399] and resolution of amino-acids either through conversion into diastereoisomeric derivatives[400] or on chiral stationary phases.[401]

Separation of underivatized amino-acids has been reported in several papers: tyrosine and tryptophan in physiological fluids,[402-407] including examples of ion-pair h.p.l.c.[404, 405] in which sodium dodecyl sulphate or other surfactant is involved, tyrosine and its analogues iodotyrosine and iodothyronines,[407] dopa and its 2- and 5-(*S*-cysteinyl) derivatives,[408] ergothioneine,[409] and *S*-adenosylmethionine and -homocysteine[410] have been under scrutiny. A mixture of amino-acids can be separated adequately within 14 min by reversed-phase h.p.l.c.[411]

Several laboratories have reported work on pre-column derivatization of amino-acid mixtures with *o*-phthalaldehyde and ethanethiol,[412] allowing analysis at even sub-picomole levels. Derivatization with 2,4-dinitrofluorobenzene[413] or through dansylation[414] has served to provide quantitative data on particular amino-acids in physiological samples, and several reports have appeared on h.p.l.c. characteristics of dansyl-amino-acids.[415] Yet more papers on h.p.l.c. of 3-phenylthiohydantoins have been published;[416, 417] a reasoned assessment of the rating of h.p.l.c. for these compounds is that this separation method is some 10—50-fold

[396] R. C. Pandey, R. Misra, and K. L. Rinehart, *J. Chromatogr.*, 1979, **170**, 498.
[397] R. L. Munier and A. M. Drapier, *Chromatographia*, 1979, **12**, 548.
[398] C.-Y. Yang, *Z. Physiol. Chem.*, 1979, **360**, 1673.
[399] M. T. W. Hearn and W. S. Hancock, *Chromatogr. Sci.*, 1979, **12**, 243.
[400] R. Audebert, *J. Liq. Chromatogr.*, 1979, **2**, 1063.
[401] T. Tamegai, M. Ohmae, K. Kawabe, and M. Tomoeda, *J. Liq. Chromatogr.*, 1979, **2**, 1229.
[402] G. M. Anderson, J. G. Young, and D. J. Cohen, *J. Chromatogr.*, 1979, **164**, 501.
[403] A. M. Krstulovic, S. Ciriello, L. Bertani-Dziedzic, and S. E. Gitlow, *J. Chromatogr.*, 1979, **164**, 495.
[404] C. M. Riley, E. Tomlinson, T. M. Jeffries, and P. H. Redfern, *J. Chromatogr.*, 1979, **162**, 153.
[405] W. S. Hancock, C. A. Bishop, and M. T. W. Hearn, *Chem. N.Z.*, 1979, **43**, 17.
[406] D. D. Koch and P. T. Kissinger, *J. Chromatogr.*, 1979, **164**, 441.
[407] N. M. Alexander and M. Nishimoto, *Clin. Chem.*, 1979, **25**, 1757.
[408] C. Hansson, G. Agrup, H. Rorsman, A. M. Rosengren, E. Rosengren, and L. E. Edholm, *J. Chromatogr.*, 1979, **162**, 7.
[409] T. Mayumi, H. Kawano, Y. Sakamoto, E. Suehisa, Y. Kawai, and T. Hama, *Chem. Pharm. Bull.*, 1978, **26**, 3772.
[410] A. Floridi, C. Fini, C. A. Palmerini, R. Mozzi, and G. Porcellati, *J. Liq. Chromatogr.*, 1979, **2**, 1003.
[411] W. S. Hancock, C. A. Bishop, and M. T. W. Hearn, *Anal. Biochem.*, 1979, **92**, 170.
[412] J. C. Hodgkin, *J. Liq. Chromatogr.*, 1979, **2**, 1047; D. W. Hill, F. H. Walters, T. D. Wilson, and J. D. Stuart, *Anal. Chem.*, 1979, **51**, 1338; P. Lindroth and K. Mopper, *Anal. Chem.*, 1979, **51**, 1667; W. S. Gardner and W. H. Miller, *Anal. Biochem.*, 1979, **101**, 61.
[413] E. W. Bachmann, J. Frei, and M. Muehlemann, *Chromatographia*, 1979, **12**, 345.
[414] G. J. Schmidt, D. C. Olson, and W. Slavin, *J. Chromatogr.*, 1979, **164**, 355.
[415] J. M. Wilkinson, *J. Chromatogr. Sci.*, 1978, **16**, 547; G. J. Schmidt, D. C. Olson, and W. Slavin, *J. Liq. Chromatogr.*, 1979, **2**, 1031.
[416] W. D. Annan, *J. Chromatogr.*, 1979, **173**, 194; P. W. Moser and E. E. Rickli, *J. Chromatogr.*, 1979, **176**, 451; W. F. MooPen, M. H. Johnson, K. C. Bechtel, and D. L. Jue, *J. Chromatogr.*, 1979, **172**, 476.
[417] M. N. Margolies and A. W. Brauer, *Chromatogr. Sci.*, 1979, **10**, 177.

more sensitive than g.l.c.,[417] and easily permits the differentiation of leucine from isoleucine, and of aspartic acid from glutamic acid.

New approaches to the resolution of partly racemized amino-acids on chiral stationary phases[418-422] include reversed-phase h.p.l.c. of Cu^{2+} or Zn^{2+} complexes with L-aspartyl-L-phenylalanine methyl ester in the mobile phase,[418] L-amino-acids grafted on to poly(acrylamide)s, and complexed with Cu^{2+} ions,[419] and a similar grafting of *N*-acetyl-L-valine to 3-aminopropylsilanized silica[420] or of (+)-9-bromomethyl-10-(1-hydroxy-2,2,2-trifluoroethyl)anthracene to silica,[421] as novel stationary phases.

Fluorimetry.—The conversion of amino-acids into fluorescent derivatives continues to attract new users, particularly the use of *o*-phthalaldehyde with 2-mercaptoethanol[423,424] or with ethanethiol[412,425] or methanethiol.[425] This system has been used recently for analysis of γ-aminobutyric acid in brain tissue,[423] cysteine and cystine,[424] and γ-carboxyglutamic acid,[426] and although it is specifically applicable only to primary amines both proline and hydroxyproline can be assayed by prior oxidation with alkaline sodium hypochlorite, down to 10 picomole and 20 picomole levels, respectively.[427] Problems exist with the use of this method with lysine, where quantum yields are reduced by intramolecular quenching of the fluorescence of the substituted isoindole which is the product of the reaction;[428] fluorescence yields are low also with cysteine and cystine but prior conversion of these amino-acids into cysteic acid or into *S*-(3-sulphopropyl) derivatives is advocated to overcome this difficulty.[424] There is a need to lay down very specific details for the use of the *o*-phthalaldehyde–thiol system, since both reaction temperature and the structure of the thiol affect fluorescence yields.[425] For fluorimetric analysis of α-branched α-amino-acids, more reliable fluorescence behaviour is achieved using higher reaction temperatures and using methanethiol or ethanethiol instead of the more commonly used 2-mercaptoethanol.[425]

The major alternative reagent for the conversion of amino-acids into fluorescent derivatives is fluorescamine, which has been involved in a tyrosine assay procedure, with special reference to sources of interference when used for rat brain or human plasma samples.[429] A tryptophan fluorimetric assay has been illustrated, again using rat brain samples.[430] Fluorescent derivatives obtained from proline and its 3- or 4-hydroxy-analogues using 7-chloro-4-nitrofurazan permit the visualization of these imino-acids on t.l.c. plates at *ca.* 10 picomole levels.[431]

[418] C. Gilon, R. Leshem, Y. Tapuhi, and E. Grushka, *J. Am. Chem. Soc.*, 1979, **101**, 7612.
[419] B. Lefebvre, R. Audebert, and C. Quiviron, *J. Liq. Chromatogr.*, 1978, **1**, 761.
[420] S. Hara and A. Dobashi, *J. Liq. Chromatogr.*, 1979, **2**, 883.
[421] W. H. Pirkle and D. W. Hause, *J. Org. Chem.*, 1979, **44**, 1957.
[422] G. Guebitz, W. Jellenz, G. Loefler, and W. Santi, *J. High Resolution Chromatogr., Chromatogr. Commun.*, 1979, **2**, 145.
[423] J. A. M. Van der Heyden and J. Korf, *J. Neurochem.*, 1978, **31**, 197.
[424] K. S. Lee and D. G. Drescher, *J. Biol. Chem.*, 1979, **254**, 6248.
[425] J. R. Cronin, S. Pizzarello, and W. E. Gandy, *Anal. Biochem.*, 1979, **93**, 174.
[426] C. M. Gundberg, J. B. Lian, and P. M. Gallop, *Anal. Biochem.*, 1979, **98**, 219.
[427] P. Bohlen and M. Mellet, *Anal. Biochem.*, 1979, **94**, 313.
[428] R. F. Chen, C. Scott, and E. Trepmann, *Biochim. Biophys. Acta*, 1979, **576**, 440.
[429] G. Evans, M. K. Hartmann, L. Wood, and M. K. Gaitonde, *J. Neurochem.*, 1979, **32**, 1303.
[430] H. D. Dell and J. Fiedler, *Z. Anal. Chem.*, 1978, **293**, 407.
[431] A. Szymanowicz, C. Cheron, and J. P. Borel, *Biochimie*, 1979, **61**, 425.

Other Separation Methods.—Thin-layer electrophoresis has been used for the micro-assay of γ-aminobutyric acid, glutamic acid, and aspartic acid in tissue samples,[432] and for γ-amino-β-hydroxybutyric acid.[433]

Swollen cyclodextrin beads have been used for inclusion chromatography separation of aromatic amino-acids from non-aromatic amino-acids and from each other.[434]

Determination of Specific Amino-acids.—This section is shorter than in previous volumes, mainly as a result of inclusion of material in earlier sections of this Chapter wherever the generality of the analytical content makes this possible.

Specific colour-forming reactions have been explored for the estimation of methionine (use of Chloramine-T, quantification at 246 nm),[435] and hydroxylysine (further studies of Ehrlich reagent colorimetry).[436] New radiometric assays for L-canaline (using ^{14}C-labelled acetone)[437] and 3′-mono-iodo-L-thyronine[438] have been reported. Bioautography of L-carnitine[439] and an enzyme assay for glycine, based on spectrophotometric quantification of the proportion of SH groups exposed by the cleavage of benzoyl–coenzyme A catalysed by glycine *N*-acyltransferase,[440] provide specific examples of methods whose principles are generally applicable.

[432] S. Sarhan, N. Seiler, J. Grove, and G. Bink, *J. Chromatogr.*, 1979, **162**, 561.
[433] K. Matsushita and L. R. Gjessing, *J. Chromatogr.*, 1979, **162**, 427.
[434] B. Zsadon, M. Szilasi, F. Tudos, E. Fenyvesi, and J. Szejtli, *Starck und Staerke*, 1979, **31**, 11.
[435] G. E. Trout, *Anal. Biochem.*, 1979, **93**, 419.
[436] N. Blumenkrantz and G. Asboe-Hansen, *Clin. Biochem.*, 1979, **12**, 157.
[437] M. Sugii, H. Miura, and K. Nagata, *Anal. Biochem.*, 1979, **92**, 265.
[438] R. C. Smallridge, L. Wartofsky, B. J. Green, F. C. Miller, and K. D. Burman, *J. Clin. Endocrinol. Metab.*, 1979, **48**, 32.
[439] C. M. Lewin and L. C. Bieber, *Anal. Biochem.*, 1979, **96**, 322.
[440] S. J. Gatley, *Anal. Lett.*, 1979, **12**, 415.

2
Structural Investigations of Peptides and Proteins

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PART IA: Protein Isolation and Characterization *by R. Harrison*

1 Introduction

This year, in a departure from the past two reports, tabulated summaries of isolation procedures are restricted in two ways. Firstly, with a few exceptions, they are confined to certain classes of proteins, the emphasis still remaining on those isolated using affinity ligands. In additon, each procedure is tabulated only once. Thus, if more than one ligand was used in the isolation, all are listed together. A further departure has been to include a summary of the characterization of each protein (see footnote to Table 1). These changes have been made in part to ease the task of the reviewer and in part to condense more information into the available space. Once again it should be emphasized that no discrimination has been made between those proteins isolated for the first time and those previously isolated using a different procedure.

2 Protein Isolation Methodology

The year saw the publication of a revised International Union of Biochemistry listing of enzyme nomenclature.[1] In addition to an historical overview and principles of classification of enzymes, a chapter on the nomenclature of electron-transport proteins was included. Recommendations for the nomenclature of iron–sulphur proteins were also published.[2] Further historical depth on protein isolation was given in a chapter entitled 'Purification and Crystallization' in 'The Origins of Modern Biochemistry: a Retrospect on Proteins'.[3] Chapters on protein purification and affinity chromatography also appeared in 'Techniques in Protein and Enzyme Biochemistry'.[4] The texts of review lectures on 'the large scale isolation of enzymes'[5] and 'molecular sieving and non-ionic adsorption in polyacrylamide gels'[6] were also published, as was the 'Proceedings of the Fourth International

[1] 'Enzyme Nomenclature—Recommendations of the Nomenclature Committee of the International Union of Biochemistry', Academic Press, New York, San Francisco, London, 1979.
[2] 'Recommendations of the Nomenclature Committee of the I.U.B.', *Biochem. J.*, 1979, **181**, 513.
[3] N. W. Pirie, *Ann. N.Y. Acad. Sci.*, 1979, **325**, 21.
[4] Techniques in the Life Sciences, Biochemistry, ed. H. L. Kornberg, Elsevier/North-Holland Biomedical Press, 1979, Vol. B1/1.
[5] M.-R. Kula, G. Johansson, and A. F. Bückmann, *Biochem. Soc. Trans.*, 1979, **7**, 1.
[6] J. Porath, *Biochem. Soc. Trans.*, 1979, **7**, 1197.

Symposium on Glycoconjugate Research', which contained many articles dealing with glycoprotein and glycosidase isolation and characterization.[7]

Affinity Chromatography Methodology.—*Ligand Investigation.* Clearly, in any affinity chromatographic procedure, the separation achieved is largely dependent on the choice of ligand. However, very few papers show much evidence of investigation of potential ligands and one is left with the impression that the first ligand used that gave some degree of success was incorporated into the overall purification, enabling the publication to be entitled 'An affinity chromatographic procedure for the isolation of . . .'. A prime consideration is whether or not the ligand binds to a specific site on the protein being investigated. In one instance this was confirmed by active-site modification; the binding of bacterial amine-metabolizing enzymes to amine-Sepharose was abolished.[8] Frequently, the fractionation found at an affinity step is no better than can be obtained, with considerably less effort, using conventional procedures. Exceptions to this were shown in the isolation of β-adrenergic receptors from turkey[9] and frog[10] erythrocyte membranes and in the isolation of two bacterial enzymes using nucleotide ligands.[11,12] Both papers on β-adrenergic receptors reported studies with alprenol derivatives regarded as potential ligands. With the turkey receptor, ease of displacement from the ligand was a major consideration.[9] The study of frog receptor binding showed that, with a long hydrophilic spacer, non-specific binding was minimized, and it verified the biospecific nature of adsorption and elution by showing that, in addition to causing elution, adrenergic agents, present during loading of the affinity matrix, blocked adsorption.[10] AMP bound *via* either the C^8- or the N^6-positions to an aminohexyl spacer arm as well as 8-(8-amino-octyl)-IMP were investigated as potential ligands for *E. coli* IMP dehydrogenase; the first named was that finally employed. Interestingly, the enzyme did not bind to either NAD^+ or Cibacron Blue ligands.[11] AMP and ATP were compared as ligands for an amidotransferase from *B. subtilis*; ATP gave a 50-fold increase in binding over AMP even though binding was thought to occur at the site of action of the allosteric effector c-AMP. This supposition was based in part on the efficiencies of various eluants; nucleotides were less effective than phosphoribosylpyrophosphate, salt, or magnesium.[12] Other ligands investigated included phosphonic and arsonic acids for human erythrocyte acid phosphatase; one which contained a bulky hydrophobic moiety separated by one atom from the phosphorus atom was most suitable.[13] Water-soluble alkyldextrans were also investigated as potential ligands for phosphorylases. Using affinity electrophoresis, an increase in strength of binding of the ligand was found as the number of methylene groups was increased from 1 to 5. Both hydroxyl and amino groups in the terminal position decreased the strength of binding.[14] Kinetic

[7] 'Glycoconjugate Research—Proceedings of the Fourth International Symposium on Glycoconjugates', ed. J. D. Gregory and R. W. Jeanloz, Academic Press, New York, San Francisco, and London, 1979, Vols. 1 and 2.

[8] M. Okada, S. Kawashima, and K. Imahori, *J. Biochem. (Tokyo)*, 1979, **85**, 1225.

[9] G. Vauquelin, P. Geynet, J. Hanoune, and A. D. Strosberg, *Eur. J. Biochem.*, 1979, **98**, 543.

[10] M. G. Caron, Y. Srinivasan, J. Pitha, K. Kociolek, and R. J. Lefkowitz, *J. Biol. Chem.*, 1979, **254**, 2923.

[11] H. J. Gilbert, C. R. Lowe, and W. T. Drabble, *Biochem. J.*, 1979, **183**, 481.

[12] J. Y. Wong and R. L. Snitzer, *Arch. Biochem. Biophys.*, 1979, **196**, 134.

[13] J. Dissing, O. Dahl, and O. Svensmark, *Biochim. Biophys. Acta*, 1979, **569**, 159.

[14] K. Nakamura, A. Kuwahara, and K. Takeo, *J. Chromatogr.*, 1979, **171**, 89.

studies of various ligands such as those with *O*-methyltransferases can also serve as a starting point for ligand choice.[15] Class-specific ligands, such as imidazole compounds for haemoproteins,[16] avidin (bound as a monomer rather than a dimer as the former has a lowered affinity for biotin) for biotin-containing enzymes,[17] and a new coenzyme A analogue with possible application in the isolation of coenzyme A-requiring enzymes, such as citrate synthase and succinic thiokinase,[18] were also described. In a welcome appreciation of the inadvisability of using 8 M-urea as an eluant, thiamine pyrophosphate was replaced by riboflavin-binding protein as the ligand used in the isolation of chicken egg-white thiamine-binding protein.[19] A precautionary note, particularly with regard to macromolecular ligands, must be sounded here. Soybean trypsin inhibitor-Sepharose, which was used to remove proteases from a rat intestinal mucosa homogenate, bound the free catalytic subunits of c-AMP-dependent protein kinases, presumably by 'subsite-specific' binding. In this instance, the unexpected finding was happily incorporated into a scheme for catalytic subunit isolation; dissociation from the regulatory subunit was induced with cyclic-AMP prior to loading the column, and a 300-fold purification was achieved.[20]

Coupling Considerations—Matrix, Spacer, Procedure, and Ligand Concentration. Matrix choice, with one exception, received little attention during the past year. In this, a porous silica gel was proposed as an alternative matrix. Two attachment procedures were described—either to the aminopropyl-derivatized gel or to the epoxy-activated gel—and used respectively to purify anti-albumin from an albumin ligand or trypsin from an aminobenzamide ligand.[21] Similarly, few papers gave much consideration to the use of spacers between ligand and matrix. In light of the findings with soybean trypsin inhibitor described above, the use of ovalbumin as an 'economic' spacer between an estradiol ligand and Sepharose must be questioned.[22] Procedures for ligand immobilization received more attention. The controlled activation of agarose by cyanogen bromide released from a dispersed organic (acetonitrile) phase into the aqueous phase (3.3 molar phosphate, pH 11.2) was studied, particularly with respect to the degree of activation achieved, the multiplicity of attachment sites with protein ligands, and the rate of 'leakage' of ligand.[23] This procedure was also used in the immobilization of aspartate prior to the isolation of plant aspartate transcarbamylases.[24] A new 8-substituted thio-cyclic AMP was coupled *via* a hydrophilic spacer to epoxy-activated agarose to give a stable ligand with little non-specific protein binding, and was used in the isolation of protein kinase regulatory subunits.[25] Stable ligand–matrix linkage was also claimed for epichlorhydrin-activated agarose. Investigation of conditions for

[15] S. K. Sharma and S. A. Brown, *Can. J. Biochem.*, 1979, **57**, 986.
[16] K. Pommerening, M. Kühn, W. Jung, K. Buttgereit, P. Mohr, J. Stamberg, and M. Benes, *Int. J. Biol. Macromol.*, 1979, **1**, 79.
[17] K. P. Henrikson, S. H. G. Allen, and W. L. Mayly, *Anal. Biochem.*, 1979, **94**, 366.
[18] E. Rieke, S. Barry and K. Mosbach, *Eur. J. Biochem.*, 1979, **100**, 203.
[19] K. Muniyappa and P. R. Adiga, *Biochem. J.*, 1979, **177**, 877.
[20] E. Alhanaty, N. Bashan, S. Moses, and S. Shaltiel, *Eur. J. Biochem.*, 1979, **101**, 283.
[21] S. K. Roy and S. K. Kunda, *Anal. Biochem.*, 1979, **98**, 238.
[22] V. Sica and F. Bresciani, *Biochemistry*, 1979, **18**, 2369.
[23] G. Kümel, H. Daus, and H. Mauch, *J. Chromatogr.*, 1979, **172**, 2211.
[24] G. S. J. Rao, H. S. Savithri, S. Seethalakshmi, and N. A. Rao, *Anal. Biochem.*, 1979, **95**, 401.
[25] W. Weber, C.-W. Vogel, and H. Hilz, *FEBS Lett.*, 1979, **99**, 62.

activation and subsequent ligand immobilization suggested epichlorhydrin (5%) in sodium hydroxide (0.4 M) acting on a 30% suspension of agarose beads for 2 h at 40 °C to be optimum. With these conditions only a low degree of agarose cross-linking was observed.[26] New procedures described included the activation of cross-linked agaroses with 1,1′-carbonyldi-imidazole. The activated matrix, containing imidazolyl carbamate, was reacted with nitrogen nucleophiles such as amines. The procedure left no charge on the matrix and, with 0.1—3 mmol reactive groups g^{-1} matrix, compared favourably with cyanogen bromide-activated agarose. In the example the authors cited, trypsin inhibitor was coupled with carbodi-imide to 6-aminohexanoic acid at 60—70% of the level seen with cyanogen bromide activation.[27] Isonitrile derivatives of polysaccharides were also used to immobilize protein and other ligands. Cellulose, Sephadex, and Sepharose were compared for isocyanide content and low and high molecular weight ligand binding; trypsin was bound *via* amino groups to Sepharose at *ca.* 80 mg g^{-1} matrix and retaining > 70% of its activity. This was claimed to be a ten-fold increase over that achieved with cyanogen bromide activation.[28] A novel regenerable ligand–matrix system was also described; *N*-acetyl-L-tyrosine was reacted with diazotized *p*-aminobenzamidoethyl-Sepharose and then with protein. Specifically bound protein was removed by reduction of the diazo linkage. The matrix was then regenerated by diazotization and reaction with further ligand. In five cycles no loss of functional groups was observed.[29] Such a system would be of value with high-affinity ligand–protein interactions, where otherwise disruptive eluants would be required to displace the protein. A similar consideration applies to procedures reported for ligand immobilization *via* an ester bond to the matrix.[30-32] Of these, two utilized thiol ester bonds but different matrices. Porous succinamidopropyl glass beads were converted to the acyl chloride using anhydryous thionyl chloride and then treated with 3-mercaptopropionic acid or mercaptoacetic acid to form a thiol ester. Ligands (in this case proteins) were bound by reaction, at around neutral pH, with their free amino groups. Binding of 10—20 mg protein g^{-1} beads, stable for 6 months, was achieved. The ligand was released by treatment with 1 molar hydroxylamine at pH 7.[30] Hydroxylamine was also used to release cholestrol or cholic acid, used in the isolation of human plasma low- and high-density lipoproteins and bound to agarose *via* a thiol ester bond. In this case the thiol ester was generated by carbodi-imide coupling of the ligand, dissolved in dioxan, to cysteamine-Sepharose.[31] The third paper again described coupling of the ligand *via* free amino groups, but to succinimydyl ester-agarose. Ligand release was achieved either by hydroxylamine treatment or by treatment with imidazole–glycine or tris buffer. This procedure emphasized the complementary nature of ester-coupled ligands to other cleavable ligands such as disulphide and vicinyl-hydroxyl-containing derivatives, pointing out the deleterious effects of free thiols or periodate on many proteins.[32] However, few cleavable ligand–matrix linkages are as

[26] I. Matsumoto, Y. Mizuno, and N. Seno, *J. Biochem. (Tokyo)*, 1979, **85**, 1091.
[27] G. S. Bethell, J. S. Ayers, W. S. Hancock, and M. T. W. Hearn, *J. Biol. Chem.*, 1979, **254**, 2572.
[28] A. Freeman, M. Sokolvsky, and L. Goldstein, *Biochim. Biophys. Acta*, 1979, **571**, 127.
[29] V. K. Joshi, K. M. Shahani, A. Kilara, and F. W. Wagner, *J. Chromatogr.*, 1979, **176**, 11.
[30] R. J. Brown, H. E. Swaisgood, and H. R. Horton, *Biochemistry*, 1979, **18**, 4901.
[31] A. Wichman, *Biochem. J.*, 1979, **181**, 691.
[32] P. Singh, S. D. Lewis, and J. A. Shafer, *Arch. Biochem. Biophys.*, 1979, **193**, 284.

attractive as that of cyanocobalamine-Sepharose. Stable at 4 °C (at which temperature cofactor-free protein is bound) the linkage was broken by incubation at 37 °C.[33] Immobilization of glycoconjugates also received considerable attention. The general procedure used was to generate aldehyde groups on the carbohydrate by periodate oxidation, and to couple these to amines (usually diamines so that a free amino group was retained) by borohydride reduction. Numerous ways of proceeding with the derivatized ligand, including choice and method of activation of the matrix, were compared.[34, 35] Periodate oxidation was also used to prepare rat liver 5S RNA for immobilization on adipic acid dihydrazide-Sepharose; the coupled RNA was used to isolate rat ribosomal proteins. The major proteins bound were L19, L8, and L6 from the 60S and S13 and S9 from the 40S subunits. *E. coli* ribosomal proteins did not bind.[36] Finally, in two cases, conditions for coupling 'subunit-exchange' ligands were examined. Clearly these are of importance as the ligand (which can be any component of an associating–dissociating system) must be bound as the monomeric unit of the system. Subunit exchange is then used as a specific purification step; matrix loading is carried out under conditions that favour association, and elution under conditions that favour dissociation. The systems investigated were α-chymotrypsin[37] and human urinary glycoprotein.[38]

Optimum Use of Affinity Matrix—Loading and Elution. The apparently simple procedure of loading, washing, and elution frequently needs considerable investigation before optimum conditions are found. This was illustrated by the loss of pig lymphocyte adenylate cyclase on concanavalin A; readdition of unbound material to that specifically eluted showed that loss of activity was due to dissociation of the enzyme.[39] Binding of milk lactoperoxidase to di-iodotyrosine was increased in the presence of cysteine or glutathione, but the free thiols interfered with binding to thiol-Sepharose.[40] A bacterial toluene dioxygenase did not bind to *p*-toluic acid unless it was first reduced. However, dithionite reduction produced an enzyme that bound irreversibly to the ligand, and NADH reduction was required for successful chromatography.[41] Non-specific binding to affinity matrices also limits their potential. In at least one instance this was decreased by limiting the amount of affinity resin available; the rationale is that the specifically bound enzyme, a sulphotransferase found in human adrenal extracts, competed for the available affinity ligand.[42] Non-specific binding of proteins to a lectin-Sepharose column was decreased by 'prewashing' the column with albumin.[43] (Albumin, containing no carbohydrate, should not bind specifically to the lectin and therefore would bind because of non-specific charge and hydrophobic interactions, hopefully saturating

[33] J. Lindemans, J. von Kapel, and J. Abels, *Biochim. Biophys. Acta*, 1979, **579**, 40.
[34] H.-Y. Hsiao and G. P. Royer, *Arch. Biochem. Biophys.*, 1979, **198**, 379.
[35] A. P. Corfield, T. L. Parker, and R. Schauer, *Anal. Biochem.*, 1979, **100**, 221.
[36] N. Ulbrich, A. Lin, and I. G. Wool, *J. Biol. Chem.*, 1979, **254**, 8641.
[37] E. Antonini, G. Carrea, P. Cremonesi, P. Pasta, M. R. Rossi Fanelli, and E. Chiancone, *Anal. Biochem.*, 1979, **95**, 89.
[38] G. Carrea, G. Lugaro, R. Niada, P. Vecchini, and E. Antonini, *FEBS Lett.*, 1979, **104**, 393.
[39] J.-C. Bonnafous, J. Dornand, and J.-C. Mani, *FEBS Lett.*, 1979, **99**, 152.
[40] J. Pommier and H. J. Cahnmann, *J. Biol. Chem.*, 1979, **254**, 3006.
[41] V. Subramanian, T.-N. Liu, W. K. Yeh, and D. T. Gibson, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 1131.
[42] J. B. Adams and D. McDonald, *Biochim. Biophys. Acta*, 1979, **567**, 144.
[43] J. H. Freed, D. W. Sears, J. L. Brown, and S. G. Nathenson, *Mol. Immunol.*, 1979, **16**, 9.

such sites on the matrix.) The substrate ligand, L-trialanine *p*-nitroanilide, was successfully used at sub-zero temperatures, where the turnover rate was low, in the isolation of porcine pancreatic elastase.[44] Such applications can only be used where the protein (and binding) are stable in the antifreeze component of the buffer (in this case hypersaline). Other ligand–protein interactions were found to be temperature-sensitive; elution was achieved either wholly or in part by raising the temperature from 2 to 22 °C.[45,46] Observations on the effect of ligands known to bind at the active centre of chymotrypsin on its binding to 4-phenylbutylamine showed different ligands to promote increased strength of binding, to exert a neutral effect, or to cause dissociation. This showed the considerable range available for 'fine-tuning' of affinity chromatography.[47] Even in the absence of stable ligand–protein interaction, affinity chromatography can, with judicious control of binding and elution conditions, provide a useful step in enzyme isolation. This was evidenced in the isolation of *Drosophila* alcohol dehydrogenase; a *concentrated* extract was applied to the column and the column effluent was monitored for enzyme activity. As soon as activity was observed the enzyme was eluted with a specific ligand and a 23-fold purification was achieved.[48] Apart from activity measurements, the properties of a protein, before and after an affinity procedure (or any step in its isolation), are frequently not followed. A suggestion that such attention might not be unwarranted was found in the alteration in binding properties of a folate-binding protein isolated from cows' whey by an affinity procedure, although in this instance the differences were regarded as due to loss of a cofactor.[49]

Affinity Chromatography versus *Affinity Elution.* Affinity elution is generally regarded as a procedure whereby proteins can be specifically eluted from non-affinity matrices by use of specific ligands. In contrast, affinity chromatography requires specific adsorption to an immobilized ligand followed by desorption using a variety of methods, frequently totally non-specific in nature as selection occurred during the loading step. Affinity elution was exemplified by the phosphate- or ATP-dependent elution of an AMP deaminase from phosphocellulose [50] and by the c-AMP- or, with improved efficiency, 8-(4-aminobutyl)-c-AMP-dependent elution of the catalytic subunit of a variety of mammalian ATP-dependent protein kinases from DEAE cellulose.[51] The latter paper pointed out some interesting comparisons of charge differences between the catalytic and regulatory subunits of the enzyme from a variety of mammalian tissues. Frequently, however, the distinction is less clear. One example was the purification of human liver alkaline phosphatase on tyraminyldiazo-*p*-aminobenzylphosphonic acid. Most of the protein contained in a crude extract was bound by the ligand, and a 400-fold purification of the enzyme was achieved by selective elution with the substrate 2-naphthylphosphate.[52] A further example was given by the binding of bovine pancreatic RNAse A and liver

[44] C. Balny, C. Le Doucen, P. Douzou, and J. G. Bieth, *J. Chromatogr.*, 1979, **168**, 133.
[45] M. F. Jett and T. R. Soderling, *J. Biol. Chem.*, 1979, **254**, 6739.
[46] V. Boggaram, T. Brobjer, K. Larson, and B. Mannervik, *Anal. Biochem.*, 1979, **98**, 335.
[47] B. M. Dunn and W. A. Gilbert, *Arch. Biochem. Biophys.*, 1979, **198**, 533.
[48] A. J. L. Brown and C.-Y. Lee, *Biochem. J.*, 1979, **179**, 479.
[49] S. I. Hansen, J. Holm, and J. Lyngbye, *Biochim. Biophys. Acta*, 1979, **579**, 479.
[50] M. Yoshino, K. Murakami, and K. Tsushima, *Biochim. Biophys. Acta*, 1979, **570**, 157.
[51] D. Kübler, M. Gagelmann, W. Pyerin, and V. Kinzel, *Z. Physiol. Chem.*, 1979, **360**, 1421.
[52] L. E. Seargeant and R. A. Stinson, *J. Chromatogr.*, 1979, **173**, 101.

glutamate dehydrogenase to folate-Sepharose. While such a ligand might be thought specific for folate-binding proteins, RNAse A could be desorbed using nucleotides.[53] In this instance, the 'non-specific' binding was thought to be related to derivatization of the agarose as cationic groups are introduced. It is less clear whether the ADP-agarose used to isolate *Acanthamoeba* myosin I was acting as an affinity matrix or not as similar conditions were used to effect adsorption onto and elution from underivatized agarose.[54] Often, particularly with ligands having a broad spectrum of affinity applications, it is not possible to differentiate between the two procedures. Thus, using affinity eluants, a number of *Drosophila* enzymes were fractionated on ATP-Sepharose.[55] In other cases the basis for binding and elution was totally unclear. Two papers that illustrate the complexity of these considerations dealt with the isolation of tryptophan synthase from *E. coli*, where the existence of dissociable subunits gives increased potential for confusion,[56] and with the isolation of various steroid-metabolizing enzymes from chicken liver on *p*-(phenoxypropoxy)aniline-Sepharose.[57] One possible theoretical way of differentiating between specific and non-specific binding to an affinity ligand appeared. A comparison of the elution of pig heart aminotransferase and albumin from affinity (2-oxoglutarate and cycloserine) and non-affinity (aminohexane) ligands showed a logarithmic relationship between elution and temperature for the specifically bound enzyme but a linear relationship between elution and temperature for the non-specifically bound protein.[58]

Specific Affinity Ligands.—*Triazine Dyes.* A noteworthy and comprehensive review of protein isolation using immobilized triazine dyes was published. One observation made, not previously generally appreciated, was that, while Sepharose CL-6B will bind 50% more ligand than does non-cross-linked 6B, the protein-binding capacity of the ligand is only 36% of that of derivatized Sepharose 6B. The one possible disadvantage of triazine dyes was that commercial preparations are frequently heterogeneous.[59] Others commented on this heterogeneity with respect to the most widely used dye, Cibacron blue F3GA. Impurities in preparations from different commercial sources were capable of cross-linking to proteins, giving misleading information in preliminary kinetic studies to evaluate the potential of the dye for specific enzyme fractionation.[60] Controversy still continues with regard to the basis of Cibacron blue binding to proteins, particularly with proteins that utilize dinucleotide cofactors. The L-arabinose-binding protein of *E. coli*, which does possess a dinucleotide-like fold super-secondary structure, did not bind specifically to the dye. Thus it cannot be regarded as a definitive probe for such a fold. (One current criterion is that substrate elution, at a ten-fold lower concentration than salt elution, from the dye is indicative of its presence.) The authors suggested that its action was derived from both cation exchange and hydrophobic

[53] F. Sawada, *Anal. Biochem.*, 1979, **98**, 184.
[54] H. Maruta, H. Gadasi, J. H. Collins, and E. D. Korn, *J. Biol. Chem.*, 1979, **254**, 3624.
[55] C.-Y. Lee, D. Charles, and D. Bronson, *J. Biol. Chem.*, 1979, **254**, 6375.
[56] H.-P. Gschwind, U. Gschwind, C. H. Paul, and K. Kirschner, *Eur. J. Biochem.*, 1979, **96**, 403.
[57] A. G. C. Renwick, S. M. Chambers, and P. Willcox, *Biochem. J.*, 1979, **177**, 401.
[58] T. K. Korpela and E. Mäkinen, *J. Chromatogr.*, 1979, **174**, 361.
[59] P. D. G. Dean and D. H. Watson, *J. Chromatogr.*, 1979, **165**, 301.
[60] B. H. Weber, K. Willeford, J. G. Moe, and D. Piszkiewicz, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 252.

properties, and that its power in enzyme fractionation is based on affinity elution rather than affinity chromatography.[61] Others also suggested that there is a strong hydrophobic component to its binding properties, based both on similarity to octyl- and pentyl-ligands in the isolation of a human growth factor [62] and on salt, but not nucleotide, elution of a rat erythrocyte glyoxylase.[63] Induced circular dichroism studies of the binding of Cibacron blue and congo red to dehydrogenases and kinases, together with studies of competition with appropriate coenzymes and substrates, showed no unique conformation, and a specific dinucleotide fold interaction was therefore regarded as unlikely. However, the study did show closely related proteins to produce similar conformations.[64] Clearly, binding does frequently involve 'active site' regions of enzymes. This was shown by the quenching of the difference spectrum of a dihydrofolate reductase with Cibacron blue by all specific eluants—NADPH, 7,8-dihydrofolate, and amethopterin, as well as by $NADP^+$.[65] A more specific illustration of this was given by *X*-ray studies of the binding of Cibacron blue to a liver alcohol dehydrogenase. Binding was found to be similar to that of NAD^+ in three of the four ring structures, with the anthroquinone ring lying in the adenine site. (Many coenzyme analogues, as well as other molecules such as salicylate, are known to bind here.) However, the binding of ring A, the aminobenzene sulphonate ring, was different. (One possible explanation given for this was that alcohol dehydrogenase acts mainly on hydrophobic uncharged substrates and that the environment required for the binding of such groups prevents the entry of ring A.)[66] Other dyes or analogues advocated for protein isolation included Procion red HE-3B, which was coupled to agarose without matrix activation,[67] and trinitrobenzene, regarded as inducing interactions similar to those of Cibacron blue.[68] A partial listing of enzymes purified using mononucleotide, dinucleotide, and triazine dye affinity ligands is given in Table 1. Others are included in listings of specific classes of proteins. No differentiation between different commercial Cibacron dye matrices is made, all being abbreviated CB. Other dyes or analogues are trinitrobenzene–TNB and Procion brilliant blue MR–PMR.

Hydrophobic Ligands. While hydrophobic ligands have gained widespread acceptance over the past few years, investigation of ligands, and of factors affecting protein binding to them, has received little attention. One exception to this was the description of 'substrate-induced' hydrophobic chromatography of rat liver phenylalanine hydroxylase; the enzyme bound to phenyl-Sepharose only in the presence of phenylalanine, and desorption was effected by omission of the substrate from the buffer.[146] The effect of acyl chain length on the chromatography of acyl–acyl carrier protein on octyl-Sepharose was also investigated, elution was effected with n-propanol.[147] The relatively narrow ligand specificity that some

[61] R. S. Beissner, F. A. Quiocho, and F. R. Rudolph, *J. Mol. Biol.*, 1979, **134**, 847.
[62] C.-H. Heldin, B. Westermark, and Å. Wasteson, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3722.
[63] J. C. Ball and D. L. Vander Jagt, *Anal. Biochem.*, 1979, **98**, 472.
[64] R. A. Edwards and R. W. Woody, *Biochemistry*, 1979, **18**, 5197.
[65] B. B. Chambers and R. B. Dunlap, *J. Biol. Chem.*, 1979, **254**, 6515.
[66] J.-F. Biellman, J.-P. Samama, C. I. Bränden, and H. Eklund, *Eur. J. Biochem.*, 1979, **102**, 107.
[67] N. D. Harris and P. G. H. Byfield, *FEBS Lett.*, 1979, **103**, 162.
[68] R. Kornbluth, P. S. Tracy, and T. P. Fondy, *Biochim. Biophys. Acta*, 1979, **568**, 273.

Table 1 *Proteins isolated using mononucleotide, dinucleotide, and triazine dye affinity ligands*

The following single letter abbreviations are used in *all* Tables to summarize the characterization of each protein: M—molecular weight; U—subunit composition; A—amino-acid composition; H—carbohydrate composition; S—sedimentation coefficient; D—diffusion coefficient; I—isoelectric point; K—kinetic characterization (frequently extremely basic); C—other chemical and immunochemical properties; P—other physical properties.

Protein (substrate)	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Dehydrogenases						
Alanine	*Halobacterium salinarium*	$NADP^+$	to remove contaminants (ADP, AMP did not)	×2.4		69
		(butyl)	decreasing Na_2SO_4, increasing NaCl gradient	×2.5		69
Alcohol	*Drosophila melanogaster*	AMP	weakly bound, elute NAD^+, pyrazole	×23.3		48
Alcohol	*Drosophila melanogaster*	ATP	NAD^+, pyrazole	×34		55
Alcohol (secondary)	*Pseudomonas*	CB	NAD^+; 67% recovery	×44	KMU	70
Aldehyde	human erythrocyte	AMP	phosphate	×8.3	K	71
Formaldehyde	pea seeds	AMP	1 M-NaCl	×1510	CIKMU	72
Formate	pea seeds	AMP	0.1 M-NaCl	×454	CIKMU	72
L-Fucose	rabbit liver	AMP	pH elution, removed other dehydrogenases	×3.9	IKM	73
Glucose 6-phosphate	rat liver cytoplasm	CB	unbound in $NADP^+$	×6.6	KMU	74
		$NADP^+$	$NADP^+$ elution	×10.4	KMU	74
Glucose 6-phosphate	*Candida boidinii*	CB	$NADP^+$ elution	×2.7	KMU	75
		ADP	0.5 M-$NADP^+$ after 0.1 mM wash	×1.1	KMU	75

[69] D. Keradjopoulos and A. W. Holldorf, *Biochim. Biophys. Acta*, 1979, **570**, 1.
[70] C. T. Hou, R. N. Patel, A. I. Laskin, N. Barnabe, and I. Marczak, *FEBS Lett.*, 1979, **101**, 179.
[71] K. Inoue, H. Nishimukai, and K. Yamasawa, *Biochim. Biophys. Acta*, 1979, **569**, 117.
[72] L. Uotila and M. Koivusalo, *Arch. Biochem. Biophys.*, 1979, **196**, 33.
[73] M. Endo and N. Hiyama, *J. Biochem. (Tokyo)*, 1979, **86**, 1559.
[74] M. L. Dao, J. L. Watson, R. Delaney, and B. C. Johnson, *J. Biol. Chem.*, 1979, **254**, 9441.
[75] N. Kato, H. Sahm, H. Schütte, and F. Wagner, *Biochim. Biophys. Acta*, 1979, **566**, 1.

Table 1 (*cont.*)

Protein (substrate)	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Dehydrogenases (cont.)						
Glyceraldehyde 3-phosphate	*E.coli*	NAD^+	NAD^+ gradient, study of mutant enzymes, immuno-chemical analysis	×3		76
α-Glycerol phosphate	*Drosophila melanogaster*	ATP	NADH elution	×83		55
Glycerol 3-phosphate	rabbit brain	TNB	NADH; NAD^+-linked; increased recovery with dihydrazide link	×867	AIK	68
				×144	AIK	
sn-Glycerol 3-phosphate: NAD^+ oxidoreductase	spinach leaves	TNB	NADH elution	×536	KM	77
Histidinol	*Saccharomyces cerevisiae*	AMP	AMP elution; His-4 gene cluster trifunctional polypeptide with histidinol deh. (60% recovery), phosphoribosyl-AMP	×17		
			cyclohydrolase (72%), and phosphoribosyl-ATP pyrophosphohydrolase (65%)	×20	MU	78
				×18		
Homoserine	maize suspension culture	CB	NADPH; threonine-sensitive	×17	KMU	79
			unbound threonine-resistant	×1.9	KMU	79
		ADP	NADPH; threonine-sensitive	×3.7	KMU	79
			NADPH; threonine-resistant	×14.2	KMU	79
		(octyl)	KCL; threonine-senstive	×3.1	KMU	79
			KCL; threonine-resistant	×4.1	KMU	79

3-Hydroxyacyl-CoA	*Mycobacterium smegmatis*	NAD^+	phosphate elution	×5.7	CKU	80
3α-Hydroxysteroid	*Pseudomonas sp.*	NAD^+	phosphate elution			81
3β-Hydroxysteroid	*Pseudomonas sp.*	NAD^+	phosphate elution			81
Isocitrate	*E. coli*	CB	$NADP^+$ (increased salt less pure); $NADP^+$-specific enzyme	×6.7	AU	82
Isocitrate	*Neurosporra crassa*	AMP	NAD^+; NAD^+-specific enzyme; high mol. wt. aggregates in absence of Triton; PMSF present	×39	SU	83
Isocitrate	*E. coli*	CB	$NADP^+$; phosphorylated $NADP^+$-specific	×4.3	CU	84
Lactate	horse muscle	AMP	reduced NAD^+-pyruvate adduct; M_4 enzyme	×21	ACKMU	85
Lipoamide	*Malbranchea pulchella*	PMR	NaCl elution	×13.6	ACKMPU	86
Malate	rat liver mitochondria	CB	NAD^+ –malate elution		AIKPU	87
Malate	rat heart mitochondria	CB	NAD^+ –malate elution		AIKPU	87
Malate	*Drosophila* mitochondria	ATP	NaCl	×13.2		55
Malate	*Drosophila* cytosol	ATP	NAD^+ –oxaloacetate	×33		55
Malate	thermophilic and mesophilic bacteria	AMP	NADH	×50—200	U	88
		NAD^+	NADH	×10—50	U	88

[76] J. D. Hillman, *Biochem. J.*, 1979, **179**, 99.
[77] G. T. Santora, R. Gee, and N. E. Tolbert, *Arch. Biochem. Biophys.*, 1979, **196**, 403.
[78] J. K. Keesey, R. Bigelis, and G. R. Fink, *J. Biol. Chem.*, 1979, **254**, 7427.
[79] T. J. Watter, J. A. Connelly, B. G. Gengenbach, and F. Wold, *J. Biol. Chem.*, 1979, **254**, 1349.
[80] T. Shimakata, Y. Fujita, and T. Kusaka, *J. Biochem. (Tokyo)*, 1979, **86**, 1191.
[81] M. Shikita and P. Talalay, *Anal. Biochem.*, 1979, **95**, 286.
[82] B. Vasquez and H. C. Reeves, *Biochim. Biophys. Acta*, 1979, **578**, 31.
[83] D. A. Nealon and R. A. Cook, *Biochemistry*, 1979, **18**, 3616.
[84] M. Garnak and H. C. Reeves, *J. Biol. Chem.*, 1979, **254**, 7915.
[85] S. Huang, C.-Y. Lee, and S. S.-L. Li, *Int. J. Biochem.*, 1979, **10**, 279.
[86] D. J. McKay and K. J. Stevenson, *Biochemistry*, 1979, **18**, 4702.
[87] K. N. Kuan, G. L. Jones, and C. S. Vestling, *Biochemistry*, 1979, **18**, 4366.
[88] I. P. Wright and T. K. Sundaram, *Biochem. J.*, 1979, **177**, 441.

Table 1 (*cont.*)

Protein (*substrate*)	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Dehydrogenases (cont.)						
NADH	*Drosophila hydei* mitochondria	CB	NaCl–increased pH; larva embryo	×5	K	89
				×4.8	K	89
6-Phosphogluconate	*Candida boidinii*	ADP	$NADP^+$ gradient	×1.2	KMU	75
Kinases						
Adenosine	rabbit liver	ATP	slightly retarded	×1.3	KMU	90
		AMP	increased pH	×39.8	KMU	90
Adenosine	human placenta	AMP	adenosine–increased pH, salt	×449	IKMU	91
Casein	human erythrocytes	ATP	KCl		KMU	92
Creatine	green sunfish	CB	pH; different isoenzymes	×4—8		93
Creatine	bovine heart mitochondria	ATP	ATP–NaCl	×1.6	AK	94
Glycogen phosphorylase	rabbit liver	CB	KCl–ethylene glycol; kinase II	×6.8	KMS	95
Malate (thiokinase)	*Pseudomonas* MA	ADP	CoA–ATP–malate; CoA–ATP wash	×1.4	KMSU	96
NAD^+	yeast	NAD^+	NAD^+ elution	×4	IKMU	97
Phosphofructokinase	human muscle	CB	ADP or ATP–fructose	×50	MU	98
	heart	CB	6-phosphate; examination	×5	MU	
	leukemic blasts	CB	of tissue isoenzymes	×142	MU	
Phosphoglycerate	bakers' yeast	CB	NaCl	×1.8		99
Protein	adrenocortical carcinoma	c-AMP	c-AMP elution after 2 M-NaCl wash	×22	KMU	100
		(hexyl)	NaCl gradient	×10.8	KMU	100
Protein	bovine heart	thio-c-AMP	kinase II regulatory subunit	×167		25

Reductases						
Aromatic aldehydes and ketones	guinea pig liver	BD	ammonium sulphate; AR1	×1.9	CKMPU	101
			AR2	×3.9	CKMPU	101
			AR3	×1.9	CKMPU	101
Aldose	porcine brain	CB	NADPH gradient; 17% recovery	×10.6	KMU	102
Flavoenzyme NADH-acceptor	*Methylococcus capsulatus*	AMP	component c of methane mono-oxygenase	×3.2	P	103
NADPH-cytochrome *c* (P-450)	*Catharanthus roseus*	ADP	2'-AMP	×90	CIKU	104
NADPH-cytochrome P-450	rabbit liver microsomes	ADP		×8	CMP	105
NADPH-cytochrome *c* (P-450)	house fly microsomes	$NADP^+$	$NADP^+$	×74	CKU	106
Glutathione	rabbit liver	CB	NADPH elution	×8.4	CMPU	107
Glutathione	porcine erythrocytes	ADP	4 M-urea, temp. 4 → 22 °C	×3048	ACDIKMPSU	46
			NADPH–glutathione; mercaptoethanol increases yield	×13.8		

[89] L. Hermans, *Biochim. Biophys. Acta*, 1979, **567**, 125.
[90] R. L. Miller, D. L. Adamczyk, and W. H. Miller, *J. Biol. Chem.*, 1979, **254**, 2339.
[91] C. M. Andres and I. H. Fox, *J. Biol. Chem.*, 1979, **254**, 11388.
[92] P. Boivin and C. Galand, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 7.
[93] S. E. Fisher and G. S. Witt, *Anal. Biochem.*, 1979, **94**, 89.
[94] N. Hall, P. Addis, and M. DeLuca, *Biochemistry*, 1979, **18**, 1745.
[95] K. Sakai, S. Matsumura, Y. Okimura, H. Yamamura, and Y. Nishizuka, *J. Biol. Chem.*, 1979, **254**, 6631.
[96] M. Elwell and L. B. Hersh, *J. Biol. Chem.*, 1979, **254**, 2434.
[97] Y.-M. Tseng, B. G. Harris and M. K. Jacobson, *Biochim. Biophys. Acta*, 1979, **568**, 205.
[98] D. Cottreau, M. J. Levin, and A. Kahn, *Biochim. Biophys. Acta*, 1979, **568**, 183.
[99] K. D. Kulbe and R. Schuer, *Anal. Biochem.*, 1979, **93**, 46.
[100] G. Shanker, H. Ahrens, and R. K. Sharma, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 66.
[101] H. Sawada, A. Hara, F. Kato, and T. Nakayama, *J. Biochem. (Tokyo)*, 1979, **86**, 871.
[102] R. A. Boghosian and E. T. McGuinness, *Biochim. Biophys. Acta*, 1979, **567**, 278.
[103] J. Kolby and H. Dalton, *Biochem. J.*, 1979, **177**, 903.
[104] K. M. Madyastha and C. J. Coscia, *J. Biol. Chem.*, 1979, **254**, 2419.
[105] J. S. French and M. J. Coon, *Arch. Biochem Biophys.*, 1979, **195**, 165.
[106] R. T. Mayer and J. L. Durrant, *J. Biol. Chem.*, 1979, **254**, 756.
[107] G. Zanetti, *Arch. Biochem. Biophys.*, 1979, **198**, 241.

Table 1 (*cont.*)

Protein (*substrate*)	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Reductases (cont.)						
3-Hydroxy-3-methylglutaryl CoA	rat liver	CB	KCl	×12.5		108
		HMG-CoA	hydroxymethylglutaryl CoA	×11.9		108
met-Myoglobin	bovine heart muscle	CB	NaCl–NADH	×16.9		109
Quinonoid	bovine kidney	CB	requires tetrahydropterin to bind; folate, amethopterin compete, NAD^+ elution	×15	K	110
UDP-*N*-Acetylenolpyruvyl-glucosamine	*E. coli*	CB	unadsorbed	×1.2	CMPU	111
		$NADP^+$	NADPH elution	×8.6	CMPU	111
Synthetases						
Acyl–acyl carrier protein	*E. coli*	CB	KSCN elution	×63	K	112
Acyl–coenzyme A	rat liver	CB	ATP wash; ATP+NaCl elution	×39	AKU	113
	rat microsomes	CB	ATP wash; ATP+NaCl elution	×12.4	AKU	113
Acyl–coenzyme A	*Candida lipolytica*	CB	ATP elution; synthetase I	×8.2	KU	114
Glutamine	*Azotobacter vinelandii*	CB	ADP elution; 75% recovery	×1.5	KMSU	115
Glutamine	*Azotobacter vinelandii*	CB	ADP elution	×39		116
Glutamine	rat kidney	ATP	ATP–$MnCl_2$ gradient	×3.5		117
Nopaline	crown gall	CB	NADPH; induced on *Helianthus* by *Agrobacterium tumefaciens*	×12.3	KMU	118
Transferases						
α-*N*-Acetyl-galactosaminide α2→6 sialyl-	porcine submaxillary gland	CDP	NaCl or CTP	×2.4, ×8	KMU	119
Adenine phosphoribosyl-	human erythrocytes	GMP	AMP elution	×307	ACMU	120

Galactosyl-	fetuin (fetal calf serum)	UDP	binds in Mn^{2+}, elute − Mn^{2+}		×327		121
UDP-glucuronyl-	rat liver microsomes	UDP	UDP-glucuronic acid; 3-methylcholanthrene-induced		×4.2	KU	122
			phenobarbital-induced		×6.5	KU	122
Glutamine phosphoribosyl-pyrophosphate amido-	*E. coli*	CB	not adsorbed; removes dehydrogenase		×1.3	AKMPU	123
Hypoxanthine-guanine phosphoribosyl-	yeast	GMP	GMP elution		×1958	IKMU	124
Phenolsulpho-	rat liver	CB	NaCl		×16.7	AKMU	125
		ATP	AMP, 5′-phosphosulphate	I	×1.3	AKMU	125
				II	×1.4	AKMU	125
GTP-AMP phospho-	bovine heart mitochondria	CB	GTP–poly(ethleneglycol)-400		×1.1	AIU	126

[108] G. C. Ness, C. D. Spindler, and M. M. Moffler, *Arch. Biochem. Biophys.*, 1979, **197**, 493.
[109] L. Hagler, R. I. Coppes, and R. H. Herman, *J. Biol. Chem.*, 1979, **254**, 6505.
[110] M. M. Chauvin, K. K. Korri, A. Tirpak, R. C. Simpson, and K. G. Scrimgeour, *Can. J. Biochem.*, 1979, **57**, 178.
[111] R. A. Anwar and M. Vlaovic, *Can. J. Biochem.*, 1979, **57**, 188.
[112] C. O. Rock and J. E. Cronan, *J. Biol. Chem.*, 1979, **254**, 7116.
[113] T. Tanaka, K. Hosaka, M. Hoshimaru, and S. Numa, *Eur. J. Biochem.*, 1979, **98**, 165.
[114] K. Hosaka, M. Mishina, T. Tanaka, T. Kaminyo, and S. Numa, *Eur. J. Biochem.*, 1979, **93**, 197.
[115] J. Siedel and E. Shelton, *Arch. Biochem. Biophys.*, 1979, **192**, 214.
[116] J. E. Lepo, G. Stacey, O. Wyss, and F. R. Tabita, *Biochim. Biophys. Acta*, 1979, **568**, 428.
[117] L. Oppenheimer, V. P. Wellner, O. W. Griffith, and A. Meister, *J. Biol. Chem.*, 1979, **254**, 5184.
[118] J. D. Kemp, D. W. Sutton, and E. Hack, *Biochemistry*, 1979, **18**, 3755.
[119] J. E. Sadler, J. I. Rearick, and R. L. Hill, *J. Biol. Chem.*, 1979, **254**, 5934.
[120] J. Holden, G. S. Meredith, and W. N. Kelley, *J. Biol. Chem.*, 1979, **254**, 6951.
[121] M. Pierce, R. D. Cummings, T. A. Cebula, and S. Roth, *Biochim. Biophys. Acta*, 1979, **571**, 166.
[122] K. W. Bock, D. Josting, W. Lilienblum, and H. Pfeil, *Eur. J. Biochem.*, 1979, **98**, 19.
[123] L. J. Messenger and H. Zalkin, *J. Biol. Chem.*, 1979, **254**, 3382.
[124] R. Schmidt, H. Wiegand, and U. Reichert, *Eur. J. Biochem.*, 1979, **93**, 355.
[125] R. O. Sekura and W. B. Jakoby, *J. Biol. Chem.*, 1979, **254**, 5658.
[126] A. G. Tomasselli, R. H. Schirmer, and L. H. Noda, *Eur. J. Biochem.*, 1979, **93**, 257.

Table 1 (*cont.*)

Protein (substrate)	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Miscellaneous proteins						
Adenylate cyclase	bovine brain	CB	ATP/KCl elution; to remove Ca^{2+}-regulatory protein	×3.3		127
Adenylate cyclase inhibitory protein	bovine brain	CB	NaCl elution; also inhibits cyclic nucleotide phosphodiesterase		IKMPU	128
'Arom' multienzyme aggregate	*Euglena gracilis*	CB	shikimic acid	×19	MPSU	129
ATP citrate lyase	rat liver	CB	ATP elution	×4.7	K	130
Contractile proteins	skeletal muscle	CB	NaCl			131
Cyclic nucleotide phosphodiesterase	bovine brain	CB	EDTA elution	×13	K	132
			c-GMP elution	×73	K	132
Deoxyuridine triphosphate nucleotidohydrolase	blast cells	CB	tris gradient	×10.9		133
		(phenyl)	decreasing ammonium sulphate–ethyleneglycol monoethylether	×3.2		133
Fatty acyl CoA ligase	rat liver mitochondria	CB	KCl elution; long chain substrate	×1.4	KM	134
Fructose 1,6-bis-phosphatase	mouse liver	CB	AMP, fructose 1,6-bisphosphate	×3	AKMPU	135
Glyoxylase I	human erythrocyte	CB	phosphate; multiple forms isolated	×30	K	136
				×85	K	136
				×100	K	136
Glyoxylase II	rat erythrocyte	CB	KCl elution	×30	M	63
		(glutathione)	KCl elution	×3.2	M	63
Guanylate cyclase	rat lung	GTP	EDTA elution; 13% recovery	×45	CKMU	137
p-Hydroxybenzoate hydroxylase	*Pseudomonas fluorescens*	CB	NaCl; replaces DEAE, 16 → 50% recovery CB coupled at 60 °C	×14.6	AKMSU	138
L-Kynurenine 3-hydroxylase	rat liver mitochondria	$NADP^+$	$NADP^+$; no affinity for L-kynurenine or AMP	×2.2	M	139

Malic enzyme	bovine heart mitochondria	$NADP^+$	$NADP^+$ elution	×600	MU	140
		ADP	$NADP^+$ at pH = 8 after $NADP^+$ pH = 7 wash	×1.5	MU	141
$NADP^+$ malic enzyme	maize leaf	CB	KCl; 48% yield	×1.2	KMSU	142
Malonyl-CoA decarboxylase	*Mycobacterium tuberculosis*	$NADP^+$	also binds *Pseudomonas* enzyme	×2.2	KM	143
Myosin I	*Acanthamoeba castellanii*	ADP	KCl; resolution of 1A, 1B, and 1C	×2—4		54
Phenylalanine hydroxylase	*Chromobacterium violaceum*	CB	KCl elution	×1.6	AKIMPSU	144
Phosphoglucose isomerase	*Drosophila*	ATP	glucose 6-phosphate	×37		55
Phosphorylase a	platelets	AMP	AMP elution: I	×4.1	KU	145
			II	×8.2	KU	145

[127] K. R. Westcott, D. C. LaPorte, and D. R. Storm, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 204.
[128] R. W. Wallace, T. J. Lynch, E. A. Tallant, and W. Y. Cheung, *J. Biol. Chem.*, 1979, **254**, 377.
[129] V. B. Patel and N. H. Giles, *Biochim. Biophys. Acta*, 1979, **567**, 24.
[130] G. E. Hoffmann, J. Schiessl, and L. Weiss, *Z. Physiol. Chem.*, 1979, **360**, 1445.
[131] A. P. Toste and R. Cooke, *Anal. Biochem.*, 1979, **95**, 317.
[132] M. E. Morrill, S. T. Thompson, and E. Stellwagen, *J. Biol. Chem.*, 1979, **254**, 4371.
[133] M. V. Williams and Y.-C. Cheng, *J. Biol. Chem.*, 1979, **254**, 2897.
[134] D. P. Philipp and P. Parsons, *J. Biol. Chem.*, 1979, **254**, 10776.
[135] Y. Tashima, H. Mizunuma, and M. Hosegawa, *J. Biochem. (Tokyo)*, 1979, **86**, 1089.
[136] C. M. Schimandle and D. L. Vander Jagt, *Arch. Biochem. Biophys.*, 1979, **195**, 261.
[137] D. L. Garbers, *J. Biol. Chem.*, 1979, **254**, 240.
[138] F. Müller, G. Voordouw, W. J. H. van Berkel, P. J. Steennis, S. Vissner, and P. J. van Rooijen, *Eur. J. Biochem.*, 1979, **101**, 235.
[139] Y. Nishimoto, F. Takeuchi, and Y. Shibata, *J. Chromatogr.*, 1979, **169**, 357.
[140] T. Caldés, H. R. Fatánia, and K. Dalziel, *Anal. Biochem.*, 1979, **100**, 299.
[141] T. Caldés, H. R. Fatánia, and K. Dalziel, *Anal. Biochem.*, 1979, **100**, 299.
[142] S. Asami, K. Inoue, K. Matsumoto, A. Murachi, and T. Akazawa, *Arch. Biochem. Biophys.*, 1979, **194**, 503.
[143] Y. S. Kim, P. E. Kolattukudy, and A. Boss, *Arch. Biochem. Biophys.*, 1979, **196**, 543.
[144] H. Nakata, T. Yamauchi, and H. Fujisawa, *J. Biol. Chem.*, 1979, **254**, 1829.
[145] P. Gergely, A. G. Castle, and N. Crawford, *Int. J. Biochem.*, 1979, **10**, 807.

proteins display was illustrated by an endoglucosaminidase that bound to phenyl- but not octyl-Sepharose.[148] A number of different ligands and eluants for hydrophobic chromatography of rat liver cytochrome *c* oxidase[149] and mouse liver non-histone proteins[150] were also studied. Table 2 lists further examples of proteins isolated in 1979 using hydrophobic affinity ligands.

Lectin Ligands. The year saw a welcome burst of activity with regard to the binding specificity of lectins. As befits its position as the most widely used ligand, concanavalin A received most attention, with two α-linked mannose residues essential for glycopeptide binding. While C-2 substituents did not necessarily prevent binding, the sequential removal of peripheral sugars from C-2 branch points did give increased affinity of binding. In contrast, an increase in the amount of α-linked mannose relative to the core disaccharide unit gave no increase in affinity. Further requirements for binding were shown by additional investigation of *N*-acetylglucosamine and galactose linkages.[182] A related proton magnetic relaxation dispersion study of mono- and oligosaccharide binding to concanavalin A was also presented.[183] Other factors affecting the affinity of glycoproteins for the lectins concanavalin A and *Ricinus communis* agglutinin were investigated using ceruloplasmin (native and desilylated) and thyroglobulin. A pH optimum of 5.0 that was relatively independent of temperature but dependent on ionic strength was found for binding, with glycoside-promoted dissociation showing a time, temperature, and ligand-concentration dependence.[184] The pH dependence (within the range 7.0—9.0) of lectin interaction with sugars was also studied using affinity electrophoresis.[185] The hydrophobic character of concanavalin A (on a variety of matrices) was tested using temperature and salt-concentration dependence of dissociation. The comparison of desorbents given here is of particular interest.[186] A hydrophobic contribution was also evidenced in the binding of a tumour virus glycoprotein; detergent affected the elution by α-methylmannoside.[187] Wheat germ agglutinin was studied using inhibition by sugars of cellular aggregation. *N*-Acetylneuraminic acid, neuramino 2 → 3 lactone, *N*-acetyl-D-glucosamine, and its β1 → 4 oligomers all inhibited agglutination, whereas neuraminic acid β-methyl ketoside, *N*-glycosyl neuraminic acid, and other acidic sugars did not.[188] A procedure for immobilization of wheat germ agglutinin in the presence of β-1,4-linked *N*-acetylglucosamine oligosaccharides, to yield a product with a 10—50-fold increase in capacity for *N*-acetylglucosamine-containing glycoproteins over other procedures, was also described. Here again there was a suggestion that binding to the lectin involved a hydrophobic component.[189] The *Ricinus communis* agglutinins RCA_I and RCA_{II} were investigated with glycopeptides containing complex β-1,3-linked *N*-acetylgalactosamine and *N*-acetylglucosamine moieties. Binding was found to be directed mainly towards β-galactose. The presence of sialic acid gave a decreased affinity of binding. Some differences between the two were found with respect to the degree of branching of the oligosaccharide; RCA_I had a two—three-fold higher affinity for di-branched oligosaccharides than RCA_{II}, and RCA_{II} had a

[146] R. Shiman, D. W. Gray, and A. Pater, *J. Biol. Chem.*, 1979, **254**, 11300.
[147] C. O. Rock and J. L. Garmin, *J. Biol. Chem.*, 1979, **254**, 7123.
[148] R. B. Trimble, A. L. Tarentino, G. Evans, and F. Maley, *J. Biol. Chem.*, 1979, **254**, 9708.
[149] T. Nagasawa, H. Nagasawa-Fujimori, and P. C. Heinrich, *Eur. J. Biochem.*, 1979, **94**, 31.
[150] D. E. Comings, A. G. Miguel, and B. H. Lesser, *Biochim. Biophys. Acta*, 1979, **563**, 253.

Table 2 *Proteins isolated using hydrophobic ligands*

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
ADP-ribosyltransferase dehydrase	turkey erythrocytes	phenyl	50% propylene glycol; NAD^+-dependent enzyme	×19	K	151
Adenosine kinase	*Lupinus luteus* seeds	aminohexyl	phosphate gradient	×57	KMU	152
δ-Aminolevulinate dehydrase	human erythrocyte	octyl	not retarded	×6	AIKMPU	153
		phenyl	ethylene glycol	×5.9	AIKMPU	153
Arginosuccinate synthetase	yeast	aminohexyl	phosphate gradient	×1.2	KMU	154
Cysteine sulphinate transaminase	rat brain	octyl	NaCl; 62% recovery	×1.5	KU	155
Estramustine-binding protein	rat prostate	octyl	ethylene glycol; 18% of total protein of ventral prostate cytosol	×1.2	AMU	156
Estrogen-binding peroxidase	human fetus	phenyl	decreasing ammonium sulphate/increasing ethylene glycol	×3.6		157
Fructose 1,6-bisphosphatase	*B. subtilis*	aminohexyl	NaCl gradient	×6.8	KMU	158
Fucokinase	porcine thyroid	octyl	decreasing KCl; 17% recovery	×1.8	KMU	159
		(fucose)	KCl; 25% recovery	×5.4	KMU	159
Galactosyltransferase		norleucine	decreasing ammonium sulphate; separate two forms on α-lactalbumin	×5.7	ACHKU	160
			with EDTA elution I	×32	ACHKU	160
			II	×25	ACHKU	160

[151] J. Moss, S. J. Stanley, and N. J. Oppenheimer, *J. Biol. Chem.*, 1979, **254**, 8891.
[152] A. Guranowski, *Arch. Biochem. Biophys.*, 1979, **196**, 220.
[153] P. M. Anderson and R. J. Desnick, *J. Biol. Chem.*, 1979, **254**, 6924.
[154] F. Hilger, J.-P. Simon, and V. Stalon, *Eur. J. Biochem.*, 1979, **94**, 153.
[155] M. Recasens, R. Benezra, M.-M. Gabellec, J.-P. Delaunoy, and P. Mandel, *FEBS Lett.*, 1979, **99**, 51.
[156] B. Forsgren, P. Björk, K. Corlström, J.-Å. Gustafsson, Å. Pousette, and B. Högberg, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3149.
[157] L. Dimitrijenic, C. Aussel, A. Mucchielli, and R. Masseyeff, *Biochimie*, 1979, **61**, 535.
[158] Y. Fujita and E. Freese, *J. Biol. Chem.*, 1979, **254**, 5340.
[159] R. D. Kilber, D. K. Shuey, and G. S. Serit, *Biochim. Biophys. Acta*, 1979, **570**, 271.
[160] D. K. Podolsky and M. W. Weiser, *J. Biol. Chem.*, 1979, **254**, 3983.

Table 2 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
1,4-α-Glucan phosphorylase	*Physarum polycephalum*	aminobutyl	phosphate gradient	×5.1	ACKMU	161
Guanylate cyclase	rat liver	hexyl	NaCl elution	×2.3	K	162
Hatching enzyme	sea urchin	4-phenyl-butylamine	NaCl–ethylene glycol	×1.1	KMU	163
Hydrogenase	*E. coli*	octyl	decreasing phosphate, ammonium sulphate; increasing Triton, deoxycholate	×4.7	KMPU	164
Hydrogenase	*Proteus mirabilis*	phenyl	membrane-bound; imidazole elution	×12.9	AMPU	165
			ethylene glycol elution	×1.3	AMPU	165
Hydroperoxidase II	*E. coli B*	aminohexyl	NaCl gradient	×1.6	MPU	166
Inositol 2-dehydrogenase	*B. subtilis*	aminohexyl	KCl	×2.4		167
Lysyl hydroxylase	fetal porcine skin	aminoethyl	NaCl gradient; I	×4.6	K	168
			II	×18.8	K	168
Phenylalanine hydroxylase	rat liver	phenyl	omit phenylalanine	×215	KU	146
Phosphatidyl ethanolamine methyltransferase	rat liver hepatocyte	octyl	Triton X-100	×2.6		169
Phosphoenolpyruvate carboxykinase	bullfrog liver	leucine	ammonium sulphate	×4.2	AKMU	170
Phosphoenolpyruvate-dependent phospho-transferase	*E. coli*	octyl	ethylene glycol gradient	×58, ×3		171
		phenyl	ethylene glycol gradient	×5.8		171
Phosphoenolpyruvate sugar phospho-transferase system	*E. coli*	hexyl	lubrol PX; mannitol-specific enzyme II of system	×24	U	172
		aminohexyl	NaCl elution	×2.2	U	172
Phospholipase D	savoy cabbage	aminopropyl	NaCl gradient	×6	K	173
Phospholipid exchange proteins	bovine heart	phenyl	decrease ionic strength/ increase pH = 5.5→7.4	×15.6	CK	174
Phosphoprotein phosphatase	calf liver nucleus	aminohexyl	NaCl elution	×2	KU	175

Phosphorylase phosphatase	bovine adrenal cortex	phenyl	decreasing KCl/increasing glycol; associates with alkaline phosphatase	×2.3	KM	176
Stearoyl CoA desaturase terminal enzyme	hen liver microsome	amino-octyl	not retained	×1.3	KU	177
Thymidylate synthetase	*E. coli K12*	amonohexyl [poly(U)]	KCl gradient	×8.1	IKMU	178
			KCl gradient	×2.8	IKMU	178
TRH-deaminase	rat brain	phenyl	decreasing ammonium sulphate; increasing ethylene glycol	×16.3	KMU	179
Tryptophan synthetase	*Saccharomyces cerevisiae*	Pentyl	decreasing ammonium sulphate, pH = 6	×3.7	MSU	180
			pH = 7.5	×10.6	MSU	180
Tyrosine aminotransferase	rat liver	octyl (tyrosine adduct)	KCl	×1.6	CMU	181
			pyridoxal 5′-phosphate-glycinate	×2.8	CMU	181

[161] W. Nader and J.-U. Becker, *Eur. J. Biochem.*, 1979, **102**, 345.
[162] J. M. Braughler, C. M. Mittal, and F. Murad, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 219.
[163] K. Takeuchi, H. Yokosuwa, and M. Hoshi, *Eur. J. Biochem.*, 1979, **100**, 257.
[164] M. W. W. Adams and D. O. Hall, *Biochem. J.*, 1979, **183**, 11.
[165] G. S. Schoenmaker, L. F. Oltmann, and A. H. Stouthamer, *Biochim. Biophys. Acta*, 1979, **567**, 511.
[166] A. Claiborne, D. P. Malinowski, and I. Fridovich, *J. Biol. Chem.*, 1979, **254**, 11664.
[167] R. Ramaley, Y. Fujita, and E. Freese, *J. Biol. Chem.*, 1979, **254**, 7684.
[168] R. L. Miller and H. H. Varner, *Biochemistry*, 1979, **18**, 5928.
[169] W. J. Schneider and D. E. Vance, *J. Biol. Chem.*, 1979, **254**, 3886.
[170] Y. Goto, J. Shimizu, T. Okazaki, and R. Shukuya, *J. Biochem. (Tokyo)*, 1979, **86**, 71.
[171] G. T. Robillard, G. Dooijewaard, and J. Lolkema, *Biochemistry*, 1979, **18**, 2984.
[172] G. R. Jacobson, C. A. Lee, and M. H. Saier, *J. Biol. Chem.*, 1979, **254**, 249.
[173] T. T. Allgyer and M. A. Wells, *Biochemistry*, 1979, **18**, 5348.
[174] P. E. DiCorleto, J. B. Warach, and D. B. Zilversmit, *J. Biol. Chem.*, 1979, **254**, 7795.
[175] M. W. Chou, E. L. Tan, and C. S. Yang, *Biochim. Biophys. Acta*, 1979, **566**, 49.
[176] H.-C. Lee, *Eur. J. Biochem.*, 1979, **102**, 363.
[177] M. R. Prasad and V. C. Joshi, *J. Biol. Chem.*, 1979, **254**, 6362.
[178] T. Haertlé, F. Wohlrab, and W. Guschlbauer, *Eur. J. Biochem.*, 1979, **102**, 223.
[179] J. H. Rupnow, W. L. Taylor, and J. E. Dixon, *Biochemistry*, 1979, **18**, 1206.
[180] M. Dettwiler and K. Kirschner, *Eur. J. Biochem.*, 1979, **102**, 159.
[181] P. A. Rubenstein and R. D. Ivarie, *Arch. Biochem. Biophys.*, 1979, **194**, 299.

two—ten-fold higher affinity for glycopeptides containing 1-, 2-, or 4-linked galactose β-1,3-*N*-acetylgalactosamine disaccharides.[190] A similar β-linked galactosyl specificity was found for tridacnin, a lectin from the small Giant Clam, *Tridacna maxima*.[191] The power of lectin ligands in the isolation of hydrolases [192] (Table 9, p. 90) as well as membrane and plasma proteins (Table 5, p. 67, and Table 7, p. 80) is well documented. Other applications are listed in Table 3.

Immunoaffinity Ligands. The applications of monoclonal antibodies have now been extended to protein isolation. Clearly their potential in this field is as yet relatively untapped. Using partially purified proteins as antigens, the procedure required to produce and isolate specific hybridomas has become routine, although laborious, technology in many laboratories. The immunoglobulin products of these are chemically homogeneous and directed against a specific antigenic determinant, thus eliminating the possibility of contamination with antibodies directed against proteins other than that to be isolated. One major advantage that they offer over complex antisera, that of selection of low affinity antibodies and therefore of protein elution using less harsh procedures, does not yet appear to have been fully appreciated. Thus the same elution conditions (0.5% deoxycholate, pH 11.5) were used to elute an Ia antigen from both complex antiserum and monoclonal antibody ligands.[202] The same eluant was used in the isolation of a rat thymocyte antigen.[203] Detergent at neutral pH [204] as well as high pH in the absence of detergent [205] were also used to dissociate histocompatibility antigens from monoclonal ligands. The former paper compared release with increased salt concentrations (0.14 M, 38%; 0.5 M, 94%; I.OM, 98%) and increased pH in the presence of detergent (pH 9, 49%; pH 10, 50%; pH 11, 94%). Sodium dodecyl sulphate released 96% of the bound protein.[204] Only in one instance was a precolumn of pre-immune IgG-Sepharose used to eliminate non-specific binding.[202] Elimination of contaminating antibodies from a complex antiserum was achieved by adsorption of the crude antiserum with choriogonadotropin-free urine in the isolation of human urinary choriogonadotropin.[206] Immunoaffinity ligands are frequently used to remove co-purifying contaminants difficult to remove by conventional procedures. Usually these are directed against specific proteins. Several examples of this usage are listed in Table 7 (p. 80). Two complement proteins were isolated using immunoaffinity columns directed against unspecified proteins. In the first, C4-deficient human serum was used as a complex antigen and the antiserum raised was used in the purification of human C4.[207] Trace contaminants were removed from human C3 by an antiserum raised in a more circuitous manner. Partially purified C3 was used to raise an antiserum against both C3 and co-purifying proteins. C3-Deficient serum was then sequentially precipitated with the antiserum, and the combined washed precipitates

[182] J. V. Baenziger and D. Fiete, *J. Biol. Chem.*, 1979, **254**, 2400.
[183] C. F. Brewer and R. D. Brown, *Biochemistry*, 1979, **18**, 2555.
[184] J. T. Dulaney, *Anal. Biochem.*, 1979, **99**, 254.
[185] K. Hauzer, M. Tichá, V. Horejs[, and J. Kocourek, *Biochim. Biophys. Acta*, 1979, **583**, 103.
[186] J. L. Ochoa, T. Kristiansen, and S. Pahlman, *Biochim. Biophys. Acta*, 1979, **577**, 102.
[187] F. Westenbrink and W. Koornstra, *Anal. Biochem.*, 1979, **94**, 40.
[188] V. P. Bhavanandan and A. W. Katlic, *J. Biol. Chem.*, 1979, **254**, 4000.
[189] G. Mintz and L. Glaser, *Anal. Biochem.*, 1979, **97**, 423.
[190] J. U. Baenziger and D. Fiete, *J. Biol. Chem.*, 1979, **254**, 9795.
[191] P. A. Gleeson, M. A. Jermyn, and A. E. Clarke, *Anal. Biochem.*, 1979, **92**, 41.
[192] M. B. Fiddler, Y. Ben-Yosef, and H. L. Nadler, *Biochem. J.*, 1979, **177**, 175.

Table 3 *Proteins isolated using lectin ligands*

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Alkaline phosphatase	bovine mammary tissue	concanavalin A	α-methylmannoside	×30	KM	193
Arabinogalactan protein	gladiolus style	tridacnin	omit calcium; 90% carbo-hydrate, 3% protein		ACHMP	194
Arabinogalactan protein	gladiolus style	tridacnin	omit calcium		H	191
Arylsulphase A	rabbit testicles	concanavalin A	α-methylmannoside	×8.4	AK	195
γ-Glutamyltranspeptidase	rat mammary tissue	concanavalin A	α-methylmannoside	×5	K	196
γ-Glutamyltranspeptidase	skimmed mouse milk membranes	concanavalin A	15% recovery	×3	M	197
γ-Glutamyltranspeptidase–glutathione oxidase		concanavalin A	α-methylmannoside; 12 bands cofocus, activity ratio constant	×4.3, ×4.7		198
Glycoprotein BGP I	human bile	concanavalin A	α-methylmannoside; cross-reacts with CEA		AHMU	199
Hyaluronidase	bovine seminal plasma	concanavalin A	α-methylmannoside	×7.5	K	200
		(heparin)	NaCl elution	×4.3	K	200
Spermine-binding protein	rat ventral prostate	concanavalin A	not bound; androgen-sensitive binding of spermine	×1.3		201

[193] R. B. O'Keefe and J. E. Kinsella, *Int. J. Biochem.*, 1979, **10**, 125.
[194] P. A. Gleeson and A. E. Clarke, *Biochem. J.*, 1979, **181**, 607.
[195] A. A. Farooqui and P. N. Srivastava, *Biochem. J.*, 1979, **181**, 331.
[196] S. Jaben and M. Mason, *Biochim. Biophys. Acta*, 1979, **568**, 331.
[197] C. Francois, C. M. Carlberg-Bacq, L. Gosselin, S. Kozma, and P. M. Osterrieth, *Biochim. Biophys. Acta*, 1979, **567**, 106.
[198] S. S. Tate and J. Orlando, *J. Biol. Chem.*, 1979, **254**, 5573.
[199] T. Svenberg, S. Hammarström, and A. Hedin, *Mol. Immunol.*, 1979, **16**, 245.
[200] P. N. Srivastava and A. A. Farooqui, *Biochem. J.*, 1979, **183**, 531.
[201] G. Mezzetti, R. Loor, and S. Liao, *Biochem. J.*, 1979, **184**, 431.

were reinjected into a rabbit. The antiserum raised in this way reacted only with proteins that co-purified with C3 over several conventional steps, thus eliminating the need for large quantities of antiserum.[208] Immunoaffinity ligands were also used to prepare the same protein from a variety of species; a pre-immune IgG column was used to eliminate non-specific binding.[209] The advantage here is that only one protein need be purified to raise the antiserum, and this is then used to isolate several related proteins. A novel protein-isolation technique dependent on secondary interaction with an antibody was used both to prepare small amounts of enzyme for analytical purposes[210] and to prepare receptor proteins.[211–213] In the former, antibody bound to protein A-Sepharose was used; the antibody–protein A interaction was disrupted using milder conditions than would be required to release the enzyme from its antibody.[210] This was acceptable as the presence of antibody did not interfere with subsequent analytical procedures. Receptors were isolated as complexes with their ligands with the antiserum directed against the ligand[211,212] or, in the case of an IgE receptor, as the receptor–IgE–hapten complex on an anti-hapten column.[213]

Other Chromatographic Procedures.—*Hydroxylapatite.* Hydroxylapatite has long been regarded as a useful, albeit somewhat unreliable, adsorption matrix. A series of papers dealing with the physical and chemical properties of different preparations,[214] sources of variability and improved methods of preparation,[215] and cation and pH effects on fractionation[216] was therefore most welcome. Different commercial preparations were found to have different crystal structures, the greater the surface area of the crystal, the greater its capacity for protein. Binding properties were unrelated to the flow-rate or calcium : phosphate ion ratio.[214] The hydroxylapatite-binding site in salivary acidic proline-rich proteins was shown to lie in the proline-poor *N*-terminal part of the protein; binding affinity increased with its phosphoserine content.[217]

High-performance Liquid Chromatography. While h.p.l.c. has been used for some time in peptide fractionation, only recently have columns capable of handling preparative amounts of protein become available. A review article of procedures applicable for preparative h.p.l.c. of proteins, using both 'normal' and reversed-phase (not for proteins denatured by organic solvents) chromatography, claimed that up to 50 mg of protein could be applied to a column.[218] (A second review, while

[202] W. R. McMaster and A. F. Williams, *Eur. J. Immunol.*, 1979, **9**, 426.
[203] C. A. Sunderland, W. R. McMaster, and A. F. Williams, *Eur. J. Immunol.*, 1979, **9**, 155.
[204] S. H. Herrmann and M. F. Mescher, *J. Biol. Chem.*, 1979, **254**, 8713.
[205] P. Parham, *J. Biol. Chem.*, 1979, **254**, 8709.
[206] Y.-M. Choy, K.-M. Lau, and C.-Y. Lee, *J. Biol. Chem.*, 1979, **254**, 1159.
[207] A. Reboul, N. Thielens, M. B. Villiers, and M. G. Colomb, *FEBS Lett.*, 1979, **103**, 156.
[208] R. A. Harrison and P. J. Lachmann, *Mol. Immunol.*, 1979, **16**, 767.
[209] R. Casey, *Biochem. J.*, 1979, **177**, 509.
[210] J. N. Davidson and D. Patterson, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1731.
[211] K. Metsikkö and H. Rajaniemi, *FEBS Lett.*, 1979, **106**, 193.
[212] P. A. Seligman, R. B. Schleicher, and R. H. Allen, *J. Biol. Chem.*, 1979, **254**, 9943.
[213] J. Kanellopoulos, G. Rossi, and H. Metzger, *J. Biol. Chem.*, 1979, **254**, 7691.
[214] M. Spencer and M. Grynpas, *J. Chromatogr.*, 1978, **166**, 423.
[215] M. Spencer, *J. Chromatogr.*, 1978, **166**, 435.
[216] M. Spencer, E. J. Neave, and N. L. Webb, *J. Chromatogr.*, 1978, **166**, 447.
[217] A. Bennick, M. Cannon, and G. Madapallimattam, *Biochem. J.*, 1979, **183**, 115.
[218] M. Rubinstein, *Anal. Biochem.*, 1979, **98**, 1.

not dealing specifically with aspects covered here, contained useful comments on techniques available.[219]) Papers dealing with gel permeation chromatography claimed rapid baseline resolution of mixtures of proteins with molecular weights ranging between 5000 and 700 000 dalton.[220-225] Those dealing with reversed-phase separations contained considerations of mobile-phase components of buffers[226-228] and included isolation of polypeptide hormones,[227] human globin chains,[229, 230] transfer factor,[231] leukocyte interferon,[232] and pro-opiocortin,[233] the latter two utilizing 80% propanol- and pyridine–formic acid-containing buffers and illustrating the need for stability in such solvents. Cation[234] and anion[235] exchange resins for h.p.l.c. were also described.

Gel Permeation. An electron microscopic examination of three commercial supports for gel filtration (Bio-Gel–polyacrylamide; Sephadex–agarose; Ultrogel–mixed), aimed at defining pore parameters, suggested that the mixed gel possessed the greater porosity and mechanical resistance and that it should therefore give increased speed of separation without loss of resolution.[236] Preferential partitioning of SDS into the bead phase (and thus initial dilution in the mobile phase) of Bio-Gel beads, thought to be due to the anomalous nature of water in the gel matrix, was reported.[237] A note of proteins that bind to underivatized agarose should also be made.[238-240]

Controlled Pore Glass. Controlled pore glass, which contains anionic silanol groups, was compared with CM-cellulose. Investigation of capacity and buffer elution conditions suggested slightly stronger adsorption to, and resolution obtained with, controlled pore glass.[241] Investigation of mouse interferon binding indicated that in addition to a charge interaction, blocked by maleylation of the protein, there was some hydrophobic interaction, overcome by ethylene glycol.[242]

[219] P. R. Brown and A. M. Krstulovic, *Anal. Biochem.*, 1979, **99**, 1.
[220] K. A. Gruber, J. M. Whitaker, and M. Morris, *Anal. Biochem.*, 1979, **97**, 176.
[221] J. L. Hodge and E. F. Rossomando, *Anal. Biochem.*, 1979, **100**, 179.
[222] K. Fukano, K. Komiya, H. Sasaki, and T. Hashimoto, *J. Chromatogr.*, 1979, **166**, 47.
[223] S. Rokushika, T. Ohkawa, and H. Hatano, *J. Chromatogr.*, 1979, **176**, 456.
[224] P. Roumeliotis and K. K. Unger, *J. Chromatogr.*, 1979, **185**, 445.
[225] J. A. Fulton, T. D. Schlabach, J. E. Kerl, and E. C. Toren, *J. Chromatogr.*, 1979, **175**, 283.
[226] E. C. Nice, M. Capp, and M. J. O'Hare, *J. Chromatogr.*, 1979, **185**, 413.
[227] S. Terabe, R. Konaka and K. Inouye, *J. Chromatogr.*, 1979, **172**, 163.
[228] M J. O'Hare and E. C. Nice, *J. Chromatogr.*, 1979, **171**, 209.
[229] L. F. Congote, H. P. J. Benett, and S. Solomon, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 851.
[230] T. A. Stroming, F. A. Garver, M. A. Gangarosa, J. M. Harrison, and T. H. J. Huisman, *Anal. Biochem.*, 1979, **96**, 113.
[231] D. R. Burger, A. A. Vandenbark, W. Dunnick, W. Kraybill, G. D. Daves, and R. M. Vetto, *J. Immunol.*, 1979, **122**, 1091.
[232] M. Rubinstein, S. Rubinstein, P. C. Familletti, R. S. Miller, A. A. Waldman, and S. Pestka, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 640.
[233] S. Kimura, R. V. Lewis, L. D. Gerber, L. Brink, M. Rubinstein, S. Stein, and S. Udenfriend, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1756.
[234] H. Aoshima, *Anal. Biochem.*, 1979, **95**, 371.
[235] A. J. Alpert and F. E. Regnier, *J. Chromatogr.*, 1979, **185**, 375.
[236] E. Grimaud, J. C. Lecoq, E. Boschetti, and M. Corgier, *J. Chromatogr.*, 1978, **166**, 37.
[237] M. Janado, R. Nakayama, Y. Yano, and H. Nakamori, *J. Biochem. (Tokyo)*, 1979, **86**, 795.
[238] P. S. Fitt and P. Baddoo, *Biochem. J.*, 1979, **181**, 347.
[239] M. B. Pepys, *Immunology*, 1979, **37**, 637.
[240] N. M. Cintrón and P. L. Pedersen, *J. Biol. Chem.*, 1979, **254**, 3439.
[241] J. Mizutani and A. Mizutani, *J. Chromatogr.*, 1979, **168**, 143.
[242] I. A. Braude, V. G. Edy, and E. De Clercq, *Biochim. Biophys. Acta*, 1979, **580**, 15.

Hydrophobic interaction was also indicated by the adsorption of barley thylakoid light-harvesting chlorophyll *a*/*b* complex, where 1 M-KCNS was required for dissociation.[243] Procollagen was isolated following adsorption to controlled pore glass.[244]

Novel Uses of Ion-exchange Resins. Ampholyte displacement chromatography from DEAE-cellulose, by use of pH 3—5 carrier ampholytes, was used in the isolation of human liver α-galactosidases A[245] and B.[246] Proteins were also concentrated by use of carboxymethyldextran displacement from both DEAE- and CM-celluloses.[247] Amberlite CG-50 (a weakly acidic cation-exchange resin) was used at pH = 4 (at which pH value the carboxyl is not dissociated) to bind proteins in a procedure described as 'hydrophobic-ionic' chromatography. Binding was independent of ionic strength and elution could be partially effected with organic solvents. Proteins not displaced were dissociated by increasing the pH of the buffer. Dissociation was independent of the p*I* of the protein, and the relative positions of elution of proteins varied with different buffers.[248]

Covalent and Chelate Chromatography. The synthesis of *p*-acrylamidophenylmercury chloride was described, polymerization of which yields a matrix with potential for high-capacity binding of thiol-containing proteins.[249] The kinetics of organomercurial reactions with model thiols was also of interest, both for the design of organomercurial ligands and for their use in protein isolation.[250] Activated thiol-Sepharose was used to separate collagen from procollagen, only the latter containing a free thiol.[251] Copper chelate chromatography, at high ionic strength, was used for lactoferrin purification. The method was of particular value as lactoferrin associates with other proteins at low ionic strength, thus precluding the use of many chromatographic procedures.[252]

Miscellaneous Column Chromatographic Procedures. Biospecific 'imphilyte' chromatography, using a ligand designed specifically to incorporate cationic, anionic, and hydrophobic features, was used in the isolation of wheat germ aspartate transcarbamylase. (The cold-lability of the enzyme was also investigated and appeared to be associated with a group, possibly a histidine residue, with a high and positive heat of ionization.)[253] The same ligand was used in association with SDS; an SDS concentration of 3.5 mM gave increased adsorption of some proteins. At concentrations > 10 mM there was a loss of capacity for protein as SDS binding to the ligand became the dominant feature.[254]

[243] P. R. Dunkley and J. M. Anderson, *Arch. Biochem. Biophys.*, 1979, **193**, 469.
[244] S. Gerard and W. M. Mitchell, *Anal. Biochem.*, 1979, **96**, 433.
[245] K. J. Dean and C. C. Sweeney, *J. Biol Chem.*, 1979, **254**, 9994.
[246] K. J. Dean and C. C. Sweeley, *J. Biol. Chem.*, 1979, **254**, 10001.
[247] A. R. Torres and E. A. Peterson, *Anal. Biochem.*, 1979, **98**, 353.
[248] I. Sasaki, H. Gotoh, R. Yamamoto, H. Hasegawa, J. Yamashita, and T. Horio, *J. Biochem.* (*Tokyo*), 1979, **86**, 1537.
[249] A. E. Reeve and L. P. Johnson, *J. Chromatogr.*, 1979, **177**, 127.
[250] G. Sanyal and R. G. Khalifah, *Arch. Biochem. Biophys.*, 1979, **196**, 157.
[251] K. Angermann and H.-J. Barrach, *Anal. Biochem.*, 1979, **94**, 253.
[252] A. R. Torres, E. A. Peterson, W. H. Evans, M. G. Mage, and S. M. Wilson, *Biochim. Biophys. Acta*, 1979, **576**, 385.
[254] R. T. Yon and R. J. Simmonds, *Biochem. J.*, 1979, **177**, 417.

Miscellaneous Non-column Procedures.—Affinity partition of proteins between aqueous two-phase systems using a polyoxyethyleneglycol-bound ligand and charged dextrans was described. Using estradiol as the ligand, a *Pseudomonas* $\Delta^{5\rightarrow4}$3-oxosteroid isomerase was retained in the upper (ligand-containing) phase. Successive washes with anionic and cationic dextran-rich phases permitted a 125-fold purification with 45% recovery.[255] With palmitate as the ligand, the partition coefficient was used as a measure of effective hydrophobicity of proteins.[256] A similar procedure using buffered isotonic ficoll-dextran partition was used to study relative hydrophobicities and to effect some resolution of human plasma proteins.[257] Mitochondrial glutamate dehydrogenase, malate dehydrogenase, and aspartate aminotransferase from bovine and rat liver as well as porcine heart were precipitated with polyethylene glycol if all were present but did not precipitate alone, suggesting that a looose complex between them might exist.[258] One highly specific procedure described was the isolation of a microtubule protein by successive polymerization cycles in GTP.[259] A method whereby cytosolic enzymes could be released from monolayers, without organelle disruption, using digitonin was also published[260] as was the isolation of D-arabitol dehydrogenase from *Klebsiella* (where it constituted 5% of the soluble protein) following transfer of the structural gene from *E.coli*.[261]

Electrophoretic Procedures.—All major preparative electrophoretic procedures were covered in a volume of the 'Journal of Chromatography Library' entitled 'Electrophoresis'.[262] Preparative aspects of electrofocusing and isotachophoresis were also covered in the proceedings of an international symposium held in 1976 but omitted from previous SPR reports.[263] The apparatus used in preparative electrophoresis was recently investigated[264] and preparative flat-bed electrofocusing in granulated gels using a natural pH gradient was described.[265] Proteins isolated using preparative flat-bed isoelectric focusing included the chick oviduct progesterone receptor.[266] High molecular weight spectrin bands were eluted from the top of a preparative slab gel following inversion of the gel after the initial separation.[267] Proteins continued to be isolated using SDS-polyacrylamide gel electrophoresis, internal dansylprotein markers (used to locate viral proteins),[268] and proteins pre-stained with Coomassie blue G250 to give band positions.[269] Protein bands were also located utilizing sodium acetate-dependent precipitation of

[255] A. Chaabouni and E. Dellacherie, *J. Chromatogr.*, 1979, **171**, 135.
[257] B. Y. Zaslavsky, N. M. Mestechkina, and S. V. Rogozhin, *Biochim. Biophys. Acta*, 1979, **579**, 463.
[258] L. A. Fahien and E. Kmiotek, *J. Biol. Chem.*, 1979, **254**, 5983.
[259] C. F. Asnes and L. Wilson, *Anal. Biochem.*, 1979, **98**, 64.
[260] J. Mackall, M. Meredith, and M. D. Lane, *Anal. Biochem.*, 1979, **95**, 270.
[261] M. S. Neuberger, R. A. Patterson, and B. S. Hartley, *Biochem. J.*, 1979, **183**, 31.
[262] 'Electrophoresis', *J. Chromatogr. Library*, ed. Z. Deyl, Elsevier Scientific, Amsterdam, New York, 1979, Vol. 18.
[263] 'Electrofocusing and Isotachophoresis'—Proceedings of the International Symposium, Hamburg, 1976, ed. B. J. Radola and D. Graesslin, Walter de Gruyter, Berlin, New York, 1977.
[264] S. Akaiwa, *Anal. Biochem.*, 1979, **92**, 205.
[265] R. L. Prestidge and M. T. W. Hearn, *Anal. Biochem.*, 1979, **97**, 95.
[266] P. A. Boyd and T. C. Spelsberg, *Biochemistry*, 1979, **18**, 3679.
[267] A. Lemay, *Anal. Biochem.*, 1979, **92**, 130.
[268] H. Schetters and B. McLeod, *Anal. Biochem.*, 1979, **98**, 329.
[269] W. Judd, *Anal. Biochem.*, 1979, **93**, 373.

non-protein-bound SDS.[270] Proteins, some functional, were recovered from SDS gels by electrophoretic procedures.[271, 272] The ability to renature proteins following isolation in SDS was claimed to depend on the source of detergent; the presence of hexadecylsulphate inhibits renaturation.[273] The electrophoretic desorption of proteins from affinity ligands received further attention with the design of an apparatus giving recovery of albumin from Cibacron blue in 85% yield[274] and in the isolation of calcium-dependent cyclic nucleotide phosphodiesterase from its regulatory protein.[275] Finally, an improved procedure for zonal electrophoresis on density gradients, giving separation of gram amounts of protein, was described.[276]

3 The Isolation of Specific Classes of Protein

Since there is some overlap between the classes listed, the following order of preference has been used: (i) plasma proteases and their inhibitors are tabulated with plasma proteins; (ii) membrane-bound proteins are, where applicable, listed with their specific class, *e.g.* proteases.

Membrane Proteins.—Most membrane protein extraction procedures depend initially on detergent solubilization; the detergent replaces phospholipid, permitting dispersion of protein molecules, and maintains solubility of the dispersed protein. Table 4 lists papers that contained discussion of the effectiveness of various detergents. Further purification often requires replacement of the extraction detergent with one that still maintains protein solubility but does not interfere with subsequent steps in its isolation. In a two-step procedure non-ionic detergents were displaced from adenylate cyclase by cholate and phospholipid, leading to recovery of dopamine sensitivity. Cholate was later removed by dialysis.[287] Triton X-100 was removed from proteins by partition in chloroform–aqueous SDS mixtures; the SDS maintained protein solubility.[288] An amphipathic matrix, consisting of deoxycholate bound *via* a poly(L-lysine) spacer to agarose, was described as specific for integral membrane proteins; hydrophilic proteins did not bind and phospholipid did not affect the amount of protein bound. Elution was performed with deoxycholate.[289] Both DNA and heparin were used as ligands for steroid receptors; similar binding properties for the rat prostate androgen receptor were observed.[290] (A simple titration procedure for quantifying heparin bound to agarose was also described.[291]) In addition, modification of the elution position of androgen receptors from DNA-cellulose was achieved by addition of carrier proteins.[292] The ATP-dependent calcium transport component of rat brain synaptosomes has been purified following incorporation at low density into phospholipid vesicles, as have transport proteins. In this instance, intra-vesicular precipitation of calcium oxalate produced a density difference which on density-gradient centrifugation gave a 100-fold purification of the receptor.[293] Differences in vesicle density were also used in the isolation of cyclopropane fatty acid synthetase, where the enzyme was bound to

[270] R. C. Higgins and M. E. Dahmus, *Anal. Biochem.*, 1979, **93**, 257.
[271] F. Hanaoka, J. L. Shaw, and G. C. Mueller, *Anal. Biochem.*, 1979, **99**, 170.
[272] B. Ahmadi, *Anal. Biochem.*, 1979, **97**, 229.
[273] S. A. Lacks, S. S. Springhorn, and A. L. Rosenthal, *Anal. Biochem.*, 1979, **100**, 357.
[274] M. R. A. Morgan, N. A. Slater, and P. D. G. Dean, *Anal. Biochem.*, 1979, **92**, 144.
[275] R. L. Kincaid and M. Vaughan, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4903.
[276] J. A. L. I. Walters, *Anal. Biochem.*, 1979, **93**, 41.

Table 4 *Membrane protein solubilization*

Protein	*Source*	*Detergent*	*Comments*	*Ref*
Acyl-CoA-lysolecithin acyltransferase	thymocyte plasma membrane	choline phosphatides	water-soluble detergents; selective solubilization of enzyme	277
Adenosine triphosphatase	inner mitochondrial membrane	chloroform	released if ATP or glycerol is present; 41% yield, 4.7-fold purification	278
β-Adrenergic receptors	canine heart, liver	various	comprehensive study of 19 different ionic and non-ionic surfactant combinations, also effect of temperature, ligand gave stability	279
Adipose tissue component	rat liver brown adipose tissue	Triton X-100 Tween 80	32000 dalton mol. wt. component increases during cold adaption; various detergents studied for eventual affinity chromatography	280
Creatine phosphokinase	rat myocardium	desoxycholate	comparison of enzyme and bulk protein release	281
2′-3′-Cyclic nucleotide 3′-phosphodiesterase	myelin, C_6 cells	various	Triton X-100 required salt also—51%; sulphobetaine DLH gave higher even without salt	282
F_1-Adenosine triphosphatase	porcine heart mitochondria	chloroform	remove extrinsic proteins first by alkaline and sonication treatments; ×4.6, ×8.5	283
Glycoproteins	human erythrocyte	Triton X-100	effect of removal of sialic acid, decreased extraction reversed by borate; conditions for optimum release discussed	284
Lipoprotein receptor	bovine adrenal cortex	octyl-α-D-glucoside	if used during purification, dilution for assay causes precipitation; use as solid phase for radiolabel binding	285
Outer membrane proteins	*Pseudomonas aeruginosa PAO*	SDS	2% SDS gives preferential release outer membrane proteins; temperature-dependent, DEGI at 35 °C, F and H at 55 °C	286

[277] H. U. Weltzien, G. Richter, and E. Ferber, *J. Biol. Chem.*, 1979, **254**, 3652.
[278] D. D. Tyler and P. R. Webb, *Biochem. J.*, 1979, **178**, 289.
[279] W. L. Strauss, G. Ghai, C. M. Fraser, and J. C. Venter, *Arch. Biochem. Biophys.*, 1979, **196**, 566.
[280] D. Ricquier, C. Gervais, J. C. Kader, and Ph. Hemon, *FEBS Lett.*, 1979, **101**, 35.
[281] B. D. Blackman and S. G. Rothstein, *Anal. Biochem.*, 1979, **98**, 278.
[282] N. R. Sims, L. B. Horvath, and P. R. Carnegie, *Biochem. J.*, 1979, **181**, 367.
[283] F. Penin, C. Godinot, and D. C. Gautheron, *Biochim. Biophys. Acta*, 1979, **548**, 63.
[284] R. S. Pratt and G. M. W. Cook, *Biochem. J.*, 1979, **179**, 299.
[285] W. J. Schneider, S. K. Basu, M. J. McPhaul, J. L. Goldstein, and M. S. Brown, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5577.
[286] T. Mizuno and M. Kageyama, *J. Biochem. (Tokyo)*, 1979, **86**, 979.

liposomes and is purified by flotation,[294] and in the enrichment of concanavalin A-binding membrane fragments.[295] The latter density difference could be reversed by α-methylmannoside treatment. Table 5 lists membrane proteins isolated during the year.

[287] F. M. Hoffmann, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 988.
[288] S. Horikawa and H. Ogawara, *Anal. Biochem.*, 1979, **97**, 116.
[289] P. Cresswell, *J. Biol. Chem.*, 1979, **254**, 414.
[290] E. Mulder, J. A. Foekens, M. J. Peters, and H. J. van der Molen, *FEBS Lett.*, 1979, **97**, 260.
[291] M. Y. Varshavskaya, A. L. Klibanov, V. S. Goldmacher, and V. P. Torchilin, *Anal. Biochem.*, 1979, **95**, 449.
[292] T. O. Fox, S. E. Bates, C. C. Vito, and S. J. Wieland, *J. Biol. Chem.*, 1979, **254**, 4963.
[293] D. Papazian, H. Rahamimoff, and S. M. Goldin, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3708.
[294] F. R. Taylor and J. E. Cronan, *Biochemistry*, 1979, **18**, 3292.
[295] J. K. Lutton, R. C. Frederich, and J. P. Perkins, *J. Biol. Chem.*, 1979, **254**, 11181.

Table 5 *Isolation of membrane proteins*

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Cytochromes						
Cytochrome *c* oxidase	rat liver	phenyl	Triton X-100; 75% recovery	×2.2	MU	149
Cytochrome P-448	rat liver	amino-octyl	Emulgen; 3,4,5,3′,4′-pentachlorobiphenyl induced	×4.4	AP	295*a*
Cytochrome P-448	rat liver	amino-octyl	Emulgen; phenobarbital and 3-methylcolanthrene-treated	×2	AKU	296
Cytochrome P-450	rat liver	amino-octyl	as above	×5.1	AKU	296
Cytochrome P-450	human liver	octyl	Emulgen 911 elution	×4.4	P	297
Cytochrome $P\text{-}450_{meg}$	*B. megatarium*	octyl	ethyleneglycol gradient	×4.1	AKPU	298
Cytochrome *c* oxidase	rat liver mitochondria	cytochrome *c*	phosphate elution	×3.8	K	299
		poly(lysine)	phosphate elution	×1.4	K	299
Receptors						
Acetylcholine receptor	*Torpedo californica*	α-neurotoxin	carbamoylcholine	×100	CU	300
β-Adrenergic receptor	turkey erythrocyte	alprenol derivative	up to 35% recovery	×2000		9
β-Adrenergic receptor	frog erythrocyte	alprenol	60% recovery; 1 cycle	×200		10
			2 cycles	×1500		10
Gonadotropin receptor	human chorion	antibody	0.5% Triton	×1500		211

[295a] N. Ozawa, S'i Yoshihara, K. Kawano, Y. Okada, and H. Yoshimura, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 1140.
[296] R. Masuda-Mikawa, Y. Fujii-Kuriyama, M. Negishi, and Y. Tashiro, *J. Biochem. (Tokyo)*, 1979, **86**, 1383.
[297] P. Beaune, J. P. Flinois, S. Columelli, D. Mansuy, and J. P. Leroux, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 826.
[298] A. Berg, M. Ingleman-Sundbuerg, and J.-Å. Gustafsson, *J. Biol. Chem.*, 1979, **254**, 5264.
[299] R. J. Rascati and P. Parsons, *J. Biol Chem.*, 1979, **254**, 1586.
[300] S. C. Froehner and S. Rafto, *Biochemistry*, 1979, **18**, 301.

Table 5 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Receptors (cont.)						
1,25-Dihydroxyvitamin D receptor	chick intestine	DNA	KCl; binds to and modifies DNA behaviour?	×6, ×2.4	M	301
		CB	KCl	×11.7	M	301
		heparin	KCl	×3.9	M	301
Epidermal growth factor receptor	human placenta	wheat germ agglutinin	*N*-acetylglucosamine	×30	M	302
		antibody	10% formic acid		M	302
Estrogen receptor	calf mammary gland	heparin	heparin; 51% yield	×115		303
Glucocorticoid receptor	rat liver	d.s.DNA	Pyridoxal phosphate elution of activated receptor	×6700	IU	304
Growth hormone receptor	rabbit liver	growth hormone (human or bovine)	urea elution; study of factors affecting yield	×70	U	305
IgE Receptor	rat basophilic leukemia cells	IgE	0.5 M-acetic acid, 1% Non-idet P-40; repetitive process, 15% yield, 90% pure		MU	306
IgE Receptor		secondary antibody interaction		×3		213
Fc Receptor (?)	human blood platelets	IgG Fc subunit	KBr elution; glycoprotein		AIMU	307
Insulin receptor	human placenta	concanavalin A	α-methylmannoside; 82%	×100	MU	308
Intrinsic factor receptor	porcine, human ileum	intrinsic factor/B12	pH elution; pretreat with B12-Sepharose	×182	IMU	309
Transferrin receptor	human placenta	transferrin	1 M-NaCl, pH = 10		AHU	212
Histocompatibility and cell surface antigens						
Cell surface protein	hamster embryo fibroblast	*Ricinus communis* lectin	galactose elution		CH	310
H-2 Alloantigens	murine lymphoblastoid cells	lentil lectin	α-methylmannoside	×6	CM	43

H-2 Antigens	mouse liver	concanavalin A	α-methylmannoside; papain solubilized	×13		311
H-2^b histo-compatibility antigen	RBL-5 mouse tumour cells	*Lens culinaris* lectin	α-methylmannoside; 76% recovery	×8.4	ACM	312
		anti-dH-2	2 M-NaSCN; 35% yield; normal serum precolumn	×2.3	ACM	312
H-2K Antigen		antibody	detergent, salt			204
HLA-A2 Antigen	human lymphoblastoid cell lines	antibody	diethylamine, pH = 11.5	×93		205
HLA-B7 Antigen	human lymphoblastoid cell lines	antibody	diethylamine, pH = 11.5	×44		205
HLA-DR Antigens	spleen, Daudi cells, and leukocytes	*Lens culinaris* lectin	α-methylmannoside; charge-shift electrophoretic characterization	×8	DIMPS	313
Ia Antigen	rat spleen	antibody	deoxycholate, pH = 11.5	×154	MU	202
Leucocyte common antigen	rat thymocytes	antibody	deoxycholate, pH = 11.5; 52%	×77		203
P1^{A1} Alloantigen	human platelets	concanavalin A	α-methylmannoside	×4		314
Rh_0(D) Antigen	erythrocytes	antibody	DMF after 1 M-acetic acid wash			315

[301] J. W. Pike and M. R. Haussler, *Proc. Natl. Acad. Sci. USA*, 1979, **73**, 5485.
[302] R. A. Hock, E. Nexø, and M. D. Hollenberg, *Nature (London)*, 1979, **277**, 403.
[303] A. Rotondi and F. Auricchio, *Biochem. J.*, 1979, **178**, 581.
[304] Ö. Wrange, J. Carlstedt-Duke, and J. Å. Gustafsson, *J. Biol. Chem.*, 1979, **254**, 9284.
[305] M. J. Waters and H. G. Fresen, *J. Biol. Chem.*, 1979, **254**, 6815.
[306] A. Kulczycki and C. W. Parker, *J. Biol. Chem.*, 1979, **254**, 3187.
[307] C. M. Cheng and J. Hawiger, *J. Biol. Chem.*, 1979, **254**, 2165.
[308] P. F. Williams and J. R. Turtle, *Biochim. Biophys. Acta*, 1979, **579**, 367.
[309] I. Kouvonen and R. Gräsbeck, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 358.
[310] W. G. Carter and S.-i. Hakomori, *Biochemistry*, 1979, **18**, 730.
[311] O. Henriksen, E. A. Robinson, and E. Appella, *J. Biol. Chem.*, 1979, **254**, 7651.
[312] M. J. Rogers, E. A. Robinson, and E. Appella, *J. Biol. Chem.*, 1979, **254**, 11126.
[313] L. Klareskog, L. Trägårdh, L. Rask, and P. A. Peterson, *Biochemistry*, 1979, **18**, 1481.
[314] T. J. Kunicki and R. H. Aster, *Mol. Immunol.*, 1979, **16**, 353.
[315] F. V. Plapp, M. M. Kowalski, L. Tilzer, P. J. Brown, J. Evans, and M. Chiga, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2964.

Table 5 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Miscellaneous						
$(Ca^{2+}$–$Mg^{2+})$-ATPase	human erythrocytes	calmodulin	EDTA elution; phosphatidyl serine in buffer to stabilize	×42	U	316
Cold adaption component	rat liver brown adipose tissue	GDP	GDP elution; 32 000 dalton		M	280
C3 Convertase inhibitor	human erythrocyte	C3 (complement)	NaCl; C3b inactivator co-factor	×9	M	317
Cyclopropane fatty acid synthase	*E.coli*		purify by flotation in lipid vesicles	×20	KMU	294
Cytochrome *c* reductase	*Neurosporra crassa*	cytochrome *c*	tris–acetate; study of detergents to solubilize and maintain solubility	×8	MU	318
Estrogen-binding protein	calf uterus	heparin	heparin	×4.4	CMPSU	22
		estradiol derivative	NaSCN elution		CMPSU	22
Organic anion binding protein	rat liver plasma membrane	bilirubin	KI, urea, or free ligand			319
		sulphobromo-phthalein	KI, urea, or free ligand			319
Succinate : ubiquinone reductase	*Neurosporra crassa*	cytochrome *c*	tris–acetate	×1.5	MU	318

[316] V. Niggli, J. T. Penniston, and E. Carafoli, *J. Biol. Chem.*, 1979, **254**, 9955.
[317] D. T. Fearon, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5867.
[318] H. Weiss and H. J. Kolb, *Eur. J. Biochem.*, 1979, **99**, 139.
[319] J. Reichen and P. D. Berk, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 484.

Nucleic-acid Metabolism, Viral and Phage-related Proteins, and Protein Synthesis.—The preferential affinity of high molecular weight, high mobility group, non-histone proteins for single-stranded DNA lead to the suggestion that they were involved in its stabilization.[320] Methods used in the extraction of RNA polymerases from rat liver nuclei were also compared.[321] Two forms of an SV40 T-antigen-related protein were separated on heparin-Sepharose, one with ATPase activity (IIa) and one with only protein kinase activity (IIb). However, it was thought possible that the form (II) applied to the column was contaminated with form (I), which also has ATPase activity, and that (IIa) and (I) were the same protein.[322] Interferon was prepared from a number of sources. Possibly the most pertinent thing that can be said about the procedures used is that they attest to the unusual nature of the protein; organic solvents,[232] SDS, and urea [323] did not affect its activity. The inability of *ca.* 60% of a human leukocyte preparation to bind to poly(I)poly(U)-Sepharose was however interpreted as evidence of denaturation.[324] A procedure whereby all 50S *E.coli* ribosomal subunit proteins, except L14, L26, L31, and L34, were resolved contained also an identification of ten distinct ribosomal protein complexes.[325] Seven *E.coli* amino-acyl tRNA synthetases (methionyl-, lysyl-, arginyl-, leucyl-, isoleucyl-, glutaminyl-, and glutamyl-) were also isolated, using both tRNA affinity and gel filtration chromatography, as a complex with molecular weight 1 000 000 dalton.[326] Other related proteins are given in Table 6.

[320] P. J. Isackson, J. L. Fishback, D. L. Bidney, and G. R. Reeck, *J. Biol. Chem.*, 1979, **254**, 5569.
[321] T. J. C. Beebee, *Biochem. J.*, 1979, **183**, 43.
[322] R. Tjian and A. Robbins, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 610.
[323] I. A. Braude and E. De Clercq, *J Biol. Chem.*, 1979, **254**, 7758.
[324] M. N. Thang, D. C. Thang, M. K. Chelbi-Alix, B. Robert-Galliot, M. J. Commoy-Chevalier, and C. Chany, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3717.
[325] G. Wystup, H. Teraoka, H. Schulze, H. Hampl, and K. H. Nierhaus, *Eur. J. Biochem.*, 1979, **100**, 101.
[326] O. Kellermann, A. Brevet, H. Tonetti, and J.-P. Waller, *Eur. J. Biochem.*, 1979, **99**, 541.
[327] A. Wadano, P. A. Hobus, and T.-H. Liao, *Biochemistry*, 1979, **18**, 4124.

Table 6 *Isolation of proteins of nucleic acid metabolism and protein synthesis*

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Proteins of nucleic-acid metabolism						
ATPase (DNA-dependent)	*E. coli*	ssDNA	NaCl; 35% yield	×3.7	KMU	328
ATPase (DNA-dependent)	calf thymus	ssDNA	NaCl	×2		329
DNA gyrase B (50K)		valine	decreasing phosphate; derived from 90K enzyme	×1.7		330
DNA polymerase	*Drosophila melanogaster*	ssDNA	phosphate gradient; 55% yield; high mol. wt. enzyme	×2.7	KMPU	331
		CB	phosphate; 25% yield	×4.4	KMPU	331
DNA polymerase I	*E. coli*	DNA	KCl	×6.6		332
DNA polymerase	human milk	poly(C)	KCl; properties of reverse transcriptase		KM	333
DNA polymerase α	mouse	oligo(dT)	NaCl	×5.3	CKU	334
DNA polymerase α	rat liver	denatured DNA	KCl	×6.4	KS	335
Deoxynucleotidyl transferase (terminal)	human leukemic lymphoblasts	CB	phosphate; high mol. wt. enzyme	×4.4	IMU	336
		dATP	dGTP did not bind, phosphate elution	×4.2	IMU	336
Endonuclease	*B. subtilis Marburg*	denatured DNA	KCl; ssDNA-specific enzyme	×4.4	KM	337
Endonuclease Bam H1	*B. amyloliquefaciens*	aminopentyl	NaCl gradient	×2.2	KMU	338
Endonuclease Pal 1	*Providencia alcalifaciens*	heparin	NaCl	×106	IKMU	339
		CB	NaCl	×4.7	IKMU	339
Endonuclease R Bgl 1	*B. globigii*	aminopentyl	KCl	×1.1	KMU	340
Histone acetyl-transferase	*Artemia salina*	DNA	KCl; highly efficient with H1 histone	×2.9	KM	341
Histone-like protein	yeast mitochondria	DNA	NaCl; binds to DNA; mol. wt. 20000 dalton if no PMSF, mol. wt. 16000 dalton		AMU	342

3-Methyladenine-DNA glycosylase	human lymphoblasts	DNA	NaCl gradient	×13		343
Poly(ADP-ribose) synthetase	calf thymus	DNA	NaCl gradient	×146	AIKPSU	344
Purine ribonucleosidase	*Leishmania donovani*	Formycin B	inosine; prewash with 2′-deoxyinosine	×24.6	IKM	345
Rec A protein	*E. coli*	ATP	gives homologous pairing of superhelical and ssDNA			346
RNAse H	cultured plant cells	CB	KCl; two enzymes—Mn^{2+}	×4.6	K	347
			Mg^{2+}	×15.8	K	347
RNAse inhibitor	human placenta	RNAse A	NaCl, decrease pH	×1538	K	348
RNA polymerase I (DNA-dependent)	*Aspergilus nidulans*	DNA	ammonium sulphate gradient	×2.7	MU	349

[328] G. T. Yarranton, R. H. Das, and M. L. Gefter, *J. Biol. Chem.*, 1979, **254**, 11997.
[329] L. M. Assairi and I. R. Johnston, *Eur. J. Biochem.*, 1979, **99**, 71.
[330] M. Gellert, L. M. Fisher, and M. H. O'Dea, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6289.
[331] G. R. Banks, J. A. Boezi, and I. R. Lehman, *J. Biol. Chem.*, 1979, **254**, 9886.
[332] G. Rhodes, K. D. Jentsch, and T. M. Jonn, *J. Biol. Chem.*, 1979, **254**, 7465.
[333] J. A. Kantor, Y.-H. Lee, J. G. Chinkjian, and W. F. Feller, *Science*, 1979, **204**, 511.
[334] Y.-C. Chen, E. W. Bohn, S. R. Planck, and S. H. Wilson, *J. Biol. Chem.*, 1979, **254**, 11678.
[335] O. Fichot, M. Pascal, M. Menchali, and A.-M. de Recondo, *Biochim. Biophys. Acta*, 1979, **561**, 29.
[336] M. R. Deibel and M. S. Coleman, *J. Biol. Chem.*, 1979, **254**, 8634.
[337] K. Matsumoto, T. Ando, H. Saito, and Y. Ikeda, *J. Biochem. (Tokyo)*, 1979, **86**, 627.
[338] L. A. Smith and J. G. Chirikjian, *J. Biol. Chem.*, 1979, **254**, 1003.
[339] K. Bakshi and G. W. Rushizky, *Anal. Biochem.*, 1979, **99**, 207.
[340] W. Johannssen, H. Schütte, F. Mayer, and H. Mayer, *J. Mol. Biol.*, 1979, **134**, 707.
[341] A. Cano and A. Pestaña, *Eur. J. Biochem.*, 1979, **97**, 65.
[342] F. Caron, C. Jacq, and J. Rouvière-Yaniv, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4265.
[343] T. P. Brent, *Biochemistry*, 1979, **18**, 911.
[344] S. Ito, Y. Shizuta, and O. Hayaishi, *J. Biol. Chem.*, 1979, **254**, 3647.
[345] G. W. Koszalka and T. A. Krenitsky, *J. Biol. Chem.*, 1979, **254**, 8185.
[346] T. Shibata, C. DasGupta, R. P. Cunningham, and C. M. Radding, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1638.
[347] Y. Sawai, S. Uchida, J. Saito, N. Sugano, and K. Tsukada, *J. Biochem. (Tokyo)*, 1979, **85**, 1301.
[348] P. Blackburn, *J. Biol. Chem.*, 1979, **254**, 12484.
[349] H. G. Stunnenberg, L. M. J. Wennekes, and H. W. J. Van den Broek, *Eur. J. Biochem.*, 1979, **98**, 107.

Table 6 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Characterization*	*Ref.*
Proteins of nucleic-acid metabolism (cont.)						
RNA polymerase	*B. subtilis*	heparin	NaCl	×28	K	350
		ssDNA	NaCl	×1.5	K	350
RNA polymerase (σ-subunit, DNA-dependent enzyme)	*E. coli*	ssDNA	NaCl		ACIMPU	351
RNA polymerase II (DNA-dependent)	pea seedling	heparin	ammonium sulphate	×3.3	KMU	352
RNA polymerase	Streptomyces antibioticus	DNA	KCl; from actinomycin producing and non-producing cells	×13	KU	353
Ribonucleotide reductase	calf thymus	dATP	ATP	×61	CKMSU	354
Viral and phage-related proteins	(*E. coli*)					
DNA polymerase	HeLa cells after Vaccinia virus induction	ssDNA (calf thymus)	phosphate gradient	×5.9	KSU	355
DNA polymerase (RNA-dependent)	A-type particles murine mammary tumour virus	poly(rC)	NaCl; two activities—I	×63	KM	356
			II	×89	KM	356
DNA polymerase I (T4)	*E. coli*	CB	dGTP and other nucleotides studied as eluants for *E. coli*			357
Gene A protein	ϕX174	DNA	NaCl	×5.7	U	358
Gene D5 protein	T5 phage	ssDNA	NaCl; dsDNA-binding protein	×23.9	AKMPSU	359
Gene 5 protein	T7 phage	hexyl	NaCl; with host (*E. coli*) thioredoxin is DNA polymerase	×1.2	KMU	360
Gene 32 protein	T4 phage	ssDNA	NaCl; EDTA required to inactivate DNAse I	×35.6	KU	361

		norleucine	decreasing phosphate	×1.2	KU	361
		phenyl	decreasing ammonium sulphate	×1.3	KU	361
Gene 41 protein	T4 phage	norleucine	decreasing ammonium sulphate	×5.6		362
Gene 43 protein	T4 phage	norleucine	decreasing ammonium sulphate	×1.4		363
Gene 43 protein	T4 phage	DNA	NaCl	×27		363
Gene 45 protein	T4 phage	norleucine	decreasing ammonium sulphate	×3.5		363
Major glycoprotein	murine mammary tumour virus	concanavalin A	α-methylmannoside			187
Replicase	poliovirus-infected HeLa cells	poly(U)	KCl elution	×4.1	K	364
Reverse transcriptase	type C RNA virus (human lymphoma cells)	poly(rC)	KCl; compared with other reverse transcriptases			365
RNA ligase	T4 phage (*E. coli*)	ADP	omit Mg^{2+} from buffer			366

[350] B. L. Davison, T. Leighton, and J. C. Rabinowitz, *J. Biol. Chem.*, 1979, **254**, 9220.
[351] P. A. Lowe, D. A. Hager, and R. R. Burgess, *Biochemistry*, 1979, **18**, 1344.
[352] Y. Sasaki, M. Ishiye, H. Goto, and T. Kamikubo, *Biochim. Biophys. Acta*, 1979, **564**, 437.
[353] G. H. Jones, *Arch. Biochem. Biophys.*, 1979, **198**, 195.
[354] Y. Engström, S. Eriksson, L. Thelander, and M. Åkerman, *Biochemistry*, 1979, **18**, 2941.
[355] M. D. Challberg and P. T. Englund, *J. Biol. Chem.*, 1979, **254**, 7812.
[356] M. Kohno and A. Ishihama, *Eur. J. Biochem.*, 1979, **97**, 257.
[357] T. D. Lindell, B. P. Nichols, J. E. Donelson, and E. Stellwagen, *Biochim. Biophys. Acta*, 1979, **562**, 231.
[358] S. Eisenberg and A. Kornberg, *J. Biol. Chem.*, 1979, **254**, 5328.
[359] A. C. Rice, T. A. Ficht, L. A. Holladay, and R. W. Moyer, *J. Biol. Chem.*, 1979, **254**, 8042.
[360] K. Hori, D. F. Mark, and C. C. Richardson, *J. Biol. Chem.*, 1979, **254**, 11591.
[361] M. Bittner, R. L. Burke, and B. M. Alberts, *J. Biol. Chem.*, 1979, **254**, 9565.
[362] C. F. Morris, L. A. Moran, and B. M. Alberts, *J. Biol. Chem.*, 1979, **254**, 6797.
[363] C. F. Morris, H. Hama-Inaba, D. Mace, N. K. Sinha, and B. Alberts, *J. Biol. Chem.*, 1979, **254**, 6787.
[364] A. DasGupta, M. H. Baron, and D. Baltimore, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2679.
[365] R. S. Goodenow, and H. S. Kaplan, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4971.
[366] M. Sugiura, M. Suzuki, E. Ohtsuka, S. Nishikawa, H. Vemura, and M. Ikehara, *FEBS Lett.*, 1979, **97**, 73.

Table 6 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Viral and phage-related proteins (cont.)						
S-Adenosylmethionine hydroxylase	T3 colivirus	*S*-adenosyl-homocysteine	adenosine–2M-NH_4Cl; Virus—17K, + host protein—49K (A)	×7.8	KMU	367
			(B)	×24.2	KMU	367
T-Antigen-related protein	SV40	heparin	NaCl; form IIa (ATPase)	×14	U	322
			form IIb (protein kinase)	×2.8	U	322
Interferons						
	human fibroblast	concanavalin A	α-methylmannoside, ethylene glycol	×10	AHMU	368
	human leukocyte	h.p.l.c. (RP-8, diol)	propanol gradients; three peaks	×10—28	AMU	232
	human leukocyte	concanavalin A	α-methylmannoside, NaCl, ethylene glycol			369
	human leukocyte	poly(I)poly(U)	NaCl; 40% maximum bound			370
	human lymphoblastoid	CB	NaCl elution	×71		371
	human lymphoblastoid	antibody	pH = 2.4 elution; sequence showed homogenous preparation		ACMU	372
		Trp-Trp	1 M-NaCl, 75% ethylene glycol	×3.5	ACMU	372
	mouse	hexyl	binds in presence SDS			373
	mouse	controlled pore glass	low pH elution			242

Proteins of protein biosynthesis						
Anti-protein synthesis inhibitor		heparin	KCl		MU	374
Arginyl-t-RNA synthetase	rat liver	aminohexyl		×3.5	HM	375
		CB		×1.5	HM	375
Cysteinyl-t-RNA synthetase	*B. stearothermophilus*	butyl	phosphate gradient	×6.7	ACKMU	376
Elongation factor I	rabbit reticulocytes	heparin	KCl; compared with eIF-2	×43	MU	377
		RNA	KCl	×3.3	MU	377
Elongation factor II	rat, pig liver	GTP, GDP	GTP elution	×9	M	378
EF-P	*E. coli*	mercurial	retarded; involved in peptide bond synthesis	×1.5	MPU	379
eIF-3	embryonic chick muscle	mRNA	KCl–LiCl–urea			380
Lysyl-t-RNA synthetase	rat liver	aminohexyl		×3.2		375
		CB		×1.5		375

[367] N. Spoerel and P. Herrlich, *Eur. J. Biochem.*, 1979, **95**, 227.
[368] Y. H. Tan, F. Barakat, W. Berthold, H. Smith-Johannsen, and C. Tan, *J. Biol. Chem.*, 1979, **254**, 8067.
[369] P. M. Krob and K. C. Chadha, *Biochemistry*, 1979, **18**, 5782.
[370] M. N. Thang, D. C. Thang, M. K. Chelbi-Alix, B. Robert-Galliot, M. J. Commoy-Chevalier, and C. Chany, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3717.
[371] J. S. Erickson and K. Paucker, *Anal. Biochem.*, 1979, **98**, 214.
[372] K. C. Zoon, M. E. Smith, P. J. Bridgen, D. zur Nedden, and C. B. Anfinsen, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5601.
[373] I. A. Braude and E. De Clercq, *J. Biol. Chem.*, 1979, **254**, 7758.
[374] H. Amesz, H. Goumans, T. Haubrich-Morree, H. O. Voorma, and R. Benne, *Eur. J. Biochem.*, 1979, **98**, 513.
[375] R. L. Glinski, P. C. Gainey, T. P. Mawhinney, and R. H. Hilderman, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1052.
[376] C. J. Bruton and L. A.-M. Cox, *Eur. J. Biochem.*, 1979, **100**, 301.
[377] S. Moretti, T. Staehelin, H. Trachsel, and J. Gordon, *Eur. J. Biochem.*, 1979, **97**, 609.
[378] H. Kapmeyer, D. A. Lappi, and N. O. Kaplan, *Anal. Biochem.*, 1979, **99**, 189.
[379] B. R. Glick, R. M. Green, and M. C. Ganoza, *Can. J. Biochem.*, 1979, **57**, 749.
[380] S. M. Heywood and D. S. Kennedy, *Arch. Biochem. Biophys.*, 1979, **192**, 270.

Table 6 (*cont.*)

Protein (*substrate*)	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Proteins of protein biosynthesis (cont.)						
m-RNA cap-binding protein	eukaryots	m^7GDP	m^7GDP; 1 M-KCl removes other proteins also			381
Ribosomal proteins	rat liver	5.8S RNA	L6, L8, L19, S9, S13			36
Valyl-t-RNA synthetase	*Euglena gracilis* chloroplasts	CB	valine–ATP	×32	K	382
Valyl-t-RNA synthetase	*Mycobacterium smegmatis*	valine	KCl elution	×3.6	KMU	383

[381] N. Sonenberg, K. M. Rupprecht, S. M. Hecht, and A. J. Shatkin, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4345.
[382] P. Imbault, V. Sorantoglou, and J. H. Weil, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 75.
[383] V. Natarajan and K. P. Gopinathan, *Biochim. Biophys. Acta*, 1979, **568**, 253.

Plasma Proteins.—A new volume entitled 'Plasma Proteins—Analytical and Preparative Techniques' was published.[384] The nine components of the classical pathway of complement activation were partially resolved on Cibacron blue-Sepharose, recoveries ranging from 17% for C1 to 128% for C9.[385] In the past, one of the major problems in isolating plasma proteins has been the removal of albumin (60% by weight of plasma protein). While for many human proteins this is overcome by adsorption of albumin to Cibacron blue, rat, mouse, and bovine albumins all had decreased affinity for the dye.[386] Similarly, much IgG (human) can be removed on *Staphylococcal* protein A. Previously regarded as being specific for subclasses G1, G2, and G4, some G3 and about 30% of human plasma IgA also bound.[387] Some success at fractionation of IgG was achieved with adsorption to aromatic rather than aliphatic ligands. The immunoglobulin remained heterogeneous however.[388] (See also Table 7.)

[384] 'Plasma Proteins—Analytical and Preparative Techniques', P. C. Allen, E. A. Hill, and A. M. Stokes, Blackwell, Oxford, 1979.
[385] A. P. Gee, T. Borsos, and M. D. P. Boyle, *J. Immunol. Methods*, 1979, **30**, 119.
[386] P. C. Kelleher, C. J. Smith, and R. Pannell, *J. Chromatogr.*, 1979, 173, 415.
[387] G. J. van Kamp, *J. Immunol. Methods*, 1979, **27**, 301.
[388] B. H. J. Hofstee, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 312.

Table 7 *Isolation of plasma proteins*

Protein	*Source*	*Ligand/Procedure*	*Comments*	*Factor*	*Character-ization*	*Ref.*
Proteins of coagulation and fibrinolysis						
Factor V	bovine	octyl; decreasing NaCl		×17.6	AMPSU	389
		phenyl; NaCl gradient		×1.2	AMPSU	389
Factor V	human	haemoglobin	to remove haptoglobin			390
Factor VII	human	heparin; NaCl	albumin, prothrombin removed by antisera	×4.8	AMU	391
Plasminogen	human	Procion red; KCl			CM	67
Prekallikrein	bovine	benzamidine; guanidine elution	Fletcher Factor	×2.7	CHMU	392
		arginyl methyl ester; NaCl gradient	Fletcher Factor	×3.2	CHMU	392
		heparin; NaCl gradient, polybrene present	Fletcher Factor	×14	CHMU	392
Prorennin		CB; NaCl elution	separates from rennin		M	393
		pepstatin; increase pH			M	393
Protein S	bovine, human	heparin			ACIMPU	394
		polyhomoarginine; guanidine–NaCl elution	in presence benzamidine, PMSF		ACIMPU	394
Complement proteins						
$C\bar{1}$ Components	human	elute from immune precipitate with EDTA ($C\bar{1}r$, $C\bar{1}s$) or high pH ($C\bar{1}q$)				395
$C1\underline{q}$	human	IgG; NaCl elution		×679		396
$C\bar{1}r$	rabbit	IgG; diaminobutane elution		×55	MU	397
C1s	bovine	gel filtration in presence and absence Ca^{2+}		×3 ×2.1	ACMU	398

C1s	human	IgG	in calcium rather than EDTA to remove amyloid P(C1t) component			399
C4	human	anti-C4def. serum	to avoid cleavage by $C\overline{1}s$; soybean trypsin inhibitor also contained in buffer	×4.5		207
C3	human	anti-impurity antiserum	yields functionally pure C3		AMU	208
C3DP (DNA-binding protein)	human	DNA; NaCl elution			IMU	400
		concanavalin A; 1-*O*-methyl-α-D-gluco-pyranoside elution			IMU	400
C5	human	protein A	to remove IgG1, 2 and 4		AMS	401
Factor D	human	heparin; NaCl elution			ACK	402
S Protein	human	antisera to remove albumin, anti-α_1 chymotrypsin	see protein S, coagulation; diff. mobility in plasma–serum; 42% recovery		AIMPU	403

[389] M. E. Nesheim, K. H. Myrmel, L. Hibbard, and K. G. Mann, *J. Biol. Chem.*, 1979, **254**, 508.
[390] P. A. Bolhuis, T. B. M. Hakvoort, K. Breederveld, I. A. Mochtar, and J. W. Ten Kate, *Biochim. Biophys. Acta*, 1979, **578**, 23.
[391] R. Flengsrud, *Eur. J. Biochem.*, 1979, **98**, 455.
[392] R. L. Heimark and E. W. Davie, *Biochemistry*, 1979, **18**, 5743.
[393] N. Yokosuwa, N. Takahashi, T. Inagami, and D. L. Page, *Biochim. Biophys. Acta*, 1979, **569**, 211.
[394] R. G. DiScipio and E. W. Davie, *Biochemistry*, 1979, **18**, 899.
[395] G. J. Arlaud, R. B. Sim, A.-M. Duplaa, and M. G. Colomb, *Mol. Immunol.*, 1979, **16**, 445.
[396] W. P. Kolb, L. M. Kolb, and E. R. Podack, *J. Immunol.*, 1979, **122**, 2103.
[397] Y. Mori, M. Kobetsu, N. Abe, and J. Koyama, *J. Biochem. (Tokyo)*, 1979, **85**, 1023.
[398] R. D. Campbell, N. A. Booth, and J. E. Fothergill, *Biochem. J.*, 1979, **183**, 579.
[399] G. R. Arlaud, A. Reboul, R. B. Sim, and M. G. Colomb, *Biochim. Biophys. Acta*, 1979, **576**, 151.
[400] C. Schröder, D. Weinblum, R. K. Zahn, and K. Dose, *Z. Physiol. Chem.*, 1979, **360**, 473.
[401] B. F. Tack, S. C. Morris, and J. W. Prahl, *Biochemistry*, 1979, **18**, 1490.
[402] A. E. Davis, C. Zalut, F. S. Rosen, and C. A. Alper, *Biochemistry*, 1979, **18**, 5082.
[403] E. R. Podack and H. J. Müller-Eberhard, *J. Biol. Chem.*, 1979, **254**, 9908.

Table 7 (*cont.*)

Protein	*Source*	*Ligand/Procedure*	*Comments*	*Factor*	*Character-ization*	*Ref.*
Protease inhibitors						
α_2-Macroglobulin		Zn-chelate chromatography; pH = 6 → 5	prolonged ammonium sulphate exposure	×9.5		404
α_2-Acute-phase macroglobulin	rat	concanavalin A; α-methyl-mannoside, α-methyl-glucoside			ACHIKMU	405
α_1-Protease inhibitor		Zn-chelate chromatography pH = 8 → 6.5 elution		×19.6		404
antithrombin III	rat, porcine, human, rabbit	heparin; NaCl elution			ACHKMU	406
Thiol proteinase inhibitor	human	phenyl; decreasing ammonium sulphate			ACIMPU	407
Thiol proteinase inhibitor	human	papain; 4 M-KSCN wash elute pH = 12	39% recovery	×218	IMU	408
Miscellaneous plasma proteins						
Adenosine deaminase complexing protein	human	antibody; 6 M-urea elution	compared with kidney protein; 48% recovery	×7857	AHMU	409
Albumin	rat	bromosulphophthalein; ligand or NaCl elution				410
Amyloid P component	mouse	binds to agarose in calcium, elute EDTA	C-reactive protein; binds to pneumococcal polysaccharide			239
Angiotensin	rat	CB; ammonium bicarbonate	80% recovery; nephrectomized plasma	×3.3	IM	411
Corticosteroid-binding globulin	guinea pig	androsterone derivative cortisol elution	pregnant serum	×133	ACHKMP	412

Fibronectin	human	gelatin; arginine elution after NaCl wash	79% recovery; 'LETS' protein	×276	A	413
		arginine; NaCl elution	80% recovery	×1.1	A	413
Fibronectin	human, rat	heparin; NaCl gradient	opsonically active cold insoluble globulin	×14		414
		antibody; pH = 2.3 elution		×765		414
Haemopexin	human	haem; pH = 2.4 elution				415
Heparin-neutralizing protein	rabbit	heparin; NaCl elution	platelet-derived; homology human platelet factor 4	×43	ACU	416
IgE	mouse	antibody; KI–NaSCN elution	antisera used to remove other γ-globulins			417
IgM, F(ab), and $(Fc)_5$		*Ricinus communis* lectin; lactose elution	separates F(ab) and $(Fc)_5$			418
Kallikrein	human	antibody	to remove IgG			419
Lactate dehydrogenase	human	*p*-aminophenyl-pyruvate or -lactate; NADH or L-phenyl-lactate elution	72% recovery	×275		420

[404] T. Kurecki, L. F. Kress, and M. Laskowski, *Anal. Biochem.*, 1979, **99**, 415.
[405] W. Nieuwenhuizen, J. J. Emeis, and J. Hemmink, *Biochim. Biophys. Acta*, 1979, **580**, 129.
[406] T. Koide, *J. Biochem. (Tokyo)*, 1979, **86**, 1841.
[407] H. C. Ryley, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 871.
[408] M. Järvinen, *FEBS Lett.*, 1979, **108**, 461.
[409] W. P. Schrader, F. J. Woodward, and B. Pollara, *J. Biol. Chem.*, 1979, **254**, 11964.
[410] A. G. Clark and S. T. Wong, *Anal. Biochem.*, 1979, **92**, 290.
[411] U. Hilgenfeldt and E. Hackenthal, *Biochim. Biophys. Acta*, 1979, **579**, 375.
[412] K. E. Mickelson and U. Westphal, *Biochemistry*, 1979, **18**, 2685.
[413] M. Vuento and A. Vatieri, *Biochem. J.*, 1979, **183**, 331.
[414] J. Molnar, F. B. Gelder, M. Z. Lai, G. E. Siefring, and R. B. Credo, *Biochemistry*, 1979, **18**, 3909.
[415] J. Suttnar, Z. Hrkal, Z. Vodrážka, and J. Rejnková, *J. Chromatogr.*, 1979, **169**, 500.
[416] M. H. Ginsberg, R. Hoskins, P. Sigrist, and R. G. Painter, *J. Biol. Chem.*, 1979, **254**, 12365.
[417] S. B. Lehrer, *Immunology*, 1979, **36**, 103.
[418] R. Kleine, M. Klapperstück, A. I. Chukhrova, and V. A. Lapuk, *Mol. Immunol.*, 1979, **16**, 421.
[419] C. F. Scott, C. Y. Liu, and R. W. Colman, *Eur. J. Biochem.*, 1979, **100**, 77.
[420] E. Brown and R. Joyeau, *Biochimie*, 1979, **61**, 437.

Table 7 (*cont.*)

Protein (*substrate*)	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Miscellaneous plasma proteins (cont.)						
Lecithin: cholesterol acyltransferase	human	CB	unretarded	×3.2		421
Riboflavin-binding protein	bovine	flavin; riboflavin elution	pregnancy-specific protein			422
Serum esterase (pseudocholine)	human	trimethylammonium; NaCl elution		×212	K	423
Testosterone-binding globulin	canine	testosterone; ligand elution	6% recovery	×141	AKMPSU	424
Transcobalamin II-cyanocobalamin	human	cyanocobalamin		×6777	CIU	33

[421] J. Chung, D. A. Abano, G. M. Fless, and A. M. Scanu, *J. Biol. Chem.*, 1979, **254**, 7456.
[422] A. H. Merrill, J. A. Froehlich, and D. B. McCormick, *J. Biol. Chem.*, 1979, **254**, 9362.
[423] T. Tsujita, K. Nagai, and H. Okuda, *Biochim. Biophys. Acta*, 1979, **570**, 88.
[424] Y. Suzuki, Y. Okamura, and H. Sinohara, *J. Biochem.* (*Tokyo*), 1979, **85**, 1195.

Proteases and Protease Inhibitors.—Conditions for the solubilization of monkey liver microsomal proteases, including detergent, salt, organic solvents, chelating agents, and enzymes, were extensively investigated,[425] as were peptides with zinc co-ordinating ligands as reversible inhibitors and potential affinity ligands for thermolysins.[426] Optimum use of organomercurial-agarose in the isolation of a low molecular weight thiol protease required dithiothreitol activation of the protein. The column capacity for thiol was initially determined in a two-step process; it was first treated with DTNB and sodium sulphite, and then with mercuric chloride to displace bound DTNB.[427] (See also Table 8.)

Lectins and Glycosidases.—Lectins and glycosidases are given in Table 9.

Miscellaneous Proteins.—Miscellaneous proteins isolated by the use of affinity ligands are given in Table 10.

4 Protein Characterization

A single tabulation of molar absorptivities (P-R) appeared during the year.[518] The retrospective view of proteins mentioned earlier contained chapters on 'The historical development of the physical chemistry of proteins',[519] on ultracentrifugation,[520] and on electrophoresis and chromatography.[521] Similarly, 'Techniques in Protein and Enzyme Biochemistry' contains chapters on the purity of proteins, protein-concentration determination, molecular-weight determination, and subunit structure.[4] Volume 61 of 'Methods in Enzymology' was the latest to be devoted to enzyme structure (part H); molecular-weight determination and related procedures are of particular relevance to the topics covered here.[522] (Parts F and G, published in 1978 but not mentioned in last year's review, also contained articles on molecular-weight determination and physical methods.[523, 524]) Procedures for the calculation of physical parameters of proteins from data derived from sucrose density gradients were also described.[525]

Estimation of Protein Concentration.—A review of the Lowry procedure cites 127 references to papers dealing with interfering substances and their elimination.[526] To these can be added yet another general procedure to circumvent detergent interference,[527] and two procedures to remove Triton X-100, by isoamylalcohol extraction [528] and by chromatography on Amberlite XAD-2 resin.[529] Low Lowry values were also found in extracts of periodate-cleaved gels, and a procedure for circumventing this was described.[530] A comparison of protein quantification using the Lowry procedure with that using Coomassie blue G250 binding showed the latter to be consistently low.[531] Coomassie blue binding was used however to quantify protein in SDS–2-mercaptoethanol–tris buffer after precipitation of the bulk of the SDS with potassium phosphate.[532] A method utilizing dye-binding (visible and fluorescent) to cellulose acetate-fixed protein was applied to quantifying 10 ng—20 μg of protein at concentrations of 0.01—10 mg ml^{-1} [533] Other

[425] K. Sogawa and K. Takahashi, *J. Biochem. (Tokyo)*, 1979, **86**, 1313.
[426] N. Nishino and J. C. Powers, *Biochemistry*, 1979, **18**, 4340.
[427] J. M. McLaughlin and M. Müller, *J. Biol. Chem.*, 1979, **254**, 1526.

Table 8 *Isolation of proteases and protease inhibitors*

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Characterization*	*Ref.*
Proteases						
Aminotripeptidase	rabbit intestinal mucosa	concanavalin A	unbound; preferential hydrolysis P—X—Y—, not X—P—Y—, or (±)—X—Y—	×2.5	CKMU	428
Asclepains	*Asclepias syriaca L* (milkweed)	organomercurial	mercuric chloride elution; two enzymes, A and B	×2.1	KMU	429
Carboxypeptidase-IIW	*B. megatarium*	peptidoglycan	NaCl elution	×3	KMU	430
Carboxypeptidase	crayfish	potato carboxy-peptidase inhibitor	binds at pH = 8, elute pH = 11.4		ACKM	431
Carboxypeptidase A	*Patella vulgata* (limpet)	aminocaproyl-phenylalanine	89% recovery; elute with pH = 10, 10 mM-phenyl-alanine	×653	KMU	432
Carboxypeptidase A	cow, horse, sheep pancreas	aminocaproyl-aminobenzene	pH/NaCl elution; arginine elutes B — cow	×31		433
			horse	×80		433
			sheep	×32		433
Carboxypeptidase A	porcine pancreas	Amberlite CG-50	organic solvents, increase pH	×8		248
Carboxypeptidase B	porcine pancreas	Amberlite CG-50	organic solvents, increase pH	×16		248
Carboxypeptidase	'Pronase'	D-arginine	HCl elution	×1.1	K	434
		tryptic digest protamine	retarded in acetate		K	434
Carboxypeptidase	*Streptomyces griseus*	aminocaproyl-aminobenzene	NaCl; pH = 6 → 8; *C*-terminal basics and esters, possible *endo–exo* protease intermediate	×17, ×2	CKMPU	435
Cathepsin D	porcine spleen	pepstatin	increase pH; separates isoenzymes	×85	ACHIMU	436
Cathepsin D	rat spleen	pepstatin	forms I and II	×45	AHIKM	437
		concanavalin A	α-methylmannoside	×1.6	AHIKM	437

α-Chymotrypsin-like protease	rat mast cells	hen ovoinhibitor	pH = 3 elution; 61% recovery		ACHIKM	438
Chymotrypsin		4-phenylbutyl-amine	various ligands			47
Collagenase	pig synovium	heparin	NaCl; extract as latent enzyme, activate before isolation	×6.9		439
		zinc chelate	pH = 4.7, NaCl	×2.5		439
Dipeptidyl amino-peptidase IV		Lys-Ala, Lys-Phe, Lys-Pro mixed	NaCl; cleaves *X*—pro-	×1.1	K	440
Elastase	porcine pancreas	L-trialanine-*p*-nitroanilide	slow turnover of ligand; elute with ethylene glycol			44
Enterokinase	bovine	pancreatic trypsin inhibitor	pH = 3	×248	AKMU	441
Intracellular serine protease	*B. subtilis* Marburg	Gramicidin S		×48	AKM	442
Kallikrein	pancreatic β-cells	aprotinin	NaCl/low pH; cleaves proinsulin			443

[428] C. Doumeng and S. Maroux, *Biochem. J.*, 1979, **177**, 801.
[429] W. Brockbank and K. R. Lynn, *Biochim. Biophys. Acta*, 1979, **578**, 13.
[430] H. DasGupta and D. P. Fan, *J. Biol. Chem.*, 1979, **254**, 5672.
[431] R. Zwilling, F. Jakob, H. Bauer, H. Neurath, and D. L. Enfield, *Eur. J. Biochem.*, 1979, **94**, 223.
[432] G. M. Hass, *Arch. Biochem. Biophys.*, 1979, **198**, 247.
[433] T. J. Bazzone, M. Sokolovsky, L. B. Cueni, and B. L. Vallee, *Biochemistry*, 1979, **18**, 4362.
[434] Y. Narahashi and K. Yoda, *J. Biochem. (Tokyo)*, 1979, **86**, 683.
[435] K. Breddam, T. J. Bazzone, B. Holmquist, and B. L. Vallee, *Biochemistry*, 1979, **18**, 1563.
[436] J. S. Huang, S. S. Huang, and J. Tang, *J. Biol. Chem.*, 1979, **254**, 11405.
[437] K. Yamamoto, N. Katsuda, M. Himeno, and K. Kato, *Eur. J. Biochem.*, 1979, **95**, 459.
[438] M. T. Everitt and H. Neurath, *Biochimie*, 1979, **61**, 653.
[439] T. E. Cawston and J. A. Tyler, *Biochem. J.*, 1979, **183**, 647.
[440] H. C. Krutzsch and J. J. Pisano, *Biochim. Biophys. Acta*, 1979, **576**, 280.
[441] J. J. Liepnieks and A. Light, *J. Biol. Chem.*, 1979, **254**, 1677.
[442] A. Y. Strongin, D. I. Gorodetsky, I. A. Kuznetsova, V. V. Yanonis, Z. T. Abramov, L. P. Belyanova, L. A. Baratova, and V. M. Stepanov, *Biochem. J.*, 1979, **179**, 333.
[443] O. ole-MoiYoi, D. C. Seldin, J. Spragg, G. S. Pinkus, and K. F. Austen, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3612.

Table 8 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Proteases (cont.)						
Kallikrein	porcine pancreas	Amberlite CG-50	organic solvents/pH	×100		248
Kallikrein	human urine	aprotinin	low pH; 89% recovery	×66	AKMU	444
Kallikrein	pig submandibular glands	pancreatic trypsin inhibitor	benzamidine elution	×80	AKMU	445
Kallikrein (kininogenase)	human colon	trasylol	0.1 M-acetic acid	×33	ACKMU	446
Ion-activated neutral protease	rabbit skeletal muscle	organomercurial	dithiothreitol	×2.4	KMU	447
Papain	*Carica papaya* latex		new procedure to remove chymopapain contamination			448
Plasminogen activator	human uterus	zinc chelate	imidazole	×31	KMU	449
		butyl	decreasing NaCl	×2	KMU	449
		concanavalin A	α-methylmannoside	×9.3	KMU	449
Plasminogen activator	human vascular	octyl	ethylene glycol	×18.4	CIMU	450
Prekallikrein	human pancreatic juice	trypsin inhibitor	acetate/pH = 4			451
Proacrosin	rabbit testis	concanavalin A	α-methylmannoside	×4.2	AHMU	452
Protease B	bakers' yeast	D-tryptophan methyl ester	low pH	×7.5	AMS	453
Rennin	dog kidney	pepstatin	lithium bromide—85% acid elution—30%	×256	AMU	454
Seminalplasmin	bovine	aminophenyl-phosphoryl-uridine phosphate	removes RNAse			455
Staphylocoagulase	*Staphylococcus aureus*	prothrombin	NaSCN	×125	AU	456
Thermolysins	*B. subtilis*	peptides with Zn-co-ordinating ligands	increased pH/calcium gradient			426
Thiol protease	*Tritrichomonas* foetus	organomercurial	dithiothreitol		K	427

Thrombocytin	*Bothrops atrox* venom	heparin	1 M-lysine elution		ACHKMU	457
Trypsin	porcine pancreas	Amberlite CG-50	increased pH	×32		248
Protease inhibitors						
Protease inhibitor	human urine	chymotrypsin	1.5 M-NaCl–50 mM-acetic acid			458
Protease B inhibitor	brewers' yeast	hexyl	NaCl	×1.2	AKMU	459
Trypsin inhibitors	winged bean seed	trypsin	0.2 M-HCl–0.5 M-KCl		AKU	460

[444] O. ole-MoiYoi, J. Spragg, and K. F. Austen, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3121.
[445] M. Lemon, F. Fiedler, B. Förg-Brey, C. Hirschauer, G. Laysath, and H. Fritz, *Biochem. J.*, 1979, **177**, 159.
[446] A. Zimmermann, R. Geiger, and H. Kortmann, *Z. Physiol. Chem.*, 1979, **360**, 1767.
[447] J.-L. Azanza, J. Raymond, J.-M. Robin, P. Cottin, and A. Ducastaing, *Biochem. J.*, 1979, **183**, 339.
[448] B. S. Baines and K. Brocklehurst, *Biochem. J.*, 1979, **177**, 541.
[449] D. C. Rijken, G. Wijngaards, M. Zaal-de Jong, and J. Welbergen, *Biochim, Biophys. Acta*, 1979, **580**, 140.
[450] B. R. Binder, J. Spragg, and K. F. Austen, *J. Biol. Chem.*, 1979, **254**, 1998.
[451] M. Amouric and C. Figarella, *Z. Physiol. Chem.*, 1979, **360**, 457.
[452] S. K. Mukerji and S. Meizel, *J. Biol. Chem.*, 1979, **254**, 11721.
[453] Y. Sanada, K. Fujishiro, H. Tanaka, and N. Katunuma, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 815.
[454] V. J. Dzau, E. E. Slater, and E. Haber, *Biochemistry*, 1979, **18**, 5224.
[455] E. S. P. Reddy and P. M. Bhargava, *Nature (London)*, 1979, **279**, 725.
[456] H. Igarashi, T. Morita and S. Iwanaga, *J. Biochem. (Tokyo)*, 1979, **86**, 1615.
[457] E. P. Kirby, S. Niewiarowski, K. Stocker, C. Kettner, E. Shaw, and T. M. Brudzynski, *Biochemistry*, 1979, **18**, 3564.
[458] A. Kessner, L. T. Hodgins, and W. Troll, *Anal. Biochem.*, 1979, **92**, 383.
[459] H. Matern, U. Weiser, and H. Holzer, *Eur. J. Biochem.*, 1979, **101**, 325.
[460] A. A. Kortt, *Biochim. Biophys. Acta*, 1979, **577**, 371.

Table 9 *Isolation of lectins and glycosidases*

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Lectins						
Agglutinin	*Ricinus communis*	L-rhamnose	separates from toxin			461
Favin	*Vicia faba*	Sephadex	glucose		ACU	462
		3-*O*-methyl-glucosamine	α-methylglucoside		ACU	462
Haemagglutinin	*Bordetella pertussis*	haptoglobin	binds pH = 6.5, elute pH = 10	×450		463
Haemagglutinin	*Wistaria floribunda*	hog gastric mucin	galactose	×8	AHI	464
Isolectins	*Polysphondilium pallidum*	acid-treated Sepharose	galactose; lactose in extraction buffer increases yield	×21	AIMU	465
Lectin	peanut	thioglycoside ligands	polyacrylamide matrix			466
Lectin	potato	triacetyl-chitotriose	0.1 M-acetic acid; 97% yield	×100		467
Lectin	rice bran	ovomucoid	0.1 M-acetic acid	×32	ACMU	468
Lectin	sainfoin	mannose	glucose		AHKMPU	469
Lectin	*Ulex europaeus*	maleylated hog stomach peptone	pH = 2.6		HMU	470
Lectin II	*Ulex europaeus*	hog A + H blood group substance	35% ethylene glycol		CU	471
		aminocaproyl-fucosylamine	L-fucose		CU	471
Lectin	*Curcubita pepo* (vegetable marrow)	chitin	0.1 M-acetic acid; specific for β-1,4-*N*-acetyl-glucosamine		AMU	472
Lectin	*Vicia sativa*	Sephadex	low pH; temperature-sensitive binding, lectin-binding protein also isolated	×53	ACMU	473

Lectin	*Vicia sativa*	Sephadex	D-glucose; 63% yield	×20	AHKMU	474
Lectin	winged bean	lactobionate			AIKMU	475
Lectin	*Wistaria floribunda*	hog gastric mucin	lactose; mitogenic, loss in mitogenic specific activity during isolation		AHMU	476
Glycosidases and related enzymes						
N-Acetylgalactosamine 6-sulphate sulphatase	human placenta	concanavalin A	α-methylmannoside	×32	K	477
β-*N*-Acetyl-hexosaminidase	rabbit sperm	desialylated mucin		×4.9	K	478
		desialylated fetuin	*N*-acetylglucosamine, salt	×9.9	K	478
Acid β-galactosidase	feline liver	concanavalin A	α-methylmannoside	×77	KMU	479
		thiogalacto-pyranoside	D-galactose; removal of other β-galactosidases	×28	KMU	479

461 V. Horejši, *J. Chromatogr.*, 1979, **169**, 457.
462 J. J. Hemperley, T. P. Hopp, J. W. Becker, and B. A. Cunningham, *J. Biol. Chem.*, 1979, **254**, 6803.
463 L. I. Irons and A. P. MacLennan, *Biochim. Biophys. Acta*, 1979, **580**, 175.
464 G. Cheung, A. Haratz, M. Katar, R. Skorov, and R. D. Poretz, *Biochemistry*, 1979, **18**, 1646.
465 S. D. Rosen, J. Kaur, D. L. Clark, B. T. Pardos, and W. A. Frazier, *J. Biol. Chem.*, 1979, **254**, 9408.
466 R. T. Lee, S. Cascio, and Y. C. Lee, *Anal. Biochem.*, 1979, **95**, 260.
467 N. N. Desai and A. K. Allen, *Anal. Biochem.*, 1979, **93**, 88.
468 M. Tsuda, *J. Biochem.* (*Tokyo*), 1979, **86**, 1451.
469 K. D. Hapner and J. E. Robbins, *Biochim. Biophys. Acta*, 1979, **580**, 186.
470 V. Hořejši, *Biochim. Biophys. Acta*, 1979, **577**, 389.
471 M. E. A. Pereira, F. Gruezo, and E. A. Kabat, *Arch. Biochem. Biophys.*, 1979, **194**, 511.
472 A. K. Allen, *Biochem. J.*, 1979, **183**, 133.
473 G. Gebauer, E. Schiltz, A. Schimpl, and H. Rüdiger, *Z. Physiol. Chem.*, 1979, **360**, 1727.
474 A. Falasca, C. Francerchi, C. A. Rossi, and F. Stirpe, *Biochim. Biophys. Acta*, 1979, **577**, 71.
475 S. G. Pueppke, *Biochim. Biophys. Acta*, 1979, **581**, 63.
476 P. M. Kaladas and R. D. Poretz, *Biochemistry*, 1979, **18**, 4806.
477 J. Glössl, W. Truppe, and K. Kresse, *Biochem. J.*, 1979, **181**, 37.
478 A. A. Farooqui and P. N. Srivastava, *Int. J. Biochem.*, 1979, **10**, 745.
479 E. W. Holmes and J. S. O'Brien, *Biochemistry*, 1979, **18**, 952.

Table 9 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Glycosidases and related enzymes (cont.)						
Acidic α-D-mannosidase	human plasma	concanavalin A	α-D-mannopyranoside; separation of multiple forms			480
endo-1,3-β-Gluconase	*Candida utilis*	concanavalin A	α-methylmannoside; removes exoenzyme	×1.04	KIMSU	481
endo-β-*N*-Acetyl-glucosaminidase L	*Streptomyces plicatus*	phenyl	decreased citrate; resolved from *endo* H by zinc/ ammonium sulphate precipitation	×1.3	AIKMU	148
α-L-Fucosidase I	almond emulsion	fucopentaosyl-amine	borate elution	×3.1		482
α-L-Fucosidase	human placenta	β-fucosylamine	L-fucose; 38% yield	×8500	KMSU	483
α-L-Fucosidase	human placenta	fucosamine	50 mM-L-fucose	×2.4	K	484
α-L-Fucosidase	monkey brain	fucose	L-fucose		M	485
α-Galactosidase A	human liver	DEAE	ampholines	×19.6	KM	245
α-Galactosidase B	human liver	DEAE	ampholines; ×98 on hydroxyl apatite	×4.2	K	246
β-Galacosidase α2 → 3 sialyltransferase	porcine submaxillary glands	CDP	NaCl	×65		486
		CDP	CTP gradient	×97		486
(α-*N*-Acetylgalactos-aminide 2 → 6 sialyltransferase)		CDP	NaCl	×26		486
Galactosylceramide β-galactosidase		concanavalin A	α-methylmannoside or *N*-acetyl-D-glucosamine; normal and Krabbe disease	×56	KM	487
		sphingosine	ethylene glycol	×3.3	KM	487
Galactosyltransferase	bovine cornea	α-lactalbumin	requires *N*-acetylglucos-amine for binding, omit to elute; keratan sulphate synthesis	×8360	K	488

Galactosyltransferase	pig thyroid	α-lactalbumin	as above; 68% with sonic extract, 37% with Triton	×1417	KMU	489
α-Glucosidase	chick embryo	glucosamine	synthesis of matrix given, glucosamine elution	×30000	KIMU	490
α-Glucosidase	*Drosophila melanogaster*	concanavalin A	α-methylmannoside; 3 forms		KMU	491
hydrolases (22 activities)	human liver	lectins	6 specificities			192

[480] S. Hirani and B. Winchester, *Biochem. J.*, 1979, **179**, 583.
[481] T. G. Villa, V. Notario, and J. R. Villanueva, *Biochem. J.*, 1979, **177**, 107.
[482] H. Yoshima, S. Takasaki, S. Ito-Mega, and A. Kobata, *Arch. Biochem. Biophys.*, 1979, **194**, 394.
[483] B. M. Turner, *Biochim. Biophys. Acta*, 1979, **578**, 325.
[484] J. Hoebeke, J. Groswasser, N. Bousard, E. Vamos, and A. D. Strosberg, *Arch. Biochem. Biophys.*, 1979, **198**, 556.
[485] T. Alam and A. S. Balasubramanian, *Biochim. Biophys. Acta*, 1979, **566**, 327.
[486] J. E. Sadler, J. I. Rearick, J. C. Paulson, and R. L. Hill, *J. Biol. Chem.*, 1979, **254**, 4434.
[487] Y. Ben-Yoseph, M. Hungerford, and H. L. Nadler, *Arch. Biochem. Biophys.*, 1979, **196**, 93.
[488] J. E. Christner, J. J. Distler, and G. W. Jourdian, *Arch. Biochem. Biophys.*, 1979, **192**, 548.
[489] S. Bouchilloux, *Biochim. Biophys. Acta*, 1979, **569**, 135.
[490] H. Hamazaki and K. Hotta, *J. Biol. Chem.*, 1979, **254**, 9682.
[491] T. Tanimura, K. Kitamura, T. Fukuda, and T. Kikuchi, *J. Biochem.* (*Tokyo*), 1979, **85**, 123.

Table 10 *Selected miscellaneous proteins isolated using affinity ligands*

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Acetylcholinesterase	cobra venom	trimethylamino-benzene	phenyltrimethylammonium iodide	× 63	MSU	492
Acid phosphatases	human erythrocyte	aminobenzyl-phosphonate	phosphate gradient	× 500		13
$(ADP\text{-}ribose)_n$ protein conjugates		boronate	phosphate	× 22		493
Adenosine deaminase conversion proteins	rabbit	adenosine deaminase	pH = 3 elution; carbo-hydrate analysis using lectins	× 757	HIMU	494
Alkaline phosphatase	*Halobacterium cutirubrum*	Sepharose	decreasing ammonium sulphate separates from phosphodiesterase	× 27	K	238
Alkaline phosphatase	human liver	aminobenzyl-phosphonate	2-naphthylphosphate	× 51		52
Alkaline phosphatases	hen tissues: yolk	aminophenyl-phenylarsonate	arsenate; 50—75% yields	× 529	MU	495
	liver			× 263	MU	495
	plasma			× 6403	MU	495
	intestine			× 4.7	MU	495
AMP deaminase	bakers' yeast	phosphocellulose	phosphate or ATP elution	× 25	KU	50
				× 160	KU	50
Aspartate transcarbamylase	*Phaseolus aureus* (mung bean)	aspartate	KCl or aspartate elution	× 21	K	24
ATPase inhibitor	rat liver	Sephadex	binds in low phosphate, elute in high phosphate	× 25	AU	240
Cholesterol oxidase	*Schizophyllum commune*	succinyl-cholesterol	deoxycholate, pH		AKMPU	496
Choline acetyltransferase	rat, cow brain	coenzyme A	NaCl gradient; rat	× 82	MU	497
			cow	× 58, × 48	MU	497
Cholyl CoA : glycine and : taurine-*N*-acyltransferase	bovine liver	cholylglycine	glycocholate; glycine: taurine ratio constant		KIMU	498

Choriogonadotropin	human urine	antibody	2 M-sodium trichloracetate after 2 M-$MgCl_2$ wash	×11	ACH	206
Creatinine aminohydrolase	*Pseudomonas putida*	sarcosine	NaCl	×42	AIKMPU	499
Dehydroepiandrosterone-sulphotransferase	human adrenals	dehydroepiandrosterone	ligand elution; 22%	×49	KMU	42
Estradiol-17 dehydrogenase	*Pseudomomas, Streptomyces*	phenoxypropoxyaniline	NaCl; other steroid enzymes followed also	×13		57
Fumarase	pig heart	phenylpentylfumarate	citrate; 54% recovery	×20		500
Glutathione reductase	mouse liver	phosphoadenosine diphosphoribose	$NADP^+$; 72%	×35	AKMPU	501
		adenosine-2′, 5′ bisphosphate	$NADP^+$ after FAD wash; 77%	×22	AKMPU	501
Glutathione *S*-transferases	rat lung	*S*-hexylglutathione	ligand elution; one form found not seen in liver	×206		502
Glycogen synthetases	rat liver	glucose 6-phosphate	glucose 6-phosphate	×26	KSU	45
Glyoxylase I	human erythrocyte	*S*-hexylglutathione	*S*-hexylglutathione, glutathione	×483	CKMU	503
Growth factor	human platelet	CB	1 M-NaCl–50% ethylene glycol	×8.6	MU	62

[492] R. Raba, A. Aaviksaar, M. Raba, and J. Siigur, *Eur. J. Biochem.*, 1979, **96**, 151.
[493] P. Adamietz, K. Klapproth, and H. Hilz, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 1232.
[494] P. P. Trotta, R. A. Peterfreund, R. Schonberg, and M. E. Balis, *Biochemistry*, 1979, **18**, 2953.
[495] I. Debruyne and J. Stockx, *Int. J. Biochem.*, 1979, **10**, 981.
[496] M. Fukuyama and Y. Miyake, *J. Biochem. (Tokyo)*, 1979, **85**, 1183.
[497] R. L. Ryan and W. O. McClure, *Biochemistry*, 1979, **18**, 5357.
[498] D. A. Vessey, *J. Biol. Chem.*, 1979, **254**, 2059.
[499] K. Rikitabe, I. Oka, M. Ando, T. Yoshimoto, and D. Tsuru, *J. Biochem. (Tokyo)*, 1979, **86**, 1109.
[500] S. Chaudhuri and E. W. Thomas, *Biochem. J.*, 1979, **177**, 115.
[501] J. López-Barea and C.-Y. Lee, *European J. Biochem.*, 1979, **98**, 487.
[502] C. Guthenberg and B. Mannervik, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1304.
[503] A.-C. Aronsson, G. Tibbelin, and B. Mannervik, *Anal. Biochem.*, 1979, **92**, 390.

Table 10 (*cont.*)

Protein	*Source*	*Ligand*	*Eluant/Comments*	*Factor*	*Character-ization*	*Ref.*
Guanine aminohydrolase	rabbit liver	aminoethoxy-phenylguanine			AIKMU	504
Lactate dehydrogenase	thermophilic and mesophilic bacteria	oxamate	binds in NADH, fructose 1,6-bisphosphate; omit to elute	×40 ×333	ACKMU ACKMU	505 505
Lactoperoxidase	milk	di-iodotyrosine	60—65% overall recovery			40
Legumin	*Pisum* (pea) seeds	antibody	low pH		ACMPSU	209
Lipoprotein lipase	rat brown fat	heparin	NaCl; 20% yield	×284	U	506
Lipoprotein lipase	rat liver	heparin	NaCl; 10% unbound, 16% requires Triton also to elute	×40	MU	507
Luciferin-binding protein		*p*-benzyl-oxaniline	NaCl gradient	×4.7	ACIMPSU	508
Methylmalonyl-CoA-pyruvate trans-carboxylase	*Arachnia, Proprionibacterium*	avidin	biotin; not on crude extract, 40—60%	×7—10		17
Myosin light chain kinase	bovine heart	calmodulin	binds in calcium, elute EDTA; calmodulin-dependent	×51.4	K	509
Orotidine-5′-phosphate decarboxylase	yeast	azuridine-5′-phosphate	ligand elution	×3158		510
Phospholipase D	*Streptomyces chromofuscus*	palmitoyl	Triton X-100; 92%	×258	IKMU	511
Phosphotransacetylase	*Clostridium kluyveri*	desulpho-CoA	coenzyme A gradient	×50		512
Prolyl-3-hydroxylase	chick embryo	denatured collagen	ethylene glycol; 43% yield	×1216	K	513
Proprionyl-CoA carboxylase	*Mycobacterium smegmatis*	avidin	biotin	×7		17
Protein kinase	rat intestinal mucosa	soybean trypsin inhibitor	c-AMP-dependent kinase 30% for catalytic subunit	×300		20

Putrescine oxidase	*Micrococcus rubens*	diaminododecone		× 40		8
S-Adenosyl-L-methionine decarboxylase	mouse mammary gland, liver	MGBG	methylglyoxal bis(guanyl-hydrazone)	× 1315	KM	514
Spermidine dehydrogen dehydrogenase	*Serratia marcescens*	diamino-octane		× 1200		8
Spermine synthase	bovine brain	spermine	spermidine	× 183	IKMPU	515
Thiamine-binding protein	chicken egg white	riboflavin-binding protein	1 M-NaCl; replaces TPP as ligand		KMU	19
Thymidylate synthetase	human leukemic cells	tetrahydro-methotextrate	KCl after dUMP wash	× 3023	AMU	516
Thymidylate synthetase	mouse L1210 cells	formyldideaza-formate	omit dUMP to elute; (in Triton X-100	× 551 × 994)	MU	517
Toluene dioxygenase	*Pseudomonas putida*	*p*-toluic acid	KCl in polyethylene glycol	× 16	P	41

[504] J. D. Bergstrom and A. L. Bieber, *Arch. Biochem. Biophys.*, 1979, **194**, 107.
[505] H.-P. Schär and H. Zuber, *Z. Physiol. Chem.*, 1979, **360**, 795.
[506] D. Guerrier and H. Pellet, *FEBS Lett.*, 1979, **106**, 115.
[507] T. Kuusi, P. K. J. Kinnunen, C. Ehnholm, and E. A. Nikkilä, *FEBS Lett.*, 1979, **98**, 314.
[508] H. Charbonneau and M. J. Cormier, *J. Biol. Chem.*, 1979, **254**, 769.
[509] M. P. Walsh, B. Vallet, F. Autric, and J. G. Demaille, *J. Biol. Chem.*, 1979, **254**, 12136.
[510] R. S. Brody and F. H. Westheimer, *J. Biol. Chem.*, 1979, **254**, 4238.
[511] S. Imamura and Y. Horiuti, *J. Biochem.* (*Tokyo*), 1979, **85**, 79.
[512] L. T. Smith and N. O. Kaplan, *Anal. Biochem.*, 1979, **95**, 2.
[513] K. Tryggvason, K. Majamaa, J. Risteli, and K. I. Kivirikko, *Biochem. J.*, 1979, **183**, 303.
[514] T. Sakai, C. Hori, K. Kano, and T. Oka, *Biochemistry*, 1979, **18**, 5541.
[515] R.-L. Pajula, A. Raina, and T. Eloranta, *Eur. J. Biochem.*, 1979, **101**, 619.
[516] A. Lockshin, R. G. Moran, and P. V. Danenberg, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 750.
[517] W. Rode, K. J. Scanlon, J. Hynes, and J. R. Bertino, *J. Biol. Chem.*, 1979, **254**, 11538.

procedures described included a biuret method for insoluble protein, requiring hydrogen peroxide oxidation and SDS–sodium hydroxide solubilization,[534] an optimized fluorescamine method,[535] and an atomic adsorption spectrophotometric procedure based on protein–copper complex formation.[536]

Determination of Molecular Weight and other Physical Parameters.—A largely forgotten double-diffusion procedure whereby diffusion coefficients of antigens can be determined was newly described.[537] A report that the partial specific volume of proteins was concentration-dependent seems largely irrelevant as the concentration at which this was found (200 mg ml^{-1}, for bovine albumin) is rarely achieved with other proteins.[538] Two possible sources of error in the determination of sedimentation coefficients were described: the increase in apparent isopotential volume of proteins at high ionic strength [539] and solvation of macromolecules producing a lowered sedimentation velocity in density gradients.[540] Procedures for analysis of interacting protein systems from sedimentation equilibrium data were also described.[541, 542] Of related interest were the methods described for characterization of unpurified proteins by sedimentation equilibrium using analysis of sequential aliquots [543] and for the analysis of active-enzyme centrifugation data at enzyme concentrations corresponding to those found *in vivo* rather than the highly diluted samples used previously.[544] Analysis by active enzyme centrifugation of pig heart fumarase showed that, at protein concentrations >0.25 mg ml^{-1}, discrete polymers greater than the tetramer (seen below 0.25 mg ml^{-1}) were formed.[545] Analysis of a soluble mitochondrial ATPase by active-enzyme sedimentation was performed at low magnesium and ATP levels; high concentrations caused a loss of activity. Chemical cross-linking confirmed that the sedimentation coefficient at

[518] D. M. Kirschenbaum, *Int. J. Pept. Protein Res.*, 1979, **13**, 479.
[519] J. T. Edsall, *Ann. N.Y. Acad. Sci.*, 1979, **325**, 53.
[520] J. W. Williams, *Ann. N.Y. Acad. Sci.*, 1979, **325**, 72.
[521] A. H. Gordon, *Ann. N.Y. Acad. Sci.*, 1979, **325**, 95.
[522] Methods in Enzymology, ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, San Francisco, London, 1979, Vol. 61 (Enzyme structure, Part H).
[523] Methods in Enzymology, ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, San Francisco, London, 1978, Vol. 48 (Enzyme structure, Part G).
[524] Methods in Enzymology, ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, San Francisco, London, 1978, Vol. 49 (Enzyme structure, Part F).
[525] J. E. Sadler, *J. Biol. Chem.*, 1979, **254**, 4443.
[526] G. L. Peterson, *Anal. Biochem.*, 1979, **100**, 201.
[527] E. Cadman, J. R. Bostwick, and J. Eichberg, *Anal. Biochem.*, 1979, **96**, 21.
[528] I. H. Mather and C. B. Tamplin, *Anal. Biochem.*, 1979, **93**, 139.
[529] P. S. J. Cheetham, *Anal. Biochem.*, 1979, **92**, 447.
[530] J. Tas, A. C. J. De Vries, and R. G. Berndsen, *Anal. Biochem.*, 1979, **100**, 264.
[531] F. Chiappelli, A. Vasil, and D. F. Haggerty, *Anal. Biochem.*, 1979, **94**, 160.
[532] Z. Zaman and R. L. Verwilghen, *Anal. Biochem.*, 1979, **100**, 64.
[533] V. Neuhoff, K. Phillip, H.-G. Zimmer, and S. Mesecke, *Z. Physiol. Chem.*, 1979, **360**, 1657.
[534] I. Goshev and P. Nedkov, *Anal. Biochem.*, 1979, **95**, 340.
[535] J. V. Kastell, M. Cervera, and R. Marco, *Anal. Biochem.*, 1979, **99**, 379.
[536] D. Davies and E. S. Holdsworth, *Anal. Biochem.*, 1979, **100**, 92.
[537] B. Pokrić and Z. Pučar, *Anal. Biochem.*, 1979, **93**, 103.
[538] H. B. Bull and K. Breese, *Arch. Biochem. Biophys.*, 1979, **197**, 199.
[539] P. Tuengler, G. L. Long, and H. Durchschlag, *Anal. Biochem.*, 1979, **98**, 481.
[540] P. Nieuwenhuysen, *Biopolymers*, 1979, **18**, 277.
[541] F. R. van der Voort, C.-Y. Ma, and S. Nakai, *Arch. Biochem. Biophys.*, 1979, **195**, 596.
[542] R. G. Clarke and G. J. Howlett, *Arch. Biochem. Biophys.*, 1979, **195**, 235.
[543] R. J. Pollet, B. A. Haase, and M. L. Standaert, *J. Biol. Chem.*, 1979, **254**, 30.
[544] G. J. Wei and W. C. Deal, *Biochemistry*, 1979, **18**, 1129.
[545] C. R. Ireland and R. G. Wolfe, *Biochim. Biophys. Acta*, 1979, **566**, 397.

both low and high substrate concentrations was in fact the same.[546] The pressure-dependent loss of ATPase activity noted here was also observed with the nucleosome-histone core complex; a 2.6 S octomer was seen at low speeds, with dissociation to a 1.6 S complex at higher speeds.[547] The advantage of analysis without isolation, seen above with active-enzyme centrifugation, was also given by radiation inactivation. The latter did require the assumption that random damage had a consistent effect on protein function as it required loss of activity to be related to target size.[548] Sepharose CL-6B continued to be praised for its performance in the sizing of polypeptides denatured in guanidine.[549, 550] H.p.l.c. on silica-based gels in both guanidine [551] and SDS [552] was also used to determine the molecular weights of polypeptides. Partial cleavage of cytochrome *c* with cyanogen bromide was used to generate calibration markers in the range 500—20 000 dalton for use in gel filtration in 10% formic acid on Sephadex G50.[553] A procedure for the calculation of molecular weights of proteins utilizing electrophoresis in polyacrylamide without detergent required a statistical analysis of migration at different times of electrophoresis, and was applicable in the range 20 000—950 000 dalton.[554]

Electrophoretic Procedures.—Two texts dealing with electrophoresis [262] and electrofocusing and isotachophoresis [263] were published during the year.

Isoelectric Focusing and Isotachophoresis. A major advance was the development of agarose that generated very low electroendosmotic flow,[555, 556] in which isoelectric focusing was possible, thus permitting analysis of large proteins (*e.g.* IgM). Ampholine–ampholine interaction was argued as the cause of cathodic drift during focusing.[557] However, the problem still existed in focusing gels utilizing no ampholine or buffer, but having a weak acid as anolyte and a weak base as catholyte.[558] Two papers dealt with focusing of membrane proteins: artefactual bands seen with membrane cytochromes were caused either by protein–protein or protein–ampholyte interaction.[559] The former would presumably be eliminated with focusing in mixtures of zwitterionic and non-ionic detergents in a procedure designed for integral membrane proteins (particularly basic proteins).[560] High-resolution focusing was claimed to separate electrophoretically identical proteins on the basis of subtle changes in effective total charge.[561] Bio-specific sample preparation for isoelectric focusing, for example of proteases adsorbed to

[546] V. Y. Chernyak, Z. E. Kozhanova, D. V. Chernyak, and I. A. Kozlov, *Eur. J. Biochem.*, 1979, **98**, 585.
[547] M. Philip, M. Jamaluddin, R. V. R. Sastry, and H. S. Chandra, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5178.
[548] E. S. Kempner and W. Schlegel, *Anal. Biochem.*, 1979, **92**, 2.
[549] D. Mahuron, *J. Chromatogr.*, 1979, **172**, 394.
[550] J. Brouwer, *J. Chromatogr.*, 1979, **179**, 342.
[551] N. Ui, *Anal. Biochem.*, 1979, **97**, 65.
[552] T. Imamura, K. Konishi, M. Yokoyama, and K. Konishi, *J. Biochem. (Tokyo)*, 1979, **86**, 639.
[553] H. Wilgus and E. Stellwagen, *Anal. Biochem.*, 1979, **94**, 228.
[554] P. Lambin and J. M. Fine, *Anal. Biochem.*, 1979, **98**, 160.
[555] C. A. Saravis and N. Zamcheck, *J. Immunol. Methods*, 1979, **29**, 91.
[556] A. Rosén, K. Ek, and P. Åman, *J. Immunol. Methods*, 1979, **28**, 1.
[557] E. Gianazza, C. Astorri, and P. G. Righetti, *J. Chromatogr.*, 1979, **171**, 161.
[558] S. Pollack, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1252.
[559] F. P. Guengerich, *Biochim. Biophys. Acta*, 1979, **577**, 132.
[560] L. M. Hjelmeland, D. W. Nebert, and A. Chrambach, *Anal. Biochem.*, 1979, **95**, 201.
[561] J. B. Whitney, G. T. Copland, L. C. Skow, and E. S. Russel, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 867.

soybean trypsin inhibitor-Sepharose, was also described; the sample was loaded to a pre-run gel at a pH at which desorption occurred (and at which, presumably, the protease remained in its native state) and the gel re-focused.[562] Finally, a critical evaluation of amino-acid spacers in isotachophoresis was presented.[563]

Polyacrylamide Gel Electrophoresis in Detergents. Examination of protein mobilities in gels of different acrylamide concentration but containing 8 M-urea and SDS showed that the urea did not affect the relationship between retardation coefficient and molecular weight; anomalous mobilities were recognized as with non-urea-containing gels.[564] However, a study (at 25 °C) of SDS binding to proteins in 8 M-urea showed the maximum SDS bound to be 50% of that in the absence of urea; the SDS concentration at which this was achieved was double that required in the absence of urea. In addition, circular dichroism studies showed other physical parameters to be altered.[565] In light of this, the resistance in 8 M-urea of the *E. coli* outer membrane protein 0-8 to dissociate beyond the trimer should be noted.[566] Also, in the presence of 4 M-urea, the relative mobilities of collagen α-chains differed from those seen in the absence of urea; α_1(I) and α_1(III) were resolved in the former instance. This phenomenon was explained on the basis of hydrophobic amino-acid content.[567] Re-evaluation of the mobilities (previously regarded anomalous) of collagen peptides in SDS showed an interesting procedural error. As the peptides contain high amounts of glycine and proline, their mean residue weight is low. By plotting the log N (the number of residues) *versus* mobility a linear relationship with other proteins was apparent.[568] A charge-dependent alteration of mobility was seen with proteins modified at cysteine residues by reaction with iodoacetamide (neutral), iodoacetic acid (acidic), or ethyleneimine (basic).[569] This strengthened the claim that denaturation of proteins in SDS–urea solutions, especially by heating, can give a loss of resolution (by carbamylation of amino groups, particularly in 'old' urea solutions).[570] An SDS–polyacrylamide gel electrophoresis system utilizing low pH and low temperatures was described.[571] Low-temperature electrophoresis was also performed in lithium dodecyl sulphate; the emphasis here was on the maintenance of chlorophyll–protein complexes. The temperature of sample preparation and electrophoresis was critical to those seen.[572] A comparison of dodecyl and tetradecyl sulphate interactions with proteins showed the latter to be more effective at dissociating dimers of like polypeptide chains, particularly at high temperatures. The same paper re-emphasized the importance of sample preparation at 100 °C; glycophorin was 75% dimer at room temperature but only 32% at 100 °C in dodecyl sulphate, in tetradecyl sulphate the figures were 62% and 7%.[573] Albumin oligomers, produced

[562] H. Amnéus, L. Näslund D. Gabel, and V. Kasche, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 1313.
[563] N. Y. Nguyen and A. Chrambach, *Anal. Biochem.*, 1979, **94**, 202.
[564] R. P. Russel and S. H. P. Chan, *Biochim. Biophys. Acta*, 1979, **576**, 109.
[565] T. Takagi and K. Kubo, *Biochim. Biophys. Acta*, 1979, **578**, 68.
[566] F. Yu, S. Ichihara, and S. Mizushima, *FEBS Lett.*, 1979, **100**, 71.
[567] T. Hayashi and Y. Nagai, *J. Biochem. (Tokyo)*, 1979, **86**, 453.
[568] J. W. Freytag, M. E. Noelken, and B. G. Hudson, *Biochemistry*, 1979, **18**, 4761.
[569] J. Svasti and N. Talupphet, *Biochim. Biophys. Acta*, 1979, **577**, 221.
[570] N. L. Anderson and B. J. Hickman, *Anal. Biochem.*, 1979, **93**, 312.
[571] R. Lichtner and H. U. Wolf, *Biochem. J.*, 1979, **181**, 759.
[572] P. Delepelaire and N.-H. Chua, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 111.
[573] J. C. Dohnal and J. E. Garvin, *Biochim. Biophys. Acta*, 1979, **576**, 393.

by peroxidizing linoleic acid, had SDS binding isotherms indistinguishable from monomeric albumin and were regarded of value as molecular-weight markers for SDS gels.[574] Analysis of the behaviour of SDS in polyacrylamide gels showed micelles and monomers to be in rapid equilibrium. However the micelle was subject to sieving and, therefore, with high-percent acrylamide gels, the effective SDS concentration decreased unless it was replenished. This occurred when SDS was included in the cathode buffer but *does not* occur in modified procedures where SDS is omitted.[575] Two procedures for the extraction of proteins from fixed and Coomassie-stained polyacrylamide gels were also described.[576, 577] The periodate-cleavable cross-linker, diallyltartardiamide, was compared with methylene-bis-acrylamide; a five-fold increase in concentration was required for comparable resolution.[578] Two papers described improvements in procedures using cetyltrimethylammonium bromide (CTAB) rather than SDS for molecular-weight determination as problems with ammonium persulphate precipitation were overcome. The method was especially effective for some highly charged peptides and membrane proteins.[579, 580] Dodecyl sulphate was removed from proteins by precipitation with CTAB and also as its organic solvent-soluble complex formed with triethyl- and tributyl-ammonium salts.[581] Comments on the use of linear polyacrylamide-impregnated cellulose acetate for SDS-protein resolution[582] and on the incorporation of Triton X-100 into sample preparation solutions[583] also appeared.

Two-dimensional Procedures. A novel and time-saving method for generating polyacrylamide gradients in slab gels, involving mixing of two different densities by tilting, was described.[584] New procedures included the incorporation of a continuous flow from a column effluent or a reaction vessel into an agarose gel while the gel was being cast. This was then set over a polyacrylamide separation gel. With this procedure considerable horizontal broadening of bands was seen.[585] Proteins were also characterized by titration curves where isoelectric focusing was combined with electrophoresis in 8 M-urea,[586] and by electrophoresis into a urea gradient running across rather than down the gel; the sample was applied as a band along the top of the gel.[587] Much-needed computer-assisted analysis of two-dimensional gels was also described.[588] Improved resolution of membrane glycoproteins[589] and clarification of apparent differences in alkaline phosphatases[590] were achieved

[574] M. Janado, Y. Yano, H. Nakamori, and T. Nishida, *J. Biochem. (Tokyo)*, 1979, **86**, 177.
[575] K. Kubo, T. Isemura, and T. Takagi, *Anal. Biochem.*, 1979, **92**, 243.
[576] L. Djondjurov and H. Holtzer, *Anal. Biochem.*, 1979, **94**, 274.
[577] D. R. Gibson and R. W. Gracy, *Anal. Biochem.*, 1979, **96**, 352.
[578] P. J. Spath and H. Koblet, *Anal. Biochem.*, 1979, **93**, 275.
[579] M. H. Eley, P. C. Burns, C. C. Kannapell, and P. S. Campbell, *Anal. Biochem.*, 1979, **92**, 411.
[580] J. J. Basch and H. M. Farrell, *Biochim. Biophys. Acta*, 1979, **577**, 125.
[581] L. E. Henderson, S. Oroszlan, and W. Konigsberg, *Anal. Biochem.*, 1979, **93**, 153.
[582] H.-J. Bode, *Anal. Biochem.*, 1979, **92**, 99.
[583] Z.-I. Ogita and C. L. Markert, *Anal. Biochem.*, 1979, **99**, 233.
[584] R. Rüchel and J. Gross, *Anal. Biochem.*, 1979, **92**, 91.
[585] K. S. Bloom and J. N. Anderson, *Anal. Biochem.*, 1979, **98**, 410.
[586] P. G. Righetti, R. Krishnamoorthy, C. Lapoumeroulie, and D. Labie, *J. Chromatogr.*, 1979, **177**, 219.
[587] T. E. Creighton, *J. Mol. Biol.*, 1979, **129**, 235.
[588] M. Capel, B. Redman, and D. P. Bourque, *Anal. Biochem.*, 1979, **97**, 210.
[589] H. Baumann and D. Doyle, *J. Biol. Chem.*, 1979, **254**, 2542.
[590] P. E. Hass, H. G. Wada, M. M. Herman, and H. H. Sussman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1164.

after neuraminidase treatment of the samples. Other modified procedures described (see also last year's review) included separation of plasma proteins without protein denaturation,[591] the use of urea in the second-dimension gel for low molecular weight proteins,[592] a reversal of procedures with an initial SDS-PAGE separation being followed by focusing in an NP-40/urea-containing gel,[593] and combinations of acidic, basic, and SDS-containing gels for ribosomal proteins.[594] Finally, Coomassie straining of isoelectric focusing gels before second-dimension electrophoresis was claimed to give improved resolution of bands, possibly because ampholines were largely removed during the fixing, straining, and destaining procedures.[595]

Crossed Immunoelectrophoresis and other Affinity Electrophoretic Procedures. Crossed immunoelectrophoresis involves electrophoresis of antigens through agarose containing specific antisera, either with (two-dimensional) or without (rocket) an initial first-dimension separation. A linear relationship between the square of the height of the precipitin line (rocket) with amount of antigen was shown,[596] and polyethylene glycol 6000 was used to give improved antigen–antibody precipitation.[597] A modification to the two-dimensional procedure required only a single agarose gel, whereby filter paper impregnated with antibody was layered over the gel after first-dimension separation.[598] Two-dimensional procedures involving initial separation in isoelectric focusing[599] and SDS-polyacrylamide gels[600] were used to characterize specific bands. The latter required antibodies specific for SDS-denatured protein[601] and an appreciation of the effect of SDS on antigen–antibody interactions.[602] Two novel procedures were described. First, crossed rocket-line immunoelectrophoresis was used to identify rennin-binding proteins of plasma. Plasma mixed with rennin was electrophoresed through a slab containing wells of different specific antisera into a slab containing a polyvalent antiserum (a peak in the second slab indicated a positive identification).[603] Secondly, crossed immuno-affino electrophoresis was used to characterize α_1-fetoprotein variants. In this method the first-dimension separation was performed in different concentrations of lectins, thus affecting the mobility of variants (expressing different binding affinities) when the amount of lectin was limiting.[604] Combinations of crossed immuno-, affino-, and hydrophobic-

[591] T. Manabe, K. Tachi, K. Kojima, and T. Okuyama, *J. Biochem. (Tokyo)*, 1979, **85**, 649.
[592] M. R. Goldsmith, E. C. Rattner, M. M. D. Koehler, S. R. Balikov, and S. C. Bock, *Anal. Biochem.*, 1979, **99**, 33.
[593] G. P. Tuszynski, C. A. Buck, and L. Warren, *Anal. Biochem.*, 1979, **93**, 329.
[594] J.-J. Madjar, S. Michel, A. J. Cozzone, and J.-P. Reboud, *Anal. Biochem.*, 1979, **92**, 174.
[595] H. Jäckle, *Anal. Biochem.*, 1979, **98**, 81.
[596] U. W. Mueller and J. M. Potter, *Anal. Biochem.*, 1979, **100**, 51.
[597] B. Teisner, J. Folkersen, P. Hindersson, J. C. Jensenuis, and J. G. Weatergaard, *Scandinavian J. Immunol.*, 1979, **9**, 409.
[598] N. Kuusi, *J. Immunol. Methods*, 1979, **31**, 361.
[599] J. Daussant and A. W. MacGregor, *Anal. Biochem.*, 1979, **93**, 261.
[600] N.-H. Chua and F. Blomberg, *J. Biol. Chem.*, 1979, **254**, 215.
[601] A.-M. Lompre, P. Bouveret, J. Leger, and K. Schwartz, *J. Immunol. Methods*, 1979, **28**, 143.
[602] G. J. Dimitriadis, *Anal. Biochem.*, 1979, **98**, 445.
[603] K. Poulsen, J. Krøll, A. Højj Nielsen, J. Jensenius, and C. Malling, *Biochim. Biophys. Acta*, 1979, **577**, 1.
[604] J.-P. Kerckaert, B. Bayard, and G. Biserte, *Biochim. Biophys. Acta*, 1979, **576**, 99.

interaction and charge-shift electrophoresis were used in the analysis of dopamine-β-hydroxylases[605] and of platelet membrane proteins.[606]

Detection of Proteins in Gels. New and potentially powerful procedures for the detection of specific proteins or classes of proteins in polyacrylamide gels were described. Analogous to the 'blotting' procedures used for nucleic acids, proteins were transferred either to nitrocellulose[607] or diazobenzyloxymethyl paper,[608] or to a cellulose filter to which an affinity ligand (lectin, antibody, *etc.*) had been coupled.[609] The former procedures were facilitated when cleavable cross-linkers were used, and required application, subsequent to transfer, of a specific probe. All procedures could be made highly sensitive either by using radiolabelled material for the initial separation or by utilizing radiolabel- or enzyme- (*e.g.* peroxidase) conjugated probes. With peroxidase-conjugated antibodies as little as 100 pg of protein were detected.[607] A somewhat complex, but highly sensitive (100 × Coomassie), silver staining procedure, based on de Olmo's neural cupric–silver stain, was described,[610, 611] as was a tannic acid staining procedure; the latter permits the recovery of active protein from gels.[612] Three commonly used dyes—Amino black, Coomassie blue, and Fast green—were compared as protein stains; particular emphasis was given to reproduction on black and white photographs.[613] The periodic acid–Schiff procedure for glycoproteins was modified to circumvent problems associated with the slow oxidation of *O*-acetylated sialic acid.[614] Glycoproteins were also detected using fluorescent[615, 616] and radiolabelled[617] lectins, by a thymol–sulphuric acid procedure (with a claimed sensitivity comparable to Coomassie staining),[618] and by a novel procedure involving transfer of ^{14}C-galactose to terminal *N*-acetylglucosamine residues.[619] Both dansylation[620] and reaction with 2-methoxy-2,4-diphenyl-3-(2*H*)-furanone[621] were used as fluorescent stains for proteins in gels. Radiolabel-staining procedures included reductive methylation[622, 623] and solid-phase lactoperoxidase-catalysed iodination.[624] Sodium salicylate, which is water soluble and inexpensive, was found in fluorographic procedures to give a sensitivity comparable to that obtained with diphenyloxazole.[625] The potential hazards of

[605] O. J. Bjerrum, K. B. Helle, and E. Bock, *Biochem. J.*, 1979, **181**, 231.
[606] I. Hagen, O. J. Bjerrum, and N. O. Solum, *Eur. J. Biochem.*, 1979, **98**, 9.
[607] H. Towbin, T. Staehelin, and J. Gordon, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4350.
[608] J. Renart, J. Reiser, and G. R. Stark, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3116.
[609] H. A. Erlich, J. R. Levinson, S. N. Cohen, and H. O. McDevitt, *J. Biol. Chem.*, 1979, **254**, 12240.
[610] R. C. Switzer, C. R. Merril, and S. Schifrin, *Anal. Biochem.*, 1979, **98**, 231.
[611] C. R. Merril, R. C. Switzer, and M. L. van Keuren, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4335.
[612] K. Aoki, S. Kajiwara, R. Shinke, and H. Nishira, *Anal. Biochem.*, 1979, **95**, 575.
[613] C. M. Wilson, *Anal. Biochem.*, 1979, **96**, 263.
[614] A. H. Sarris and G. E. Palade, *J. Biol. Chem.*, 1979, **254**, 6724.
[615] J. D. Lane, H.-G. Zimmer, and V. Neuhoff, *Z. Physiol. Chem.*, 1979, **360**, 1405.
[616] M. Furlan, B. A. Perret, and E. A. Beck, *Anal. Biochem.*, 1979, **96**, 208.
[617] L. J. McIntyre, R. H. Quarles, and R. O. Brady, *Biochem. J.*, 1979, **183**, 205.
[618] D. Racusen, *Anal. Biochem.*, 1979, **99**, 474.
[619] B. Wallenfels, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3223.
[620] P. Tijssen and E. Kurstak, *Anal. Biochem.*, 1979, **99**, 97.
[621] S. Chen-Kiang, S. Stein, and S. Udenfriend, *Anal. Biochem.*, 1979, **95**, 122.
[622] R. L. Wilder, C. C. Yuen, B. Subbarao, V. L. Woods, C. B. Alexander, and R. G. Mage, *J. Immunol. Methods*, 1979, **28**, 255.
[623] R. Kumarasamy and R. H. Symons, *Anal. Biochem.*, 1979, **95**, 359.
[624] R. G. Parsons and P. Kowal, *Anal. Biochem.*, 1979, **95**, 568.
[625] J. P. Chamberlain, *Anal. Biochem.*, 1979, **98**, 132.

both methods were discussed. Procedures for the differentiation of ^{3}H and ^{14}C[626, 627] and for differentiation of any two of ^{3}H, ^{14}C, ^{35}S, ^{32}P, and ^{125}I in double-labelled gels were described. The latter required colour-negative film and was relatively insensitive.[628]

Immunological Characterization of Proteins.—Antisera were used in two ways—in identification of cross-reacting proteins and evaluation of evolutionary relationships and in the identification of precursor proteins. Problems associated with the former were discussed in an immunological characterization of lysozyme.[629] Identifications made using the latter procedure were frequently verified by peptide mapping. Such verification was required as control procedures, such as lack of precipitation by pre-immune sera, competition with specific antigen,[630] or lack of precipitation in antigen-deficient preparations,[631] were rarely carried out. Table 11 summarizes some immunological characterizations reported during the year.

[626] E. H. McConkey, B. J. Taylor, and D. Phan, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6500.
[627] E. H. McConkey, *Anal. Biochem.*, 1979, **96**, 39.
[628] L. H. Kronenberg, *Anal. Biochem.*, 1979, **93**, 189.
[629] I. M. Ibrahimi, E. M. Prager, T. J. White, and A. C. Wilson, *Biochemistry*, 1979, **18**, 2736.
[630] J. G. Conboy, F. Kalousek, and L. E. Rosenberg, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5724.
[631] F. J. Bollum and M. Brown, *Nature, (London)*, 1979, **278**, 191.

Table 11 *Immunological characterization—antigenically-related proteins*

Protein/Source	*Comments*	*Ref.*
Torpedo californica and *Electrophorus electricus* acetylcholine receptors	^{125}I-labelled proteins, antisera to native and denatured, slight cross-reaction of native α, β, and γ chains, especially α, no cross-reaction of denatured proteins	632
Human alkaline phosphatase isoenzymes	two immunological groups, bone, kidney, and liver or intestine and placenta	633
Limulus sperm 95K protein and muscle α-actinin	no cross-reaction	634
Mouse C4 and slp (sex-limited protein)	antigenically distinct precursors shown by sequential precipitation	635
Intestinal and pancreatic glucagon-like peptides	detected antigenically	636
Various seed lectins	cross-reaction, not due to carbohydrate, ?common function	637
Avian sarcoma virus pp60^{src} protein	normal chicken, quail, rat, and human cells contain cross-reacting protein therefore gene 32 causes modification of normal protein?	638
Stratum corneum basic protein of newborn rat epidermis	cross-reacts with 52K protein of less differentiated cells	639
Alkaline phosphatases of mouse teratoma stem cells	immunologically cross-reactive with enzymes from various tissues	590
Precursor proteins		
Drosophila chorion proteins	cell-free translation, precursor A_1 A_2 B_1 B_2, anti-B antisera showed further functionally unrelated precursor protein	640
Bovine hypothalamus ACTH and β-endorphin	pituitary-derived 31K precursor	641
Camel pituitary pro-opiocortin	retained by anti-α-corticotropin, precursor of opinoids and corticotropin	233
Human placenta opinoid precursor	antisera against ACTH(1—24) gives molecule with ACTH, LPH, α-LPH, and β-endorphin antigens, 48 000 and 36 000 dalton polypeptides	642

[632] J. Lindstrom, B. Walter (Nave), and B. Einarson, *Biochemistry*, 1979, **18**, 4470.
[633] M. J. McKenna, T. A. Hamilton, and H. H. Sussman, *Biochem. J.*, 1979, **181**, 67.
[634] I. Mabuchi, *J. Biochem. (Tokyo)*, 1979, **86**, 1785.
[635] K. L. Parker, M. H. Roos, and D. C. Schreffler, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5853.
[636] H. S. Tagar and J. Markese, *J. Biol. Chem.*, 1979, **254**, 2229.
[637] J. Howard, J. Kindinger, and L. M. Shannon, *Arch. Biochem. Biophys.*, 1979, **192**, 457.
[638] H. Oppermann, A. D. Levinson, H. E. Varmus, L. Levintow, and J. M. Bishop, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1804.
[639] B. A. Dale and S. Y. Ling, *Biochemistry*, 1979, **18**, 3539.
[640] G. Thireos, R. G. Shea, and F. C. Kafatos, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6279.
[641] A. S. Liotta, D. Gildersleeve, M. J. Brownstein, and D. T. Kreiger, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1448.
[642] E. Odagiri, B. J. Sherrell, C. D. Mount, W. E. Nicholson, and D. N. Orth, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2027.

Toad pituitary opinoid precursor	anti-ACTH and anti-β-endorphin gave 32 K and 29.5 K polypeptides with both sequences; pulse chase gave corticotropin-reactive 23 K, 21 K, 13 K, 4.3 K; endorphin-reactive 11.7 K and 3.5 K	643
Mouse submaxillary glands, epidermal growth factor	9K precursor	644
Horse F_1-ATPase subunits	extramitochondrial precursor, processed and imported into mitochondria	645
Bovine hypothalamus neurophysins	25K oxytocin plus vasopressin precursor	646
Mouse hypothalamus vasopressin	immuno-reactive high mol. wt. form	647
Mouse hypothalamus neurophysin, somatostatin	immuno-reactive high mol. wt. form, protease inhibitors affect yield	648
Mouse hypothalamus somatostatin	high mol. wt. immunoreactive forms 15K, 10K, 6K, and 1.6K	649
Rat ornithine transcarbamoylase	cell-free translate immunoreactive 43K, mature enzyme 39K	630
Human and primate parotid proline-rich proteins	immunochemical techniques showed common precursor molecule	650
Human lymphoblastoid terminal deoxynucleotidyl transferase	high mol. wt. form, 58K; calf thymus 32K (24K + 8K)	631
Avian very low density lipoproteins	all derived from single precursor, plasma plus protease inhibitors gave single band, serum gave multiple bands, largest 350 000 dalton	651

[643] Y. P. Loh, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 796.
[644] P. Frey, R. Forand, T. Maciag, and E. M. Shooter, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6294.
[645] M.-L. Maccecchini, Y. Rudin, G. Blobel, and G. Schatz, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 343.
[646] L. C. Giudice and I. M. Chaiken, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3800.
[647] M. Camier, M. Lauber, J. Möhring, P. Cohen, and C. Fahy, *FEBS Lett.*, 1979, **108**, 369.
[648] M. Lauber, M. Camier, and P. Cohen, *FEBS Lett.*, 1979, **97**, 343.
[649] M. Lauber, M. Camier, and P. Cohen, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6004.
[650] E. E. Kousvelari and F. G. Oppenheim, *Biochim. Biophys. Acta*, 1979, **578**, 76.
[651] D. L. Williams, *Biochemistry*, 1979, **18**, 1056.

Peptide Mapping.—*Thin-layer Chromatography.* A 'Laboratory Handbook of Paper and Thin-Layer Chromatography' contained comprehensive sections on detection methods, but little on peptide separation procedures.[652] Two innovations made during the year were the use of reductive methylation to radiolabel peptides, with little affect on peptide mobility,[653] and the addition of 2,5-diphenyloxazole to chromatography buffers used with cellulose thin-layer mapping techniques, thus allowing immediate application of fluorographic detection procedures.[654] A comparison of digests of radiolabelled proteins, prepared either by filtration in guanidine or by electrophoresis in SDS, showed minor differences, particularly with respect to intensities of spots.[655]

Liquid Chromatography. H.p.l.c. and peptide analyser mapping of peptides are now widespread. Some recent applications are given in Table 12a.

Gel Electrophoresis. There has been an explosion in mapping of partial digests of proteins on gels; the procedure was first reported in Volume 10 of this series. Some applications are listed in Table 12b.

[652] J. Gasparič and J. Churácek, 'Laboratory Handbook of Paper and Thin-Layer Chromatography', Ellis Horwood, Chichester, 1978.
[653] L. P. Nelles and J. R. Bamburg, *Anal. Biochem.*, 1979, **94**, 150.
[654] S. E. Zweig and S. J. Singer, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1147.
[655] M. L. Bryant, R. P. Nalewaik, V. L. Tibbs, and G. J. Todaro, *Anal. Biochem.*, 1979, **96**, 84.

Table 12 *Peptide mapping procedures*

Protein	*Method and comments*	*Ref.*
(*a*) *Liquid chromatography*		
	peptide analyser, Aminex resin, pH elution in pyridine acetate buffers	656
	h.p.l.c., reverse phase, tryptic digests at sub-nanomole level, fluorescamine	657
	h.p.l.c., reverse phase, phosphoric acid/acetonitrile, 4—30 residues, 1 h recycle	658
Mouse C4, slp	peptide analyser of tryptic maps of α- and β-chains different	635
Bovine fibrinopeptides	h.p.l.c., reverse phase, ammonium phosphate/acetonitrile, 10^{-10} mol detection	659
	h.p.l.c., reverse phase, low pH and high buffer molarity increases reproducibility	228
	h.p.l.c., reverse phase, acetonitrile–ammonium acetate elution of CNBr peptides	230
Ia antigens	h.p.l.c., nitrile column, acetone-gradient elution, ^{3}H-labelled peptides	660
Camel pro-opiocortin	h.p.l.c., reverse phase, pyridine–formic–propanol gradient, tryptic peptides	233

656 G. F. Rohrmann, T. J. Bailey, B. Brimhall, R. R. Becker, and G. S. Beaudreau, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4976.
657 M. Rubinstein, S. Chen-Kiang, S. Stein, and S. Udenfriend, *Anal. Biochem.*, 1979, **95**, 117.
658 C. S. Fullmer and R. H. Wasserman, *J. Biol. Chem.*, 1979, **254**, 7208.
659 R. A. Martinelli and H. A. Scheraga, *Anal. Biochem.*, 1979, **96**, 246.
660 M. McMillan, J. M. Cecka, L. Hood, D. B. Murphy, and H. O. McDevitt, *Nature (London)*, 1979, **227**, 663.

Proteins	*Digestion method*	*Comments*	Ref.
(*b*) *Gel electrophoresis*			
	proteolytic	two-dimensional, whole gel strip re-applied, overlayered with protease, compare bands from first dimension to check for proteolysis	661
	proteolytic; V8	stop electrophoresis with protease in stacking gel, partial digestion then re-apply voltage	662
	proteolytic	as above, electrophoresis of protease into stack, electrophoresis stopped for 30 min then continued	663
	CNBr	relevant slice treated then loaded onto second gel, optimum digestion conditions described	664
Chicken, porcine, and mouse brain σ-proteins	chymotryptic	proteins compared, digestion in solution or in gel	665
Alkaline phosphatase, mouse stem cells	V8 protease	^{32}P-labelled protein digested, differences in electrophoretic variants due to sialic acid	590
Alkaline phosphatase, human tissue isoenzymes	proteolytic	liver and kidney identical, placenta, and intestine different	633
Limulus sperm 95K protein and muscle α-actinin	V8, papin	proteins different	634
Mouse Ss and C4 proteins	V8 protease	electrophoresis into stack, stop, warm to 37 °C to digest, then re-apply current	666
Rat liver microsomal cytochrome P-450s	V8, chymotrypsin, papain	all cytochromes different	667
Maize chloroplast and type II Nuclear DNA-dependent RNA polymerases	proteolytic	proteins compared	668
Desmin from muscle and non-muscle cells	V8 protease	proteins compared	669

[661] C. Bordier and A. Crettol-Järvinen, *J. Biol. Chem.*, 1979, **254**, 2565.
[662] A. Barrieux and M. G. Rosenfeld, *J. Biol. Chem.*, 1979, **254**, 8087.
[663] N. M. Nathanson and Z. W. Hall, *Biochemistry*, 1979, **18**, 3392.
[664] V. Nikodem and J. R. Fresco, *Anal. Biochem.*, 1979, **97**, 382.
[665] D. W. Cleveland, B. M. Spiegelman, and M. W. Kirschner, *J. Biol. Chem.*, 1979, **254**, 12670.
[666] M. C. Carroll and J. D. Capra, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4641.
[667] E. F. Johnson, M. C. Zounes, and U. Müller-Eberhard, *Arch. Biochem. Biophys.*, 1979, **192**, 282.
[668] G. H. Kidd and L. Bogorad, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4890.
[669] D. L. Gard, P. B. Bell, and E. Lazarides, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3894.

Table 12 (*cont.*)

Proteins	*Digestion method*	*Comments*	*Ref.*
F_1-ATPase subunits, yeast	V8 protease	fingerprint of subunits, extra-mitochondrially synthesized, processed, imported into mitochondria	645
Silk fibroin	V8 protease	mRNA translate analysed, SDS first dimension, V8 then second dimension to identify 'pause' sites	670
Ligandin and glutathione-*S*-transferase B, rat liver	chymotrypsin	both lithocholic acid-binding proteins, different	671
Myosin isoenzymes	V8, chymotrypsin	embryonic form of heavy chain detected, makes point that digestion of native protein could be affected by protein–protein interactions therefore denature	672
Myosin isoenzymes, *Acanthamoeba castehlanii*	V8 protease, CNBr, cyanylation	1C isoenzyme is 1A plus 20000 dalton piece, 1B is a true isoenzyme	673
Chicken adult, embryonic, and dystrophic *myosins*	V8 protease, papain	papain activated with 2-mercaptoethanol before use	674
3 Major nuclear envelope proteins (78K, 74K, 68K) src gene product of avian sarcoma virus $pp60^{src}$	chymotrypsin, hydroxylamine	78K and 68K showed homology	675
	V8 protease	not seen in *Drosophila*, seen in normal frog, chicken, rat tumour cells	676
Prolyl hydroxylase subunits	V8, chymotrypsin	partial digestion in gel stack	677
Spectrin-binding protein	V8 protease	slice equilibrated and digested in well, search for precursors	678

[670] P. M. Lizardi, V. Mahdavi, D. Shields, and G. Candelas, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6211.
[671] J. D. Hayes, R. C. Strange, and I. W. Percy-Robb, *Biochem. J.*, 1979, **181**, 699.
[672] R. G. Whalen, K. Schwartz, P. Bouveret, S. M. Sell, and F. Cros, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5197.
[673] H. Gadasi, H. Maruta, J. H. Collins, and E. D. Korn, *J. Biol. Chem.*, 1979, **254**, 3631.
[674] J. I. Rushbrook and A Stracher, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4331.
[675] K. S. Lam and C. B. Kasper, *J. Biol. Chem.*, 1979, **254**, 11713.
[676] L. R. Rohrschneider, R. N. Eisenman, and C. R. Leitch, *Proc. Natl. Acad. Sci USA*, 1979, **76**, 4479.
[677] R. A. Berg, N. L. Kedersha, and N. A. Guzman, *J. Biol. Chem.*, 1979, **254**, 3111.
[678] E. J. Luna, G. H. Kidd, and D. Branton, *J. Biol. Chem.*, 1979, **254**, 2526.

Miscellaneous Structural Characterization.—An h.p.l.c. procedure for the simultaneous separation and detection of amino-acids, amino-sugars, and neutral sugars (18 amino-acids and 19 sugars separated in 390 min) was described.[679] Glycopeptides were also analysed in polyacrylamide gels by use of tris–borate buffer; neutral sugars give charged borate complexes.[680] In addition, structural requirements for *N*-glycosidic bonds in glycoproteins were discussed.[681] Papers reporting amino-acid composition determination by h.p.l.c. (specifically, improved thiol determination),[682,683] single-column resolution of imino-acids, amino-sugars, and amino-acids following hydrolysis of collagen-like proteins in 3 molar methane-sulphonic acid,[684] and a re-discovery of composition determination using [^{3}H]-dansyl chloride and [^{14}C]-amino-acid standards[685] have appeared. 3-Diazonium-1,2,4-triazole (3-DT) was described as being uniquely reactive at acid pH with indoles, and [^{14}C]-3-DT used to quantify tryptophan in a number of proteins.[686] Examination of the effective hydrophobicity of proteins using both partition on charge-free hydrophobic gels and partition in two-phase systems (the methods gave close correlation) showed no significant relationship between the average hydrophobicity calculated from amino-acid composition and the effective hydrophobicity found experimentally.[687] Comparison of homologous sequences of enzymes from thermophiles and mesophiles showed that residues giving increased helix stability and hydrophobicity were favoured in thermophiles; common replacements for Gly, Ser, Lys, and Asp are Ala, Ala or Thr, Arg, and Glu.[688] Finally, two proteins were reported to contain covalently bound lipid, binding possibly being mediated by an ester linkage either to a protein hydroxyl group (Ser, Thr, or Tyr)[689] or to a thiol group.[690]

PART IB: Primary Structures *by J. Gagnon and D. L. Christie*

1 Chain Cleavage

Chemical Cleavages.—Cleavage of methionyl bonds with CNBr is commonly employed for the fragmentation of proteins. Certain bonds such as methionyl–seryl and methionyl–threonyl are sometimes resistant to cleavage. Human serum albumin and bovine α-lactalbumin both contain a methionyl–cystinyl bond and it has recently been shown that treatment of these non-reduced proteins with CNBr results in the conversion of methionine to homoserine without chain cleavage.[1] A

[679] M. M. Tikhomirov, A. Y. Khorlin, W. Voelter, and H. Bauer, *J. Chromatogr.*, 1978, **167**, 197.
[680] S. Weitzman, V. Scott, and K. Keegstra, *Anal. Biochem.*, 1979, **97**, 438.
[681] G. W. Hart, K. Brew, G. A. Grant, R. A. Bradshaw, and W. J. Lennarz, *J. Biol. Chem.*, 1979, **254**, 9747.
[682] K. S. Lee and D. G. Drescher, *J. Biol. Chem.*, 1979, **254**, 6248.
[683] J. R. Cronin, S. Pizzarello, and W. E. Gandy, *Anal. Biochem.*, 1979, **93**, 174.
[684] J.-P. Vergnes and I. L. Freeman, *Anal. Biochem.*, 1979, **99**, 427.
[685] J. Airhart, J. Kelley, J. E. Brayden, R. B. Low, and W. S. Stirewalt, *Anal. Biochem.*, 1979, **96**, 45.
[686] M. C. De Traglia, J. S. Brand, and A. M. Tometsko, *Anal. Biochem.*, 1979, **99**, 464.
[687] E. Keshavarz and S. Nakai, *Biochim. Biophys. Acta*, 1979, **576**, 269.
[688] P. Argos, M. G. Rossmann, U. M. Grau, H. Zuber, G. Frank, and J. D. Tratshin, *Biochemistry*, 1979, **18**, 5698.
[689] M. F. G. Schmidt, M. Bracha, and M. J. Schlesinger, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1687.
[690] T. Mizuno and M. Kageyama, *J. Biochem. (Tokyo)*, 1979, **85**, 115.
[1] N. Doyen and C. Lapresle, *Biochem. J.*, 1979, **177**, 251.

mechanism is postulated in which there is intramolecular interaction between the sulphur atom of the cystine and the iminolactone formed during treatment with CNBr, giving a bicyclic structure. Opening of the first ring gives rise to homoserine, while opening of the second ring results in the formation of a thioester or peptide linkage, in both cases leaving the polypeptide chain uncleaved.

Cleavage of aspartyl–prolyl bonds of proteins with dilute acid risks the potential oxidation of sensitive amino-acids, particularly methionine. When this procedure was used during sequence determination of the histocompatibility antigen HLA-B7, conditions were modified to overcome this problem.[2] The digest was carried out in formic acid (70%) containing β-mercaptoethanol (0.01%) and nitrogen was bubbled through the solution. This procedure prevented oxidation of methionine residues, permitting further cleavage with CNBr.

A new, simple procedure for the selective cleavage of tryptophanyl peptide bonds has been reported.[3] Treatment of *S*-alkylated proteins with *o*-iodosobenzoic acid gave cleavage in yields of 90—95%, except when the tryptophan residue preceded valine or isoleucine, in which case yields dropped to 65—70%. The yields were shown to be greater than those obtained with the BNPS–skatole reagent. The procedure did however result in the oxidation of free cysteine and some methionine was converted to the sulphoxide. A modification of this procedure has been used in sequence studies of HLA-B7.[4] In this case, *o*-iodosobenzoic acid was pretreated with *p*-cresol which acts as a free-radical scavenger, thus protecting residues such as tyrosine from iodination.

An unwanted side-reaction of many chemical cleavage procedures is the oxidation of susceptible amino-acids. A systematic study of the reaction conditions for the reduction of methionine sulphoxide and the analogous oxidation product of *S*-carboxymethylcysteine has been carried out.[5] *N*-Methylmercaptoacetamide was found to be the reagent of choice and its effectiveness was relatively independent of pH. Complete reduction of methionine sulphoxide occurred in 12—24 h, while the sulphoxide form of *S*-carboxymethylcysteine took approximately five times longer.

Enzymatic Cleavages.—New enzymes are being added to the list of those available for the preparation of fragments suitable for sequence analysis at an ever increasing rate. Clostripain, which has a specificity for bonds *C*-terminal to arginine residues, has been used for both the limited proteolysis of immunoglobulin-like HLA antigen[6] and for complete digestion of H5 Histone from goose erythrocytes.[7] A thombin-like enzyme from *Agkistrodon contortrix* (pit viper) venom has been used for the limited cleavage of human fibrin.[8] Two other proteases of unusual specificity have also proved useful. *Armillaria mellea* proteinase which cleaves on the amino-side of lysine residues was used in the sequence study of chymosin.[9] Another fungal

[2] J. A. Lopez de Castro, H. T. Orr, R. J. Robb, T. G. Kostyk, D. L. Mann, and J. L. Strominger, *Biochemistry*, 1979, **18**, 5704.
[3] W. C. Mahoney and M. A. Hermodson, *Biochemistry*, 1979, **18**, 3810.
[4] H. T. Orr, J. A. Lopez de Castro, D. Lancet, and J. L. Strominger, *Biochemistry*, 1979, **18**, 5711.
[5] R. A. Houghten and C. H. Li, *Anal. Biochem.*, 1979, **98**, 36.
[6] L. Tragardh, L. Rask, K. Wiman, J. Fohlman, and P. A. Petersen, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5839.
[7] M. Yaguchi, C. Roy, and V. L. Seligy, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 1400.
[8] A. Henschen, F. Lottspeich, and B. Hessel, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1951.
[9] B. Foltmann, V. B. Pedersen, D. Kauffman, and G. Wybrandt, *J. Biol. Chem.*, 1979, **254**, 8447.

spore protease with a specificity for glutamyl isoleucyl bonds was used in a sequence study of Protein A from spores of *Bacillus megaterium*.[10]

Enzymatic hydrolysis of polypeptides with dipeptidyl aminopeptidases has never been widely used for sequence determination. However, in combination with the relatively new gas chromatography–mass spectrometric techniques enabling easy determination of dipeptides, it has become a more interesting procedure. Recently the preparation of dipeptidyl aminopeptidase IV from swine kidney has been described.[11] This enzyme releases X-Pro from the *N*-terminus of polypeptides and is suitable for use in sequence analysis, complementing the specificity of dipeptidyl aminopeptidases available previously.

2 Separation of Peptides

Reversed-phase High-pressure Liquid Chromatography.—Enzymatic or chemical cleavage of proteins and peptides inevitably results in difficulties in separating progressively smaller quantities of material. Traditionally this has involved gel filtration, ion exchange, and paper electrophoresis. The technique of high-pressure liquid chromatography (h.p.l.c.) on reversed-phase columns has developed to a stage where it is highly suited to this task. It has the advantage that results obtained with 1 nmole of material are virtually identical to those obtained with 100 nmole, enabling preparative separations to be worked out easily. This technique was used to isolate three peptides from 53 nmole of a CNBr digest of the α-chain of human haemoglobin for sequence analysis in yields of 60—100%.[12]

Some of the theoretical aspects and practical considerations of this type of chromatography are discussed in two reviews.[13, 14] The column packings employed for reversed-phase h.p.l.c. are silica-based, chemically bonded with alkyl side-chains. The two common derivatives are octadecylsilanyl (C_{18}) or cyanopropyl-silanyl (CN). The high resolution obtained with these columns is due to the particle size of 10 μm or smaller. Separations are dependent on the relative hydrophobicity of peptides. Thus, larger peptides generally have longer retention times although the addition of a few hydrophilic residues decreases the retention time, despite increased molecular weight.[15] Protonation of carboxy-groups at low pH increases hydrophobicity. It is also possible with the use of hydrophilic or hydrophobic ion-pairing reagents, at the appropriate pH, to increase or decrease retention times.[13] Thus, unique chromatographic molecular species can be generated, enabling the separation of complex mixtures.

Table 1 presents some recent applications of reversed-phase h.p.l.c. It is important to appreciate that these separations were generally complete within 1 h. As can be seen, a wide variety of buffer systems have been employed. In all cases, elution is dependent on the presence of organic solvent, commonly acetonitrile or n-propanol. Both mobile phases of constant composition (isocratic) and gradients of increasing organic-solvent concentration have proved useful. Ideally, the mobile

[10] P. Setlow and J. Ozols, *J. Biol. Chem.*, 1979, **254**, 11938.
[11] H. C. Krutzsch and J. J. Pisano, *Biochim. Biophys. Acta*, 1979, **576**, 280.
[12] G. J. Hughes, K. H. Winterhalter, and K. J. Wilson, *FEBS Lett.*, 1979, **108**, 81.
[13] M. T. W. Hearn and W. S. Hancock, *Trends Biochem. Sci.*, 1979, **4**, 58.
[14] M. Rubinstein, *Anal. Biochem.*, 1979, **98**, 1.
[15] M. Rubinstein, S. Chen-Kiang, S. Stein, and S. Udenfriend, *Anal. Biochem.*, 1979, **95**, 117.

Table 1 *Peptide separation by h.p.l.c.*

Application	*Column*	*Buffer system*	*Ref.*
Tryptic peptides of α-chain of goldfish and human haemoglobin, β-chain of goldfish haemoglobin, and CNBr-peptides of α-chain of human haemoglobin	LiChrosorb RP 18	Multi-step gradient of pyridine formate (0.5 M, pH = 3.0) or pyridine acetate (1.0 M, pH = 5.5) and pyridine acetate (1.0 M, pH = 5.5)–propan-1-ol (4 : 6 v/v)	*a*
Tryptic mapping of ovalbumin, rat and camel β-endorphin, cross-reacting protein, and small subunit of prolyl hydroxylase. Opioid peptides	LiChrosorb RP 18	Linear gradient of n-propanol (0—20%) in pyridine (1.0 M)—acetic acid (0.5 M)	*b, c*
Peptide mapping of γ-chain of human foetal haemoglobin. Separation of $^{A}\gamma$ and $^{G}\gamma$ CNBr peptides	μ Bondapak C_{18}	Linear gradient of acetonitrile (10—25%) in ammonium acetate (0.01 M, pH = 6.0)	*d, e*
Bovine fibrinopeptides *A* and *B*	μ Bondapak C_{18}	Isocratic elution with ammonium phosphate (0.083 M, pH = 3)—acetonitrile (81.6 : 18.4)	*f*
Peptide mapping of murine I*a* antigens from I—E region	Zorbax-CN	Multi-step gradient of acetonitrile in sodium phosphate (0.1 M, pH = 2.1)	*g*
Bovine vasoactive intestinal peptide and gastrin releasing peptide	μ Bondapak C_{18}	Isocratic elution with ammonium acetate (5 mM, pH = 4.55)–ethanol (65 : 35)	*h, i*
Pancreatic somatostatin	μ Bondapak C_{18} μ Bondapak CN	Isocratic elution with phosphoric acid (0.083 M), adjusted to pH = 2.5 or 3 with triethylamine: acetonitrile (75 : 25)	*j*
Hydrophobic peptides from bacterio-rhodopsin	μ Bondapak C_{18}	Linear gradient (40—75%) of formic acid (5%) in water and formic acid (5%) in ethanol	*k*

(*a*) G. J. Hughes, K. H. Winterhalter, and K. J. Wilson, *FEBS Lett.*, 1979, **108**, 81. (*b*) M. Rubinstein, S. Chen-Kiang, S. Stein, and S. Udenfriend, *Anal. Biochem.*, 1979, **95**, 117. (*c*) R. V. Lewis, S. Stein, and S. Udenfriend, *Int. J. Pept. Protein Res.*, 1979, **13**, 493. (*d*) G. D. Effremov, J. B. Wilson, and T. H. J. Huisman, *Biochim. Biophys. Acta*, 1979, **579**, 421. (*e*) T. A. Stoming, F. A. Garver, M. A. Gangarosa, J.

phase should be non-absorbent in the u.v. down to 200 nm to facilitate the detection of peptides. Non-volatile buffers can be used and the purified components subsequently desalted by h.p.l.c., using a volatile buffer.[16] Greater flexibility in the choice of mobile phase is available if a fluorescent detection system is used. Fluorescamine was preferred to *o*-phthaldialdehyde as peptides must contain lysine residues to be detected by the latter reagent.[12]

High-pressure Gel-permeation Chromatography.—Silica-based aqueous gels of the TSK SW type enabled high-speed gel-filtration to be carried out in 6M-guanidine hydrochloride, providing a convenient means of estimating molecular weight.[17] Proteins of molecular weight less than 70 000 dalton and those with molecular weights of up to 160 000 dalton were fractionated on G 3000 SW and G 4000 SW columns, respectively. The resolution was superior to that obtained with soft gels. A possible disadvantage is that only relatively small volumes can be applied to the columns (*ca.* 0.2 ml) although the technique is of obvious value where the supply of material is a limiting factor. In another report, a linear separation of substances in the molecular-weight range 5000—700 000 dalton was achieved on Synchropak GPC.[18] It was evident that this separation reflected in part liquid–liquid partition. Pituitary extracts, tumour-associated foetal antigens, and oestrogen receptors were chromatographed on this column, proving its suitability for the separation of biological samples.

Peptide Mapping.—A new method of preparative peptide mapping was developed during the course of primary structural analysis of rabbit α-lactalbumin.[19] Solvents contained 2,5-diphenyloxazole (PPO) at a concentration of $5\,g\,l^{-1}$ and peptides were visualized by observing the plates under u.v. light at 254 nm. Each peptide had a characteristic colour. PPO had no effect on the mobility of peptides, their extraction from the plates, nor subsequent amino-acid or sequence analysis. Chromatograms could still be developed with ninhydrin and this showed the PPO procedure to be accurate as no additional peptides could be detected.

3 Manual Sequencing

The decrease in the average yield per cycle is one of the limitations of sequence determination by the Edman procedure. The extractive loss of peptide as well as the efficiency of extracting the cleaved thiazolinones are both parameters which affect manual sequencing. A study of various solvents, not generally used in sequence determination, has been carried out in an attempt to improve this procedure.[20]

[16] J. Spiess, J. E. Rivier, J. A. Rodkey, C. D. Bennett, and W. Vale, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2974.
[17] N. Ui, *Anal. Biochem.*, 1979, **97**, 65.
[18] K. A. Gruber, J. M. Whitaker, and M. Morris, *Anal. Biochem.*, 1979, **97**, 176.
[19] T. P. Hopp and K. R. Woods, *Biochemistry*, 1979, **18**, 5182.
[20] M. Boehnert and D. H. Schlesinger, *Anal. Biochem.*, 1979, **96**, 469.

M. Harrison, and T. H. J. Huisman, *Anal. Biochem.*, 1979, **96**, 113. (*f*) R. A. Martinelli and H. A. Scheraga, *Anal. Biochem.*, 1979, **96**, 246. (*g*) M. McMillan, J. M. Cecka, L. Hood, D. B. Murphy, and H. O. McDevitt, *Nature*, 1979, **227**, 663. (*h*) M. Carlquist, V. Mutt, and H. Jörnvall, *FEBS Lett.*, 1979, **108**, 457. (*i*) T. J. McDonald, H. Jörnvall, G. Nilsson, M. Vagne, M. Ghatei, S. R. Bloom, and V. Mutt, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 227. (*j*) J. Spiess, J. E. Rivier, J. A. Rodkey, C. D. Bennett, and W. Vale, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2974. (*k*) G. B. Gerber, R. J. Anderegg, W. C. Herlihy, C. P. Gray, K. Biemann, and H. G. Khorana, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 227.

Improved repetitive yields were obtained when benzene was used both for removing excess reagents and for extracting the thiazolinone derivatives.

A method has been developed for the convenient deproteinization of enzymic hydrolysates.[21] It is particularly useful for aminopeptidase or carboxypeptidase digests of polypeptides carried out during sequence determination. The use of a volatile buffer and precipitation of protein with cold acetone eliminate difficulties in subsequent analyses caused by a high salt concentration and the presence of strong acid when trichloroacetic acid precipitation is used.

4 Liquid-phase Sequencing

A simple procedure for improving and maintaining the purity of the solvents and Quadrol buffer used in automated liquid-phase amino-acid sequence determination is described by Frank.[22] The main contaminants present in Quadrol are probably aldehydes. The method consists of removing these impurities by allowing them to react with insoluble amino-groups, prior to the delivery of the buffer to the reaction cup. For this purpose aminoethylcellulose is added to the buffer reservoir and the reagent is passed through a sintered-glass filter prior to delivery. It is also recommended to store the Quadrol in the presence of the same ion exchanger. The solvents benzene, ethyl acetate, and butyl chloride are stored in the presence of aluminium oxide and this is also present in the reservoirs containing benzene and ethyl acetate. To ensure good reducing conditions, DL-dithiothreitol ($100\,mg\,l^{-1}$) and tributylphosphine ($100\,\mu l^{-1}$) are added to butyl chloride, freshly decanted into its reservoir to remove the aluminium oxide. Following these simple precautions, repetitive yields of 98% were obtained over an extended number of cycles.

A modification of the cleavage procedure is introduced by Inglis *et al.*[23] to eliminate problems associated with inaccurate delivery of pentafluoropropionic acid and movement of the protein film. The reaction cup is kept stationary during the delivery of acid, the duration of the cleavage reaction, and the first drying stage. The *N*-terminal amino-acid sequence (69 of 82 residues) of turkey apovitellin I is determined by this procedure.

After extended repetitive phenylisothiocyanate degradations, two inherent limitations in the methodology become very acute. The problems of increasing overlap and background have been reduced with the use of a larger reaction cup and a high-speed drive.[24] The idea is to obtain a more uniform protein film by increasing the surface area of the cup by 75% and the speed drive up to 1800—3600 r.p.m. With a more uniform film the efficiency of the coupling reaction is increased, thus lowering the overlap. A more efficient cleavage reaction allows a reduction in the time the protein sample is exposed to acid and as a result reduces the background from non-specific cleavage of peptide bonds. This procedure appears to be most useful for studying larger proteins since proportionally larger quantities of material are required.

[21] A. Castel, J. M. De Graaf, and B. Kraal, *J. Chromatogr.*, 1979, **174**, 474.
[22] G. Frank, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 997.
[23] A. S. Inglis, P. M. Strike, W. C. Osborne, and R. W. Burley, *FEBS Lett.*, 1979, **97**, 179.
[24] T. Fairwell and H. B. Brewer, *Anal. Biochem.*, 1979, **99**, 242.

5 Solid-phase Sequencing

Chemical cleavage of tryptophanyl bonds generates peptides containing at their *C*-terminus a lactone derivative of tryptophan, namely dioxindolylalanine spirolactone. It has been shown that spirolactone-containing peptides can be coupled to 3-aminopropyl-glass (APG) selectively and almost quantitively by a procedure similar to that used for homoserine-containing peptides.[25] Using peptides generated from cytochrome *c*, myoglobin, glucagon, and melittin by treatment with a mixture of dimethyl sulphoxide (DMSO) and halogenoacids (HBr, HCl), attachment yields of 85—95% and a repetitive yield of 90% for the Edman degradations were achieved. The method offers interesting possibilities with hydrophobic proteins.

In order to optimize the solid-phase degradation of peptidyl–resin products obtained by peptide synthesis using the Merrifield procedure, two different peptidyl–resin linkages were compared.[26] The peptidyl–oxy–methylphenylacetamidomethyl linkage was preferable to the peptidyl–oxymethyl linkage. The former was more stable to strong acid preventing the loss of peptide from the resin during sequential degradation.

6 Microsequencing

The use of 4-*NN'*-dimethylaminoazobenzene 4'-isothiocyanate (DABITC) in combination with phenylisothiocyanate (PITC) in sequence analysis has increased the sensitivity of the Edman degradation. The products of the step-wise degradations are coloured DABTH derivatives which can be detected at the sub-nanomole level. Their extinction coefficients are about twice that of their PTH analogues and their absorption maximum is at a higher wavelength, making detection easier. One of the drawbacks of this procedure, however, is the low recovery of serine and threonine–thiohydantoin derivatives. Some of the destruction products have been partially identified and can be of help during protein-sequence analysis.[27] The difficulty of separating DABTH derivatives of leucine and isoleucine previously encountered by t.l.c. has been overcome using a 10% formic acid–ethanol (10 : 9) system.[28] Both an isocratic and a gradient h.p.l.c. system have been used for the identification of DABTH amino-acids which were detected by their absorbance at 436 nm.[29]

A manual method using DABITC involving the attachment of the peptide or protein to *p*-phenylene di-isothiocyanate-activated amino-glass (DITC-glass) has been described.[30] It is an inexpensive way of doing solid-phase sequencing. It is sensitive (1—10 nmole of peptide) but has the disadvantage of possible losses of peptide due to inefficient coupling to the solid support. The advantage is that it allows for better extraction of reagents and products than is possible by standard manual procedures.

[25] E. Wachter and R. Werham, *Anal. Biochem.*, 1979, **97**, 56.
[26] G. R. Matsueda and M. N. Margolies, *FEBS Lett.*, 1979, **106**, 89.
[27] J. Y. Chang, *Biochim. Biophys. Acta*, 1979, **578**, 175.
[28] C.-Y. Yang, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1673.
[29] K. J. Wilson, K. Rodger, and G. J. Hughes, *FEBS Lett.*, 1979, **108**, 87.
[30] J. Y. Chang, *Biochim. Biophys. Acta*, 1979, **578**, 188.

The DABITC–PITC procedure has also been successfully applied to automated solid-phase and liquid-phase sequencing.[31, 32] In both cases, DMF is used as the solvent for the DABITC in a 0.5% solution. Methodology for the spinning cup sequencer involves a chlorobutane wash to remove excess DABITC after the coupling reaction. Sequence of peptides at the 10 nmole level are obtained by this procedure.

7 Phenylthiohydantoin Amino-acids

High-pressure liquid chromatography on reversed-phase columns is the most suitable procedure for the identification of PTH amino-acids during sequence determination. The quantitative identification of PTH amino-acid derivatives during extended automated degradations has been reviewed.[33] Being a non-destructive technique, h.p.l.c. permits the identification of phenylthiohydantoins of derivatized amino-acids such as aminoethyl cysteine.[34] The pH value of the eluant has a marked effect on the retention times of acidic PTH amino-acids while the presence of various anions alters the mobility of basic PTH amino-acids.[35]

A method has been described for the separation of the common PTH amino-acids in a single run on a Zorbax CN column.[36] A complex multilinear gradient formed from sodium acetate (0.024 M, pH = 5.4) and methanol–acetonitrile (17 : 3) is used. This column has advantages over C_{18} columns, providing greater durability, reproducibility, and sensitivity.

In a different system, using a Spherisorb S5-ODS column, all the common PTH amino-acids as well as the derivatives of hydroxyproline, *S*-carboxymethyl- and *S*-carboxyamidomethyl-cysteine were identified.[37] This separation involves a multilinear gradient formed with lithium acetate (0.02 M, pH = 5.2)–acetonitrile (20 : 80). A temperature between 35 and 39 °C gave an optimal separation.

The step-wise chemical degradation of polypeptide chains from the *C*-terminal end is still in its developmental stage. To assist in these studies, a h.p.l.c. system has been devised to separate thiohydantoin amino-acids which are the derivatives generated by the ammonium thiocyanate method. The separation procedure is rapid and sensitivity of 1 nmole has been achieved.[38]

8 Mass Spectrometry

Amino-acid sequencing by mass spectrometry is a technique not readily available to most protein chemistry laboratories. However, while less sensitive than automated Edman degradation methods, it offers advantages in some specific problems encountered in sequence determination, such as blocked *N*-termini. Short blocked *N*-terminal peptides generated by enzymatic cleavage are relatively easy to isolate by ion-exchange chromatography. Once the peptide has been characterized by amino-acid analysis and derivatized to increase its volatility, structural analysis can

[31] G. J. Hughes, K. H. Winterhalter, H. Lutz, and K. J. Wilson, *FEBS Lett.*, 1979, **108**, 92.
[32] K. J. Wilson, P. Hunziker, and G. J. Hughes, *FEBS Lett*, 1979, **108**, 98.
[33] M. N. Margolies and A. W. Brauer, *Chromatogr. Sci.*, 1979, **10**, 177.
[34] W. F. Moo-Penn, M. H. Johnson, K. C. Bechtel, and D. L. Jue, *J. Chromatogr.*, 1979, **172**, 476.
[35] W. D. Annan, *J. Chromatogr.*, 1979, **173**, 194.
[36] N. D. Johnson, M. W. Hunkapiller, and L. E. Hood, *Anal. Biochem.*, 1979, **100**, 335.
[37] P. W. Moser and E. E. Rickli, *J. Chromatogr.*, 1979, **176**, 451.
[38] D. H. Schlesinger, J. Weiss, and T. K. Audhya, *Anal. Biochem.*, 1979, **95**, 494.

be performed by mass spectrometry. The *N*-termini of subunit VII of beef heart cytochrome *c* oxidase[39] and of shark myoglobin[40] have been sequenced in this way. This type of approach was also used in the case of Rhodotorucine *a*, a peptide containing a novel lipophilic *C*-terminus. Mass spectrometry, in combination with chemical synthesis and proton magnetic resonance revealed this residue to be *S*-farnesylcysteine.[41]

Mass spectrometry is also a useful technique for the identification of dipeptides resulting from enzymatic hydrolysis of polypeptides. This technique enables differentiation between dipeptides of identical composition but different sequence. For example, dipeptides containing phenylalanine in both the *N*-terminal and *C*-terminal positions have been studied. The results showed that the values for the ratios of metastable peaks to daughter ions are influenced by the sequence.[42]

Another approach is the use of computerized pattern recognition for the interpretation of the dipeptide mass spectra obtained by gas chromatography–mass spectrometry (g.c.–m.s.).[43] In this procedure, the fragmentation patterns obtained by analysis of derivatized dipeptides are identified by nearest-neighbour analysis. In the method described it is not necessary to have the pattern of all 400 possible dipeptides stored in the computer memory. Using only a limited number of patterns, the authors have observed an overall accuracy of 100% and 97% for the recognition of *N*-terminal and *C*-terminal amino-acids, respectively.

Finally, g.c.–m.s. has also been employed for the elucidation of the structure of cylic peptides. After non-specific cleavage of the peptide by partial acid hydrolysis, the mixture of smaller peptides is resolved after derivatization by g.c. and analysed directly by m.s. The original structure can be reassembled from that of the large number of smaller overlapping peptides. The structure of the antibiotic Permetin *a* from *Bacillus circulans* was shown to be a cyclic acyl nonapeptide by this technique.[44] Another example is that of the cyclic pentapeptide cyclochlorotine, a toxin from *Penicillium islandicum*.[45]

9 New Strategy

Tryptophan is easily oxidized, which can cause problems in automated sequence analysis, and its dansyl derivative is destroyed during the hydrolysis step of the dansyl procedure. It has been suggested that prior oxidation of tryptophan to stable derivatives would facilitate its identification during sequence analysis.[46] Two such derivatives, oxindolyl-3-alanine and kynurenine, are formed by treatment with dimethyl sulphoxide–HCl and sodium periodate, respectively. These formed stable PTH derivatives which could be separated from common PTH amino-acids. Kynurenine also formed a dansyl derivative stable to acid hydrolysis. The

[39] G. C. M. Steffens, G. J. Steffens, G. Buse, L. Witte, and H. Nau, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1633.
[40] W. K. Fisher and E. O. P. Thompson, *Aust. J. Biol. Sci.*, 1979, **32**, 277.
[41] Y. Kamiya, A. Sakurai, S. Tamura, N. Takahashi, E. Tsuchiya, K. Abe, and S. Fukui, *Agric. Biol. Chem.*, 1979, **43**, 363.
[42] Z. V. I. Zaretskii and P. Dan, *Biomed. Mass. Spectrom.*, 1979, **6**, 45.
[43] J. N. Ziemer, S. P. Perone, R. M. Caprioli, and W. E. Seifert, *Anal. Biochem.*, 1979, **51**, 1732.
[44] Y. Takeuchi, A. Murai, Y. Takahara, and M. Kainosho, *J. Antibiot.*, 1979, **32**, 121.
[45] R. J. Anderegg, K. Biemann, A. Manmade, and A. C. Ghosh, *Biomed. Mass. Spectrom.*, 1979, **6**, 129.
[46] A. S. Inglis, D. E. Rivett, and T. W. McMahon, *FEBS Lett*, 1979, **104**, 115.

disadvantage is that periodate can cause oxidation of cystine and partial oxidation of histidine and tyrosine, as well as *N*-terminal serine and threonine residues.

γ-Carboxyglutamic acid (Gla) is a residue resulting from a post-translational modification of specific glutamic acid residues by vitamin K-dependent carboxylation. This residue is important with respect to Ca^{2+}-binding and is found in an increasing number of proteins, the most notable of which are involved in blood-clotting. Gla residues are not stable during sequence analysis and their identification has relied on amino-acid analysis from *in vitro* labelling with $^{14}CO_2$. Recently, a method has been developed for the incorporation of radioactivity specifically into Gla residues.[47] Proteins and peptides are equilibrated with tritiated water of high specific activity in ^2HCl (0.05 M) and lyophilized. Subsequent heating gives decarboxylation and the transfer of tritium to the γ-carbon of the newly formed glutamic acid residue. Dialysis against or repeated lyophilization from HCl (0.05 M) gives the back-exchange of tritium incorporated at other sites, to give a specifically labelled product. Sequencing of Gla-containing peptides labelled by this method would result in a discrete pattern of radioactivity at former Gla-sites.

Sequence studies of membrane proteins frequently encounter problems due to the hydrophobicity of such proteins. Methodology particularly suited to this task has been developed during the course of sequence studies of bacteriorhodopsin.[48] The success of this approach resides in the amalgamation of a range of procedures. Cleavage of the protein with CNBr and chymotrypsin gave mainly hydrophobic peptides. These were separated by both gel-filtration on LH-20 and LH-60 and by reversed-phase h.p.l.c. Both systems used solvents consisting of mixtures of formic acid and ethanol. In most cases, *N*-terminal sequence analysis was carried out using automated procedures. In one case, however, a fraction containing two peptides was subjected to g.c.–m.s. and the sequence of both obtained. The ability of g.c.–m.s. to obtain certain peptide derivatives in very complex mixtures was particularly useful for the alignment of the CNBr peptides. Analysis of a partial acid hydrolysate by g.c.–m.s. gave the sequence of four of the five methionine-containing peptides required for this. Pyroglutamic acid was also identified as the *N*-terminal amino-acid by this procedure.

The automated liquid-phase sequencer and g.c.–m.s. were found to complement each other effectively and the results obtained should be more reliable than those obtained with either technique alone.

10 Primary Structures

The various amino-acid sequences reported in 1979 are listed in Table 2.

[47] P. V. Hauschka, *Biochemistry*, 1979, **18**, 4992.

[48] G. E. Gerber, R. J. Anderegg, W. C. Herlihy, C. P. Gray, K. Biemann, and H. G. Khorana, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 227.

Table 2 *Amino-acid sequences reported during 1979*

Protein	*Source*	*Comments*	*Ref.*
Enzymes			
Alanine amino-transferase	porcine heart	sequence of peptide containing pyridoxal phosphate binding site, 20 residues	49
Alcohol dehydro-genase	*Saccharomyces cerevisiae*	partial sequence of two major isoenzymes	50
	Drosophila melanogaster	sequence of peptide containing the ADFf/ADF sub-stitution, 37 residues	51
α-Amylase 1	porcine	sequence of 3 CNBr fragments, first 115 residues	52
α-Amylase	*Bacillus subtilis*	*N*-terminal, 7 residues	53
L-Asparaginase	*E. coli*	sequence of all 27 tryptic peptides, 321 residues	54
Aspartate amino-transferase	bovine heart mitochondria	*N*-terminal, 12 residues	55
	turkey liver mitochondria	partial sequence and alignment by homology to pig mitochondrial enzyme, 410 residues	56
	chicken heart	complete sequence, 412 residues	57
	E. coli B	*N*-terminal sequence, 33 residues, and sequence of peptide containing pyridoxal binding site, 5 residues	58
Aspartate carbamoyl transferase	*E. coli*. mutant	substitution Gly→ Asp from wild type	59

[49] S. Tanase, H. Kojima, and Y. Morino, *Biochemistry*, 1979, **18**, 3002.
[50] C. Wills and H. Jörnvall, *Eur. J. Biochem.*, 1979, **99**, 323.
[51] A. D. Retzios and D. R. Thatcher, *Biochimie*, 1979, **61**, 701.
[52] L. Pasero, B. Abadie, Y. Mazzei, D. Moinier, J. P. Biz-Zozero, M. Fougereau, and G. Marchis-Mouren, *Ann. Biol. Anim. Biochim., Biophys.*, 1979, **19**, 1033.
[53] P. Mäntsälä and H. Zalkin, *J. Biol. Chem.*, 1979, **254**, 8540.
[54] T. Maida, K. Morokuma, and G. Matsuda, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1483.
[55] S. Capasso, A. M. Garzillo, G. Marino, L. Mazzarella, P. Pucci, and G. Sannia, *FEBS Lett.*, 1979, **101**, 351.
[56] D. Barra, F. Martini, G. Montarani, S. Doonan, and F. Bossa, *FEBS Lett.*, 1979, **108**, 103.
[57] S. V. Shlyapnikov, A. N. Myasnikov, E. S. Severin, M. A. Myagkova, Y. M. Torchinsky, and A. E. Braunstein, *FEBS Lett.*, 1979, **106**, 385.
[58] H. Kagamiyama and T. Yagi, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1347.
[59] K. A. Wall and H. K. Schachman, *J. Biol. Chem.*, 1979, **254**, 11917.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Aspartokinase I–homoserine dehydrogenase I	*E. coli* K12	sequence of CNBr peptide CB-3, 79 residues	60
ATP phosphoribosyl transferase	*Salmonella typhimurium*	complete sequence, 299 residues	61
Carboxypeptidases A, B_1, and B_2	human	*N*-terminal, 20 residues of A, B_1, and B_2	62
Carboxypeptidase	crayfish	*N*-terminal, 19 residues	63
Chloramphenicol acetyltransferase	bacteria	*N*-terminal of 9 variants, 12 to 25 residues	64
	chloramphenicol resistant *staphylococci*	*N*-terminal of strains A, B, C, and D, 16 residues	65
	specified by plasmid JR 66b	complete sequence, 218 residues	66
Chorismate mutase–prephenate dehydratase	*E. coli* K12	sequence of 28 tryptic peptides	67
Chymosin	calf	complete sequence, 323 residues, 189 residues identical to corresponding residues of porcine pepsin	68
Cytochrome oxidase subunit II	bovine heart	complete sequence, 227 residues	69
subunit 2	*Neurospora crassa*	*N*-terminal, 39 residues	70
subunit IV	bovine heart	alignment and partial sequence of CNBr peptides	71
	bovine heart	complete sequence, 147 residues	72

[60] L. Sibilli, G. Le Bras, P. Cossart, M.-A. Chalvignac, G. Le Bras, P. A. Briley, and G. N. Cohen, *Biochimie*, 1979, **61**, 733.
[61] D. Piszkiewicz, B. E. Tilley, T. Rand-Meir, and S. M. Parsons, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1589.
[62] D. V. Marinkovic, *Biochem. Med.*, 1979, **22**, 11.
[63] R. Zwilling, F. Jakob, H. Bauer, H. Neurath, and D. N. Enfield, *Eur. J. Biochem.*, 1979, **94**, 223.
[64] Y. Zaidenzaig, J. E. Fitton, L. C. Packman, and W. V. Shaw, *Eur. J. Biochem.*, 1979, **100**, 609.
[65] J. E. Fitton and W. V. Shaw, *Biochem. J.*, 1979, **177**, 575.
[66] W. V. Shaw, L. C. Packman, B. D. Burleigh, A. Dell, H. R. Morris, and B. S. Hartley, *Nature* (*London*), 1979, **282**, 870.
[67] G. S. Baldwin and B. E. Davidson, *Biochim. Biophys. Acta*, 1979, **579**, 483.
[68] B. Foltmann, V. B. Pedersen, D. Kauffman, and G. Wybrandt, *J. Biol. Chem.*, 1979, **254**, 8447.
[69] G. J. Steffens and G. Buse, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 613.
[70] W. Machleidt and S. Werner, *FEBS Lett.*, 1979, **107**, 327.
[71] R. Sacher, G. Buse, and G. J. Steffens, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1377.
[72] R. Sacher, G. J. Steffens, and G. Buse, *Hoppe-Seyler's Z Physiol. Chem.*, 1979, **306**, 1385.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
subunit V	bovine heart	complete sequence, 109 residues	73
subunit VII	bovine heart	complete sequence, 85 residues	74
Dihydrofolate reductase	porcine liver	complete sequence, 186 residues	75
	mouse lymphoma L1210	complete sequence, 186 residues	76
	specified by R-plasmid R67 in trimethoprim resistant *E. coli*	complete sequence, 78 residues	77
Epoxide hydrase	rat liver microsomes	*N*-terminal, 20 residues	78
Fatty-acid synthetase, malonyl and palmitoyl transferase	yeast	sequence of active site peptides, 7 residues, both peptides are homologous	79
Fructose-1,6-diphosphatase	rat liver	sequence of peptide containing phosphorylation site, 8 residues	80
Fructose-1,6-diphosphate aldolase	rabbit muscle, sturgeon muscle, trout muscle, ox liver	sequence around the active site Lys, 75 residues	81
	Drosophila melanogaster	sequence of four Cys-containing peptides	82
Glutamate dehydrogenase	human liver	partial sequence, 454 residues	83
Glutathione reductase	human	sequence of *C*-terminal CNBr peptide, 25 residues	84

[73] M. Tanaka, M. Haniu, K. T. Yasunobu, C.-A. Yu, L. Yu, Y.-H. Wei, and T. E. King, *J. Biol. Chem.*, 1979, **254**, 3879.
[74] G. C. M. Steffens, G. J. Steffens, and G. Buse, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1641.
[75] S. L. Smith, P. Patrick, D. Stone, A. W. Phillips, and J. J. Burchall, *J. Biol. Chem.*, 1979, **254**, 11475.
[76] D. Stone, S. J. Paterson, J. H. Raper, and A. W. Phillips, *J. Biol. Chem.*, 1979, **254**, 480.
[77] D. Stone and S. L. Smith, *J. Biol. Chem.*, 1979, **254**, 10857.
[78] G. C. DuBois, E. Apella, R. Armstrong, W. Levin, A. Y. H. Lu, and D. M. Jerina, *J. Biol. Chem.*, 1979, **254**, 6240.
[79] H. Engeser, K. Hübner, J. Straub, and F. Lynen, *Eur. J. Biochem.*, 1979, **101**, 413.
[80] E. Humble, U. Dahlquist-Edberg, P. Ekman, E. Netzel, U. Ragnarsson, and L. Engström, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 1064.
[81] P. A. Benfield, B. G. Forcina, I. Gibbons, and R. N. Perham, *Biochem. J.* 1979, **183**, 429.
[82] O. Brenner-Holzach, *Arch. Biochem. Biophys.*, 1979, **194**, 328.
[83] J. H. Julliard and E. L. Smith, *J. Biol. Chem.*, 1979, **254**, 3427.
[84] R. Untucht-Grau, G. E. Schulz, and R. H. Schirmer, *FEBS Lett.*, 1979, **105**, 244.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
	human	sequence of CNBr peptide linking all 3 domains, 128 residues	85
Glycogen synthase	rabbit skeletal muscle	identification of site phosphorylated by phosphorylase kinase as Ser-7	86
	rabbit skeletal muscle	sequence of site phosphorylated by glycogen synthase kinase-2	87
Lactate dehydrogenase	mouse testis	tentative sequence based on electron density map and homology, 298 residues	88
Lipase	porcine pancreas	*N*-terminal, 234 residues, carbohydrate linked to Asn-166, Tyr-49 reactive to di-isopropyl phosphorofluoridate	89
	porcine pancrease	sequence between residues 235 and 307	90
Luciferase	*Beneckea harveyi* and *Photobacterium fischeri*	*N*-terminal of α- and β-subunits, 15 and 25 residues, sequences are homologous between two species	91
Lysozyme	california quail	complete sequence, 129 residues	92
	ring-necked pheasant	complete sequence, 130 residues, an extra Gly at *N*-terminus	93
Mast cell protease (α-chymotrypsin-like)	rat peritoneal mast cell	*N*-terminal, 47 residues	94

[85] E. Schiltz, R. Blatterspiel, and R. Untucht-Grau, *Eur. J. Biochem.*, 1979, **102**, 269.
[86] T. R. Soderling, V. S. Sheorain, and L. H. Ericsson, *FEBS Lett.*, 1979, **106**, 181.
[87] D. B. Rylatt and P. Cohen, *FEBS Lett.*, 1979, **98**, 71.
[88] W. D. L. Musick and M. G. Rossmann, *J. Biol. Chem.*, 1979, **254**, 7621.
[89] J. D. Bianchetta, J. Bidaud, A. A. Guidoni, J. J. Bonicel, and M. Rovery, *Eur. J. Biochem.*, 1979, **97**, 395.
[90] A. Guidoni, J. Bonicel, J. Bianchetta, and M. Rovery, *Biochimie*, 1979, **61**, 841.
[91] T. O. Baldwin, M. M. Ziegler, and D. A. Powers, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 4887.
[92] I. M. Ibrahimi, E. M. Prager, T. J. White, and A. C. Wilson, *Biochemistry*, 1979, **18**, 2736.
[93] J. Jollès, I. M. Ibrahimi, E. M. Schoentgen, P. Jollès, and A. C. Wilson, *Biochemistry*, 1979, **18**, 2744.
[94] M. T. Everitt and H. Neurath, *Biochimie*, 1979, **61**, 653.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Peroxidase C	horseradish	complete sequence, 308 residues	95
Phosphofructo-kinase	rabbit muscle	sequence of peptide near ATP inhibitory site, 5 residues	96
	sheep heart	*N*-terminal, 11 residues	97
	Bacillus stearo-thermophilus	complete sequence, 316 residues	98
Phosphoglycerate kinase	horse muscle	complete sequence, 416 residues	99
Phospholipase A_2 isoenzyme	porcine pancreas	location of 4 substitutions Ala-12 → Thr, His-17 → Asp, Met-20 → Leu, Glu-71 → Asn	100
Protein kinase (adenosine 3′:5′-monophosphate dependent)	bovine cardiac muscle	sequence of 2 phosphopeptides, phosphate is linked to Ser in one and Thr in the other	101
Protocatechuate 3,4-dioxygenase	*Pseudomonas aeruginosa*	sequence of 14 tryptic peptides of α-subunit, 199 residues	102
	Pseudomonas aeruginosa	complete sequence of α-subunit, 200 residues	103
	Pseudomonas aeruginosa	complete sequence of β-subunit, 237 residues	104
Pyruvate dehydrogenase	porcine heart	sequence around 3 sites of phosphorylation	105
	E. coli	sequence of lipoyl-, lysine-containing peptide, 7 residues	106

[95] K. G. Welinder, *Eur. J. Biochem.*, 1979, **96**, 483.
[96] K. Nagata, K. Suzuki and K. Imahori, *J. Biochem.* (*Tokyo*), 1979, **86**, 1179.
[97] A. M. Fordyce, G. G. Midwinter, and C. H. Moore, *Biochem. Soc. Trans.*, 1979, **7**, 721.
[98] P. J. Hudson, H. Hengartner, E. Kolb, and J. I. Harris, *Proc. FEBS Meet.*, 1979, **52**, 341.
[99] R. D. Banks, C. C. F. Blake, P. R. Evans, R. Haser, D. W. Rice, G. W. Hardy, M. Merret, and A. W. Phillips, *Nature* (*London*), 1979, **279**, 773.
[100] W. C. Puijk, H. M. Verheij, P. Wietzes, and G. H. De Haas, *Biochim. Biophys. Acta*, 1979, **580**, 411.
[101] S. Shoji, K. Titani, J. C. Demaile, and E. H. Fischer, *J. Biol. Chem.*, 1979, **254**, 6211.
[102] N. A. Kohlmiller and J. B. Howard, *J. Biol. Chem.*, 1979, **254**, 7302.
[103] N. A. Kohlmiller and J. B. Howard, *J. Biol. Chem.*, 1979, **254**, 7309.
[104] M. Iwaki, H. Kagamiyama, and M. Nozaki, *J. Biochem.* (*Tokyo*), 1979, **86**, 1159.
[105] P. H. Sudgen, A. L. Kerbey, P. J. Randle, C. A. Waller, and K. B. M. Reid, *Biochem. J.*, 1979, **181**, 419.
[106] G. Hale and R. N. Perham, *FEBS Lett.*, 1979, **105**, 263.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Pyruvate kinase	bovine muscle	sequence of peptide containing E-trinitrophenylated lysine, 34 residues, this modification inactivates the enzyme	107
Ribonuclease	*Bacillus intermedius* 7P	complete sequence, 109 residues	108
	hamster pancreas	complete sequence, 124 residues	109
	bovine semen	pairing of 4 intrachain disulphide bonds	110
	mouse pancreas	complete sequence, 124 residues	111
Ribulose-1,5-bis-phosphate carboxylase	barley	*N*-terminal sequence, 46 residues, sequence of 8 of 10 CNBr peptides for a total of 210 residues	112
	spinach	complete sequence of small subunit, 120 residues, Tyr and Pro at position 91	113
	Chlamydomonas reinhardtii	*N*-terminal sequence of precursor of small subunit and mature form, 55 and 12 residues	114
Serine Protease (intracellular)	*Bacillus subtilis* strain Marburg 168	*N*-terminal, 19 residues	115
Sulphite oxidase 'b_5-like'	chicken	sequence of haem-binding domain, 97 residues	116
Thymidylate synthetase	*Lactobacillus casei*	sequence of CNBr peptides 1, 2, 3, and 5, 208 residues	117

[107] S. C. Johnson, T. Bailey, R. R. Becker, and J. M. Cardenas, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 525.
[108] G. A. Afanasenko, S. M. Dudkin, L. B. Kaminir, I. B. Leshchinskaya, and E. S. Severin, *FEBS Lett.*, 1979, **97**, 77.
[109] P. A. Jekel, H. J. Sips, J. A. Lenstra, and J. J. Beintema, *Biochimie*, 1979, **61**, 827.
[110] A. Di Donato and G. D'Alessio, *Biochim. Biophys. Acta*, 1979, **579**, 303.
[111] J. A. Lenstra and J. J. Beintema, *Eur. J. Biochem.*, 1979, **98**, 399.
[112] C. Poulsen, B. Martin, and I. Svendsen, *Carlsberg Res. Commun.*, 1979, **44**, 191.
[113] P. G. Martin, *Aust. J. Plant Physiol.*, 1979, **6**, 401.
[114] G. W. Schmidt, A. Devillers-Thiery, H. Desruisseaux, G. Blobel, and N.-H. Chua, *J. Cell. Biol.*, 1979, **83**, 615.
[115] A. Y. Strongin, D. I. Gorodetsky, I. A. Kuznetsova, V. V. Yanonis, Z. T. Abramov, L. P. Belyanova, L. A. Baratova, and V. M. Stepanov, *Biochem. J.*, 1979, **179**, 333.
[116] B. Guiard and F. Lederer, *Eur. J. Biochem.*, 1979, **100**, 441.
[117] G. F. Maley, R. L. Bellisario, D. U. Guarino, and F. Maley, *J. Biol. Chem.*, 1979, **254**, 1288.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
	Lactobacillus casei	sequence of active site CNBr peptide, 102 residues	118
	Lactobacillus casei	complete sequence, 316 residues	119
Transaminase B	*E. coli* K12	*N*-terminal, 10 residues	120
Transcarboxylase	*Propioni bacterium shermanii*	complete sequence of biotinyl-subunit, 123 residues	121
Trypsinogens	dog	*N*-terminal of anionic and cationic forms, 35 and 15 residues, both have the sequence $(Asp)_4$–Lys of other trypsinogens	122
Tryptophan synthetase, β_2 protein	*Serratia marcescens*	Sequence of peptide binding pyridoxal-phosphate, 23 residues	123
Hormones			
Crinia-angiotensin	Australian frog	complete sequence, 11 residues	124
Endorphin	salmon pituitary	complete sequence, 29 residues	125
Gastric intrinsic factor	hog	*N*-terminal, 22 residues	126
Gastrin-releasing peptide	porcine gastric and intestinal tissue	complete sequence, 27 residues	127
Luteinizing hormone, β-subunit	human	reinvestigation of complete sequence, 112 residues	128
α-Melanocyte-stimulating hormone	bovine pituitary	variant containing *NO*-diacetylserine as *N*-terminal	129

[118] R. L. Bellisario, G. F. Maley, D. U. Guarino, and F. Maley, *J. Biol. Chem.*, 1979, **254**, 1296.
[119] G. F. Maley, R. L. Bellisario, D. U. Guarino, and F. Maley, *J. Biol. Chem.*, 1979, **254**, 1301.
[120] C. W. Adams, R. P. Lawther, and G. W. Hatfield, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 650.
[121] W. L. Maloy, B. U. Bowien, G. K. Zwolinski, K. G. Kumar, and H. G. Wood, *J. Biol. Chem.*, 1979, **254**, 11615.
[122] A. Borgstroem, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 657.
[123] V. Rocha, M. Deeley and I. P. Crawford, *J. Bacteriol.*, 1979, **137**, 700.
[124] V. Erspamer, P. Melchiorri, T. Nakajima, T. Yasuhara, and R. Endean, *Experientia*, 1979, **35**, 1132.
[125] H. Kawauchi, M. Tubokawa, and K. Muramoto, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1249.
[126] D. Bucher, J. Thomsen, and E. Nexo, *Comp. Biochem. Physiol. B*, 1979, **62B**, 175.
[127] T. J. McDonald, H. Joernvall, G. Nilsson, M. Vagne, M. Ghatei, S. R. Bloom, and V. Mutt, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 227.
[128] H. T. Keutmann, R. M. Williams, and R. J. Ryan, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 842.
[129] D. Rudman, R. K. Chawla, and B. M. Hollins, *J. Biol. Chem.*, 1979, **254**, 10102.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Neuropeptide, egg-laying hormone	mollusc (*Aplysia californica*)	complete sequence, 36 residues, by radiosequencing	130
Neurophysin, VLDV	porcine and bovine	complete sequence, 93 residues, differ only by 6 substitutions	131
Neurophysins, MSEL and VLDV	human	*N*-terminal sequence	132
Pro-adrenocortico-tropic hormone, endorphin glycoprotein	mouse tumour cell	positions of radio-labelled Cys and Trp align with sequence predicted from cDNA	133
Somatostatin	pigeon pancreas	complete sequence, 14 residues, identical to mammalian hypothalamic somatostatin	134
Substance P		complete sequence, 11 residues, *C*-terminal is Met-amide	135
Vasoactive intestinal peptide (VIP)	bovine hypothalamus	complete sequence, 28 residues, *C*-terminal is Asn-amide	136
Muscle Proteins			
Actin	bovine skeletal muscle, cardiac muscle, and aorta tissue, rabbit slow skeletal muscle	sequence by comparison with rabbit skeletal muscle actin	137
Actin	chicken breast, skeletal muscle, and gizzard smooth muscle calf spleen	comparison of both actins, differ at 6 positions	138

[130] A. Y. Chiu, M. W. Hunkapiller, E. Heller, D. K. Stuart, L. E. Hood, and F. Strumwasser, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6656.
[131] M. T. Chauvet, P. Codogno, J. Chauvet, and R. Acher, *FEBS Lett.*, 1979, **98**, 37.
[132] M. T. Chauvet, J. Chauvet, R. Acher, and A. G. Robinson, *FEBS Lett.*, 1979, **101**, 391.
[133] H. T. Keutmann, B. A. Eipper, and R. E. Mains, *J. Biol. Chem.*, 1979, **254**, 9204.
[134] J. Spiess, J. E. Rivier, J. A. Rodkey, C. D. Bennett, and W. Vale, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2974.
[135] R. Carraway and S. E. Leeman, *J. Biol. Chem.*, 1979, **254**, 2944.
[136] M. Carlquist, V. Mutt, and H. Jörnvall, *FEBS Lett.*, 1979, **108**, 457.
[137] J. Vandekerckhove and K. Weber, *Differentiation* (*Berlin*), 1979, **14**, 123.
[138] J. Vandekerckhove and K. Weber, *FEBS Lett.*, 1979, **102**, 219.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Profilin	calf spleen	complete sequence, 142 residues, no homology with actin	139
Tropomyosin	rabbit cardiac muscle and skeletal muscle (β-tropomyosin)	complete sequence, 284 residues differ at 39 positions, cardiac tropomyosin is identical to skeletal α-tropomyosin	140
Histones			
H2	wheat germ	*N*-terminal sequence of 3 variants $H2A_1$, $H2A_2$, and $H2A_3$, 54, 37, and 37 residues	141
H2A and H2B	calf thymus	partial sequence of peptides cross-linked by u.v. irradiation, Pro-26 of H2A and Tyr-37, 40, or 42 of H2B are involved in cross-linking	142
H2B	*Drosophila melanogaster*	complete sequence, 122 residues	143
H2B	mollusc (*Patella granatina*)	complete sequence, 121 residues	144
	human spleen	complete sequence, 125 residues, identical to calf thymus H2B	145
	rat thymus	sequence by homology of tryptic peptide with sequence of calf thymus H2B, phosphorylation sites at Ser-32, -36, -87, and -91	146
H4	*Tetrahymena thermophila*	partial sequence, 66 residues	147

[139] L. E. Nystroem, U. Lindberg, J. Kendrick-Jones, and R. Jakes, *FEBS Lett.*, 1979, **101**, 161.
[140] A. S. Mak, W. G. Lewis, and L. B. Smillie, *FEBS Lett.*, 1979, **105**, 232.
[141] J. De A. Rodrigues, W. F. Brandt, and C. Von Hault, *Biochem. Biophys. Acta*, 1979, **578**, 196.
[142] R. J. DeLange, L. C. Williams, and H. G. Martinson, *Biochemistry*, 1979, **18**, 1942.
[143] S. C. R. Elgin, J. Schilling, and L. E. Hood, *Biochemistry*, 1979, **18**, 5679.
[144] P. D. Van Helden, W. N. Strickland, W. F. Brandt, and C. Von Holt, *Eur. J. Biochem.*, 1979, **93**, 71.
[145] Y. Ohe, H. Hayashi, and K. Iwai, *J. Biochem. (Tokyo)*, 1979, **85**, 615.
[146] A. Martinage, P. Mangeat, P. Sautierre, G. Marchis-Mouren, and G. Biserte, *Biochimie*, 1979, **61**, 61.
[147] C. V. C. Glover and M. A. Gorovsky, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 585.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
H5	goose erythrocytes	complete sequence, 193 residues	148
Toxins			
Cholera toxin subunit A_1	*Vibrio cholerae* strain 569B	alignment of the 4 CNBr peptides, tentative sequence, 68 residues	149
Cyclochlorotine	*Penicillium islandicum*	sequence of cyclic pentapeptide	150
Diphtheria toxin, fragment A	*Corynebacterium diphtheriae*	sequence of 6 tryptic peptides, 193 residues	151
		sequence of 5 CNBr peptides, 193 residues	152
		complete sequence, 193 residues	153
Myotoxin a	prairie rattlesnake (*Crotalus viridis viridis*)	complete sequence, 42 residues, assignment of the 3 disulphides	154
Neurotoxin III	scorpion (*Androctonus australis hector*	complete sequence, 64 residues	155
Neurotoxin (postsynaptic)	australian tiger snake (*Notechis scutatus scutatus*)	complete sequence, 73 residues	156
Proteins C_8S_2 and C_9S_3	green mamba (*Dendroaspic angusticeps*	complete sequence of both toxin C_8S_2 and C_9S_3, 62 and 63 residues	157
Protein S_2C_4	Jameson's mamba (*Dendroaspis jamesoni kaimosae*)	complete sequence, 62 residues	158
Proteins S_5C_1 and S_5C_{10}	Jameson's mamba	complete sequence of both toxin S_5C_1 S_5C_{10}, 61 and 58 residues	159

[148] M. Yaguchi, C. Roy, and V. L. Seligy, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 1400.
[149] C. Y. Lai, F. Cancedda, and D. Chang, *FEBS Lett.*, 1979, **100**, 85.
[150] R. J. Anderegg, K. Biemann, A. Manmade, and A. C. Ghosh, *Biomed. Mass. Spectrom.*, 1979, **6**, 129.
[151] R. J. DeLange, L. C. Williams, and R. J. Collier, *J. Biol. Chem.*, 1979, **254**, 5827.
[152] R. E. Drazin, R. J. Collier, L. C. Williams, and R. J. DeLange, *J. Biol. Chem.*, 1979, **254**, 5832.
[153] R. J. DeLange, L. C. Williams, R. E. Drazin, and R. J. Collier, *J. Biol. Chem.*, 1979, **254**, 5838.
[154] J. W. Fox, M. Elzinga, and A. T. Tu, *Biochemistry*, 1979, **18**, 678.
[155] C. Kopeyan, G. Martinez, and H. Rochat, *Eur. J. Biochem.*, 1979, **94**, 609.
[156] J. Halpert, J. Fohlman, and D. Eaker, *Biochimie*, 1979, **61**, 719.
[157] F. J. Joubert and C. C. Viljoen, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1075.
[158] F. J. Joubert and N. Taljaard, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 571.
[159] F. J. Joubert and N. Taljaard, *Biochim. Biophys. Acta*, 1979, **579**, 228.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Ribosomal proteins			
'A' protein	*Vibrio costicola* and NRCC 11227	*N*-terminal sequence of each, 38 residues homologous to 'A' protein from *E. coli* (EL-12)	160
eL12/eL12-P	*Artemia salina*	complete sequence, 111 residues, Ser-98 is partially phosphorylated	161
L20	*E. coli*	complete sequence, 117 residues	162
L21	*E. coli*	complete sequence, 103 residues	163
L23	*E. coli*	complete sequence, 99 residues	164
L24	*E. coli*	complete sequence, 103 residues	165
S1 and A protein	*E. coli*	*N*-terminal of S1 and A protein, 23 and 24 residues	166
S3	*E. coli*	complete sequence, 232 residues	167
S7	*E. coli* K and B	complete sequence of S7K and S7B, 177 and 153 residues, sequences identical but for 24 additional residues at *C*-terminal of S7K	168
Globins			
Haemoglobins:			
fetal, γ-chain	pig-tailed macaque	complete sequence, 146 residues	169
α- and β-chains	brown lemur	sequence of tryptic peptides, alignment by homology to human haemoglobin	170

[160] P. Falkenberg, M. Yaguchi, C. F. Rollin, A. T. Matheson, and R. Wydro, *Biochem. Biophys. Acta*, 1979, **578**, 207.
[161] R. Amons, W. Pluijms, and W. Moeller, *FEBS Lett.*, 1979, **104**, 85.
[162] B. Wittmann-Leibold and C. Seib, *FEBS Lett.*, 1979, **103**, 61.
[163] I. Hieland and B. Wittmann-Liebold, *Biochemistry*, 1979, **18**, 4605.
[164] B. Wittmann-Liebold and B. Greuer, *FEBS Lett.*, 1979, **108**, 69.
[165] B. Wittmann-Liebold, *FEBS Lett.*, 1979, **108**, 75.
[166] A. R. Subramanian, B. Wittman-Liebold, A. W. Geissler, G. Stöffler, and M. Giesen, *FEBS Lett.*, 1979, **99**, 357.
[167] D. Brauer and R. Röming, *FEBS Lett.*, 1979, **106**, 352.
[168] J. Reinbolt, D. Tritsch, and B. Wittmann-Liebold, *Biochimie*, 1979, **61**, 501.
[169] P. E. Nute and W. C. Mahoney, *Biochemistry*, 1979, **18**, 467.
[170] T. Maita, M. Setoguchi, G. Matsuda, and M. Goodman, *J. Biochem.* (*Tokyo*), 1979, **85**, 755.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
α-chains, 2 forms	Malaysian pig-tailed macaque	complete sequence of both forms, 141 residues, at position-78 αI has Gln and αII has His	171
α- and β-chains	guinea pig and dromedary	complete sequence of both α- and β-chains, 141 and 146 residues	172
α- and β-chains	European hedgehog	sequence of tryptic peptides, alignment by homology to human haemoglobin	173
α- and β-chains	oppossum	complete sequence of both α- and β-chains, 141 and 146 residues	174
α- and β-chains	greylag goose	complete sequence of both α- and β-chains, 141 and 146 residues	175
β-chains, 2 forms	carp	complete sequence of both β_A- and β_B-chain, 147 residues, differ by 4 residues	176
HbIII	hagfish	complete sequence, 148 residues	177
2 main components	river lamprey	complete sequence of both, 149 residues, in one the *N*-terminal Pro is formylated	178
4 chains	*Phoronopsis viridis*	*N*-terminal sequence of 4 chains Hb-Ia, -Ic, -IIa, and -IIb, 34, 15, 14, and 26 residues, respectively	179
CTT III	diptera (*Chironomus thumi thumi*)	complete sequence, 136 residues, Ile and Thr at position 57	180
CTT VI	diptera	complete sequence, 147 residues	181

[171] W. C. Mohoney and P. E. Nute, *Arch. Biochem. Biophys.*, 1979, **196**, 64.
[172] G. Braunitzer, B. Schrank, A. Stangl, and H. Wiesner, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1941.
[173] T. Maita, A. Araya, G. Matsuda, and M. Goodman, *J. Biochem (Tokyo)*, 1979, **85**, 259.
[174] P. Stenzel, B. Brimhall, R. T. Jones, J. A. Black, A. McLachlan, and D. Gibson, *J. Biol. Chem.*, 1979, **254**, 2071.
[175] G. Braunitzer and W. Oberthuer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 679.
[176] B. Grujic-Injac, G. Braunitzer, and A. Stangl, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 609.
[177] G. Liljeqvist, G. Braunitzer, and S. Paleus, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 125.
[178] M. Zelenik, V. Rudloff, and G. Braunitzer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1879.
[179] R. L. Garlick, B. J. Williams, and A. F. Riggs, *Arch. Biochem. Biophys.*, 1979, **194**, 13.
[180] G. Buse, G. J. Steffens, G. Braunitzer, and W. Steer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 89.
[181] H. Aschauer, Z. H. Zaidi, and G. Braunitzer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1513.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
CTT VII B	diptera	complete sequence, 145 residues	182
CTT X	diptera	complete sequence, 151 residues, Thr and Ala at position 48	183
myoglobin	Pacific spotted dolphin	complete sequence, 153 residues	184
	shark	complete sequence, 148 residues	185
	yellowfin tuna	sequence of 14 tryptic peptides	186
	Pacific sei whale	complete sequence, 153 residues	187
Electron-transport proteins			
Cytochromes:			
b_5	rabbit	sequence of *C*-terminal membrane binding segment, 43 residues, 91—133	188
c and *c'*	*Rhodopseudomonas gelatinesa* and *Rhodospirillum tenue*	complete sequence of both cytochromes *c*-511 and *c'*	189
c'	*Chromatium vinosum*	complete sequence, 131 residues, haem attached at Cys-121 and -124	190
c_2	*Rhodospirilla ceae* family	complete sequence of cytochrome c_2 from 10 species	191
c_3	*Desulphovibrio desulphuricans*, strain Norway	complete sequence, 116 residues	192
c-553	*Desulphovibrio desulphuricans*	*N*-terminal sequence, 14 residues	193
c-556	*Agrobacterium tumefaciens*	*N*-terminal sequence, 26 residues	194

[182] D. Sladic-Simic, T. Kleinschmidt, and G. Braunitzer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 115.
[183] R. Lalthantluange and G. Braunitzer, *Biochimie*, 1979, **61**, 725.
[184] B. N. Jones, C.-C. Wang, F. E. Dwulet, L. D. Lehman, J. L. Meuth, R. A. Bogardt, and F. R. N. Gurd, *Biochim. Biophys. Acta*, 1979, **577**, 454.
[185] W. K. Fisher and E. O. P. Thompson, *Aust. J. Biol. Sci.*, 1979, **32**, 277.
[186] R. H. Rice, D. A. Watts, and W. D. Brown, *Comp. Biochem. Physiol. B.*, 1979, **62B**, 481.
[187] B. N. Jones, T. M. Rothgeb, R. D. England, and F. R. N. Gurd, *Biochim. Biophys. Acta*, 1979, **574**, 464.
[188] K. Kondo, S. Tajima, R. Sato, and K. Narita, *J. Biochem.* (*Tokyo*), 1979, **86**, 1119.
[189] R. P. Ambler, T. E. Meyer, and M. D. Kamen, *Nature* (*London*), 1979, **278**, 661.
[190] R. P. Ambler, M. Daniel, T. E. Meyer, R. G. Bartsch, and M. D. Kamen, *Biochem. J.*, 1979, **177**, 819.
[191] R. P. Ambler, M. Daniel, J. Hermoso, T. E. Meyer, R. G. Bartsch, and M. D. Kamen, *Nature* (*London*), 1979, **278**, 659.
[192] R. Haser, M. Pierrot, M. Frey, F. Payan, and J. P. Astier, *Nature* (*London*), 1979, **282**, 806.
[193] G. Fauque, M. Bruschi, and J. Le Gall, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1020.
[194] J. Van Beeumen, C. Van der Branden, P. Tempst, and J. De Ley, *FEBS Lett.*, 1979, **101**, 336.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
P-450	rat liver	*N*-terminal sequence of 3 forms of P-450, *a*, *b*, and *c*, 19 residues each	195
Desulphoredoxin	*Desulphovibrio gigas*	complete sequence, 36 residues	196
Ferredoxin	wheat	complete sequence, 97 residues, Cys-39, -44, -47, and -77 necessary for iron binding	197
	Mycobacterium smegmatis	complete sequence, 106 residues	198
	Desulphovibrio	revision of complete sequence, 57 residues	199
	Rhodospirillum tenue	complete sequence, 63 residues	200
Haemocyanin	scorpion	*N*-terminal sequences of 7 subunits	201
	horseshoe crab	*N*-terminal sequence of subunit IV	201
	vineyard snail	*N*-terminal sequence of a functionally active domain, 6 residues	202
C-Phycocyanin	*Cyanidium caldarium*	partial sequence of regions of α- and β-subunits	203
Plastocyanin	flowering plants	*N*-terminal sequence of 40 members of 10 angiosperm families, 40 residues	204
Lectins			
Favin	fava beans (*Vicia faba*)	complete sequence of β-chain, 185 residues, homologous to Con A	205
	fava beans	complete sequence of α-chain, 51 residues	206

[195] L. H. Botelho, D. E. Ryan, and W. Levin, *J. Biol. Chem.*, 1979, **254**, 5635.
[196] M. Bruschi, I. Moura, J. Le Gall, A. V. Xavier, and L. C. Sieker, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 596.
[197] I. Takruri and D. Boulter, *Biochem. J.*, 1979, **179**, 373.
[198] T. Hase, S. Wakabayashi, H. Matsubara, T. Imai, T. Matsumoto, and J. Tobari, *FEBS Lett.*, 1979, **103**, 224.
[199] M. Bruschi, *Biochem. Biophys. Res. Commun.*, 1979, **61**, 623.
[200] S. M. Tedro, T. E. Meyer, and M. D. Kamen, *J. Biol. Chem.*, 1979, **254**, 1495.
[201] J. Jollès, P. Jollès, J. Lamy, and J. Lamy, *FEBS Lett.*, 1979, **106**, 289.
[202] M. Brouwer, M. Wolters, and E. F. J. Van Bruggen, *Arch. Biochem. Biophys.*, 1979, **193**, 487.
[203] A. S. Brown, G. D. Offner, M. E. Ehrhardt, and R. F. Troxler, *J. Biol. Chem.*, 1979, **254**, 7803.
[204] D. Boulter, D. Peacock, A. Guise, J. T. Gleaves, and G. Eastabrook, *Phytochemistry*, 1979, **18**, 603.
[205] B. A. Cunningham, J. L. Hemperly, T. P. Hopp, and G. M. Edelman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3218.
[206] J. J. Hemperly, T. P. Hopp, J. W. Becker, and B. A. Cunningham, *J. Biol. Chem.*, **254**, 6803.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Lectins	*Vicia cracca*	*N*-terminal sequence of 2 lectins, 25 residues	207
Structural proteins			
Basement membrane collagen	mouse tumour (EHS sarcoma)	*N*-terminal sequence of 2 peptic peptides, 18 residues	208
Collagen type III	human	partial sequence of tryptic peptide (T9) involved in cross-linking	209
	calf skin	sequence of *N*-terminal CNBr peptides CB-3A, -3B, -3C, -7, and -6, positions 1—222	210
	calf skin	sequence of CNBr peptides CB-1, -8, -10, and -2, positions 223—402	211
	calf skin	sequence of CNBr peptide CB5, positions 403—551	212
	calf skin	sequence of CNBr peptide CB-5, positions 552—788	213
	calf skin	sequence of CNBr peptide CB-9A, positions 789—927	214
	calf skin	sequence of CNBr peptide CB-9B, positions 928—1028	215
Collagen (α_2)	chick skin	sequence of CNBr peptide, 112 residues, includes collagenase cleavage site	216
Procollagen type I (α1)	calf skin	*N*-terminal sequence, 139 residues	217

[207] C. Baumann, H. Rüdiger, and A. D. Strosberg, *FEBS Lett.*, 1979, **102**, 216.
[208] R. Timpl, P. Bruckner, and P. Fietzek, *Eur. J. Biochem.*, 1979, **95**, 255.
[209] W. Henkel, J. Rauterberg, and R. W. Glanville, *Eur. J. Biochem.*, 1979, **96**, 249.
[210] P. P. Fietzek, H. Allmann, J. Rauterberg, W. Henkel, E. Wachter, and K. Kuehn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 809.
[211] H. Dewes, P. P. Fietzek, and K. Kuehn, *Hoppe-Seyler's Z Physiol. Chem.*, 1979, **360**, 821.
[212] H. Bentz, P. P. Fietzek, and K. Kuehn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 833.
[213] H. Lang, R. W. Glanville, P. P. Fietzek, and K. Kuehn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 841.
[214] H. Dewes, P. P. Fietzek, and K. Kuehn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 851.
[215] H. Allmann, P. P. Fietzek, R. W. Glanville, and K. Kuehn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 861.
[216] S. N. Dixit, C. L. Mainardi, J. M. Seyer, and A. H. Kang, *Biochemistry*, 1979, **18**, 5416.
[217] D. Hoerlein, P. P. Fietzek, E. Wachter, C. M. Lapiere, and K. Kuehn, *Eur. J. Biochem.*, 1979, **99**, 31.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Procollagen type I	sheep skin	*N*-terminal sequence, 98 residues	218
Procollagen type I (α_1) (precursor)	chick cranial bone	*N*-terminal sequence of protein synthesized *in vitro*, 19 residues	219
Tropoelastin (precursor)	chick aorta	*N*-terminal sequence, 11 residues	220
Viral proteins			
Coat protein	alfalfa mosaic virus (strain VRU)	complete sequence using homology with strain 425 coat protein, 219 residues	221
	bacteriophage PRR1	complete sequence, 131 residues	222
Glycoprotein PE_2	sindbis virus	partial *N*-terminal sequence, 11 of 25 residues	223
Haemagglutinin	influenza virus	*N*-terminal sequence, 45 residues, and signal sequence, 14 residues	224
	influenza virus	sequence of CNBr peptide CN2 of lightchain, 98 residues	225
	influenza virus	sequence of 2 CNBr peptides CN-3 and CN-4 of light chain, 18 and 17 residues, and *N*-terminal sequence of CN-1, 20 residues	226
	influenza virus	*N*-terminal sequence of HA_1 and HA_2 of 6 variants	227

[218] H. Rohde, E. Wachter, W. J. Richter, P. Bruckner, O. Helle, and R. Timpl, *Biochem. J.*, 1979, **179**, 631.
[219] R. D. Palmiter, J. M. Davidson, J. Gagnon, D. W. Rowe, and P. Bornstein, *J. Biol. Chem.*, 1979, **254**, 1433.
[220] R. B. Rucker, C. S. Heng-Khoo, M. Dubick, M. Lefevre, and C. E. Cross, *Biochemistry*, 1979, **18**, 3854.
[221] A. Castel, B. Kraal, J. M. De Graaf, and L. Bosch, *Eur. J. Biochem.*, 1979, **102**, 125.
[222] P. Dhaese, J. S. Vandekerckhove, and M. C. Van Montagu, *Eur. J. Biochem.*, 1979, **94**, 375.
[223] S. Bonatti and G. Blobel, *J. Biol. Chem.*, 1979, **254**, 12261.
[224] J. McCauley, J. Bye, K. Elder, M. J. Gething, J. J. Skehel, A. Smith, and M. D. Waterfield, *FEBS Lett.*, 1979, **108**, 422.
[225] C. W. Ward and T. A. A. Dopheide, *Virology*, 1979, **95**, 107.
[226] T. A. A. Dopheide and C. W. Ward, *Virology*, 1979, **92**, 230.
[227] M. D. Waterfield, K. Espelie, K. Elder, and J. J. Skehle, *Br. Med. Bull.*, 1979, **35**, 57.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
	influenza virus (A/Memphis/ 102/72)	*C*-terminal sequence of HA_1-chain, 160 residues, and *N*-terminal sequence of HA_2-chain, 153 residues	228
Membrane protein	influenza virus (A/WSN)	*N*-terminal sequence of 5 CNBr peptides	229
Phosphoprotein p12	mouse leukemia virus	*N*-terminal sequence of 3 p12 proteins, 15 residues each	230
Polyhedrin	baculoviruses	*N*-terminal sequence of 2 polyhedrin, 40 residues each	231
Structural protein p34	bovine leukemia virus	*N*-terminal sequence, 55 residues	232
Blood-clotting proteins			
Antithrombin	bovine	sequence of thrombin cleavage site, 12 residues	233
Factor IX	bovine	complete sequence, 416 residues, comparison to Factor X, protein C, and Prothrombin	234
Fibronectin	human	*N*-terminal sequence of gelatin-binding fragment, 17 residues	235
Fibrinogen α-chain	human	preliminary report of complete sequence	236
	human	sequence of large CNBr peptide, 236 residues, corresponding to positions 241—476	237

[228] C. W. Ward and T. A. Dopheide, *Br. Med. Bull.*, 1979, **35**, 51.
[229] B. H. Robertson, A. S. Bhown, R. W. Compans, and J. C. Bennett, *J. Virol.*, 1979, **30**, 759.
[230] L. E. Henderson, R. V. Gilden, and S. Oroszlan, *Science*, 1979, **203**, 1346.
[231] G. F. Rohrmann, T. J. Bailey, B. Brimhall, R. R. Becker, and G. S. Beaudreau, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4976.
[232] S. Oroszlan, T. D. Copeland, L. E. Henderson, J. R. Stephenson, and R. V. Gilden, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2996.
[233] H. Jörnvall, W. W. Fish, and I. Björk, *FEBS Lett.*, 1979, **106**, 358.
[234] K. Katayama, L. H. Ericsson, D. L. Enfield, K. A. Walsh, H. Neurath, E. W. Davie, and K. Titani, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 4990.
[235] L. I. Gold, A. Garcia-Pardo, B. Frangione, E. C. Franklin, and E. Pearlstein, *Proc. Natl. Acad. Sci., USA*, 1979, **76**, 4803.
[236] R. F. Doolittle, B. A. Cottrell, D. Strong, and K. W. K. Watt, *Thromb. Res.*, 1979, **14**, 787.
[237] D. D. Strong, K. W. Watt, B. A. Cottrell, and R. F. Doolittle, *Biochemistry*, 1979, **18**, 5399.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
	human	location of cross-linking acceptor sites, Gln-328 and -366	238
	human	complete sequence, 610 residues	239, 240
Fibrinogen α- and β-chains	bovine	*N*-terminal sequence of CNBr peptides-α and -β from α- and β-chains, 30 and 32 residues	241
Fibrin α-chain	human	sequence of middle section of α-chain, 184 residues	242
Fibrinogen β- and γ-chains	human	*N*-terminal sequence of γ-chain, 87 residues, and tryptic glycopeptide of β-chain, 21 residues, carbohydrate attachment site at positions 52 of γ-chain and 18 of β-chain peptide	243
Fibrinogen γ-chain	human	complete sequence, 410 residues	244
Fibrinogen β-chain	human	complete sequence, 461 residues, carbohydrate attachment site at position 364	245
Fibrinogen and fibrin, β-chain	human	*N*-terminal sequence of Bββ- and β-chains, 118 and 104 residues, respectively	246
Prekallikrein	bovine	*N*-terminal sequence, 15 residues	247
Kallikrein	human	*N*-terminal sequence, 31 residues	248

[238] B. A. Cottrell, D. D. Strong, K. W. K. Watt, and R. F. Doolittle, *Biochemistry*, 1979, **18**, 5405.
[239] K. W. K. Watt, B. A. Cottrell, D. D. Strong, and R. F. Doolittle, *Biochemistry*, 1979, **18**, 5410.
[240] R. F. Doolittle, K. W. K. Watt, B. A. Cottrell, D. D. Strong, and M. Riley, *Nature (London)*, 1979, **280**, 464.
[241] R. A. Martinelli, A. S. Inglis, M. R. Rubira, T. C. Hageman, J. G. R. Hurrell, S. J. Leach, and H. A. Scheraga, *Arch. Biochem. Biophys.*, 1979, **192**, 27.
[242] A. Henschen, F. Lottspeich, and B. Hessel. *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1951.
[243] E. Toepfer-Petersen, F. Lottspeich, and A. Henschen, *Thromb. Haemostasis*, 1979, **41**, 671.
[244] A. Henschen, F. Lottspeich, E. Toepfer-Petersen, and R. Warbinek, *Thromb. Haemostasis*, 1979, **41**, 662.
[245] K. W. K. Watt, T. Takagi, and R. F. Doolittle, *Biochemistry*, 1979, **18**, 68.
[246] B. Hessel, M. Makino, S. Iwanaga, and B. Blombaeck, *Eur. J. Biochem.*, 1979, **98**, 521.
[247] R. L. Heinmark and E. W. Davie, *Biochemistry*, 1979, **18**, 5743.
[248] F. Lottspeich, R. Geiger, A. Henschen, and C. Kutzbach, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1947.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Protein S	human and bovine	*N*-terminal sequence, 13 residues, both have 10 Gla residues	249
Protein S	bovine	*N*-terminal sequence, 13 residues	250
Complement proteins			
C2 and factor B	human	*N*-terminal sequence of C2a, C2b, Ba, and Bb, 10 residues each	251
$C1_q$	human	sequence of collagen-like region of A-, B-, and C-chains of $C1_q$, 110, 107, and 96 residues, respectively	252
C3	human	*N*-terminal sequence of C3α, C3β, and C3b d^1, 15, 10, and 20 residues, respectively	253
C4	human	*C*-terminal sequence of C4a (anaphylatoxin), 3 residues	254
C4a	bovine	*N*-terminal sequence, 13 residues	255
C5	human	*N*-terminal sequence of C5, C5d, and C5β, 10 residues, each	256
Factor B	human	*N*-terminal sequence of Bb, *C*-terminal sequence of Ba and Bb, 8, 3, and 2 residues, respectively	257
Factor D	human	*N*-terminal sequence, 9 residues	258

[249] R. G. Di Scipio and E. W. Davie, *Biochemistry*, 1979, **18**, 899.
[250] J. Stenflo and M. Jönsson, *FEBS Lett.*, 1979, **101**, 377.
[251] M. A. Kerr, *Biochem. J.*, 1979, **183**, 615.
[252] K. B. M. Reid, *Biochem. J.*, 1979, **179**, 367.
[253] B. F. Tack, S. C. Morris, and J. W. Prahl, *Biochemistry*, 1979, **18**, 1497.
[254] J. P. Gorski, T. E. Hugli, and H. J. Müller-Eberhard, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5299.
[255] N. A. Booth, R. D. Campbell, M. A. Smith, and J. E. Fothergill, *Biochem. J.*, 1979, **183**, 573.
[256] B. C. Tack, S. C. Morris and J. W. Prahl, *Biochemistry*, 1979, **18**, 1490.
[257] P. H. Lesavre, T. E. Hugli, A. F. Esser, and H. J. Müller-Eberhard, *J. Immunol.*, 1979, **123**, 529.
[258] A. E. Davis III, C. Zalut, F. S. Rosen, and C. A. Alper, *Biochemistry*, 1979, **18**, 5082.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
HLA antigens and antibodies			
Antiarsonate antibody	mouse (A/J strain)	*N*-terminal sequence of L- and H-chains, 30 and 10 residues	259
Anti-*p*-azophenyl-arsonate antibody	mouse (A/J strain)	sequence of H-chain variable region	260
Anti-*p*-azophenyl arsonate monoclonal antibody	mouse (A/J strain)	*N*-terminal sequence of L- and H-chains, 30 residues	261
Antibody K29-213	rabbit	sequence of L-chain variable region, 120 residues	262
Antibody K49-501	rabbit	sequence of L-chain variable region, 150 residues	263
Antidextran antibody B1355	mouse (BALB/c)	*N*-terminal sequence of 2γ and 2μ chains	264
Antiphosphocholine antibody	mouse (CBA/J and C57Bl/6J)	*N*-terminal sequence of H-chain variable region, 36 residues	265
Bence–Jones protein (Kue)	human	sequence of k-chain variable region, 108 residues	266
Bence–Jones cryoprotein (TOG)	human	complete sequence, 217 residues	267
β_2-microglobulin	rabbit	complete sequence, 99 residues	268
Pre-β_2 microglobulin	mouse	*N*-terminal extension of protein synthesized *in vitro*, 19 residues	269
H2-2^a antigen	mouse	*N*-terminal sequence of H-chain, 27 residues	270
H2-H-2K^b antigen	mouse (EL4)	partial sequence, 156 of 170 residues	271

259 J. J. Marchalonis, G. W. Warr, P. Smith, G. S. Begg, and F. J. Morgan, *Biochemistry*, 1979, **18**, 560.
260 J. D. Capra and A. Nisonoff, *J. Immunol.*, 1979, **123**, 279.
261 P. Estess, A. Nisonoff, and J. D. Capra, *Mol. Immunol.*, 1979, **16**, 1111.
262 H. Huser and D. G. Braun, *Scand. J. Immunol.*, 1979, **9**, 105.
263 D. G. Braun, H. Huser, R. Knecht, and J. K. Wright, *Hoppe-Seyler's Z. Physiol Chem.*, 1979, **360**, 663.
264 J. Schilling, D. Hansburg, J. M. Davie, and L. Hood, *J. Immunol.*, 1979, **123**, 384.
265 J. L. Claflin and S. Rudikoff, *J. Immunol.*, 1979, **122**, 1402.
266 M. Eulitz, H.-P. Kley, and H.-J. Zeitler, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 725.
267 Y. Nabeshima and T. Ikenaka, *Mol. Immunol.*, 1979, **16**, 439.
268 F. T. Gates, tert., J. E. Coligan, and T. J. Kindt, *Biochemistry*, 1979, **18**, 2267.
269 V. R. Lingappa, B. A. Cunningham, S. M. Jazwinski, T. P. Hopp, G. Blobel, and G. M. Edelman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3651.
270 O. Henriksen, E. A. Robinson, and E. Appella, *J. Biol. Chem.*, 1979, **254**, 7651.
271 J. E. Coligan, T. J. Kindt, B. M. Ewenstein, H. Uehara, J. M. Martinko, and S. G. Nathenson, *Mol. Immunol.*, 1979, **16**, 3.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
HLA antigen	human	*N*-terminal sequence of papain-solubilized H-chain, 30 residues	272
	human	sequence of H-chain fragment, 90 residues	273
	human	*N*-terminal sequence of 2 CNBr peptides and 4 partial acid cleavage peptides, 9—10 residues each	274
	human	*N*-terminal sequence of 2 partial acid cleavage peptides from H-chain, 41 and 31 residues	275
HLA-B7 antigen	human lympho-blastoid line JY	complete sequence of papain-solubilized H-chain, 271 residues, carbohydrate attachment site at Asn-86, disulphide bonds at Cys-101—164 and -203—259	276
	human	comparison of an 88 residues fragment with Ig constant domains	277
HLA-B7 and HLA-A2 antigens	human	complete sequence of HLA-B7, 271 residues, and *N*-terminal sequence of HLA-A2, 167 residues	278
Ia antigens	guinea pig	partial *N*-terminal sequence of 3 β-chains	279

[272] L. Trägårdh, B. Curman, K. Wiman, L. Rask, and P. A. Peterson, *Biochemistry*, 1979, **18**, 2218.
[273] L. Trägårdh, L. Rask, K. Wiman, J. Fohlman, and P. A. Peterson, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5839.
[274] L. Trägårdh, K. Wiman, L. Rask, and P. A. Peterson, *Biochemistry*, 1979, **18**, 1322.
[275] K. Wiman, L. Trägårdh, L.Rask, and P. A. Peterson, *Eur. J. Biochem.*, 1979, **95**, 265.
[276] H. T. Orr, J. A. Lopez de Castro, D. Lancet, and J. L. Strominger, *Biochemistry*, 1979, **18**, 5711.
[277] H. T. Orr, D. Lancet, R. J. Robb, J. A. Lopez de Castro, and J. L. Strominger, *Nature (London)*, 1979, **282**, 266.
[278] H. T. Orr, J. A. Lopez de Castro, P. Parham, H. L. Ploegh, and J. L. Strominger, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4395.
[279] M. J. Waxdal, T. Y. Basham, L. T. Clement, E. M. Shevach, and B. D. Schwartz, *Mol. Immunol.*, 1979, **16**, 61.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
I-E antigens	mouse	partial *N*-terminal sequence of α- and β-chains from 2 strains of mice, 32 residues	280
Immunoglobulin H-chains	mouse myeloma	sequence of variable region of 3 H-chains, 120 residues	281
Immunoglobulin (k-type) L-chains	mouse myeloma	*N*-terminal sequence of 27 k L-chains, 23 residues each	282
Immunoglobulin μ-chain	mouse myeloma	complete sequence, 576 residues	283
Immunoglobulin, post-γ-globulin	human	*N*-terminal sequence of 2 electrophoretic forms, 52 and 29 residues	284
Immunoglobulin precursors	rat and mouse	sequence of *N*-terminal extensions of 9 precursors of L- and H-chains	285
Immunoglobulin A	mouse myeloma (MOPC 47A)	complete sequence of H(α)-chain, 330 residues	286
Immunoglobulin IgA1 (Bur)	human	sequence of 52 chymotryptic peptides of α 1-chain	287
	human	sequence of 66 thermolytic peptides of α 1-chain	288
	human	sequence of 28 tryptic peptides and CNBr peptides of α 1-chain	289
	human	complete sequence of α 1-chain, 472 residues	290

[280] J. Silver, L. E. Walker, R. A. Reisfeld, M. A. Pellegrino, and S. Ferrone, *Mol. Immunol.*, 1979, **16**, 37.
[281] D. N. Rao, S. Rudikoff, H. Krutzsch, and M. Potter, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2890.
[282] E. Loh, B. Black, R. Riblet, M. Weigert, J. M.Hood, and L. Hood, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1395.
[283] M. Kehry, C. Sibley, J. Fuhrman, J. Schilling, and L. E. Hood, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2932.
[284] C. Tonnelle, A. Colle, M. Fougereau, and Y. Manuel, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 613.
[285] I. Schecter, O. Wolf, R. Zemell, and Y. Burstein, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 1839.
[286] E. A. Robinson and E. Apella, *J. Biol. Chem.*, 1979, **254**, 11418.
[287] Y.-S. V. Liu and F. W. Putnam, *J. Biol. Chem.*, 1979, **254**, 2839.
[288] Y.-S. V. Liu, T. L. K. Low, and F. W. Putnam, *J. Biol. Chem.*, 1979, **254**, 2859.
[289] T. L. K. Low, Y.-S. V. Liu, and F. W. Putnam, *J. Biol. Chem.*, 1979, **254**, 2850.
[290] F. W. Putnam, Y.-S. V. Liu, and T. L. K. Low, *J. Biol. Chem.*, 1979, **254**, 2865.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
	human	complete sequence of λ L-chain, 214 residues	291
Immunoglobulin IgA2 [A2m (1) allotype]	human myeloma	sequence of α 2 H-chains constant region, 339 residues	292
Immunoglobulin IgG, anti-S III antibody	rabbit	sequence of b4 L-chain variable region, 128 residues	293
Immunoglobulin IgG	rabbit (b4b5)	partial sequence of b5 L-chain variable region, positions 29—71	294
Immunoglobulin IgG	domestic rabbit, hare, cottontail, rabbit, and pike	sequence of tryptic peptides (T-19), 16 residues, differences correlated to serological polymorphism	295
Immunoglobulin (Cryo-) IgG1 Hil	human myeloma	sequence of H-chain variable region, 125 residues	296
Immunoglobulin IgM, μ-chain	canine	sequence of CH_1 and CH_2 domains, completes the sequence of the μ-chain	297
Myeloma protein Dob, IgG	human	sequence of H-chain variable region, 129 residues	298
	human	sequence of hinge region, 15 residues	299
Myeloma protein Tro, Monoclonal IgA1	human	characterization of disulphide bonds, 7 in L-chain and 17 in H-chain	300
	human	complete sequence of L-chain, 216 residues	301

[291] A. J. Infante and F. W. Putnam, *J. Biol. Chem.*, 1979, **254**, 9006.
[292] Y. Tsuzukida, C.-C. Wang, and F. W. Putnam, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1104.
[293] A. Chersi, E. Appella, S. Carta, and R. Mage, *Mol. Immunol.*, 1979, **16**, 589.
[294] A. Chersi, R. G. Mage, and C. B. Alexander, *Mol. Immunol.*, 1979, **16**, 833.
[295] J. Teherani, J. D. Capra, S. Aggarwal, and W. J. Mandy, *Eur. J. Immunol.*, 1979, **9**, 690.
[296] Y.-Y. H. Chiu, J. A. Lopez de Castro, and R. J. Poljak, *Biochemistry*, 1979, **18**, 553.
[297] L. J. McCumber and J. D. Capra, *Mol. Immunol.*, 1979, **16**, 565.
[298] L. A. Steiner, A. Garcia-Pardo, and M. N. Margolies, *Biochemistry*, 1979, **18**, 4068.
[299] L. A. Steiner and D. A. Lopes, *Biochemistry*, 1979, **18**, 4054.
[300] C.-Y. Yang, H. Kratzin, H. Götz, and N. Hilschmann, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1919.
[301] R. Scholz, C.-Y. Yang, and N. Hilschmann, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1903.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Myeloma proteins U-61 and E-109	mouse (BALB/c)	sequence of k-chain variable region, 108 residues	302
Myeloma protein Zie, IgG2	human	sequence of 3 H-chain constant domains, positions 113—202 (CH1), 253—341 (CH2), and 342—446 (CH3)	303
Thy-1 antigen	rat brain	partial sequence, positions 19—33 and 61—105, homology with Eu myeloma IgG constant domains	304
Miscellaneous			
Acetylcholine receptor	*Torpedo californica*	*N*-terminal sequence of major subunit, 25 residues	305
	Torpedo marmorata	*N*-terminal sequence of 40 000 subunit, 20 residues	306
Apovitellenin I	turkey egg yoke	complete sequence, 82 residues	307
Bacteriorhodopsin	*Halobacterium*	*N*-terminal and *C*-terminal sequence, 83 and 39 residues, respectively	308
	Halobacterium halobium	review of complete sequence, 247 residues	309
	Halobacterium halobium	partial *N*-terminal and *C*-terminal sequence, 102 and 39 residues, respectively	310
	Halobacterium halobium	complete sequence, 248 residues	311

[302] M. Vrana, S. Rudikoff, and M. Potter, *J. Immunol.*, 1979, **122**, 1905.
[303] G. E. Connell, D. M. Parr, and T. Hofmann, *Can. J. Biochem.*, 1979, **57**, 758.
[304] D. G. Campbell, A. F. Williams, P. M. Bayley, and K. B. M. Reid, *Nature (London)*, 1979, **282**, 341.
[305] M. W. Hunkapiller, C. D. Strader, L. Hood, and M. A. Raftery, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 164.
[306] A. Deviller-Thiery, J.-P. Changeux, P. Paroutaud, and A. D. Strosberg, *FEBS Lett.*, 1979, **104**, 99.
[307] A. S. Inglis, P. M. Strike, W. C. Osborne, and R. W. Burley, *FEBS Lett.*, 1979, **97**, 179.
[308] J. E. Walker, A. F. Carne, and H. W. Schmitt, *Nature (London)*, 1979, **278**, 653.
[309] Y. A. Ovchinnikov, N. G. Abdulaev, M. Y. Feigina, A. V. Kiselev, and N. A. Lobanov, *FEBS Lett.*, 1979, **100**, 219.
[310] G. E. Gerber, R. J. Anderegg, W. C. Herlihy, C. P. Gray, K. Biemann, and H. G. Khorana, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 227.
[311] H. G. Khorana, G. E. Gerber, W. C. Herlihy, C. P. Gray, R. J. Anderegg, K. Nihel, and K. Biemann, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5046.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Calcium-binding protein	porcine intestine	complete sequence, 78 residues	312
Carboxypeptidase inhibitor	potato	determination of 3 disulphide bonds, Cys-8—24, -12—27, and -18—34	313
β-casein	ovine milk	complete sequence of β_1-casein, 207 residues, and partial sequence of β_2-casein, 2 multiphosphorylated β-casein components	314
β-casomorphins (opioid peptides)	bovine milk	heptapeptide and pentapeptide derived from β-casein, both with opioid activity	315
Ceruloplasmin	human	sequence of a histidine-rich proteolytic fragment, 159 residues	316
Chromosomal protein HMG 14	calf thymus	complete sequence, 100 residues	317
C-reactive protein	human	complete sequence, 187 residues	318
DNA-binding protein HU	*Cyanobacterium synechocystis*	*N*-terminal and *C*-terminal sequence, 27 and 4 residues, respectively	319
Elongation factor 2 (EF-2)	bovine and wheat	sequence of peptides from beef and wheat EF-2 containing the ADP-ribosylation site, 15 and 18 residues, respectively	320
Elongation factor TU	*E. coli*	location of methylation site at Lys-56	321
Fimbrial colonization Factor CFA/1	*E. coli*	*N*-terminal sequence, 22 residues	322

[312] T. Hofmann, M. Kawakami, A. J. W. Hitchman, J. E. Harrison, and K. J. Dorrington, *Can. J. Biochem.*, 1979, **57**, 737.
[313] T. R. Leary, D. T. Grahn, H. Neurath, and G. M. Hass, *Biochemistry*, 1979, **18**, 2252.
[314] B. C. Richardson and J. C. Mercier, *Eur. J. Biochem.*, 1979, **99**, 285.
[315] A. Henschen, F. Lottspeich, V. Brantl, and H. Teschemacher, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1217.
[316] I. B. Kingston, B. L. Kingston, and F. W. Putnam, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1668.
[317] J. M. Walker, G. H. Goodwin, and E. W. Johns, *FEBS Lett.*, 1979, **100**, 394.
[318] E. B. Oliveira, E. C. Gotschlich, and T.-Y. Liu, *J. Biol. Chem.*, 1979, **254**, 489.
[319] A. Aitken and J. Rouviere-Yaniv, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 461.
[320] B. A. Brown and J. W. Bodley, *FEBS Lett.*, 1979, **103**, 253.
[321] J. J. L'Italien and R. A. Laursen, *FEBS Lett.*, 1979, **107**, 359.
[322] P. Klemm, *FEBS Lett.*, 1979, **108**, 107.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
K88 Fimbrial proteins	*E. coli*	*N*-terminal and *C*-terminal sequence of 4 variants, 23 and 24 residues, respectively, also *N*-terminal sequence of CNBr peptides	323
Folate-binding protein	bovine milk	*N*-terminal, *C*-terminal, and *N*-terminal sequence of a CNBr peptide, 62, 31, and 31 residues, respectively	324
Glycinin	soya bean seeds	*N*-terminal sequence of the 10 subunits	325
Glycinin and legumin	soya bean and broad bean	*N*-terminal sequence of both basic subunits, 29 residues each	326
Glycopeptide	bovine, ovine, and porcine pituitary	complete sequence, 39 residues each, strong homology	327
Glycophorin	porcine	partial sequence of chymotryptic and a tryptic peptide, 19 and 36 residues, respectively	328
Major glycoprotein (BR-PO protein)	bovine myelin	sequence of a glyco-peptide, 5 residues	329
Initiation Factor IF-1	*E. coli*	complete sequence, 71 residues	330
Inter-α-trypsin inhibitor (ITI)	human	sequence of the two Kunitz-type domains, 125 residues	331
α-Lactalbumin	goat	complete sequence, 123 residues	332
	rabbit	complete sequence, 122 residues	333

[323] W. Gaastra, P. Klemm, J. M. Walker, and F. K. De Graaf, *FEMS Microbiol. Lett.*, 1979, **6**, 15.
[324] I. Svendsen, B. Martin, T. G. Pedersen, H. S. Ingemann, J. Holm, and J. Lyngbye, *Carlsberg Res. Commun.*, 1979, **44**, 89.
[325] M. A. Moreira, M. A. Hermodson, B. A. Larkins, and N. C. Nielsen, *J. Biol. Chem.*, 1979, **254**, 9921.
[326] J. Gilroy, D. J. Wright, and D. Boulter, *Phytochemistry*, 1979, **18**, 315.
[327] D. G. Smyth and D. E. Massey, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1006.
[328] K. Honma, M. Tomita, and A. Hamada, *Biochem. Biophys. Acta*, 1979, **580**, 210.
[329] K. Kitamura, A. Suzuki, M. Suzuki, and K. Uyemura, *FEBS Lett.*, 1979, **100**, 67.
[330] C. L. Pon, B. Wittmann-Liebold, and C. Gualerzi, *FEBS Lett.*, 1979, **101**, 157.
[331] E. Wachter and K. Hochstrasser, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1305.
[332] R. T. A. MacGillivary, K. Brew, and K. Barnes, *Arch. Biochem. Biophys.*, 1979, **197**, 404.
[333] T. P. Hopp and K. R. Woods, *Biochemistry*, 1979, **18**, 5182.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
	rat	partial sequence of tryptic glycopeptide responsible for charge heterogeneity, 50 residues	334
Lactogen precursor	human placenta	sequence of *N*-terminal extension of precursor synthesized *in vitro*, 25 residues	335
β-Lactoglobulin	goat	complete sequence, 162 residues	336
α_2-Macroglobulin	human	*N*-terminal sequence, 13 residues, the protein is composed of 4 identical polypeptide chains	337
	human	sequence of chymotryptic peptide containing the alkylamine-sensitive site, 9 residues	338
Macromomycin	*Streptomyces macromomyceticus*	*N*-terminal sequence, 45 residues	339
Membrane protein I	*E. coli* (B/r)	complete sequence, 340 residues	340
Non-histone protein C23	rat (Novikoff hepatoma)	sequence of acidic peptide, 42 residues, phosphorylated at Ser-8, -25, and -28	341
Non-histone protein H6	rainbow trout testis	complete sequence, 69 residues	342
Non-histone high-mobility-group protein 2 (HMG-2)	calf thymus	*N*-terminal sequence of CNBr peptides	343
	calf thymus	*N*-terminal sequence of tryptic peptide glutamic and aspartic acid-rich, 16 residues	344

[334] R. Prasad, B. G. Hudson, R. Butkowski, J. W. Hamilton, and K. E. Ebner, *J. Biol. Chem.*, 1979, **254**, 10607.
[335] L. M. Sherwood, Y. Burstein, and I. Schechter, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3819.
[336] G. Préaux, G. Braunitzer, B. Schrank, and A. Stangl, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1595.
[337] R. P. Swenson and J. B. Howard, *J. Biol. Chem.*, 1979, **254**, 4452.
[338] R. P. Swenson and J. B. Howard, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4313.
[339] T. H. Sawyer, K. Guetzow, M. O. J. Olson, H. Busch, A. W. Prestayko, and S. T. Crooke, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1133.
[340] R. Chen, C. Krämer, W. Schmidmayr, and U. Henning, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5014.
[341] M. D. Mamrack, M. O. J. Olson, and H. Busch, *Biochemistry*, 1979, **18**, 3381.
[342] D. C. Watson, N. C. W. Wong, and G. H. Dixon, *Eur. J. Biochem.*, 1979, **95**, 193.
[343] J. M. Walker, K. Gooderham, and E. W. Johns, *Biochem. J.*, 1979, **181**, 659.
[344] J. M. Walker, K. Gooderham, and E. W. Johns, *Biochem. J.*, 1979, **179**, 253.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Permetin A	*Bacillus circulans*	complete sequence of cyclic-acyl nonapeptide	345
Phosphatidylcholine exchange protein	bovine liver	*N*-terminal sequence of peptide containing binding site, 38 residues	346
Preproalbumin	bovine	sequence of *N*-terminal extension of protein synthesized *in vitro*, 18 residues	347
Progesterone-induced secretory protein (Preuteroglobin)	rabbit	sequence of *N*-terminal extension of protein synthesized *in vitro*, 21 residues	348
Proline-rich peptides	human saliva	sequence of 2 peptides, P-A has 22 Pro residues and P-B 30	349
Proline-rich phosphoprotein	human saliva	complete sequence, 106 residues, including 24 Pro residues	350
Protease inhibitor	egg plant	complete sequence, 52 residues	351
Protein A	*Bacillus megaterium*	complete sequence, 61 residues	352
M Protein	streptococcal	*N*-terminal sequence of CNBr peptides, homology with mammalian tropomyosin	353
Proteinase inhibitor I	adzuki bean	complete sequence, 82 residues	354
Proteinase inhibitor II and II^1	adzuki bean	complete sequence of both, 81 and 72 residues, II^1 is identical to II but lacks 9 *N*-terminal residues	355
Proteinase inhibitor DE-2 and DE-4	leguminosae seeds	complete sequences of both inhibitors, 76 residues each	356

[345] Y. Takeuchi, A. Murai, Y. Takahara, and M. Kainosho, *J. Antibiot.*, 1979, **32**, 121.
[346] P. Moonen, H. P. Haagsman, L. L. M. Van Deenen, and K. W. A. Wirtz, *Eur. J. Biochem.*, 1979, **99**, 439.
[347] R. T. A. MacGillivray, D. W. Chung, and E. W. Davie, *Eur. J. Biochem.*, 1979, **98**, 477.
[348] M. L. Malsky, D. W. Bullock, J. J. Willard, and D. N. Ward, *J. Biol. Chem.*, 1979, **254**, 1580.
[349] S. Isemura, E. Saitoh, and K. Sanada, *J. Biochem.* (*Tokyo*), 1979, **86**, 79.
[350] R. S. C. Wong, T. Hofman, and A. Bennick, *J. Biol. Chem.*, 1979, **254**, 4800.
[351] M. Richardson, *FEBS Lett.*, 1979, **104**, 322.
[352] P. Setlow and J. Ozols, *J. Biol. Chem.*, 1979, **254**, 11938.
[353] B. Hosein, M. McCarty, and V. A. Fischetti, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3765.
[354] C. Ishikawa, S. Nakamura, K. Watanabe, and K. Takahashi, *FEBS Lett.*, 1979, **99**, 97.
[355] M. Yoshikawa, T. Kiyohara, T. Iwasaki, Y. Ishii, and N. Kimura, *Agric. Biol. Chem.*, 1979, **43**, 787.
[356] F. J. Joubert, H. Kruger, G. S. Townshend, and D. P. Botes, *Eur. J. Biochem.*, 1979, **97**, 85.

Table 2 (*cont.*)

Protein	*Source*	*Comments*	*Ref.*
Proteolipid	bovine brain	*N*-terminal sequence of 4 CNBr peptides	357
Pseudomurein	*Methanobacterium thermoautrophicum*	sequence of peptide moiety	358
rec A protein	*E. coli*	*N*-terminal sequence, 31 residues	359
Retinol-binding protein (RBP)	human	*N*-terminal sequence, 18 residues	360
	human	*N*-terminal sequence, 119 residues, complete sequence of 4 out of 5 CNBr peptides	361
	human	complete sequence, 182 residues	362
Rhodotorucine A	*Rhodosporidium toruloides*	complete sequence, 11 residues, the *C*-terminal residue is *S*-farnesylcysteine	363
5-S RNA binding protein	*Saccharomyces cerevisiae*	*N*-terminal sequence of intact protein and a CNBr peptide, 30 and 48 residues, respectively	364
Sillucin	*Mucor pusillus*	complete sequence, 30 residues	365
Thaumatin I	fruit of *Thaumatococcus daniellii*	complete sequence, 207 residues, sweet-tasting protein	366
Thymosin	calf thymus	complete sequence of thymosin α_1 and β_1, 28 and 74 residues, respectively	367
Transferrin	rabbit	sequence of tryptic glycopeptide, 5 residues	368

[357] J. Jollès, F. Schoentgen, P. Jollès, M. Vacher, C. Nicot, and A. Alfsen, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 619.
[358] H. Koenig and O. Kandler, *Arch. Microbiol.*, 1979, **121**, 271.
[359] P. T. Emmerson, F. D. Northrop, J. E. Walker, and S. C. West, *FEBS Lett.*, 1979, **106**, 349.
[360] B. K. Seon and D. Pressman, *Cancer Res.*, 1979, **39**, 4423.
[361] Y. Kanda and D. S. Goodman, *J. Lipid Res.*, 1979, **20**, 865.
[362] L. Rask, H. Anundi, and P. A. Peterson, *FEBS Lett.*, 1979, **104**, 55.
[363] Y. Kamiya, A. Sakurai, S. Tamura, N. Takahashi, E. Tsuchiya, K. Abe, and S. Fukui, *Agric. Biol. Chem.*, 1979, **43**, 363.
[364] R. N. Nazar, M. Yaguchi, G. E. Willick, C. F. Rollin, and C. Roy, *Eur. J. Biochem.*, 1979, **102**, 573.
[365] W. A. Bradley and G. A. Somkuti, *FEBS Lett.*, 1979, **97**, 81.
[366] R. B. Iyengar, P. Smits, F. Van der Ouderaa, H. Van der Wel, J. Van Brouwershaven, P. Ravestein, G. Richters, and P. D. Van Wassenaar, *Eur. J. Biochem.*, 1979, **96**, 193.
[367] T. L. K. Low and A. L. Goldstein, *J. Biol. Chem.*, 1979, **254**, 987.
[368] D. K. Strickland, J. W. Hamilton, and B. G. Hudson, *Biochemistry*, 1979, **18**, 2549.

Protein	*Source*	*Comments*	*Ref.*
Tryptophan-containing peptide	human uraemic fluid	complete sequence, 5 residues, corresponds to positions 123—127 of fibrinogen β-chain	369
α-Tubulin	porcine brain	*C*-terminal sequence, 78 residues	370
Uteroglobin	rabbit	*N*-terminal sequence, 50 residues, sequence of *N*-terminal extension of protein synthesized *in vitro*, 21 residues	371
Vitamin D binding protein (Group-specific component)	human serum	*N*-terminal sequence of 3 types, 20 residues	372

Part IC: Chemical Modification of Proteins

by D. M. Moran and A. J. Garman

1 Introduction

A comprehensive list of proteins which have been modified, apart from those discussed in the narrative section, is given in the Table. Proteins cited in the main text are listed in alphabetical order in the Appendix, together with their text references.

[369] T. Abiko, I. Onodera, and H. Sekino, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 813.
[370] H. Ponstingl, M. Little, E. Krauhs, and T. Kempf, *Nature (London)*, 1979, **282**, 423.
[371] M. Atger, J.-C. Mercier, G. Haze, F. Fridlansky, and E. Milgrom, *Biochem. J.*, 1979, **177**, 985.
[372] J. Svasti, A. Kurosky, A. Bennett, and B. H. Bowman, *Biochemistry*, 1979, **18**, 1611.

Table *Chemical modification of proteins (see also Appendix)*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Acetylcholinesterase	*Torpedo californica*	[1-(dimethylamino)naphthalene-5-sulphonamido]pentylmethyl phosphonofluoridate	Ser	conformational changes	1
Acetylcholinesterase	*Electrophorus electricus*	fluorinated aldehydes and ketones		quasi-substrate inhibition	2
Acetylcholinesterase	electric eel	various 1,3,2-dioxaphosphorinan 2-oxides		unstable covalent intermediates formed	3
Acetylcholinesterase	human erythrocyte	di-imidates	Lys	molecular forms identified	4
Acetylcholine receptor	*Torpedo californica*	CH_3NH_2–NaOH		phosphoserine residues identified	5
Acetylcholine receptor	*Torpedo californica*	various thiol and disulphide reagents	Cys	effect on affinity states	6
Acetylcholine receptor	rat mitochondria	DTNB	Cys	effect on agonist-specific state transitions	7
Acetylcholine receptor	bovine nucleus caudatus	*N*-[2′,3′-$^3H_2$]propyl-*N*-2-aziridinium methylbenzilate		effect of detergents on labelled receptor	8
Acetylcholine receptor	cat muscle, *Torpedo* electric organ	bromoacetylcholine		used to isolate receptor, subunits labelled compared	9
Acid phosphatase	human prostate	potassium (*N*-ethyl-5-phenylisoxalium-3′-sulphonate)	carboxyl	essential carboxylic acid	10

[1] D. J. Epstein, H. A. Berman, and P. Taylor, *Biochemistry*, 1979, **18**, 4749.
[2] U. Brodbeck, K. Schweikert, R. Gentinetta, and M. Rottenberg, *Biochim. Biophys. Acta*, 1979, **567**, 357.
[3] Y. Ashani and H. Leader, *Biochem. J.*, 1979, **177**, 781.
[4] C. R. Römer-Lüthi, J. Hajdu, and U. Brodbeck, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 929.
[5] R. L. Vandlen, C. S. Wilson, J. C. Eisenach, and M. A. Raftery, *Biochemistry*, 1979, **18**, 1845.
[6] H. Hsiao-Ping Moore and M. A. Raftery, *Biochemistry*, 1979, **18**, 1907.
[7] R. J. Luckas, H. Morimoto, and E. L. Bennett, *Biochemistry*, 1979, **18**, 2384.
[8] K. P. Ruess and M. Liefländer, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 627.
[9] A. Lyddiatt, K. Sumikawa, J. M. Wolosin, J. O. Dolly, and E. A. Barnard, *FEBS Lett.*, 1979, **108**, 20.
[10] M. E. Saini and R. L. Van Etten, *Biochim. Biophys. Acta*, 1979, **568**, 370.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Acid protease	*Endothia parasitica*	1,2-epoxy-3-(*p*-nitrophenoxy)-propane	Asp-220 Asp-33	structural studies	11
Actin	chicken gizzard	glutaraldehyde ($\pm Ca^{2+}$)	Lys	evidence for actin-linked regulation	12
Actin	various sources	2-nitro-5-thiocyanobenzoic acid	Cys	Cys residue in similar position on all actins	13
Actin (F)	rabbit muscle	glutaraldehyde	Lys	interaction with heavy meromyosin studied	14
Actinidin	*Actinidia chinensis*	2,2′-dipyridyl disulphide	Cys	kinetic study	15
Acyl phosphatase	horse muscle	O_2, sodium selenite	Cys	dimer studies	16
Adenosine deaminase	calf intestinal mucosa	(i) 9-(4-bromoacetamidobenzyl)-adenine, (ii) 9-(2-bromoacetamidobenzyl)-adenine	(i) Lys (ii) His	affinity labelling	17
Adenosine deaminase	human erythrocyte	various cross-linking reagents	Lys	stoicheiometry of binding complex examined	18
Adenylate cyclase	bovine corpus luteum	(i) 3,3′-dithiobispropionimidate, (ii) methyl acetimidate	Lys	(i) inhibition at >0.02 mM; (ii) no inhibition at 10 mM	19
Adenylate cyclase	yeast	butane-2,3-dione, cyclohexane-1,2-dione, and phenylglyoxal	Arg	inactivation	20
Adenyl cyclase	rat adipocytes	various thiol reagents	$(Cys)_2$,Cys	activity partially regulated by oxidation–reduction status	21
β-Adrenergic receptor	turkey erythrocyte	*N*-ethylmaleimide		effect on agonist binding	22
β-Adrenergic receptor	turkey erythrocyte	dithiothreitol	$(Cys)_2$	effect on antagonist binding	23
Adrenodoxin	bovine adrenocortical mitochondria	(i) *N*-(2,2,5,5,-tetramethyl-3-carbonylpyrroline-1-oxyl)-imidazole (ii) *N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl) maleimide	(i) Tyr (ii) Cys (Cys-46?)	spin-labelling study	24
Agglutinin	*Ricinus communis*	various thiol reagents and protease inhibitors	Ser,Cys	effect on SDS-heat induced dissociation	25
Alanine aminotransferase	porcine heart	3-chloro-L-alanine		affinity labelling	26

Albumin	bovine	(i) tetranitromethane (ii) phenylglyoxal	(i) Tyr (ii) Arg	antigenicity studies	27
Albumin	bovine	linoleic acid–ascorbic acid		oligomer formation	28
Albumin	bovine	8-methoxypsoralen, u.v. irradiation	Tyr(?)	photoconjugation	29
Albumin	bovine	various steroid *N*-hydroxy-succinimide esters	Lys	conjugate preparations	30
Albumin	bovine	butyric anhydride	Lys	delayed hypersensitivity studies	31
Albumin	bovine, human	bilirubin–photo-irradiation	His, Trp (Tyr)	u.v. and fluorescence study	32
Albumin	human	*N*-(2,5-dinitro-4-fluorophenyl)-4-amino-2,2,6,6-tetramethyl-piperidinoxy radical	Lys, Tyr	2 Lys particularly reactive	33

[11] C. Wong, T. J. Lee, T. Lee, T. Lu, and I. Yang, *Biochemistry*, 1979, **18**, 1638.
[12] T. Mikawa, *J. Biochem.* (*Tokyo*), 1979, **85**, 879.
[13] T. Nakamura, M. Yamaguchi, and T. Yanagisawa, *J. Biochem.* (*Tokyo*), 1979, **85**, 627.
[14] E. Próchniewica, *Biochim. Biophys. Acta.*, 1979, **579**, 346.
[15] K. Brocklehurst, T. Stuchburg, and J. P. G. Malthouse, *Biochem. J.*, 1979, **183**, 233.
[16] M. Stefani, A. Berti, G. Camici, G. Mana, G. Cappugi, and G. Ramponi, *Int. J. Pept. Protein Res.*, 1979, **14**, 227.
[17] A. Lucacchini, A. D. Bertolini, G. Ronca, D. Segnini, and C. A. Rossi, *Biochim. Biophys. Acta.*, 1979, **569**, 220.
[18] P. E. Daddona and W. N. Kelley, *Biochim. Biophys. Acta.*, 1979, **580**, 303.
[19] J. L. Young, *FEBS Lett.*, 1979, **104**, 294.
[20] K. Varimo and J. Londesborough, *FEBS Lett.*, 1979, **106**, 153.
[21] S. P. Mukherjee and W. S. Lynn, *Biochim. Biophys. Acta*, 1979, **568**, 224.
[22] S. Bottari, G. Vanquelin, O. Durieu, C. Klutchko, and A. D. Strosberg, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1311.
[23] G. Vanquelin, S. Bottari, L. Kanarek, and A. D. Strosberg, *J. Biol. Chem.*, 1979, **254**, 4462.
[24] R. Miura, T. Sugiyama, and T. Yamano, *J. Biochem.* (*Tokyo*), 1979, **85**, 1107.
[25] D. B. Cawley and L. L. Houston, *Biochim. Biophys. Acta*, 1979, **581**, 51.
[26] Y. Morino, H. Kojima, and S. Tanase, *J. Biol. Chem.*, 1979, **254**, 279.
[27] A. L. Kazim, A. F. S. A. Habeeb, and M. Z. Atassi, *Mol. Immunol.*, 1979, **16**, 457.
[28] M. Janado, Y. Yano, H. Nakamori, and T. Nishida, *J. Biochem.* (*Tokyo*), 1979, **86**, 177.
[29] K. Yoshikawa, N. Mori, S. Sakakibara, N. Hizunio, and P. Song, *Photochem. Photobiol.*, 1979, **29**, 1127.
[30] H. Hosoda, Y. Sabai, H. Yoshida, S. Miyairi, K. Ishii, and T. Nambara, *Chem. Pharm. Bull.*, 1979, **27**, 742.
[31] G. Drach and J. Chen-Marotel, *Int. Arch. Allergy Appl. Immunol.*, 1979, **59**, 28.
[32] F. F. Rubaltelli and G. Jori, *Photochem. Photobiol.*, 1979, **29**, 991.
[33] J. T. Greig, J. D. Reinheimer, and G. H. Robinson, *Biochim. Biophys. Acta*, 1979, **579**, 409.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Albumin	human	tetranitromethane	Tyr	binding of L-Trp and benzodiazepine studied	34
Albumin	human	di-isopropylfluorophosphate	Tyr	single Tyr residue modified	35
Albumin	human	[^{3}H]bilirubin–photo-irradiation		covalent conjugate formed	36
Albumin	human, rat	D-[6-^{3}H] glucose	Lys	glycosylation *via* Amadori rearrangement	37, 38
Albumin	human	dansyl chloride	Lys	binding of L-Trp and bilirubin studied	39
Alcohol dehydrogenase	*Bacillus stearothermophilus*	4-(3-bromoacetylpyridino)-butylphosphoadenosine	Cys-38	selective reaction of reactive Cys	40
Alcohol dehydrogenase	horse liver, yeast	(*R*-)- and (*S*-)-2-chloro-3-(5-imidazoyl) propionic acid and methyl esters	Cys	stereoselective metal-directed affinity labelling	41, 42
Alcohol dehydrogenase	horse liver	iodoacetic acid	Cys-46	effect on ^{109}Cd binding	43
Alcohol dehydrogenase	horse liver, yeast	4-(3-bromoacetylpyridino)-butylphosphoadenosine	Cys-46, Cys-43	inactivation of enzymes; differential effects of added Cys	44
Alcohol dehydrogenase	horse liver	bromo[1-^{13}C]acetate	Cys-46	NAD^+ and NADH binding studies	45
Aldehyde dehydrogenase	ovine liver	2,2′-dithiopyridine–NAD^+, 2,2′-dithiobis-(5-nitropyridine)	Cys	classes of thiol groups studied	46
Aldehyde reductase	porcine kidney	various reagents	Arg	essential Arg for NADPH interaction	47
Aldehyde reductase	human liver	various n-alkane methyl imidates and bisimidoesters	Lys	inactivation by intramolecular cross-links	48
Aldolase	rabbit muscle	cyclohexane-1,2-dione	Arg	Arg-55 in C-1 phosphate binding site	49
Alkaline phosphatase	porcine kidney	butane-2,3-dione, phenylglyoxal	Arg	inactivation studies	50
Alkaline phosphatase	*E. coli*, calf intestine, bovine liver	*p*-nitrophenol phosphate	Ser	substrate specificity patterns	51
Amine oxidase	*Aspergillus niger*	*β*-bromo-[^{14}C]ethylamine	Cys	2 moles label per mole enzyme	52
D-Amino-acid transaminase	*Bacillus sphaericus*	various thiol reagents	Cys	alkylation kinetics of thiol groups	53

α-Aminoisobutyrate decomposing enzyme	*Pseudomonas sp.*	(i) phenylglyoxal (ii) ethoxyformic anhydride	(i) Arg (ii) His	enzyme-inactivation studies	54
δ-Aminolevulinic acid dehydratase	bovine liver	(i) DTNB (ii) dibromoacetone–photo-irradiation	(i) Cys (ii) His	essential Cys and His residues studies	55
AMP deaminase	rat skeletal muscle	periodate-oxidised ATP, $NaBH_4$		inactivation	56
c-AMP-binding protein	*Dictyostelium discoideum cells*	8-azido-[^{32}P]-c-AMP		3 proteins labelled	57
c-AMP-binding protein	porcine kidney	N^6-(ethyl-2-diazomalonyl)-AMP		affinity label	58
c-AMP-receptor protein	*E. coli*	*o*-phenylenedimaleimide	Cys	receptor-conformation studies	59

[34] K. J. Fehske, W. E. Muller, and U. Wollert, *Biochim. Biophys. Acta,* 1979, **577**, 346.
[35] G. E. Means and Hua-Lin Wu, *Arch. Biochem. Biophys.,* 1979, **194**, 526.
[36] D. W. Hutchinson and D. S. Mutopo, *Biochem. J.,* 1979, **181**, 779.
[37] J. F. Day, S. R. Thorpe, and J. W. Baynes, *J. Biol. Chem.,* 1979, **254**, 595.
[38] J. F. Day, R. W. Thornburg, S. R. Thorpe, and J. W. Barnes, *J. Biol. Chem.,* 1979, **254**, 9394.
[39] C. Jacobsen and J. Jacobsen, *Biochem. J.,* 1979, **181**, 251.
[40] R. Jeck, C. Woenckhaus, J. I. Morris, and M. J. Runswick, *Eur. J. Biochem.,* 1979, **93**, 57.
[41] K. H. Dahl, J. S. McKinley-McKee, H. C. Beyerman, and A. Noordam, *FEBS Lett.,* 1979, **99**, 308.
[42] K. H. Dahl, J. S. McKinley-McKee, H. C. Beyerman, and A. Noordam, *FEBS Lett.,* 1979, **99**, 313.
[43] A. J. Sytkowski and B. L. Vallee, *Biochemistry,* 1979, **18**, 4095.
[44] C. Woenckhaus, R. Jeck, and H. Jornvall, *Eur. J. Biochem.,* 1979, **93**, 65.
[45] R. G. Khalifah and W. M. Sutherland, *Biochemistry,* 1979, **18**, 391.
[46] T. M. Kitson, *Biochem. J.,* 1979, **183**, 751.
[47] W. S. Davidson and T. G. Flynn, *J. Biol. Chem.,* 1979, **254**, 3724.
[48] B. Wermuth, J. D. B. Munch, J. Hajdu, and J.-P. von Wartburg, *Biochim. Biophys. Acta,* 1979, **566**, 237.
[49] L. Patthy, A. Váradi, J. Thész, and K. Kovács, *Eur. J. Biochem.,* 1979, **99**, 309.
[50] M. N. Woodrooft and P. J. Butterworth, *Biochem. J.,* 1979, **181**, 137.
[51] M. H. Jensen, *Biochim. Biochem. Acta,* 1979, **571**, 55.
[52] H. Kumagai, H. Uchida, and H. Yamada, *J. Biol. Chem.,* 1979, **254**, 10913.
[53] T. S. Soper, W. M. Jones, and J. M. Manning, *J. Biol. Chem.,* 1979, **254**, 10901.
[54] S. Sato, M. Honma, and T. Shimanura, *Agric. Biol. Chem.,* 1979, **42**, 2341.
[55] I. Tsukamoto, T. Yoshinaga, and S. Sano, *Biochim. Biophys. Acta,* 1979, **570**, 167.
[56] A. Raggi and M. Ranieri-Raggi, *Biochim. Biophys. Acta,* 1979, **566**, 353.
[57] L. J. Wallace and W. A. Frazier, *Proc. Natl. Acad. Sci. USA,* 1979, **76**, 4250.
[58] J. A. Near, F. C. Szoka, R. Olsen, and M. J. Elfinger, *Biochim. Biophys. Acta,* 1979, **587**, 522.
[59] C. Pampeno and J. S. Krakow, *Biochemistry,* 1979, **18**, 1519.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
α-Amylase	*Bacillus subtilis*	diazo-arsanilic acid	Lys	delayed hypersensitivity suppressed	60
Antigen E	ragweed pollen	maleimidobenzoyl-*N*-hydroxy-succinimide ester	Lys	preparation of protein–polymer conjugates	61
Antithrombin	bovine	*N*-bromosuccinimide	Trp	heparin-binding affected	62
Apolipoprotein A-1	human, rhesus monkey serum	dansyl chloride	Asp (N_2), Lys (N_6)	solution properties studied	63
Aquomethaemoglobin	human	iodoacetamide, *N*-(1-oxyl-2,6,6-tetramethyl-4-piperidinyl)-iodoacetamide	Cys	relaxation processes examined	64
L-Arabinose binding protein	*E coli*	2-chloromercuri-4-nitrophenol, DTNB	Cys	sugar-binding site identified	65
Arginase	bovine liver	polyethyleneglycol, cyanuric chloride	Lys	selective reduction in antigenicity	66
Argininosuccinate synthetase	rat liver	DTNB	Cys	4- and 16-SH groups identified in absence and presence of SDS	67
Arginyl-t-RNA synthetase	*E. coli*	*p*-hydroxymercuribenzoate	Cys	essential Cys	68
Arylsulphatase A	human liver	butane-2,3-dione, phenylglyoxal	Arg	*inactivation studies*	69
Aspartate transaminase	rat liver	various thiol reagents	Cys	uptake of enzyme into mitochondrion	70
Aspartate transaminase	porcine heart	potassium cyanate	Lys-258	stereochemical studies	71
Aspartate transcarbamoylase	*E. coli*	tartryl diazide	Lys	conformational states examined	72
Aspartate transcarbamylase	*E. coli*	2-chloromercuri-4-nitrophenol, mercaptoethanol	Cys	reversible modification	73, 74
ATPase	bovine heart	(i) 2-mercaptopropionylglycine; (ii) carbonylcyanide-*p*-trifluoromethoxy phenylhydrazone	Cys	(i) restored activity after treatment with (ii)	75
ATPase	bovine heart	various carbodi-imides	carboxyl	binding sites of [^{14}C]DCCD on β-subunit	76

ATPase	bovine heart	arylazido-β-alanyl-ATP		photoaffinity label	77
ATPase F_1	bovine heart	(i) [^{3}H]arylazido-ATP and ADP (ii) 4-chloro-7-nitrobenzofurazan		(i) affinity labelling (ii) protects β- but not α-site	78
ATPase	*E. coli*	4-chloro-7-nitrobenzofurazan	Tyr, Lys	at neutral pH binds to Tyr; upon alkalinization transfers to Lys	79
ATPase	*E. coli*	dicyclohexylcarbodi-imide	carboxyl	inactivation	80
ATPase	ovine kidney	2-(4′-maleimidylanilino)-naphthalene sulphonic acid	Cys	inactivation studies	81
ATPase	ovine kidney	butane-2,3-dione	Arg	inactivation studies	82
ATPase F_1	porcine heart	various reagents	His, Lys	inhibition	83
ATPase F_1	porcine heart	5-*p*-fluorosulphonylbenzoyl-adenosine		affinity label	84

[60] S. Nakashima, Y. Sakai, Y. Umeda, and K. Takatsu, *Microbiol. Immunol*, 1979, **23**, 105.
[61] Fu-Tong Liu and D. H. Katz, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1430.
[62] I. Björk and K. Nordling, *Eur. J. Biochem.*, 1979, **102**, 497.
[63] D. L. Barbeau, A. Jonas, Teng Ta-Lee, and A. M. Scanu, *Biochemistry*, 1979, **18**, 362.
[64] A. Bracht, R. R. Enfinger, A. Redhardt, and J. Schlitter, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 585.
[65] D. M. Miller, M. E. Newcower, and F. A. Quiocho, *J. Biol. Chem.*, 1979, **254**, 7521.
[66] K. V. Savoca, A. Abuchowski, T. Van Es, F. F. Davis, and N. C. Palczuk, *Biochim. Biophys. Acta*, 1979, **578**, 47.
[67] S. Takada, T. Kusumi, T. Saheki, M. Tsudu, and T. Katsunuma, *J. Biochem. (Tokyo)*, 1979, **86**, 1353.
[68] J. Charlier and E. Gerlo, *Biochemistry*, 1979, **18**, 3171.
[69] G. T. James, *Arch. Biochem. Biophys.*, 1979, **197**, 57.
[70] E. Marra, S. Passarella, S. Doonan, C. Saccone, and E. Quagliariello, *Arch. Biochem. Biophys.*, 1979, **195**, 269.
[71] M. Martinez-Carrion, J. C. Slebe, and M. Gonzalez, *J. Biol. Chem.*, 1979, **254**, 3160.
[72] C. A. Enns and W. W.-C. Chan, *J. Biol. Chem.*, 1979, **254**, 6180.
[73] M. H. Baron, W. N. Lipscomb, and D. R. Evans, *J. Biol. Chem.*, 1979, **254**, 10686.
[74] D. R. Evans and W. N. Lipscomb, *J. Biol. Chem.*, 1979, **254**, 10679.
[75] G. Zimmer, L. Mainka, and I. Berger, *FEBS Lett.*, 1979, **107**, 217.
[76] P. Pougeois, M. Satre, and P. V. Vignais, *Biochemistry*, 1979, **18**, 1408.
[77] J. J. Cossen and R. J. Guillory, *J. Biol. Chem.*, 1979, **254**, 2946.
[78] J. Lunardi and P. V. Vignais, *FEBS Lett.*, 1979, **102**, 23.
[79] J. Lunardi, M. Satre, M. Bof, and P. V. Vignais, *Biochemistry*, 1979, **18**, 5311.
[80] M. Satre, J. Lunardi, R. Pougeois, and P. V. Vignais, *Biochemistry*, 1979, **18**, 3134.
[81] S. S. Gupte and L. K. Lane, *J. Biol. Chem.*, 1979, **254**, 10362.
[82] C. M. Grisham, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 229.
[83] C. Godinot, F. Penin, and D. C. Gautheron, *Arch. Biochem. Biophys.*, 1979, **192**, 225.
[84] A. Di Pietro, C. Godinot, J.-C. Martin, and D. C. Gautheron, *Biochemistry*, 1979, **18**, 1738.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
ATPase	rabbit kidney	dimethyl 3,3′-dithiobispropionimidate	Lys	reversible inactivation	85
ATPase		copper(II) 1 : 10 phenanthroline	Cys	polymerization	86
ATPase	rabbit muscle	[^{14}C]dicyclohexylcarbodi-imide	carboxyl	tetramer minimal functional unit	87
ATPase	rabbit muscle	I_2, dimethylsuberimidate, copper(II) 1 : 10 phenanthroline	Cys	polymerization	88
ATPase	rat liver	(i) fluorescamine (ii) dicyclohexylcarbodi-imide	(i) Lys (ii) carboxyl	activity increases after modification with (i)	89
ATPase	rat brain, eel electroplax, canine kidney	acetic anhydride, trinitrobenzenesulphonic acid	Lys	inactivation	90
ATPase F_0–F_1	*Thermophilic bacterium PS3*	various reagents		proton-conduction studies	91, 92
ATP sulphurylase	*Penicillium chrysogenum*	various reagents	Arg, His, Tyr	evidence for essential residues	93
Azurin	*Pseudomonas aeroginosa*	bromoacetate	Met	low pH study	94
Bacteriophage λ receptor	*E. coli*	various cross-linking reagents	Lys	present as trimer	95
Bacteriorhodopsin	*Halobacterium halobium*	dansyl chloride	Lys-40	protein-folding studies	96
Bacteriorhodopsin	*Halobacterium halobium*	*trans*[15-^{3}H]retinal	Lys	NH_2 labelling	97
Bacteriorhodopsin	*Halobacterium halobium*	tetranitromethane	Tyr	proton pumping increased if <4 residues modified	98
Bacteriorhodopsin	*Halobacterium halobrium*	phenylglyoxal, butane-2,3-dione, various carbodi-imides–GlyOMe	Arg-carboxyl	Arg and carboxyl residues involved in decay of M_{412} species	99
Bacteriorhodopsin	*Halobacterium halobium*	various mono- and di-imidoesters	Lys	inactivated only by bifunctional reagents	100
Bence–Jones protein	human	dithiothreitol–iodoacetamide	$(Cys)_2$ Cys	conformational studies	101
Calmodulin	bovine brain	tetranitromethane	Tyr	spectroscopic study	102

Capsid proteins	Polio virus	*N*-succinimidyl 2-[3-^{3}H]-propionate, *N*-succinimidyl 3-(4-hydroxy-5-^{125}I-iodophenyl) propionate, various di-imidoesters	Lys	topographical studies	103
Carbonic anhydrase	bovine	diazosulphanilic acid	His	Raman spectral study	104
Carbonic anhydrase	spinach	DTNB, bromopyruvate, bromo-acetazolamide, bromoacetate	Cys (His)	inactivation studies	105
Carboxypeptidase	*Streptomyces griseus*	(i) tetranitromethane, *N*-(bromoacetyl)-*N*-methyl-L-Phe (ii) phenylglyoxal	(i) Tyr (ii) Arg	enzyme intermediate between endo and carboxy-peptidase	106
Carboxypeptidase A	bovine pancreas	diazotized arsonilic acid–cobalt(II) EDDA	Tyr-248	differential enzyme activities	107

[85] J. J. H. M. De Pont, *Biochim. Biophys. Acta*, 1979, **567**, 247.
[86] R. J. Baskin and S. Hanna, *Biochim. Biophys. Acta*, 1979, **576**, 61.
[87] U. Pick and E. Racker, *Biochemistry*, 1979, **18**, 108.
[88] G. M. Hebdon, L. W. Cunningham, and N. M. Green, *Biochem. J.*, 1979, **179**, 135.
[89] S. Tu, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 483.
[90] J. D. Robinson and M. S. Flastner, *Arch. Biochem. Biophys.*, 1979, **196**, 350.
[91] N. Sone, M. Yoshida, H. Hirata, and Y. Kagawa, *FEBS Lett.*, 1979, **97**, 61.
[92] N. Sone, M. Yoshida, H. Hirata, and Y. Kagawa, *J. Biochem. (Tokyo)*, 1979, **85**, 503.
[93] J. R. Farley, E. A. Christie, P. A. Senbert, and I. H. Segel, *J. Biol. Chem.*, 1979, **254**, 3537.
[94] R. H. L. Marks and R. D. Miller, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 661.
[95] E. T. Palva and P. Westermann, *FEBS Lett.*, 1979, **99**, 77.
[96] G. Harris, R. Renthel, J. Turkey, and N. Robinson, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 926.
[97] E. Mullen, M. G. Grove, and M. Akhtor, *Biochem. J.*, 1979, **183**, 175.
[98] M. Campos-Carieres, T. A. Moore, and R. N. Perham, *Biochem. J.*, 1979, **179**, 233.
[99] L. Packer, S. Tristram, J. M. Herz, C. Russell, and C. L. Borders, *FEBS Lett.*, 1979, **108**, 243.
[100] T. Konishi, S. Tristram, and L. Packer, *Photochem. Photobiol.*, 1979, **29**, 353.
[101] Y. Goto and K. Hamaguchi, *J. Biochem. (Tokyo)*, 1979, **86**, 1433.
[102] W. D. McCubbin, M. T. Hincke, and C. M. Kay, *Can. J. Biochem.*, 1979, **57**, 15.
[103] K. Wetz and K.-O. Habermehl, *J. Gen. Virol.*, 1979, **44**, 525.
[104] T. Y. Li, J. F. Chen, K. L. Walters, and J. T. McFarland, *Arch. Biochem. Biophys.*, 1979, **197**, 477.
[105] D. L. Cybulsky, A. Nagy, S. I. Kandel, M. Kandel, and A. G. Gornall, *J. Biol. Chem.*, 1979, **254**, 2032.
[106] K. Breddam, T. J. Bazzone, B. H. Imquist, and B. L. Vallee, *Biochemistry*, 1979, **18**, 1565.
[107] M. S. Urdea and J. I. Legg, *J. Biol. Chem.*, 1979, **254**, 11868.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Carboxypeptidase Y	yeast	various alkyl and aryl mercurials	Cys	—SH role in enzyme studies	108
Carboxypeptidase inhibitor	potato	*N*-methylnicotinamide chloride	Trp, Tyr	aromatic residues exposed	109
Cathepsin D	rabbit, bovine	diazoacetyl-D,L-norleucine methyl ester/copper(II),1,2-epoxy-3-(*p*-nitrophenoxy) propane		inhibition characteristics	110
Cell adhesion and spreading factor	fetal calf serum	various reagents	(i) Asp, Glu, Tyr, Trp; (ii) Cys, Lys, Met	(i) inhibition (ii) no effect	111
Cholera toxin	*Vibrio cholerae* 569B	*N*-ethylymaleimide, iodoacetic acid, iodocetamide	Cys	Cys not essential	112
Cholera toxin	*Vibrio cholerae* 569B	various thiol reagents	$(Cys)_2$	interaction of functional regions studies	113
Cholera toxin	*Vibrio cholerae*	12-(4-azido-2-nitrophenoxy)-stearoyl[1-^{14}C]glucosamine		A1 subunit labelled within membrane bilayer	114
α-Chymotrypsin	bovine	methyl *p*-nitrobenzene sulphonate	His-57 Met-192	refolding transition studies	115
α-Chymotrypsin	bovine	*R*(+)- and *S*(−)-3 carboxy-2,2,5,5-tetramethyl pyrrolinyl-1-oxyl-*p*-nitrophenyl ester	Ser-195	e.s.r. single-crystal study	116
α-Chymotrypsin	bovine	*N*-benzoyl-L-phenylalaninal	Ser-195	neutral hemiacetal	117
α-Chymotrypsin	bovine	*N*-acetyl-L-phenylalaninal, *N*-benzoyl-L-phenylalaninal	Ser-195	n.m.r. study	118
α-Chymotrypsin	bovine	acrolein–$NaBH_4$, succinic and acetic anhydride	Lys	1—2 of 15 NH_2 groups essential	119
α-Chymotrypsin	bovine	*N*-acetylhomocysteinethiolactone, DTNB, α,ω-dithio-alkanes	Lys(·SH)	intramolecular cross-links studied	120, 121
α-Chymotrypsin	bovine	(i) phenylmethanesulphonyl fluoride, (ii) α-bromo-*p*-nitroacetophenone	(i) Ser-195 (ii) Met-192	thermodynamics of analogue association studied	122

Citrate synthase	*E. coli*	DTNB, 4,4′-dithiopyridine	Cys	activity studies	123
Collagen	calf, human	[^{125}I]-*p*-hydroxyphenylpropionic acid	Lys	radioimmunoassay	124
Collagen peptidase	rabbit	(i) phenylmethylsulphonylfluoride, (ii) *p*-hydroxymercuribenzoate	(i) Ser (ii) Cys	(i) essential Ser; (ii) slowly inhibitory	125
Concanavalin A	Jack bean	1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide/EDTA, *p*-azidophenyl-α-D-mannopyranoside	carboxyl	cross-linking studies	126
Coupling factor	spinach chloroplasts	5′-*p*-fluorosulphonylbenzoyl-adenosine		ATPase activity inhibited	127
Coupling factor	spinach chloroplasts	(i) 3′-*o*-{3-[*N*-(4-azido-2-nitrophenyl)amino]propionyl}-ATP		(i) affinity label (ii) fluorescence studies	128
		(ii) 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole	(ii) Tyr		

[108] Y. Bai and R. Hayashi, *J. Biol. Chem.*, 1979, **254**, 8473.
[109] T. R. Leary, D. T. Grahn, H. Neurath, and G. M. Hass, *Biochemistry*, 1979, **18**, 2252.
[110] T. Y. Lin and H. R. Williams, *J. Biol. Chem.*, 1979, **254**, 11876.
[111] F. Grimell and D. Minter, *Biochim. Biophys. Acta*, 1979, **550**, 92.
[112] J. J. Malabanos, R. J. Collier, and W. R. Romig, *J. Biol. Chem.*, 1979, **254**, 5855.
[113] M. Tomasi, A. Battistini, A. Araco, L. G. Roda, and G. D'Agnolo, *Eur. J. Biochem.*, 1979, **93**, 621.
[114] B. J. Wisnieski and J. S. Bramhall, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 308.
[115] J. Stoesz and R. W. Lumry, *Biophys. Chem.*, 1979, **10**, 105.
[116] R. S. Bauer and L. J. Berliner, *J. Mol. Biol.*, 1979, **128**, 1.
[117] W. P. Kennedy and R. M. Schultz, *Biochemistry*, 1979, **18**, 349.
[118] R. Chen, D. G. Gorenstein, W. P. Kennedy, G. Lowe, D. Nurse, and R. M. Schultz, *Biochemistry*, 1979, **18**, 921.
[119] V. P. Torchilin, A. V. Maksimenko, V. N. Smirnov, I. V. Berezin, A. M. Klibanov, and K. Martinek, *Biophys. Acta*, 1979, **567**, 1.
[120] V. P. Torchilin, A. V. Maksimenko, V. N. Smirnov, I. V. Berezin, and K. Martinek, *Biochim. Biophys. Acta*, 1979, **568**, 1.
[121] V. P. Torchilin, A. V. Maksimenko, and K. Martinek, *Bioorg. Khim.*, 1979, **5**, 295.
[122] R. M. Schultz, P. Varma-Nelson, J. R. Peters, and W. J. Treadway, *J. Biol. Chem.*, 1979, **254**, 12411.
[123] M. M. Talgoy, A. W. Bell, and H. W. Duckworth, *Can. J. Biochem.*, 1979, **57**, 822.
[124] F. J. Roll, J. A. Madri, and H. Furthmayr, *Anal. Biochem.*, 1979, **96**, 489.
[125] M. Nagelschmidt, T. Unger, and H. Struck, *Biochem. Biophys. Acta*, 1979, **571**, 105.
[126] M. Beppu, T. Terao, and T. Osawa, *J. Biochem. (Tokyo)*, 1979, **85**, 1275.
[127] E. De Benedetti and A. Jagendorf, *Biochem. Biophys. Res. Commun.*, 1979, **18**, 3452.
[128] M. F. Carlier, D. A. Holowka, and G. G. Hammes, *Biochemistry*, 1979, **18**, 3452.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Coupling factor	spinach chloroplasts	pyridoxal phosphate	Lys	1 Lys residue results in complete inactivation	129
Coupling factor F_1	bovine heart	(i) butane-2,3-dione (ii) 7-chloro-4-nitrobenzo-2-oxa,-1,3-diazole	(i) Arg (ii) Tyr	ATP catalytic-site studies	130
Creatine kinase	rabbit muscle	various iodoalkanes	Cys	reactive Cys residues in active site analysed	131
Creatine kinase	rabbit muscle	various derivatives of iodoacetamide	Cys	—SH group reactivities probed	132
Creatine kinase	rabbit muscle	2-nitro-5-thiocyanobenzoic acid	Cys	non-equivalent Cys residues in dimeric structure	133
Creatine kinase	rabbit muscle	ethoxyformic acid anhydride	His (Lys, Cys)	5 of 16 His residues modified	134
Creatinase	*Pseudomonas putida*	(i) *N*-bromosuccinimide (ii) ethoxyformic anhydride	(i) Met (ii) His	inactivation	135
Cytochrome b_5	bovine liver	1-ethyl-3-(3-dimethylaminopropyl)-carbodi-imide–various nucleophiles	carboxyl	carboxyl groups involved in interaction with cyt-b_5 reductase	136
Cytochrome *c*	bovine heart	^3H_2O	His-33	His-18 and His-26 buried in hydrophobic interior	137
Cytochrome *c*	various sources	ascorbic acid		single-site redox reaction	138
Cytochrome *c*	horse heart	4-carboxy-2,6-dinitrochlorobenzene	Lys (7,25, 73,86)	kinetics of modified cyt-*c* with beef mitochondrial reductase studied	139
Cytochrome *c*	horse heart	maleic anhydride	Lys	conformational stability examined	140
Cytochrome *c* oxidase	bovine heart	dicyclohexylcarbodi-imide	carboxyl	non-ATP-lined proton-pump demonstrated	141
Cytochrome *c* oxidase	bovine heart	1-ethyl-3-(3-dimethylaminopropyl)-carbodi-imide	carboxyl	polylysine binding study	142
Cytochrome *c* oxidase	bovine heart	[^{35}S]diazobenzenesulphonate		disposition across membrane examined	143
Dihydrofolate reductase	*Lactobacillus casei*	pentane-2,4-dione	Lys	inactivation when 3 Lys residues modified	144

DNA polymerase	*E. coli*	thymidine triphosphate γ-4-(*N*-2-chloroethyl-*N*-methylamino)benzylamidate		affinity label	145
DNase	bovine	*N*-acetyl-D,L-homocysteine thiolactone, 4,4′-dithiopyridine	Cys	hybrid enzyme formation with RNase	146
Dynein	*Tetrahymena pyriformis*	(i) trinitrobenzonesulphonic acid (ii) various thiol reagents	(i) Lys (ii) Cys	ATPase activity increased by $-NH_2$ and reduced by $-SH$ modifications	147, 148
Elastase	porcine pancreas	1-oxyl-2,2,5,5-tetramethyl-4-piperidinylethyl phosphonofluoridate	Ser-195	spin-label study	149
Elastase	porcine pancreas	trifluoroacetylpeptide chloromethyl ketones		^{19}F n.m.r. study	150

[129] Y. Sugiyama and Y. Mukohata, *FEBS Lett.*, 1979, **98**, 276.
[130] R. C. Steinmeier and J. H. Wang, *Biochemistry*, 1979, **18**, 11.
[131] S. R. R. Reddy and D. C. Watts, *Biochim. Biophys. Acta*, 1979, **569**, 109.
[132] N. C. Price, *Biochem. J.*, 1967, **177**, 603.
[133] Y. Degani and C. Degani, *Biochemistry*, 1979, **18**, 5917.
[134] D. E. Clarke and N. C. Price, *Biochem. J.*, 1979, **181**, 467.
[135] K. Rikitake, I. Oka, M. Ando, T. Yoshimoto, and D. Tsuru, *J. Biochem.* (*Tokyo*), 1979, **86**, 1109.
[136] H. A. Dailey and P. Strittmatter, *J. Biol. Chem.*, 1979, **254**, 5388.
[137] S. Kitmura, H. Matsuo, and K. Narita, *Int. J. Pept. Protein Res.*, 1979, **14**, 472.
[138] A. I. Al-Ayash and M. T. Wilson, *Biochem. J.*, 1979, **177**, 641.
[139] S. H. Speck, S. Ferguson-Miller, N. Osheroff, and E. Margoliash, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 155.
[140] A. Schejter, M. Zuckerman, and I. Aviram, *J. Biol. Chem.*, 1979, **254**, 7042.
[141] R. P. Casey, M. Thelen, and A. Azzi, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1044.
[142] C. H. A. Seiter, R. Margalit, and R. A. Perreult, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 473.
[143] B. Ludwig, N. W. Dowrer, and R. A. Capaldi, *Biochemistry*, 1979, **18**, 1401.
[144] H. B. Otwell, K. L. Cipollo, and R. B. Dunlap, *Biochim. Biophys. Acta*, 1979, **568**, 297.
[145] V. N. Bunera, N. V. Kudryashova, and V. A. Kurbatov, *Biokhimiya*, 1978, **43**, 2261.
[146] D. Wang, *Biochemistry*, 1979, **18**, 4449.
[147] T. Shimizu, *J. Biochem.* (*Tokyo*), 1979, **86**, 1139.
[148] T. Shimizu, *J. Biochem.* (*Tokyo*), **85**, 1421.
[149] J. L. Dimicoli, M. Nakache, and J. M. Lhoste, *Biochim. Biophys. Acta*, 1979, **571**, 294.
[150] J. L. Dimicoli, A. Renaud, P. Lestienne, and J. G. Bieth, *J. Biol. Chem.*, 1979, **254**, 5208.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Elastase	human leucocytes	trifluoroacetylpeptide chloromethyl ketones		inactivation	151
Elongation factor T_s	*E. coli*	butane-2,3-dione	Arg	inactivation	152
Elongation factor T_u	*B. stearothermophilus*	[^{14}C]*N*-ethylmaleimide	Cys	—SH not essential for GDP binding	153
Elongation factor T_u	*Thermus thermophilus*	photo-oxidation–Rose Bengal	His	essential His involved in substrate binding	154
Endonuclease Bgl I	*Bacillus globigii*	(i) pyridoxal 5′-phosphate, (ii) butane-2,3-dione	(i) Lys (ii) Arg	(i) activity and binding reduced; (ii) only activity affected	155
Enkephalin receptor	rat	dimethyl suberimidate	Lys	enkephalin receptor complex prepared	156
Enolase	yeast	various carbodi-imides	carboxyl	essential carboxyl groups	157
Enolase	yeast	(i) ethoxyformic anhydride (ii) pyridoxal 5′-phosphate, trinitrobenzenesulphonic acid	(i) His (ii) Lys	inactivation follows modification of 6 His residues per sub-unit	158
Enolase	yeast	photo-oxidation–Rose Bengal	His	1 essential His (His-180?)	159
Enolase	rabbit muscle	butane-2,3-dione	Arg	inactivation	160
D-Erythrulose reductase	bovine liver	photo-oxidation–Rose Bengal	His	$NADP^+$ prevents photoinhibition	161
Estradiol 17β-dehydrogenase	human placenta	3-chloroacetylpyridine-ADP		affinity label	162
Fatty-acid synthetase	rat mammary gland	5-(4-bromo-2,3-dioxobutyl)-CoA		affinity label; two sites per enzyme dimer	163
Fatty-acid synthetase	yeast	dimethylmaleic anhydride	Lys	reversible dissociation	164
Ferredoxin–$NADP^+$ reductase	spinach	various α-dicarbonyl compounds	Arg	2 Arg required for maximum activity	165
Ferredoxin–$NADP^+$	*Bumilleriopsis filiformis*	butane-2,3-dione	Arg	inhibition of $NADP^+$ binding	166
Fibroblast surface	human	[^{125}I]–lactoperoxidase	Tyr(His)	gel-electrophoresis study	167
Fibronectin	hamster fibroblast	various disulphide and thiol reagents	$(Cys)_2$, Cys	domain structure investigated	168

Fibronectin	human	various reagents	carboxyl, Trp,Lys	secondary or tertiary structure not essential for activity	169
Fibrinogen	human	[^{125}I]lactoperoxidase	Tyr-119	Tyr labelling patterns	170
Fibrinogen	human	dimethyl suberimidate	Lys	cross-linking study	171
β-Fibrinogenase	*Trimeresurus gramineus*	phenylmethanesulphonyl fluoride	Ser	comparative inhibition studies of α- and β-forms	172
Follicle-stimulating hormone	human	2-hydroxy-5-nitrobenzyl bromide	Tyr, Met, Arg	α-subunit exposed; essential Arg	173
Follitropin	human	potassium cyanate, maleic anhydride	Lys	α-subunit NH_2 groups essential	174

[151] P. Lestienne, J. L. Dimicoli, A. Renaud, and J. G. Bieth, *J. Biol. Chem.*, 1979, **254**, 5129.
[152] A. H. Marschel and J. W. Bodley, *J. Biol. Chem.*, 1979, **254**, 1816.
[153] A. Wittinghofer and R. Leberman, *FEBS Lett.*, 1979. **101**, 153.
[154] A. Nakane, T. Miyazawa, S. Nakamura, and Y. Kaziro, *Arch. Biochem. Biophys.*, 1979, **196**, 233.
[155] Y. H. Lee and J. G. Chirikjian, *J. Biol Chem.*, 1979, **254**, 6838.
[156] R. S. Zukin and R. M. Kream, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1593.
[157] A. L. George, jun. and C. L. Borders, jun., *Biochem. Biophys. Res. Commun.*, 1979, **87**, 59.
[158] A. L. George, jun. and C. L. Borders, jun., *Biochim. Biophys. Acta*, 1979, **569**, 63.
[159] J. I. Elliott and J. M. Brewer, *Arch. Biochem. Biophys.*, 1979, **192**, 203
[160] C. L. Borders, jun. and J. A. Zurcher, *FEBS Lett.*, 1979, **108**, 415.
[161] K. Uekara, S. Mannen, and S. Hosomi, *J. Biochem. (Tokyo)*, 1979, **85**, 1003.
[162] J. F. Biellmann, P. R. Goulas, J. C. Nicolas, B., Descomps, and A. Crastes de Paulet, *Eur. J. Biochem.*, 1979, **99**, 81.
[163] P. R. Clements, R. E. Barden, P. M. Ahmad, and F. Ahmad, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 278.
[164] F. Wieland, L. Renner, C. Verfürth, and F. Lynen, *Eur. J. Biochem.*, 1979, **94**, 189.
[165] G. Zanetti, C. Gozzer, G. Sacchi, and B. Curti, *Biochim. Biophys. Acta*, 1979, **568**, 127.
[166] G. Bookjans and P. Böger, *Arch. Biochem. Biophys.*, 1979, **194**, 387.
[167] D. E. Comings and L. W. Cohen, *Biochim. Biophys. Acta*, 1979, **578**, 61.
[168] D. D. Wagner and R. O. Hynes, *J. Biol. Chem.*, 1979, **254**, 6746.
[169] L. I. Gold and E. Pearestein, *Biochim. Biophys. Acta*, 1979, **581**, 237.
[170] J. L. York and B. Blombäck, *J. Biol. Chem.*, 1979, **254**, 8786.
[171] J. Ross and G. D. Kemp, *Biochem. Soc. Trans.*, 1979, **7**, 695.
[172] C. Ouyang and Huang Tur-Fu, *Biochim. Biophys. Acta*, 1979, **571**, 270.
[173] P. Rathnam and B. B. Saxena, *Biochim. Biophys. Acta*, 1979, **576**, 81.
[174] M. R. Sairam and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, **13**, 394.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Follitropin	ovine	potassium cyanate, maleic anhydride	Lys	inactivation when all NH_2 modified	175
Formaldehyde dehydrogenase	*Pseudomonas putida*	*p*-chloromercuribenzoate, phenylmethanesulphonyl fluoride		inactivation; first report of NAD^+ linked but GSH-independent properties	176
Formate dehydrogenase	bacterial origin	DTNB, iodoacetamide	Cys	NAD protects enzyme from inactivation	177
Fructose-1,6-bisphosphatase	porcine kidney	8-azido-AMP		3.7 mole reagent : mole enzyme incorporated	178
Fructose-1,6-bisphosphatase		periodate oxidized-AMP	Lys	enzyme not inhibited	179
Fucosyl transferase	human	*N*-ethylmaleimide, dithiothreitol	$(Cys)_2$, Cys	enzyme-activity profiles	180
β-D-Galactosidase	*E. coli*	methylmercaptobutyrimidate–*NN'-o*-phenylenedimaleimide	Lys(—SH)	antibody conjugate	181
β-D-Galactosidase	*E. coli*	*NN-o*-phenylenedimaleimide	Cys	antibody conjugate	182, 183
β-D-Galactosidase	*E. coli*	*N*-hydroxysuccinimide esters of various steroids	Lys	immunoassay reagents	184
Gelatin-specific proteinase	human leucocytes	various thiol reagents	Cys	activation studies	185
Glucagon	porcine	trinitrobenzenesulphonic acid	His(αNH_2)	antagonist for c-AMP induction	186
Glucokinase	rat	various *N*-bromoacetylated monosaccharides	Cys	affinity labelling	187
α-Glucosidase	buck wheat	*N*-acetylimidazole	Tyr	protecting action of various saccharides examined	188
β-Glucosidase	sweet almonds	poly(vinylalcohol) bearing aromatic azido groups–photoirradiation		enzyme immobilized; retained 45% activity	189
β-Glucosidase	sweet almonds	4,4'-diazidostilbene-2,2'-disodium sulphonate, photoirradiation		immobilization procedure studied	190
Glutamate decarboxylase	*E. coli*	phenylglyoxal	Arg	apoenzyme inactivated	191

Glutamate dehydrogenase		glutaraldehyde	Lys	enzyme–bovine serum albumin polymers produced	192
Glutamate dehydrogenase	bovine liver	[5′-^{3}H]-5′-*p*-fluorosulphonyl-benzoyl-guanosine		affinity labelling	193
Glutamate dehydrogenase	bovine liver	spin-labelled *p*-chloromercuri-benzoate		conformational changes	194
Glutamate dehydrogenase	bovine liver, porcine heart	dimethyl-3,3′-dithiobispro-pionimidate	Lys	hybrid complex formed with malate dehydrogenase	195
Glutamine synthetase	*Bacillus stearothermo-philus*	ethyl acetimidate	Lys	V_{max} affected only	196
Glutamine synthetase	*Bacillus stearothermo-philus*	dimethyl malonimidate, adipimidate, suberimidate	Lys	allosteric properties unchanged and thermostability increased	197

[175] M. R. Sairam, *Arch. Biochem. Biophys.*, 1979, **194**, 79.
[176] M. Ando, T. Yoshimoto, S. Ogushi, K. Rikitake, S. Shibata, and D. Tsura, *J. Biochem.* (*Tokyo*), 1979, **85**, 1165.
[177] V. O. Popov and A. M. Egorov, *Biokhimiya*, 1979, **44**, 207.
[178] F. Marcus and B. E. Haley, *J. Biol. Chem.*, 1979, **254**, 259.
[179] R. B. Maccioni, E. Hubert, and J. C. Slebe, *FEBS Lett.*, 1979, **102**, 29.
[180] D. Kessel and T. H. Chow, *Biochem. J.*, 1979, **181**, 767.
[181] M. J. O'Sullivan, E. Gnemmi, D. Morris, G. Chieregatti, A. D. Simmonds, M. Simmonds, J. W. Bridges, and V. Marks, *Anal. Biochem.*, 1979, **100**, 100.
[182] S. Yoshitake, Y. Hamaguchi, and E. Ishikawa, *Scand. J. Immunol.*, 1979, **10**, 81.
[183] Y. Hamaguchi, S. Yoshitake, E. Ishikawa, Y. Endo, and S. Ohtaki, *J. Biochem.* (*Tokyo*), 1979, **85**, 1289.
[184] H. Mosoda, Y. Sakai, M. Yoshida, and T. Nambora, *Chem. Pharm. Bull.*, 1979, **27**, 2147.
[185] I. Sopata and J. Wize, *Biochim. Biophys. Acta*, 1979, **571**, 305.
[186] T. E. Cote and R. M. Epand, *Biochim. Biophys. Acta*, 1979, **582**, 295.
[187] B. A. Connolly and I. P. Trayer, *Biochem. Soc. Trans.*, 1979, **7**, 713.
[188] K. Kanaya, S. Chiba, and T. Shimomura, *Agric. Biol. Chem.*, 1979, **43**, 781.
[189] S. Mikairi, *Biochim. Biophys. Acta*, 1979, **571**, 374.
[190] S. Miyairi, H. Tanaka, M. Sugiura, and S. Fukui, *Anal. Biochem.*, 1979, **97**, 320.
[191] S. Cheung and M. L. Fonda, *Arch. Biochem. Biophys.*, 1979, **198**, 541.
[192] J. N. Barbotin and B. Thomassett, *Biochim. Biophys. Acta*, 1979, **570**, 11.
[193] P. K. Pal and R. F. Colman, *Biochemistry*, 1979, **18**, 838.
[194] A. Zantena, M.-J. de Smet, and G. T. Robillard, *Eur. J. Biochem.*, 1979, **96**, 453.
[195] L. A. Fahian, E. Kmiotek, and L. Smith, *Arch. Biochem. Biophys.*, 1979, **192**, 33.
[196] T. Sekiguchi, S. Oshiro, E. M. Goingo, and Y. Nosoh, *J. Biochem.* (*Tokyo*), 1979, **85**, 75.
[197] T. Sekiguchi, S. Oshiro, E. M. Goingo, and Y. Nosoh, *J. Biochem.* (*Tokyo*), 1979, **86**, 447.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
γ-Glutamyl transpeptidase	rat	6-diazo-5-oxo-D-norleucine		affinity label	198
Glutathione reductase	bovine liver	trinitrobenzenesulphonic acid	Cys	inactivation	199
Glutathione-*S*-transferase	rat	various thiol reagents	Cys	inactivation	200
Glutathione-*S*-transferase	rat	various thiol reagents	Cys	4-SH group present; inactivated if 3 or more modified	201
Glycinin	soybean	succinic anhydride	Lys	dissociation effected	202
Glyceraldehyde-3-phosphate dehydrogenase	rabbit muscle	iodoacetic acid	Cys	NAD^+ binding	203
Glyceraldehyde-3-phosphate dehydrogenase	rabbit	various platinum complexes	Cys	essential SH groups	204
Glyceraldehyde-3-phosphate dehydrogenase	rabbit muscle	various aralkyl and alkyl isothiocyanates	Cys	reversible modification of essential Cys	205
Glyceraldehyde-3-phosphate dehydrogenase	*Scenedesmus obliquus*	dithiothreitol	$(Cys)_2$	increase in NADPH-dependent activity	206
Glyceraldehyde-3-phosphate dehydrogenase	various lower vertebrates	DTNB	Cys	inter-species activity	207
Glycerol-3-phosphate dehydrogenase	rabbit	iodo[2-^{14}C]acetic acid	Cys	2 essential Cys residues in native dimeric enzyme	208
Glycogen phosphorylase	rabbit	8-[*m*-(*m*-fluorosulphonylbenzamido)-benzylthio]adenine		inter-subunit transmission of ligand effects studied	209
Glycogen phosphorylase	rabbit muscle	various di-imidates	Lys	cross-linking study	210
Growth hormone	bovine	trinitrobenzenesulphonic acid	Lys	α-NH_2 group most reactive	211
Haemoglobin	canine	various organomercurials	Cys	4 SH groups react; only 2 react with non-mercurials	212
Haemoglobin	feline	ethoxyformic anhydride, iodoacetamide phenylmercuric acetate, Methylene Blue–photo-oxidation	Cys, His	triethylin binding measured	213
Haemoglobin	human	photo-oxidation–Methylene Blue		cross-links formed	214
Haemoglobin	human	sodium nitrite	Cys(α-93)	Protects haem against oxidation	215

Haemoglobin	human	potassium cyanate	Val(1-α)	*X*-ray diffraction studies	216
Haemoglobin	human	[^{14}C]glucose–$NaBH_4$	Lys	non-enzymic glycosylation	217
Haemoglobin	human	*p*-chloromercuribenzoate	Cys	enhances proteolysis	218
Haemoglobin	human	ω-aminoenantic acid–L-Phe	carboxyl	peroxidase activity diminished	219
Haemoglobin	human	dimethyl adipimidate	Lys	effect on O_2 affinity examined	220
Haemoglobin	human	menadione	Cys(β-93)	accompanied by reduction of methaemoglobin	221
Haemoglobin	human	glutaraldehyde	Lys	co-operativity increased and oxygen affinity decreased	222
Haptoglobin	human	*N*-bromosuccinimide, 2-hydroxy-5-nitrobenzyl bromide	Trp	loss of Hb binding and antigenicity studies	223

[198] M. Inoue, S. Horiuchi and Y. Morino, *Euro. J. Biochem.*, 1979, **99**, 169.
[199] I. Carlberg and B. Mannervik, *FEBS Lett.*, 1979, **98**, 263.
[200] R. Morgenstern, J. W. De Pierre, and L. Ernster, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 657.
[201] T. Carne, E. Tipping, and B. Ketterer, *Biochem. J.*, 1979, **177**, 433.
[202] A. G. Appu Rao and M. S. Narasinga Rao, *Int. J. Pept. Protein Res.*, 1979, **14**, 307.
[203] C. H. Reynolds and K. Dalziel, *Biochim. Biophys. Acta*, 1979, **567**, 287.
[204] J. L. Aull, R. L. Allen, A. R. Bapat, H. H. Daron, M. E. Friedman, and J. F. Wilson, *Biochim. Biophys. Acta*, 1979, **571**, 352.
[205] P. Germeiner and L. Drobnica, *Experientia*, 1979, **35**, 857.
[206] S. Woodrow, M. J. O'Brien, J. S. Easterby, and R. Powls, *Eur. J. Biochem.*, 1979, **98**, 425.
[207] D. Ya Leibman and V. P. Nesterov, *Int. J. Biochem.*, 1979, **10**, 645.
[208] R. E. Smith and R. MacQuarrie, *Biochim. Biophys. Acta*, 1979, **567**, 269.
[209] M. K. Battersby and G. K. Radda, *Biochemistry*, 1979, **18**, 3774.
[210] J. Hajdu, G. B. Dombradi, and P. Friedrich, *Biochemistry*, 1979, **18**, 4037.
[211] M. J. Biscoglio de Jimenez Bonino, O. Casone, and J. A. Santome, *Int. J. Pept. Protein Res.*, 1979, **13**, 107.
[212] K. Okonjo, A. Taiwo, M. Balogun, and O. B. Ekisola, *Biochim. Biophys. Acta*, 1979, **576**, 30.
[213] B. Elliot, W. N. Aldridge, and J. W. Bridges, *Biochem. J.*, 1979, **177**, 461.
[214] A. W. Girotti, S. Lyman and M. R. Deziel, *Photochem. Photobiol.*, 1979, **29**, 1119.
[215] A. Mansouri, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 441.
[216] S. O'Donnell, R. Mandaro, and T. M. Schuster, *J. Biol. Chem.*, 1979, **254**, 12204.
[217] H. F. Bunn, R. Shapiro, M. McManus, L. Garrick, M. J. McDonald, P. M. Gallop, and K. H. Gabbay, *J. Biol. Chem.*, 1979, **254**, 3892.
[218] H. Kimura, N. Takahashi, H. Murate, and H. Uematsu, *J. Biochem. (Tokyo)*, 1979, **85**, 1047.
[219] V. M. Belostotskij and A. P. Andreeva, *Biokhimiya*, 1979, **44**, 240.
[220] R. Pennathur-Das, L. E. Vickery, W. Mentzer, and B. H. Lubin, *Biochim. Biophys. Acta*, 1979, **580**, 356.
[221] C. C. Winterbourn, J. K. French, and F. C. Claridge, *Biochem. J.*, 1979, **179**, 665.
[222] J. W. O. Tam and L. Y. Cheng, *Biochim. Biophys. Acta*, 1979, **580**, 75.
[223] I. Katnik and W. Dobryszycka, *Acta. Biochim. Pol.*, 1978, **25**, 325.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Hemerythrin	*Phascolopsis gouldii, Thermiste dyscritum*	various reagents	Cys-9, Cys-50	subunit interactions	224
Hexokinase type II	rat muscle	*N*-bromoacetylglucosamine	Cys	affinity label	225
Hexokinase	yeast	phenylglyoxal	Arg	essential Arg	226
High-density (apo) lipoproteins	human	[^{3}H]azido fatty acids		hydrophobic interactions probed	227
Histocompatibility antigens	human	2-nitro-5-thiocyanobenzoic acid	Cys	cleavage studies	228
Histones	bovine kidney	various cross-linking agents	Lys	H_1 cross-linked to 4 histones in nucleosome core	229
Histones	bovine	*N*-(iodoacetylaminoethyl)-8-naphthylamine-1-sulphonic acid	Met-84	nucleosome structure probe	230
Histones	calf thymus	various di-imidoesters	Lys	cross-linking sites of four histone sequences identified	231
Histones	calf, rabbit	acetic anhydride	Lys	70% residues modified	232
Histones	calf thymus	tetranitromethane, formaldehyde, methylacetimidate, u.v. irradiation	Tyr, Lys	histone interactions examined	233, 234
Histones 2A, 2B	calf thymus	u.v. irradiation	Tyr, Pro	Tyr-37 interaction with Tyr-40 or -42 for 2A or Pro-26 for 2B	235
Histones	calf	dimethyl suberimidate, dithiobis(succinylpropionate)	Lys	DNA–histone cross-linking	236
Histones	chicken erythrocyte	I_2–chloramine T, lacto-peroxidase	Tyr	conformational studies	237
Histones–protamines	herring, salmon	fluorescamine	Lys	DNA–basic protein interactions	238
Histones	leek	tetranitromethane, u.v. irradiation	Tyr	same cross-links as mammalian histones	239
Histones	mouse	1-ethyl-3-(3-dimethylamino-propyl)carbodi-imide	Lys, carboxyl	histone 1 adjacent to 2A and A_{24}	240
Horseradish peroxidase		various anhydrides of dicarboxylic acids	Lys	stability studied	241

Horseradish peroxidase		sodium periodate		compounds II → I, oxidation	242
Hyaluronidase	bovine testes	butane-2,3-dione	Arg	essential Arg	243
β-Hydroxybutyrate dehydrogenase	rat liver	N-methylmaleimide	Cys	1 molecule per dimer inhibits	244
15-Hydroxyprostaglandin dehydrogenase	swine	u.v. irradiation	Trp	inactivation	245
Immunoglobulin	rabbit	glutaraldehyde	Lys	coupling to liposomes	246
Immunoglobulin	rabbit	dithiothreitol, $HgCl_2$	$(Cys)_2$, Cys	antigen binding changes	247

[224] S. E. Clarke, L. C. Sieker, R. E. Stenkamp, and J. S. Loehr, *Biochemistry*, 1979, **18**, 684.
[225] B. A. Connolly and I. P. Trayer, *Eur. J. Biochem.*, 1979, **93**, 375.
[226] M. Philips, D. B. Pho, and L. A. Pradel, *Biochim. Biophys. Acta*, 1979, **566**, 296.
[227] W. Stoffel and P. Metz, *Hoppe-Seyler's Z. Physiol Chem.*, 1979, **360**, 197.
[228] W. S. Ferguson, T. T. Cox, R. J. Robb, and J. L. Strominger, *Mol. Immunol.*, 1979, **16**, 23.
[229] D. Ring and R. D. Cole, *J. Biol. Chem.*, 1979, **254**, 11688.
[230] P. N. Lewis, *Eur. J. Biochem.*, 1979, **99**, 315.
[231] M. Suda and K. Iwai, *J. Biochem. (Tokyo)*. 1979, **86**, 1659.
[232] L. O. Tack and R. T. Simpson, *Biochemistry*, 1979, **18**, 3110.
[233] H. G. Martinson, R. True, C. K. Lau, and M. Mehrabian, *Biochemistry*, 1979, **18**, 1075.
[234] H. G. Martinson, R. True, and J. B. E. Burch, *Biochemistry*, 1979, **18**, 1083.
[235] R. J. DeLange, L. C. Williams, and H. G. Martinson, *Biochemistry*, 1979, **18**, 1942.
[236] J. O. Thomas and P. Oudet, *Nucleic Acid Res.*, 1979, **7**, 611.
[237] G. R. Griffiths and P. C. Huang, *J. Biol. Chem.*, 1979, **254**, 8057.
[238] J. Bode, *Anal. Biochem.*, 1979, **99**, 274.
[239] H. G. Martinson and R. J. True, *Biochemistry*, 1979, **18**, 1947.
[240] W. M. Bonner and J. D. Stedman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2190.
[241] N. N. Ugarova, G. D. Rozhkova, and I. V. Berezin, *Biochim. Biophys. Acta*, 1979, **570**, 31.
[242] A. Nadezhdin and H. B. Dunford, *Can. J. Biochem.*, 1979, **57**, 1080.
[243] P. Gracesa, K. S. Dodgson, and A. H. Olavesen, *Biochem. Soc. Trans.*, 1979, **7**, 1058.
[244] M. Levy and M. Joncant, *C. R. Hebd. Seances Acad. Sci.*, 1979, **288**, 363.
[245] M. Sun, S. Zigman, and H. Tai, *Photochem. Photobiol.*, 1979, **29**, 63.
[246] V. P. Torchilin, B. A. Khaw, V. N. Smirnov, and E. Haber, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1114.
[247] S. Vuk-Parlovic, D. E. Isenman, G. A. Elgarish, A. Gafni, A. Licht, and I. Pecht, *Biochemistry*, 1979, **18**, 1125.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Immunoglobulin	porcine	(i) 2,2,6,6-tetramethyl-*N*-1-oxylpiperidine-4-amino-(*N*-dichlorotriazine)	(i) Lys	segmental flexibility of anti-hapten antibodies studied	248
		(ii) 2,2,6,6-tetramethyl-*N*-1-oxylpiperidine-4-iodo-acetamide	(ii) His		
Immunoglobulin G	human	reducing agents, DTNB	Cys	controlled disulphide cross-linking of proteins	249
Immunoglobulin M	human	I_2–lactoperoxidase	Tyr	topographical investigation	250
Insulin	bovine	1-fluoro-2-nitro-4-trimethyl-aminobenzene iodide	Tyr(A_{14}) Gly(A_1)	crystalline derivative prepared and receptor-binding studies	251, 252
Insulin	bovine	4-pyridylmethyl chloride	Cys	reversible protecting group	253
Insulin	bovine	ω-toluenesulphone, 2,3-benzo-1,4-butane sultone	Cys	reversible protecting group	254
Insulin	porcine	[1-^{14}C]acetic anhydride, [^{3}H]acetic anhydride	Lys(εNH_2), Phe(αNH_2) Gly($\alpha NH)_2$)	amino-group reactivities studied	255
Isocitrate dehydrogenase	human	iodoacetic acid, iodacetamide	Met	essential Met	256
Δ^5-3-Ketosteroid isomerase	*Pseudomonas testosteroni*	β-spiro-3-oxiranyl-5α-androstan-17β-ol		irreversible inhibition; covalent bond probably formed	257
Kinase (cAMP dependent)		8-azido-ATP		affinity label	258
α-Lactalbumin	bovine, human	ethoxyformic anhydride	His-32, His-68	His-32 essential residue	259
β-Lactamase	*Staphylococcus aureus*	clavulanic acid		inactivation	260
β-Lactamase	various sources	6-β-bromopenicillanic acid		inactivation	261
β-Lactamase	*Bacillus cereus*	6-α-bromo[3H]penicillanic acid	Ser-44	essential Ser-44	262
β-Lactamase	*Bacillus cereus*	various bifunctional reagents	Lys	native conformation stabilized	263

Lactate dehydrogenase	porcine heart	methylmethanethiosulphonate	Cys-165	full catalytic activity retained	264
Lactate dehydrogenase	porcine	*O*-methylisourea; ethyl-acetimidate, 1-ethyl-(3-dimethylaminopropyl)-carbodi-imide	Lys, carboxyl	thermostability increased	265
Lactate dehydrogenase	*Streptococcus cremoris*	DTNB, [^{14}C]KCN	Cys	2 cleavage products obtained	266
Lactogen	human	$NaBH_4$, [^{3}H]iodoacetic acid	Cys	dimeric forms involves Cys-182, Cys-189 residues	267
β-Lactoglobulin		*O*-methylisourea, succinic anhydride, trinitrobenzene sulphonic acid	Lys	kinematic viscosity measured	268
β-Lactoglobulin	bovine	tetracyanoaurate	Cys	heavy-atom incorporation for *X*-ray studies	269

[248] Y. K. Sykulev, V. P. Timofeev, R. S. Nezlin, A. Y. Misharin, and F. Franek, *FEBS Lett.*, 1979, **101**, 27.
[249] P. Laidler, L. Konieczny, and K. Bobrzecka, *Mol. Immunol.*, 1979, **16**, 141.
[250] B. K. Seon and D. Pressman, *Biochim. Biophys. Acta*, 1979, **577**, 248.
[251] B. R. D. Easter and S. E. Drewes, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **260**, 1335.
[252] M. Lesniak, J. Gliemann, J. Roth, B. R. D. Easter, D. A. Sutton, and S. E. Drewes, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 467.
[253] U. Th-Ruegg, D. Jarvis, and J. Rudinger, *Biochem. J.*, 1979, **179**, 119.
[254] U. Th-Ruegg, D. Jarvis, and J. Rudinger, *Biochem. J.*, 1979, **179**, 127.
[255] M. G. Sheffer and H. Kaplan, *Can. J. Biochem.*, 1979, **57**, 489.
[256] G. F. Seelig and R. F. Colman, *J. Biol. Chem.*, 1979, **254**, 1191.
[257] R. M. Pollack, R. H. Kayser, and C. L. Bevins, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 783.
[258] J. Hoppe and W. Freist, *Eur. J. Biochem.*, 1979, **93**, 141.
[259] J. P. Preels, J. E. Bell, M. Schindler, F. J. Castellino, and R. L. Hill, *Biochemistry*, 1979, **18**, 1771.
[260] C. Reading and P. Hepburn, *Biochem. J.*, 1979, **179**, 67.
[261] V. Knott-Hunziker, B. S. Orlek, P. G. Sammes, and S. G. Waley, *Biochem. J.*, 1979, **177**, 365.
[262] V. Knott-Hunziker, S. G. Waley, B. S. Orlek, and P. G. Sammes, *FEBS Lett.*, 1979, **99**, 59.
[263] Y. Klemes and N. Citri, *Biochim. Biophys. Acta*, 1979, **567**, 401.
[264] D. P. Bloxham, R. P. Sharma, and D. C. Wilton, *Biochem. J.*, 1979, **177**, 769.
[265] N. Minotani, T. Sekiguchi, J. G. Bautisha, and Y. Nosoh, *Biochim. Biophys. Acta*, 1979, **581**, 334.
[266] L. G. Crossley, G. R. Jago, and B. E. Davidson, *Biochim. Biophys. Acta*, 1979, **581**, 342.
[267] A. B. Schneider, K. Kowalski, J. Russell, and L. M. Sherwood, *J. Biol. Chem.*, 1979, **254**, 3782.
[268] U. Freimuth, F. Tebling, F. Bohme, and E. Ludwig, *Nahr. Chem. Biochem. Mikrobiol. Technol.*, 1979, **23**, 215.
[269] L. Sawyer and D. W. Green, *Biochim. Biophys. Acta*, 1979, **579**, 234.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Lactose permease	*E. coli*	photo-irradiation		inactivation maximal at 290 nm	270
Lactose repressor protein	*E. coli*	*N*-ethylmaleimide, two *N*-maleimide spin labels, *N*-(3-pyrene)maleimide	Cys(140, 107, 281)	DNA binding interactions studied	271, 272
Lactose repressor protein	*E. coli*	*N*-bromosuccinimide	Cys, Met	inactivation in the presence of DNA	273
Lactose transport protein	*E. coli*	ethoxyformic anhydride	His	essential His in translocation complex	274
Lens proteins	bovine, human	8-methoxypsoralen–u.v. irradiation	Trp	photoaddition product	275
Leucyl-tRNA synthetase	*E. coli*	6-amino-7-chloro-dioxoquinoline, *N*-ethylmaleimide	Cys	quinone masks reactive SH groups	276
Lipoamide dehydrogenase	porcine heart	*N*-(1-anilinonaphthyl-4)maleimide	Cys	fluorescence depolarizations study	277
Liver supernatant enzyme	rabbit	iodoacetamide	Cys	inactivation	278
Luteinizing hormone	human	2-hydroxy-5-nitrobenzyl bromide	Arg	essential Arg	279
Lutropin	ovine	photoirradiation–Rose Bengal	His	essential His	280
Lutropin	bovine	dithiothreitol, [^{14}C]iodoacetic acid	$(Cys)_2$, Cys	binding and circular dichroism study	281
Lutropin	bovine	1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide	carboxyl, Lys	substantial activity retained	282, 283
Lysozyme	hen egg-white	*N*-bromosuccinimide	Trp	Raman spectral study	284
Lysozyme	hen egg-white	ozone–HCl	Trp-62	Kyn-62 lysozyme formed	285
Lysozyme	hen egg-white	iodoacetate	His-15	^{13}C n.m.r. study of protein conformation	286
Lysyl hydroxylase	porcine	*p*-chloromercuribenzoate, *N*-ethyl-maleimide, iodoacetamide	Cys	inactivation; reversed with dithiothreitol	287
α_2-Macroglobulin	human	methylamine	Glu (Gln?)	γ-glutamylmethylamide is formed	288
Malate dehydrogenase (cytoplasmic)	porcine heart	iodoacetamide	His (Cys, Met)	essential His	289
Malate dehydrogenase (mitochondrial)	porcine heart	active ester of thyroxine		inhibition; reactivation with anti-thyroxine Ab	290
Malate dehydrogenase (mitochondrial)	porcine heart	5′-[*p*-(fluorosulphonyl)benzoyl]-adenosine	Lys	Lys near or at binding site	291

Malic enzymes	*E. coli*	*N*-ethylmaleimide, *p*-hydroxymercuribenzoate, 5,5′-dithiobis-(2-nitrobenzoate), iodoacetate	Cys	4 thiols per subunit	292
Malic enzymes	*E. coli*	various —SH reagents	Cys	role of thiols in conformation	293
Mandelate racemase	*Pseudomonas putida*	α-phenylglycidate		affinity labelling	294
Membrane proteins	bovine heart mitochondria	3′-*O*-3-[*N*-(4-azido-2-nitrophenyl)amino]propionyl-ATP		affinity labelling	295
Membrane proteins	bovine heart mitochondria	[^{14}C]dicyclohexylcarbodi-imide		binding studies	296

[270] F. T. Robb and M. J. Peak, *Photochem. Photobiol.*, 1979, **30**, 379.
[271] R. D. Brown and K. S. Matthews, *J. Biol. Chem.*, 1979, **254**, 5128.
[272] R. D. Brown and K. S. Matthews, *J. Biol. Chem.*, 1979, **254**, 5135.
[273] S. P. Hanly and K. S. Matthews, *J. Biol. Chem.*, 1979, **254**, 3341.
[274] E. Padan, L. Patel, and H. R. Kaback, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6621.
[275] S. Lerman, J. Megaw, and I. Willis, *Photochem. Photobiol.*, 1979, **31**, 235.
[276] K. Wiebaner, A. Ogilvie, and W. Kerstein, *J. Biol. Chem.*, 1979, **254**, 327.
[277] H. Fukazawa and K. Aki, *J. Biochem. (Tokyo)*, 1979, **86**, 849.
[278] G. W. Rafter and G. G. Harmison, *Biochim. Biophys. Acta*, 1979, **567**, 18.
[279] P. Rathnam and B. B. Saxena, *Biochim. Biophys. Acta*, 1979, **576**, 81.
[280] B. B. Aggarawal and H. Papkoff, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 169.
[281] L. C. Guidice and J. G. Pierce, *J. Biol. Chem.*, 1979, **254**, 1164.
[282] T. F. Parsons and J. G. Pierce, *J. Biol. Chem.*, 1979, **254**, 6010.
[283] J. A. Weare and L. E. Reichert, jun., *J. Biol. Chem.*, 1979, **254**, 6964.
[284] H. Schmidt and L. Bieker, *Arch. Biochem. Biophys.*, 1979, **195**, 205.
[285] M. Yamasaki, T. Tsujita, T. Eto, S. Masuda, K. Mizuno, and F. Sakiyama, *J. Biochem. (Tokyo)*, 1979, **86**, 1291.
[286] W. J. Goux and A. Allerhand, *J. Biol. Chem.*, 1979, **254**, 2210.
[287] R. L. Miller and H. S. Varner, jun., *Biochemistry*, 1979, **18**, 5929.
[288] R. P. Swenson and J. B. Howard, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4313.
[289] T. E. Aspray, G. M. Rühimaki, and R. G. Wolfe, *J. Biol. Chem.*, 1979, **254**, 1576.
[290] E. F. Ulman, R. A. Yoshida, J. I. Blackemore, E. Maggio, and R. Leute, *Biochim. Biophys. Acta*, 1979, **567**, 66.
[291] S. Roy and R. F. Colman, *Biochemistry*, 1979, **18**, 4683.
[292] M. Yamaguchi, *J. Biochem. (Tokyo)*, 1979, **86**, 325.
[293] M. Iwakura, M. Tokushige, and H. Katsuki, *J. Biochem. (Tokyo)*, 1979, **86**, 1239.
[294] B. L. Kenyan and G. D. Hegeman, *Adv. Enzymol.*, 1979, **50**, 325.
[295] J. J. Cosson and R. J. Guillory, *J. Biol. Chem.*, 1979, **254**, 2946.
[296] P. Svoboda, J. Kopecký, J. Moňstěk, and Z. Drahota, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 981.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Membrane proteins	bovine heart mitochondria	[^{14}C]dicyclohexylcarbodi-imide		binding studies	297
Membrane proteins	*Dictyostelium discoideum*	[^{32}P]8-azido-cAMP, [^{32}P]8-azido-AMP		membrane associated actin	298
Membrane proteins	*E. coli*	12-(4-azido-2-nitrophenoxy)-stearoyl[1-^{14}C]glucosamine		integral membrane proteins labelled	299
Membrane proteins	*E. coli*	dithiobis(succinimidyl propionate)	Lys	nearest-neighbour study	300
Membrane proteins	*E. coli*	dimethylsuberimidate	Lys	hetero- and homo-trimers of proteins 08 and 09	301
Membrane proteins	*E. coli*	tartryl diazide, tartryl bis-(glycylazide)	Lys	protein interactions investigated	302
Membrane proteins	*E. coli*	ethoxyformic anhydride	His	lactose transport studies	303
Membrane proteins	mouse L1210	8-azido-AMP		inactivation of carrier protein system	304
Membrane proteins	mouse pancreatic islet cells	chloromercuribenzene-*p*-sulphonic acid	Cys	involved in phosphate efflux	305
Membrane proteins	human erythrocyte	3-azido-(2,7)-naphthalene disulphonate		fluorescent surface-labelling probe	306
Membrane proteins	human erythrocyte	azido[^{3}H]benzene-1-azido-4-*iodo*[^{3}H]benzene		hydrophobic probes	307, 308
Membrane proteins	human erythrocyte	[^{3}H]*N*-ethylmaleimide	Cys	disposition of band 3 polypeptide	309
Membrane proteins	human erythrocyte	[^{3}H]*N*-ethylmaleimide	Cys	reactive SH groups identified	310
Membrane proteins	human peripheral lymphocyte	fluorescamine	Lys	12 labelled proteins identified	311
Membrane proteins	rabbit small intestine brush border	various —SH reagents	Cys	effect on D-glucose transport studied	312
Membrane proteins	rat jejunal brush border vesicles	chloromercuribenzene-*p*-sulphonic acid	Cys	non-electrolyte transport studies	313
Membrane proteins	rat adipocytes	[^{125}I]diazotized di-iodo-sulphanilic acid		non-permeable probe	314

Membrane proteins	rat liver	fluorescamine	Lys	effect on uncouplers studied	315
Membrane proteins	rat thymus	pyridoxal phosphate–$NaBH_4$	Lys	conformation altered	316
Membrane proteins	*Rhodopseudomonas sphaeroides*	2-azido[^{3}H]anthroquinone		affinity labelling	317
Membrane proteins	*Salmonella typhimurium*	various cross-linking reagents		present as trimer	318
Membrane proteins	*Sarcophaga bullata mitochondria*	*p*-hydroxymercuribenzoate	Cys	32 000 dalton protein on phosphate carrier system	319
Membrane proteins	ovine erythrocyte	dimethylsuberimidate	Lys	antibody coating procedure	320
Membrane proteins	Simian virus transformed cells	benzaldehyde		inhibits growth	321

[297] G. A. Blondin, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1087.
[298] J. J. Wallace and W. A. Frazier, *J. Biol. Chem.*, 1979, **254**, 10109.
[299] V. W. Hu and B. J. Wisnieski, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5460.
[300] R. Endermann and U. Henning, *FEBS Lett.*, 1979, **97**, 339.
[301] S. Ichihara and S. Mizushima, *Eur. J. Biochem.*, 1979, **100**, 321.
[302] E. T. Palva, *Eur. J. Biochem.*, 1979, **93**, 495.
[303] E. Padan, L. Patel, and H. R. Kaback, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6221.
[304] G. B. Henderson, E. M. Zevely, and F. M. Huennteus, *J. Biol. Chem.*, 1979, **254**, 9973.
[305] K. Asplund, J. Sehlin, and I. B. Täljedal, *Biochim. Biophys. Acta*, 1979, **588**, 232.
[306] M. E. Dockter, *J. Biol. Chem.*, 1979, **254**, 2161.
[307] E. Wells and J. B. C. Findlay, *Biochem. J.*, 1979, **179**, 257.
[308] E. Wells and J. B. C. Findlay, *Biochem. J.*, 1979, **179**, 265.
[309] A. Rao, *J. Biol. Chem.*, 1979, **254**, 3503.
[310] A. Rao and R. A. F. Reithmeier, *J. Biol. Chem.*, 1979, **254**, 6144.
[311] J. Lambris, M. Papamichail, and P. Fessas, *J. Immunol. Meths.*, 1979, **27**, 55.
[312] J. Biber and H. Hauser, *FEBS Lett.*, 1979, **108**, 451.
[313] P. C. Will and U. Hopfer, *J. Biol. Chem.*, 1979, **254**, 3806.
[314] D. S. Lewis, M. D. Celluci, E. J. Masoro, and B. P. Yu, *Anal. Biochem.*, 1979, **96**, 236.
[315] E. Lam and S. Tu, *FEBS Lett.*, 1979, **106**, 226.
[316] J. A. Cidlowski and J. W. Thanassi, *Biochemistry*, 1979, **18**, 2378.
[317] T. D. Marinetti, M. T. Okamura, and G. Feher, *Biochemistry*, 1979, **18**, 3126.
[318] E. T. Palva and P. Westermann, *FEBS Lett.*, 1979, **99**, 77.
[319] H. Wohlrab, *Biochemistry*, 1979, **18**, 2098.
[320] N. R. Ling, G. Stephens, P. Bratt, and H. S. Dhaliwal, *Mol. Immunol.*, 1979, **16**, 637.
[321] T. Miyokaura, J. Lue Zundel, and K. Sakaguchi, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1024.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Membrane proteins	yeast cell organelles	[^{14}C]ethidinium bromide, photo-irradiation		covalent complexes	322
β-Methylcrotonyl-CoA carboxylase	human fibroblast	phenylglyoxal	Arg	inhibition	323
β_2-Microglobulin	human urine	*N*-(*N*$^{\alpha}$-iodoacetyl-*N*$^{\varepsilon}$-dinitrophenyl-L-lysyl)-*p*-aminophenyl β-lactoside	Cys	carrier for reactive lactosyl groups; antibody-binding studies	324
Mitochondrial phosphate transporter	rat, *Sarcophaga bullata*	*N*-ethylmaleimide		reactive protein identified	325
Myeloma protein M315	mouse	tetranitromethane	Tyr-34$_L$	not involved in DNP binding site	326
Myofibrillar protein	rat muscle lysosomes	[2,3-^{14}C]*N*-ethylmaleimide		intracellular protein-degradation studies	327
Myoglobulin	sperm whale	3-sulphophenyl isothiocyanate	Val	*N*-terminus removed	328
Myoglobulin	sperm whale	copper(II)protoporphyrin IX		reporter group	329
Myosin	bovine heart	phenylglyoxal	Arg	2 essential Arg per myosin head	330
Myosin	chicken gizzard	*o*-iodosobenzoate,*N*-ethylmaleimide	Cys	ATPase activity studied	331
Myosin	chicken gizzard, porcine heart	trinitrobenzenesulphonic acid	Lys	cardiac and smooth muscle compared	332
Myosin	rabbit muscle	[^{14}C]phenylglyoxal	Arg	essential Arg	333
Myosin	rabbit muscle	trinitrobenzenesulphonic acid	Lys	differential modification	334
Myosin	rabbit muscle	DTNB	Cys	ATPase active site thiol studied	335
Myosin	rabbit muscle	*N*-(iodoacetyl)-*N*-(5-sulphonaphthyl)ethylenediamine, methylmethanethiosulphonate	Cys	SH not involved in ATPase activity or actin binding	336
Myrosinase	*Brassica juncea*	various reagents	Cys, Lys	active-centre studies	337
Myotoxin *a*	*Crotalus viridus viridus*	iodoacetic acid	Cys	disulphide-bridge positions identified	338
NADH–Co Q reductase	bovine heart	arylazide-β-alanyl NAD$^+$		affinity labelling	339
Neurophysin	bovine	various reagents		fluorescence of native and modified protein studied	340

Neutral proteinase	monkey liver microsomes	di-isopropylphosphorofluoridate, phenylmethanesulphonyl fluoride and other reagents	Ser	*inhibition*, essential Ser	341
Nucleocapsids	*Sendai* virus	various cross-linking agents		topographical study	342
Ovalbumin		maleic anhydride	Lys	13 Lys modified	343
Ovalbumin		*m*-maleimidobenzoyl-*N*-hydroxy-succinimide ester, poly(D-Glu,D-Lys)		preparation of conjugates	344
Ovomucoid	penguin, turkey	NaOH, NaCN	$(Cys)_2$	cross-linking study	345
Ovomucoid, ovotransferrin	chicken	various carbonyl reagents, $NaBH_4$	Lys	substitution compared	346

[322] M. Fukunaga and K. L. Yielding, *Biochim. Biophys. Acta*, 1979, **585**, 293.
[323] B. Wolf, F. Kalonsek, and L. E. Rosenberg, *Enzyme*, 1979, **24**, 302.
[324] F. Karush, M. M. Chua, and J. D. Rodwell, *Biochemistry*, 1979, **18**, 2226.
[325] H. Wohlrab, *Biochemistry*. 1979, **18**, 2098.
[326] M. Garish, Y. Ben Neriah, R. Zakut, D. Givol, R. A. Dwek, and W. R. C. Jackson, *Mol. Immunol.*, 1979, **16**, 957.
[327] K. W. Gerard and D. L. Schneider, *J. Biol. Chem.*, 1979, **254**, 11798.
[328] R. D. DiMarchi, G. W. Neireiter, W. H. Garner, and F. R. N. Gurd, *Biochemistry*, 1979, **18**, 3101.
[329] K. Alston and C. B. Storm, *Biochemistry*, 1979, **18**, 4292.
[330] E. Markin, I. L. Flink, and S. K. Banergee, *J. Biol. Chem.*, 1979, **254**, 12647.
[331] J. C. Seidel, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 958.
[332] S. K. Srivastara, Y. Tonomura, and A. Inoue, *J. Biochem.* (*Tokyo*), 1979, **86**, 725.
[333] D. Mornet, P. Pantel, E. Audemard, and R. Kassab, *Eur. J. Biochem.*, 1979, **100**, 421.
[334] T. Mryanishi, A. Inoue, and Y. Tonomura, *J. Biochem.* (*Tokyo*), 1979, **85**, 747.
[335] T. Horigarne and T. Yamashita, *J. Biochem.* (*Tokyo*), 1979, **85**, 221.
[336] J. Bolts, K. Ue, T. Hozumi, and J. Samet, *J. Biochem.* (*Tokyo*), 1979, **18**, 5157.
[337] M. Ohtsuru and T. Hata, *Biochim. Biophys. Acta*, 1979, **567**, 384.
[338] J. W. Fox, M. Elzinga, and A. T. Tu, *Biochemistry*, 1979, **18**, 678.
[339] S. Chen and R. J. Guillory, *J. Biol. Chem.*, 1979, **254**, 7220.
[340] S. S. Sur, L. D. Rabbani, L. Libman, and E. Breslo, *Biochemistry*, 1979, **18**, 1026.
[341] K. Sogawa and K. Takahashi, *J. Biochem.* (*Tokyo*), 1979, **86**, 1313.
[342] R. Raghow, D. W. Kingsbury, A. Portner, and S. George, *J. Virol.*, 1979, **30**, 701.
[343] M. A. Qasim and A. Salahuddin, *J. Biochem.* (*Tokyo*), 1979, **85**, 1029.
[344] F.-T. Liu, M. Zinnecker, T. Hamaaka, and D. H. Katz, *Biochemistry*, 1979, **18**, 690.
[345] R. G. Walsh, A. S. Nashef, and R. E. Feeney, *Int. J. Pept. Protein Res.*, 1979, **14**, 290.
[346] K. Freitheim, S. Iwai, and R. E. Fenney, *Int. J. Pept. Protein Res.*, 1979, **14**, 450.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Papain	papaya latex	2-chloromethyl-4-nitrophenyl-*N*-carbobenzoxyglycinate		reporter group; adjacent Trp-177	347
Phenylalanine ammonia-lyase	*Rhodotorula glutinis*	activated polyethyleneglycol	Lys	immunogenicity decreased, activity retained	348
Phenylalanine-tRNA synthetase	*E. coli*	*N*-chlorambucilyl [^{14}C]phenylalanyl-tRNA		activity in α-subunit	349
Phenylalanyl-tRNA synthetase	yeast	u.v. irradiation, periodate-oxidized Phe-tRNA		affinity labelling	350
Phosphoenolpyruvate carboxykinase	porcine liver	*N*-(iodoacetylaminoethyl)-5-naphthylamine-1-sulphonic acid		affinity labelling	351
Phosphoenolpyruvate carboxylase	*E. coli*	trinitrobenzenesulphonic acid	Lys	5 out of 37 modified; inactivation	352
Phosphoenolpyruvate carboxylase	*E. coli*	[^{14}C]bromopyruvate	Cys	1 Cys modified with inhibition	353
Phosphodiesterase activator	rat renal cortex	(i) iodoacetic acid, $HgCl_2$ (ii) N-ethylmaleimide	Cys	(i) inhibition (ii) no inhibition	354
Phosphofructokinase	rabbit muscle	N^6-(6-bromoacetamidohexyl)AMP, PCP		affinity labelling	355
Phospholipase A	*Laticauda semifasciata*	*N*-bromosuccinimide	Trp	activity reduced 10-fold	356
Phospholipase A_2	various sources	tetranitromethane	Tyr	inactivation	357, 358
Plasminogen	human	potassium cyanate	Lys, α-NH_2	urokinase gives active plasmin	359
Plasminogen activator	HeLa cells	X-GlyArgCH_2Cl		differential protease specificity examined	360
Plasminogen activator	human uterus	[^{3}H]di-isopropylphosphofluoridate	Ser	active site Ser in 31 000 dalton chain	361
Plastocyanin	spinach chloroplasts	tetranitromethane	Tyr(-80?)	fully active	362
Pollen proteins	Timothy grass	photo-oxidation	Met, Tyr, Trp	antigenic studies	363
Post-proline cleaving enzyme	lamb kidney	di-isopropylphosphofluoridate, 2-Gly-Pro-CH_2Cl		inhibition	364

Prenyltransferase	avian liver	various photoreactive substrates		affinity labelling	365
Progesterone receptor	avian oviducts	pyridoxal-5'-phosphate	Lys	inhibits binding to ATP–Sepharose	366
Progesterone receptor	chick oviduct	4-mercaptobutyrimidate	Lys (Cys)	7S material identified (AB dimer?)	367
Prolactin	bovine, ovine	ethoxyformic anhydride	His	His-27 and His-30 affect binding	368
Prolactin	bovine, ovine	2-nitrophenylsulphenyl chloride	Trp	loss of receptor affinity and antigenicity	369
Prolactin	ovine	dithiothreitol, iodoacetamide	$(Cys)_2$	activity lost (teleost fish assay)	370
Prolactin	ovine	tetranitromethane	Tyr	5 exposed Tyr not essential	371

[347] S. T. Chang and H. R. Horton, *Biochemistry*, 1979, **18**, 1559.
[348] K. J. Wieder, N. C. Palank, T. Van Es, and F. F. Dars, *J. Biol. Chem.*, 1979, **254**, 12579.
[349] S. N. Khodyrera, *Biokhimiya*, 1979, **44**, 570.
[350] M. Baltzinger, F. Fasiolo, and P. Remy, *Eur. J. Biochem.*, 1979, **97**, 481.
[351] R. Silverstein, A. B. Rawitch, and D. A. Grainger, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 911.
[352] A. Naide, K. Izui, T. Yoshinaga, and H. Katsuki, *J. Biochem. (Tokyo)*, 1979, **85**, 423.
[353] I. Kameshita, M. Tokushige, K. Izui, and H. Katsuki, *J. Biochem. (Tokyo)*, 1979, **86**, 1251.
[354] G. J. Strewler and V. C. Marganiello, *J. Biol. Chem.*, 1979, **254**, 11891.
[355] K. Nagata, K. Suzuki, and K. Imahori, *J. Biochem. (Tokyo)*, 1979, **86**, 1179.
[356] H. Yoshida, T. Kudo, W. Shinkai, and N. Tamiya, *J. Biochem. (Tokyo)*, 1979, **85**, 379.
[357] H. Meyer, H. Verhoef, F. F. A. Hendriks, A. J. Slotboom, and G. H. de Haas, *Biochemistry*, 1979, **18**, 3582.
[358] H. Meyer, W. C. Puijk, R. Dijkman, M. M. E. L. Foda van der Hoorn, F. Pattus, A. J. Slotboom, and G. H. de Haas, *Biochemistry*, 1979, **18**, 3589.
[359] U. Christensen, *Biochim. Biophys. Acta*, 1979, **570**, 324.
[360] P. Coleman, C. Kettner, and E. Shaw, *Biochim. Biophys. Acta*, 1979, **569**, 41.
[361] D. C. Rijken, G. Wijngaards, M. Saal-DeJong, and J. Welbergen, *Biochim. Biophys. Acta*, 1979, **580**, 140.
[362] D. J. Davis and A. San Pietro, *Anal. Biochem.*, 1979, **95**, 254.
[363] A. Malley, D. Begley, and A. Forsham, *Mol. Immunol.*, 1979, **16**, 929.
[364] T. Yoshimoto, K. Ogita, R. Walter, M. Koida, and D. Tsuru, *Biochim. Biophys. Acta*, 1979, **569**, 184.
[365] D. N. Brems and H. C. Rilling, *Biochemistry*, 1979, **18**, 860.
[366] H. Nishigori and D. Toft, *J. Biol. Chem.*, 1979, **254**, 9155.
[367] M. E. Birnbaumer, W. T. Schrader, and B. W. O'Malley, *Biochem. J.*, 1979, **181**, 201.
[368] T. Andersen and K. E. Ebner, *J. Biol. Chem.*, 1979, **254**, 10995.
[369] H. Kochman, J. Garner, and K. Kochman, *Biochim. Biophys. Acta*, 1979, **578**, 125.
[370] B. A. Doneen, T. A. Bewley, and C. H. Li, *Biochemistry*, 1979, **18**, 4851.
[371] T. T. Andersen, M. M. Zamierowski, and K. E. Ebner, *Arch. Biochem. Biophys.*, 1979, **192**, 112.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Propionyl CoA carboxylase	human fibroblast	phenylglyoxal	Arg	inhibition	372
Protease B	*Streptomyces griseus*	maleic anhydride	Lys-125, α-NH_2	Lys-125 essential?	373
Proteases	*Bacillus subtilis*	[^{3}H]phenylmethylsulphonyl fluoride		specificity for serine proteases questioned	374
Protease inhibitor	bovine colostrum	potassium cyanate	Lys	reactive Lys studied	375
Protease inhibitor	human	*N*-chlorosuccinimide	Met	inactivation	376
Proteases (serine)	various sources	di-isopropyl phosphofluoridate	Ser	^{31}P n.m.r. study	377
α_1-Protease inhibitor	human plasma	chloramine T	Met	reaction prevents complex with elastase	378
Protease inhibitor	chick pea	various reagents		differential inhibition of trypsin and chymotrypsin	379
Protease inhibitor	soybean, lima bean	*N*-acetylimidazole	Tyr	some loss of activity	380
Protease disulphide oxidoreductase	bovine liver	iodoacetamide, *N*-ethylmaleimide	Cys	interconversion of oxidized and reduced enzymes studied	381
Protein kinase	bovine heart	*p*-fluorosulphonylbenzoyl-5′-adenosine		affinity labelling	382
Protein kinase	porcine muscle	*p*-fluorosulphonyl[^{14}C]benzoyl-5′-adenosine	Lys	inhibition	383
Protein kinase	rabbit muscle	N^{α}-tosyl-L-lysine chloromethylketone		affinity labelling	384
Protein kinase		8-azido-ATP		high-affinity site localized	385
Protein kinase inhibitor	rabbit muscle	various reagents		*N*-terminal portion at substrate binding site	386
Pterin deaminase	*Bacillus megaterium*	*p*-chloromercuribenzoic acid	Cys	inhibition	387
Purine nucleoside phosphorylase	bovine thyroid	(i) *p*-chloromercuribenzoic acid, *N*-ethylmaleimide (ii) photo-oxidation (iii) pyridoxal-5′-phosphate/$NaBH_4$	(i) Cys (ii) His (iii) Lys	(i), (ii) inosine binding affected (iii) V_{max} reduced	388
Pyocin R1	*Pseudomonas aeruginosa*	*p*-chloromercuribenzoic acid, 4-(*p*-sulphophenyl-azo)-2-mercuriphenol	Cys	important for adsorption to cells	389

Pyridine dinucleotide transhydrogenase	*Rhodospirillum rubrum*	*N*-ethylmaleimide, pentane-2,4-dione	Lys	NADP-binding site studies	390
Pyridoxal kinase	porcine brain	photo-oxidation–bound riboflavin	His	irreversible inactivation	391
Pyridoxamine-5′-phosphate oxidase	rabbit liver	ethoxyformic anhydride	His	1 essential His	392
Pyruvate carboxylase	yeast	dimethylsuberimidate	Lys	tetrameric structure demonstrated	393
Pyruvate decarboxylase	*Thermus aquatious*	DTNB	Cys	10 Cys per subunit	394
Pyruvate dehydrogenase	*E. coli*	various disulphide cross-linking agents	Lys	spatial arrangements studied	395
Pyruvate dehydrogenase	*E. coli*	methyl[1-^{14}C]acetimidate	Lys	use for determining polypeptide chain ratios	396

[372] B. Wolf, F. Kalonsek, and L. E. Rosenberg, *Enzyme*, 1979, **24**, 302.
[373] S. Shinar and A. Gertler, *Int. J. Pept. Protein Res.*, 1979, **13**, 218.
[374] V. Sekar and J. H. Hageman, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 474.
[375] D. Cechova and M. Havranova, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1745.
[376] D. Johnson and J. Travis, *J. Biol. Chem.*, 1979, **254**, 4022.
[377] M. A. Porubcan, W. M. Westler, I. B. Ibanee, and J. L. Markley, *Biochemistry*, 1979, **18**, 4108.
[378] S. Satoh, T. Kurecki, L. F. Kress, and M. Laskowski, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 130.
[379] P. Smirnoff, S. Khalef, Y. Birk, and S. W. Applebaum, *Int. J. Pept. Protein Res.*, 1979, **14**, 186.
[380] E. Kay, *J. Biol. Chem.*, 1979, **254**, 7648.
[381] D. F. Carmichael, M. Keefe, M. Face, and J. E. Dixon, *J. Biol. Chem.*, 1979, **254**, 8386.
[382] C. S. Hixon and E. G. Krebs, *J. Biol. Chem.*, 1979, **254**, 7509.
[383] M. J. Zoller and S. S. Taylor, *J. Biol. Chem.*, 1979, **254**, 8363.
[384] A. Kupfer, V. Gani, J. S. Jimenez, and S. Shaltial, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3073.
[385] J. Hoppe and W. Freist, *Eur. J. Biochem.*, 1979, **93**, 141.
[386] J. G. Demaille, C. Ferraz, and E. H. Fischer, *Biochim. Biophys. Acta*, 1979, **586**, 374.
[387] S. Takikawa, C. Kitayana-Yokokawa, and M. Tsusue, *J. Biochem. (Tokyo)*, 1979, **85**, 785.
[388] J. D. Carlson and A. G. Fischer, *Biochim. Biophys. Acta*, 1979, **571**, 21.
[389] T. Hasegawa, K. Terayama, M. Azegami, and S. Ishii, *J. Biochem. (Tokyo)*, 1979, **85**, 977.
[390] E. Jacobs and R. R. Fisher, *Biochemistry*, 1979, **18**, 4315.
[391] F. Kwok and J. E. Churchich, *J. Biol. Chem.*, 1979, **254**, 6489.
[392] K. Moriike, H. Tsuge, and D. B. McCormick, *J. Biol. Chem.*, 1979, **254**, 6638.
[393] N. D. Cohen, M. F. Utter, N. G. Wrigley, and A. N. Barrett, *Biochemistry*, 1979, **18**, 2197.
[394] S. Libor, T. K. Sundaram, R. Warwick, J. A. Chapman, and S. M. W. Grundy, *Biochemistry*, 1979, **18**, 3647.
[395] R. A. De Abreu, J. De Vries, A. De Kok, and C. Veeger, *Eur. J. Biochem.*, 1979, **97**, 379.
[396] G. Hale, E. A. Hooper, and R. N. Perham, *Biochem. J.*, 1979, **177**, 129.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Pyruvate kinase		trinitrobenzenesulphonic acid	Lys	reactive Lys identified	397
Pyruvate kinase	*Neurospora crassa*	7-chloro-4-nitro-benzo-2-oxa-1,3-diazole	Cys	effect of ligands on accessibility	398
Pyruvate kinase	rabbit muscle	butane-2,3-dione	Arg	essential Arg	399
Pyruvate kinase	yeast	bromopyruvate	Cys	inactivation	400
Pyruvate kinase	yeast	DTNB	Cys	not essential for catalysis	401
Pyruvate oxidase	*E. coli*	*N*-ethylmaleimide	Cys	inactivation	402
Restriction endonuclease	*Providencia alcalifaciens*	*p*-chloromercuribenzoic acid	Cys	complete inhibition	403
Rhodanase	bovine liver	bis-(2-amino-2-carboxypropyl)-trisulphide		^{35}S transfer achieved	404
Rhodopsin	ovine	various cross-linking agents		organization in membrane	405
Ribonuclease	*Aspergillus saitoi*	cyclohexane-1,2-dione, phenylglyoxal	Arg	1 essential Arg	406
Ribonuclease	*Aspergillus saitoi*	iodoacetic acid	Glu	active site Glu (?)	407
Ribonuclease	bovine pancreas	bromoacetic acid	His	His-12, His-119 in active site	408
Ribonuclease	bovine pancreas	malonic dialdehyde	Arg	selective modification	409
Ribonuclease	bovine pancreas	potassium ferrate	His-119	target site inactivation	410
Ribonuclease	bovine pancreas, bovine semen	iodoacetic acid	Cys	structure and stability study	411
Ribonuclease	bovine semen	*N*-acetyl-DL-homocysteine thiolactone		product cross-linked DNase	412
Ribonuclease	*Rhizopus sp.*	photo-oxidation, ethoxyformic anhydride	His	inactivation	413
Ribonuclease	*Ustilago sphaerogena*	(i) iodoacetic acid (ii) 2-methoxy-5-nitrotropone	(i) Glu (ii) α-NH_2	Glu-61 essential, Cys-1 not essential	414
RNA-polymerase	*E. coli*	dimethylsuberimidate, dimethyladipimidate	Lys	structural studies	415
RNA-polymerase	*E. coli*	ethoxyformic anhydride	His	inactivation, complex formation investigated	416
RNA-polymerase	*E. coli*	I_2–lactoperoxidase	Tyr	topographical study	417
RNA-polymerase	*E. coli*	4-chloro-7-nitrobenzofurazan	Cys	50—60% activity loss	418

Ribonucleotide reductase	*E. coli*	dithiothreitol	$(Cys)_2$	membrane activity enhanced, solubilized decreased	419
Ribosomes		aurintricarboxylic acid, *NN'-p*-phenylenedimaleimide	Lys	poly-U interactions studied	420
Ribosomes	*E. coli*	2,3-dimethylmaleic anhydride	Lys	gives protein-deficient ribosomal particles	421
Ribosomes	*E. coli*	puromycin, photoirradiation		affinity labelling	422, 423
Ribosomes	*E. coli*	dimethylsuberimidate	Lys	differential Lys reactivities studied	424

[397] M. P. Russell, R. N. Harkins, and L. A. Fothergill, *Biochem. Soc. Trans.*, 1979, **7**, 714.
[398] M. Kapoor and C. L. Parfett, *Int. J. Biochem.*, 1979, **10**, 47.
[399] E. Cardemil and J. Eyzaguirre, *Arch. Biochem. Biophys.*, 1979, **192**, 533.
[400] S. Yun and C. H. Suelter, *J. Biol. Chem.*, 1979, **254**, 1811.
[401] S. Yun and C. H. Suelter, *J. Biol. Chem.*, 1979, **254**, 1806.
[402] R. L. Houghton, *Int. J. Biochem.*, 1979, **10**, 205.
[403] K. Baksi and G. W. Rushizky, *Anal. Biochem.*, 1979, **99**, 207.
[404] R. Abdolrasulnia and J. L. Wood, *Biochim. Biophys. Acta*, 1979, **567**, 135.
[405] M. Brett and J. B. C. Findlay, *Biochem. J.*, 1979, **177**, 215.
[406] H. Watanabe, K. Ohgi, and M. Irie, *J. Biochem. (Tokyo)*, 1979, **85**, 1315.
[407] K. Ohgi and M. Irie, *J. Biochem. (Tokyo)*, 1979, **86**, 35.
[408] E. P. Lennette and B. V. Plapp, *Biochemistry*, 1979, **18**, 3938.
[409] A. V. Muramor and N. N. Modyanov, *Bioorg. Khim.*, 1979, **5**, 210.
[410] J. Steezko, D. E. Walter, M. Hermodson, and B. Axelrod, *J. Biol. Chem.*, 1979, **254**, 3254.
[411] C. Grandi, E. D'Allesio, and A. Fontana, *Biochemistry*, 1979, **18**, 3413.
[412] D. Wang, *Biochemistry*, 1979, **18**, 4449.
[413] A. Sanda and M. Irie, *Chem. Pharm. Bull.*, 1979, **27**, 2310.
[414] S. Minato and A. Mirai, *J. Biochem. (Tokyo)*, 1979, **85**, 327.
[415] A. Ishihama, M. Aiba, T. Saitoh, and S. Takahashi, *Biochemistry*, 1979, **18**, 972.
[416] T. A. Rozovskaya and R. Sh. Beabealashvilli, *Mol. Biol.*, 1979, **13**, 388.
[417] V. M. Lipkin, N. N. Modyanov, O. Yu. Chertov, S. A. Nikiforov, and A. N. Lebedev, *Bioorg. Khim.*, 1979, **5**, 929.
[418] S. C. Bratcher, K. Nitta, and M. J. Kronman, *Biochem. J.*, 1979, **183**, 255.
[419] G. Viswanathan and J. M. Noronha, *Biochim. Biophys. Acta*, 1979, **567**, 325.
[420] K. Terau and K. Ogata, *J. Biochem. (Tokyo)*, 1979, **86**, 597.
[421] J. A. Pintor-Toro, D. Vazgnez, and E. Palacian, *Biochemistry*, 1979, **18**, 3219.
[422] P. G. Grant, W. A. Strycharz, E. N. Jaynes, and B. S. Cooperman, *Biochemistry*, 1979, **18**, 2149.
[423] P. G. Grant, B. S. Cooperman, and W. A. Strycharz, *Biochemistry*, 1979, **18**, 2154.
[424] G. Allen, R. Capasso, and C. Gualerzi, *J. Biol. Chem.*, 1979, **254**, 9800.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Ribosomes	*E. coli*	2-iminothiolane	Lys, Cys	30S protein S9 linked to 50S proteins	425
Ribosomes	*E. coli*	photo-oxidation	His	peptidyl transferase proteins identified	426
Ribosomes	*E. coli*	2-iminothiolane	Lys, Cys	15 protein pairs identified	427
Ribosomes	*E. coli*	cyanuric chloride	Lys	cross-links to RNA	428
Ribosomes	*E. coli*	I_2–KI	Tyr	topographical study	429
Ribosomes		chemically reactive A-U-G analogues		affinity labelling within initiation complexes	430
Ribosomes	*E. coli*	I_2–lactoperoxidase	Tyr	effect of Mg^{2+} and polyamines studied	431
Ribosomes	*E. coli*	*N*-ethylmaleimide	Cys	conformation study	432
Ribosomes	*E. coli*	u.v. irradiation		cross-linking of *N*-acetyl-tRNA	433
Ribosomes	*E. coli*	*N*-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolidinyl)maleimide, [^{14}C]*N*-ethylmaleimide	Cys	spin labelling	434
Ribosomes	*E. coli*, yeast	I_2–chloramine T	Tyr	2-D gel electrophoretic comparison	435
Ribosomes	L-cells	u.v. irradiation		cross-linking to mRNAs	436
Ribosomes–initiation factor eIF-2	rat liver	dimethyl-4,7-dioxo-5,6-dihydroxy-3,8-diazadecanbisimidate	Lys	binding protein identified	437
Ribosomes	rat liver	Phe–tRNA *N*-acylated by 2-nitro-4-azidobenzoic acid		affinity labelling	438
Ribulose biphosphate carboxylase	*Rhodospirillum rubrum*	tetranitromethane	Tyr	49% activity when 2 Tyr modified	439
Ribulose biphosphate carboxylase	spinach	pyridoxal-5′-phosphate	Lys	inactivation with HCO_3^-	440
Ricin	*Ricinus communis*	glutaraldehyde	Lys	conjugation to lysozyme	441
Ricin	*Ricinus communis*	*NN′-o*-phenylenedimaleimide	Cys	toxicity reduced	442
RNA polymerase	mouse spleen	haematoporphyrin, photoirradiation		inactivation	443
Saccharopine dehydrogenase	yeast	various —SH reagents	Cys	inactivation	444

Sialoglycoprotein	human erythrocyte	1-azido-4-iodo[^{3}H]benzene		intramembrane region labelled	445
Subtilisin inhibitor	*Streptomyces algogriseolus*	(i) H_2O-soluble carbodi-imide–GlyOMe; (ii) tetranitromethane	Asp Tyr	Asp-76 or Asp-68 is involved	446
Succinate dehydrogenase	bovine heart	3-nitropropionate	Cys	inactivation	447
Succinate semialdehyde dehydrogenase	porcine brain	pyridoxal-5′-phosphate,*o*-phthalaldehyde, $NaBH_4$	Lys	inhibition	448
Succinyl-CoA-synthetase	*E. coli*	[^{3}H]ethoxyformic anhydride	His	1 essential His per $\alpha\beta$-catalytic unit	449
Superoxide dismutase	bovine	several diazonium reagents	His	2 active site His per subunit	450
Superoxide dismutase	various sources	phenylglyoxal	Arg-14	inactivation	451

425 J. W. Kenny, T. G. Fanning, J. M. Lambert, and R. R. Traut, *J. Mol. Biol.*, 1979, **135**, 151.
426 F. Dohme and S. R. Fahnestock, *J. Mol. Biol.* 1979, **129**, 63.
427 J. W. Kenny and R. R. Traunt, *J. Mol. Biol.*, 1979, **127**, 243.
428 C. Oste and R. Brimacombe, *Mol. Gen. Genet.*, 1979, **168**, 81.
429 M. K. T. Lam, L. M. Changchien, and G. R. Craven, *J. Mol. Biol.*, 1979, **128**, 561.
430 O. Pongs, H. U. Petersen, M. Grunberg-Manago, E. Lanka, R. Bald, and G. Stoffer, *J. Mol. Biol.*, 1979, **134**, 329.
431 C. J. Michalski, S. M. Boyle, and B. H. Sells, *Can. J. Biochem.*, 1979, **57**, 250.
432 N. Ghosh and P. B. Moore, *Eur. J. Biochem.*, 1979, **93**, 147.
433 J. Ofengand, R. Lion, J. Kohut, I. Schwartz, and R. A. Zimmerman, *Biochemistry*, 1979, **18**, 4322.
434 H. Dugas, A. Rodriguez, and N. Brisson, *Can. J. Biochem.*, 1979, **57**, 1407.
435 C. Bernabeu, D. Vazquez, and F. P. Conde, *Biochim. Biophys. Acta*, 1979, **577**, 400.
436 J. R. Greenberg, *Nucl. Acid. Res.*, 1979, **6**, 715.
437 P. Westermann, W. Heumann, U.-A. Bommer, H. Bielka, O. Nygard, and T. Hultin, *FEBS Lett.*, 1979, **97**, 101.
438 J. Stahl, H. Bohm, V. A. Pozdnjakov, and A. S. Girshovich, *FEBS Lett.*, 1979, **103**, 273.
439 P. D. Robson and F. R. Tabita, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 85.
440 W. B. Whitman, C. Colletti, and F. R. Tabita, *FEBS Lett.*, **101**, 249.
441 S. Pillai, A. Surolia and B. K. Báchhawat, *Biochim. Biophys. Acta*, 1979, **580**, 216.
442 T. Oda and G. Funatsu, *Agric. Biol. Chem.*, 1979, **43**, 547.
443 B. R. Munson, *Int. J. Biochem.*, 1979, **10**, 957.
444 M. Ogaura, M. Okamoto, and M. Fujioka, *J. Biol. Chem.*, 1979, **254**, 7030.
445 E. Wells and J. B. C. Findlay, *Biochem. J.*, 1979, **179**, 265.
446 K. Inoue, B. Tonomura, and K. Heromi, *J. Biochem. (Tokyo)*, 1979, **85**, 1115.
447 C. J. Coles, D. E. Edmonson, and T. P. Singer, *J. Biol. Chem.*, 1979, **254**, 5161.
448 W. S. Blaner and J. Churchich, *J. Biol. Chem.*, 1979, **254**, 1794.
449 G. E. Collier and J. S. Nishimura, *J. Biol. Chem.*, 1979, **254**, 10925.
450 D. P. Malinowski and I. Fridovich, *Biochemistry*, 1979, **18**, 237.
451 D. P. Malinowski and I. Fridovich, *Biochemistry*, 1979, **18**, 5909.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Taipoxin	*Oxyuranus s. scutellatus*	*p*-bromophenacyl bromide	His	effect on phospholipase activity, neurotoxicity, and acetylcholine	452
T-antigen	Simian virus	reduction, *N*-ethylmaleimide	$(Cys)_2$	effect on physical properties	453
Thiolsubtilisin		Z-Ala-Gly-PheCH_2Cl	Cys	inhibition	454
Thrombin	human	Val-Ile-Pro-Arg-chloromethyl ketone		antithrombin III-heparin/thrombin complex studies	455
α-Thrombin	human	pyridoxal-5′-phosphate–$NaBH_4$	Lys	binding studies with heparin	456
Thrombocytin	*Bothrops atrox*	di-isopropyl fluorophosphate, several Arg-chloromethyl ketones		inhibition; Ser and His in active site	457
Thymidylate synthetase	*Lactobacillus casei*	DTNB, *N*-ethylmaleimide	Cys	1 essential reactive Cys	458
Thymidylate synthetase	*Lactobacillus casei*	phenylglyoxal	Arg	1 essential Arg	459
Thymidylate synthetase	*Lactobacillus casei*	5(α-bromoacetyl)-2′-deoxyuridine-5′-phosphate		affinity labelling	460
Thyroglobulin	human	I_2–lactoperoxidase–chloramine T	Tyr	kinetics compared	461
Thyroliberin deaminating enzyme	bovine pituitary	(i) *N*-ethylmaleimide (ii) di-isopropylfluorophosphate	(i) Cys (ii) Ser	(i) essential Cys (ii) essential Ser	462
Thyroid-stimulating hormone	human	HIO_4, horseradish peroxidase		conjugate for TSH assay	463
Thyroid-stimulating hormone	bovine	dansyl chloride	Lys, Tyr	thyroid plasma membrane binding studies	464
Thyrotropin	bovine	1-ethyl-3-(3-dimethylaminopropyl-carbodi-imide	Lys, carboxyl	substantial activity retained	465
Thyroxine-binding globulin	human	*p*-iodophenylsulphonyl chloride	Lys	binding inhibited when 1 Lys modified	466
Transhydrogenase	*E. coli*	phenylglyoxal, butane-2,3-dione	Arg	2 conformations proposed	467
Transketolase	yeast	diacetyl, $NaBH_4$	Arg	functional Arg in active centre	468
Trehalase	*Rhynochosciara americana* midgut	1-ethyl-3-(3-dimethylaminopropyl)-carbodi-imide	carboxyl	2 essential carboxyls	469
Troponin C	rabbit muscle, bovine heart muscle	tetranitromethane	Tyr	nitrotyrosine as spectroscopic probe	470

Troponin C	rabbit muscle	*N*-ethylmaleimide, *N*-[*p*-(2-benzimidazolyl)phenyl] maleimide	Cys	kinetics of conformational change studied	471
Trypsin	bovine pancreas	various hydrophobic polymer anhydrides		physical and enzymic properties	472
Trypsin	bovine pancreas	leupeptin sulphate	Ser	mechanistic study	473
Trypsin	bovine pancreas	methyl acetimidate	Lys	autolysis of modified enzyme studied	474
Trypsin	bovine pancreas	activated polyethylene glycol	Lys	antigenicity abolished, activity largely unaltered	475
Trypsin	bovine pancreas	Ehrlich ascites tumour cell inhibitor, sodium periodate		kinetics of inhibition and reactivation	476

[452] J. Fohlman, D. Eaker, M. J. Dowdall, R. Lullman-Rauch, T. Sjodin, and S. Leander, *Eur. J. Biochem.*, 1979, **94**, 531.
[453] L. V. Crawford and P. Z. O'Farrell, *J. Virol.*, 1979, **29**, 587.
[454] I.-H. Tsai and M. L. Bender, *Biochemistry*, 1979, **18**, 3764.
[455] M. J. Griffith, H. S. Kingdon, and R. L. Lundblad, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 686.
[456] M. J. Griffith, *J. Biol. Chem.*, 1979, **254**, 3401.
[457] E. P. Kirby, S. Niewiarowski, K. Stocker, C. Kettner, E. Shaw, and T. M. Brudzynski, *Biochemistry*, 1979, **18**, 3564.
[458] K. D. Danenberg and P. V. Danenberg, *J. Biol. Chem.*, 1979, **254**, 4345.
[459] K. L. Cipollo and R. B. Dunlap, *Biochemistry*, 1979, **18**, 5537.
[460] C. B. Brouilette, C. T. C. Chang, and M. P. Hertes, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 613.
[461] L. Lamas, *Eur. J. Biochem.*, 1979, **96**, 93.
[462] H. Knisatschek and K. Bauer, *J. Biol. Chem.*, 1979, **254**, 10936.
[463] N. Kato, H. Naruse, M. Irie, and A. Tsuji, *Anal. Biochem.*, 1979, **96**, 419.
[464] S. M. Aloj, G. Lee, E. Consiglio, S. Formisane, A. P. Minton, and L. D. Kohn, *J. Biol. Chem.*, 1979, **254**, 9030.
[465] T. F. Parsons and J. G. Pierce, *J. Biol. Chem.*, 1979, **254**, 6010.
[466] J. S. Siegel, L. Korcek, and M. Tabachnick, *FEBS Lett.*, 1979, **102**, 306.
[467] M. Homyk and P. D. Bragg, *Biochim. Biophys. Acta*, 1979, **571**, 201.
[468] L. E. Meshalkina and O. E. Volkovitskaya, *Dokl. Akad. Nauk SSSR*, 1979, **245**, 1259.
[469] W. R. Terra, L. C. M. Terra, C. Ferreira, and A. G. de Bianchi, *Biochim. Biophys. Acta*, 1979, **571**, 79.
[470] W. D. McCubbin, M. T. Hincke, and C. M. Kay, *Can. J. Biochem.*, 1979, **57**, 15.
[471] T. Lio and H. Kondo, *J. Biochem. (Tokyo)*, 1979, **86**, 1883.
[472] R. A. G. Smith, *Biochem. J.*, 1979, **181**, 111.
[473] H. Kuramochi, H. Nakata, and S. Ishii, *J. Biochem. (Tokyo)*, 1979, **86**, 1403.
[474] T. Kumazaki and S. Ishii, *J. Biochem. (Tokyo)*, 1979, **85**, 581.
[475] A. Abuchowski and F. F. Davis, *Biochim. Biophys. Acta*, 1979, **578**, 41.
[476] F. S. Stevens and A. Al-Habib, *Biochim Biophys. Acta*, 1979, **568**, 408.

Table (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Trypsin	bovine pancreas	dansyl-Ala-Lys-CH_2Cl		active site probed	477
β-Trypsin	bovine pancreas	photoirradiation	Trp	role of indole triplet in photolysis	478
Trypsinogen	bovine pancreas	glutathione	Cys	mixed disulphide	479
Trypsinogen	bovine pancreas	$NaBH_4$, dithiothreitol, [^{14}C]iodoacetic acid	$(Cys)_2$	role in refolding	480
Trypsin inhibitor (basic pancreatic)		$NaBH_4$ (and proteolysis), iodoacetamide	$(Cys)_2$	conformation studied by ^{1}H n.m.r.	481
Tryptophanase	*E. coli*	ozone	Trp	essential Trp	482
Tryptophanase	*E. coli*	light–pyridoxal-5′-phosphate and other sensitizers	His	inactivation	483
Tryptophan synthase	*E. coli*	DTNB	Cys	conformational study	484
Tryptophanyl-tRNA synthetase	bovine pancreas	*p*-azido anilides of ADP, GDP, ATP, GTP, and CTP		binding sites present	485
Tubulin	ovine brain	[β,γ-^{32}P]- and [γ-^{32}P]-8-azido-guanosine-5′-triphosphate		role of GTP in microtubule assembly	486
Tyrosinase	mushroom	*NN*-di(2-chloroethyl)-*p*-phenylene-diamine	Tyr	activity doubled	487
Tyrosine chemoreceptor	*Pseudomonas aeruginosa*	*p*-azidophenylalanine, t-butoxy-carbonyl-*p*-azidophenylalanine		affinity labelling	488
Tyrosine decarboxylase	*Streptococcus facaelis*	Na[^{3}H]borohydride		stereospecificity of reduction studied	489
Uricase	*Candida utilis*	2-*O*-methoxypolyethylene glycol-4,6-dichloro-*s*-triazine	Lys	antigenicity eliminated activity partially retained	490
Uricase	hog liver	glutaraldehyde, albumin	Lys	stable conjugate	491
UDP-galactose 4-epimerase	*E. coli*	*p*-(bromoacetamido)phenyl-UDP		inactivation; 80% activity on diphosphopyridine nucleotide addition	492, 493
Urolanase	*Pseudomas putida*, bovine liver	[^{3}H]$NaBH_4$		inactivation, essential nicotinamide	494
Urokinase	human	Pro-Gly-Arg-CH_2Cl		affinity labelling	495

Viral proteins	*Densonucleosis* virus	dansyl chloride	Lys	gel electrophoretic method	496
Viral glycoprotein	*Sendai* virus	dithiothreitol, reduced glutathione, 2-mercaptoethanol	$(Cys)_2$	loss of activity	497
Virus envelope proteins	Newcastle disease virus	12-(4-azido-2-nitrophenoxy)-stearoyl[1-^{14}C]glycosamine		photoreactive glycolipid probe	498
Xanthine dehydrogenase	chicken liver	hydrogen peroxide		inactivation	499

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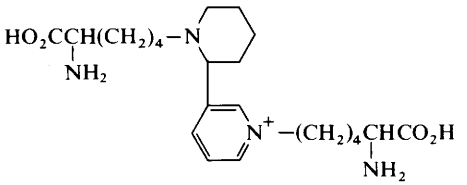
[477] G. S. Penny, D. F. Dyckes, and B. D. Burleigh, *Int. J. Pept. Protein Res.*, 1979, **13**, 95.
[478] N. Ramachandran and C. A. Ghiron, *Photochem. Photobiol.*, 1979, **29**, 67.
[479] T. W. Odorzynski and A. Light, *J. Biol. Chem.*, 1979, **254**, 4291.
[480] A. Light and T. W. Odorzynski, *J. Biol. Chem.*, 1979, **254**, 9162.
[481] G. Wagner, H. Tschesche, and K. Wüthrich, *Eur. J. Biochem.*, 1979, **95**, 239.
[482] M. Tokushige, Y. Fukada, and Y. Watanabe, *Biochem. Biophys, Res. Commun.*, 1979, **86**, 976.
[483] T. Nihira, T. Toraya, and S. Fukui, *Eur. J. Biochem.*, 1979, **101**, 341.
[484] M. E. Goldberg and A. Högberg-Raibaud, *J. Biol Chem.*, 1979, **254**, 7752.
[485] G. A. Nevinsky, O. I. Lavrik, O. O. Farorova, and L. L. Kisseler, *Bioorg. Khim.*, 1979, **5**, 332.
[486] R. L. Geahlen and B. E. Haley, *J. Biol. Chem.*, 1979, **254**, 11982.
[487] Ts. K. Ivanova, Z. D. Raikov, and S. Kovacheva, *Dokl. Bolg. Akad. Nauk*, 1979, **31**, 1463.
[488] R. Barber, S. Primrose, and G. Dodd, *FEBS Lett.*, 1979, **105**, 43.
[489] J. C. Vederas, I. D. Reingold, and H. W. Sellers, *J. Biol. Chem.*, 1979, **254**, 5053.
[490] H. Nishimura, Y. Ashihara, A. Matsushima, and Y. Inada, *Enzyme*, 1979, **24**, 261.
[491] M. J. Poznansky, *Life Sci.*, 1979, **24**, 153.
[492] Y. Wong, F. B. Winer, and P. A. Frey, *Biochemistry*, 1979, **18**, 5332.
[493] Y. Wong and P. A. Frey, *Biochemistry*, 1979, **18**, 5337.
[494] V. Keul, F. Kaeppli, C. Ghosh, T. Krebs, J. A. Robinson, and J. Retey, *J. Biol. Chem.*, 1979, **254**, 843.
[495] C. Kettner and E. Shaw, *Biochim. Biophys. Acta*, 1979, **569**, 31.
[496] P. Tijssen and E. Kurstak, *Anal. Biochem.*, 1979, **99**, 97.
[497] M. Ozawa, A. Asano, and Y. Okada, *J. Biochem. (Toyko)*, 1979, **86**, 1361.
[498] J. S. Bramhall, M. A. Shiflett, and B. J. Wisnieski, *Biochem. J.*, 1979, **177**, 765.
[499] S. L. Betcher-Lange, M. P. Coughlan, and K. V. Rajagoplan, *J. Biol. Chem.*, 1979, **254**, 8825.

2 Re-investigation of Known Reagents and Reactions

Cyanogen Bromide.—Evidence is presented [500] to show that only partial cleavage of methionine–cystine bonds occurs when human serum albumin and bovine α-lactalbumin are treated with CNBr. The data illustrate that Met-123 in the human albumin is converted into homoserine, probably *via* an intramolecular reaction of the cystine sulphur and the iminolactone intermediate formed during the CNBr treatment; as a consequence, the peptide bond remains uncleaved. The earlier suggestion of methionine omission at position 123 in some human albumins has been correspondingly revised.

Butane-2,3-dione.—The reagent has been shown to sensitize the photoinactivation of α-chymotrypsin, reversing the conclusion of earlier negative findings.[501] The first-order rate constant for the butane-2,3-dione-sensitized photoinactivation was found to decrease linearly with increasing pH; this appears to be the first such case reported. A similar novel photo-oxidation reaction of NADH in the presence of butane-2,3-dione has also been reported,[502] under conditions of normal laboratory lighting.

Glutaraldehyde.—In support of an earlier proposal, modification of proteins with glutaraldehyde has been shown[503] to involve the formation of quarternary pyridinium compounds from lysine residues. Following reaction at pH = 4.7 with ovalbumin and complete acid hydrolysis, two main products were isolated with characteristic absorption maxima near 265 nm. The first product was shown to be 1-(5-amino-5-carboxylpentyl)pyridinium chloride and the second 1-(5-amino-5-carboxy pentyl)-3-[1-(5-amino-5-carboxypentyl-2-piperidyl)]pyridinium chloride or anabilysine (1). The latter moiety is proposed as the important cross-linking elements in glutaraldehyde reactions with proteins.



(1)

2-Nitro-5-thiocyanobenzoic acid (NTCB).—This reagent is known to cleave proteins at cysteine residues.[504] Reaction of NCTB with native bovine DNase has been shown in the present studies[505, 506] to modify serine and threonine residues with fragmentation of the enzyme at Thr-14, Ser-40, and Ser-135. The reaction scheme proposed by the authors is analogous to that described for cysteine cleavage. NCTB is proposed as a possible novel specific inhibitor of DNase I.

[500] N. Doyen and C. Lapresle, *Biochem. J.*, 1979, **177**, 251.
[501] H. Fliss and T. Viswanatha, *Can. J. Biochem.*, 1979, **57**, 1267.
[502] M. Homyk and P. D. Bragg, *Can. J. Biochem.*, 1979, **57**, 977.
[503] P. M. Hardy, G. J. Hughes, and H. N. Rydon, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2288.
[504] Y. Eshdat and A. Lemay, *Biochim. Biophys. Acta*, 1979, **577**, 360.
[505] Ta-Hsiu Liao and L. J. McKenzie, *J. Biol. Chem.*, 1979, **254**, 9598.
[506] Ta-Hsiu Liao and A. Wadano, *J. Biol Chem.*, 1979, **254**, 9602.

2-(2′-Pyridylmercapto)mercuri-4-nitrophenol.—Synthesis and further characterization of the reagent are reported.[507, 508] This 2-pyridyl mercurial offers advantages over the typical 2-chloromercuri-4-nitrophenol class of nitrophenol mercurials, in that the 2-thiopyridyl leaving group allows reactions with thiol compounds to be quantified more easily over a wide pH range. Caution is recommended, however, when using such organomercurials as thiol reactivity probes because of easy ligand-exchange reactions.[509]

1-Fluoro-2,4-dinitrobenzene.—Discordant results have been reported [510] for the influence of 1-fluoro-2,4-dinitrobenzene reaction on ox liver glutamate dehydrogenase activity. In this study, the pattern of modification and protection of specific binding sites in the presence of nucleotide ligands is shown to be considerably more complex than indicated by earlier reports. The results suggest that the effects of dinitrophenylation are not due to direct blocking of nucleotide binding sites, as indicated previously, but rather to modification of that part of the proteins responsible for transmitting information between various binding sites. The authors discuss and question the strategy of using a combination of relatively non-specific modifying reagents and selective protecting agents to identify residues involved in various regulatory sites in allosteric enzymes.

5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB).—Inactivation studies of yeast pyruvate kinase with DTNB confirm [511] earlier results that the enzyme exhibits three classes of thiol groups. The distribution of groups reported in each category, however, is again at variance with published data. The complication caused by the possible formation of disulphide bridges with this reagent is indicated. A similar point is illustrated [512] in the reaction of cysteine residues of histone H3 and DTNB. A more reliable value for the molar extinction coefficient of the 2-nitro-5-thiobenzoate anion has been reported,[513] *viz.* $\varepsilon_{412} = 14\,150$.

Sodium Cyanoborohydride.—Optimal conditions for labelling proteins by reductive methylation using formaldehyde (labelled as ^{14}C or ^{13}C) and sodium cyanoborohydride (labelled as ^{3}H) are described.[514, 515] Evidence is presented to show that minimal side reactions occur, confirming the superiority of this reagent over sodium borohydride.

4-Chloro-7-nitrobenzofurazan.—The reaction of this reagent (Nbf-Cl) with simple thiols at pH values above 5 has been further characterized.[516] Results confirm that products other than the 4-thio-derivative are formed; one of these materials is thought to be a reversible complex of the Meisenheimer type. A second product, was shown to inactivate *E. coli* RNA polymerase irreversibly. This latter moiety is suggested to result from further reaction of the thiol compound with the

[507] B. S. Baines and K. Brocklehurst, *Biochem. J.*, 1979, **179**, 701.
[508] K. Brocklehurst, *Int. J. Biochem.*, 1979, **10**, 259.
[509] R. G. Khalifah, G. Sanyal, D. J. Strader, and W. McI. Sutherland, *J. Biol. Chem.*, 1979, **254**, 602.
[510] K. G. Gould and P. C. Engel, *Arch. Biochem. Biophys.*, 1979, **196**, 284.
[511] S. Yun and C. H. Suelters, *J. Biol. Chem.*, 1979, **254**, 1806.
[512] J. Bode, *Anal. Biochem.*, 1979, **94**, 465.
[513] P. W. Riddles, R. L. Blakeley, and B. Zerner, *Anal. Biochem.*, 1979, **94**, 75.
[514] N. Jentoft and D. G. Dearborn, *J. Biol. Chem.*, 1979, **254**, 4359.
[515] J. E. Jentoft, N. Jentoft, T. A. Gerken, and D. G. Dearborn, *J. Biol. Chem.*, 1979, **254**, 4366.
[516] K. Nitta, S. C. Bratcher and M. J. Kronman, *Biochem. J.*, 1979, **177**, 385.

Meisenheimer complex. The reaction pattern of Nbf-Cl with thiol compounds would appear to complicate the use of this reagent in characterizing thiol-group reactivities in proteins.

2-Carboxy-4,6-dinitrochlorobenzene.—Further studies with this relatively new arylating agent (CDNCB) have been described.[517] CDNCB is claimed to display high reactivity and selectivity toward Lys-41 in ribonuclease. The reagent appears to react sluggishly with low molecular weight model compounds but is reported to be useful for probing unusually reactive amino-groups.

Fluorescamine.—Re-examination of the reaction conditions of primary amino-groups and fluorescamine is reported.[518] Comparisons of the utility of this reagent, *o*-phthalaldehyde, and other conventional protein assay procedures are described.

3 New Reagents and Techniques

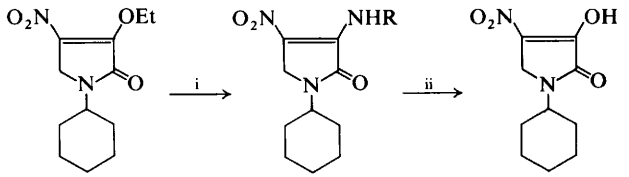
A general strategy has been proposed,[519] using [^{14}C]sucrose covalently bound to protein *via* a cyanuric chloride bridging group, for assessing the catabolism of plasma proteins by lysosomal mechanisms. Sucrose acts as a suitable marker group since it is metabolized poorly, if at all, by mammalian tissues and, secondly, is sufficiently large such that it escapes from lysosomes only very slowly. Incomplete degradation of the modified protein, however, possibly limits the scope of the method. A similar approach has been described[520] using [G-^{3}H]raffinose coupled to protein *via* a reductive methylation procedure.

Further details have appeared[521] on the synthesis and properties of the chromophoric agent, 4-hydroxy-3-nitrophenylglyoxal (HNPG); the reagent supplements the range of available arginine residue probes. In studies on the inactivation kinetics of creatine kinase, HNPG was shown to possess selectivity characteristics similar to phenylglyoxal. A new procedure to cleave tryptophanyl peptide bonds has been described,[522] using *o*-iodosobenzoic acid. Unwanted side reactions, such as the expected oxidation of cysteine, can be readily prevented by *S*-alkylation; methionine sulphoxide formation is likely, however. The reaction would seem to be particularly valuable for sequence analysis, since it is claimed to be highly specific, nearly quantitative, and directed at a residue that is relatively rare in many proteins. In acidic media, 3-diazonium-1,2,4-triazole has also been reported[523] to react specifically with tryptophan residues. The observation has been exploited, using the corresponding ^{14}C-labelled triazole, to provide an additional and sensitive means of determining protein tryptophan levels.

A means of converting γ-carboxyglutamic acid residues in proteins to glutamic acid has been described.[524] The procedure is based on the thermal stability of dry proteins and involves heating the dried material *in vacuo* at 110 °C following freeze-drying from an acid solution.

[517] J. Bello, H. Iijima, and G. Kartha, *Int. J. Pept. Protein Res.*, 1979, **14**, 199.
[518] J. V. Castell, M. Cervera, and R. Marco, *Anal. Biochem.*, 1979, **99**, 379.
[519] R. C. Pittman, R. Green, A. D. Attie, and D. Steinberg, *J. Biol. Chem.*, 1979, **254**, 6876.
[520] J. Van Zile, L. A. Henderson, J. W. Baynes, and S. R. Thorpe, *J. Biol. Chem.*, 1979, **254**, 3547.
[521] C. J. L. Borders, L. J. Pearson, A. E. McLaughlin, M. E. Gustafson, J. Vasiloff, F. Y. Au, and D. J. Morgan, *Biochim. Biophys. Acta*, 1979, **568**, 491.
[522] W. C. Mahoney and M. A. Hermodson, *Biochemistry*, 1979, **18**, 3810.
[523] M. C. DeTraglia, J. S. Brand, and A. M. Tomesko, *Anal. Biochem.*, 1979, **99**, 464.
[524] J. W. Poser and P. A. Price, *J. Biol. Chem.*, 1979, **254**, 431.

Several reversible protecting groups have been developed. Reductive alkylation of protein amino-groups with an α-hydroxy aldehyde or ketone in the presence of sodium borohydride has been shown[525] to produce structures of the type $R^1NHCHR^2C(OH)<$ (R^2 = H or alkyl). This grouping is readily attacked by periodate at pH values in the range 7—9 to regenerate the amino-function; best results were obtained with acetol, CH_3COCH_2OH. A new reversible amino-group reagent, 4-nitro-1-cyclohexyl-3-ethoxy-2-oxo-3-pyrroline (2), has been reported.[526] Protein amino-groups are readily modified with this agent at pH = 9.0 to yield a structure which is stable at neutral pH ($t_{\frac{1}{2}}$ = 167 h, pH = 7.0, 20 °C) (Scheme 1). Deprotection is effected rapidly in alkaline media (pH > 8.0) and can



(2)

Scheme 1

Reagents: i, RNA_2 (pH = 9.0), dialyse pH = 3—4; ii, pH > 8

be quantified by u.v. spectroscopy (λ_{max} = 363 nm). Mild conditions have been described[527] for the removal of halogenoacetyl groups from protein amino-groups, thereby affording the possibility of using *N*-halogenoacetoxy succinimide derivatives as reversible protecting agents.

Oxidation of methionine occurs relatively commonly as an undesired side reaction in a number of protein modification procedures, and perhaps most especially in radio-iodinations. The importance of establishing optimal iodination conditions in order to avoid this possibility has been emphasized,[528] following studies with a variety of common oxidants; for this work, a new chromatographic technique to estimate methionine oxidation was developed. An investigation[529] of the means of converting the sulphoxide to methionine has identified *N*-methylmercaptoacetamide as the reducing agent of choice. Over a wide range of pH, methionine sulphoxide in protein is reported to be completely reduced during 12—24 h at 37 °C with this reagent.

Further progress has been made in the development of procedures for studying the location and interaction of membrane proteins. The synthesis of a lipophilic photolabel, [^{3}H]pyrenesulphonyl azide, has been described[530, 531] and used to detect the components of the acetylcholine receptor molecule in contract with the hydrophobic region of *Torpedo californica* electroplax membranes. This moiety

[525] K. F. Geoghegan, D. M. Ybarra, and R. E. Feeney, *Biochemistry*, 1979, **18**, 5392.
[526] D. J. Negri, P. L. Southwick, and W. E. Brown, *Biochim. Biophys. Acta*, 1979, **579**, 31.
[527] J. D. Glass, M. Pelzig, and C. S. Pande, *Int. J. Pept. Protein Res.*, 1979, **13**, 28.
[528] R. A. Rosenberg and T. M. Murray, *Biochim. Biophys. Acta*, 1979, **584**, 261.
[529] R. A. Houghten and C. H. Li, *Anal. Biochem.*, 1979, **98**, 36.
[530] V. Sâtor, J. M. Gonzales-Ros, P. Calvo-Fernandez, and M. Martinez-Carrion, *Biochemistry*, 1979, **18**, 1200.
[531] J. M. Gonzales-Ros, V. Sâtor, P. Calvo-Fernandez, and M. Martinez-Carrion, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 214.

complements the range of available membrane-penetrating reporter reagents. An improved phosphorescence-depolarization probe, erythrosin (tetraiodofluorescein), has been synthesized[532] and employed to investigate the rotational mobility of erythrocyte membrane proteins. This agent has a quantum yield for phosphorescence some 3—5 times higher than eosin. Excited-state life-time studies have also been applied[533] to the evaluation of conformational changes in the *E. coli* maltose receptor. The methodology employed in this study, utilizing the reagent 5-(iodoacetamido)fluorescein, extends the distant reporter-group technique to proteins with more than one tryptophan residue.

A versatile methodology has been developed[534] which facilitates the coupling of macromolecules to cell surfaces. In neutral and alkaline media, glutaraldehyde has been shown to polymerize and produce colloidal microspheres. Such materials display a high concentration of surface aldehyde groups which bind proteins with good capacity and react readily with cell membranes. These convenient immunoreagents are suitable for a wide range of applications, including the separation of individual cell populations. By carrying out the polymerization reaction in the presence of iron oxide, magnetic microspheres can be produced which improves the utility of the materials still further. A second novel procedure has been described[535] for attaching exogeneous protein to membranes, using the expedient of bonding the macromolecule to a hydrophobic anchor group. For this purpose, a new phospholipid-containing alkylating reagent, *N*-(N^{α}-iodoacetyl, N^{ε}-dansyl-lysyl)-phosphatidylethanolamine, has been synthesized. Other recent developments in cell-surface labelling procedures are reported[536] in the collected proceedings of an international workshop.

Appropriate modifications to a previously published technique has demonstrated that proteins can be labelled directly and efficiently with tritium gas.[537] At tritium pressures as low as 10^{-3}Torr, thermolysin and collagen were labelled without denaturation to specific activity levels ranging from 1—74 Ci mole^{-1} and 90—5600 Ci mole^{-1}, respectively. Antithrombin III preparations afforded an average specific activity of 10 Ci mole^{-1}. This gas-phase procedure offers particular advantages for labelling internal, and possibly solvent-inaccessible, sites within the protein structure. A successful synthesis of ^{13}C-labelled RNase at Met-29 has been described,[538] *via* a sulphonium salt reaction at acid pH with [^{13}C]methyl iodide. Judicious choice of reactant concentrations allows the mercaptoethanol-induced demethylation reaction to predominate over disulphide interchanges.

Selective carboxymethylation of bioaffinity-bound alcohol dehydrogenase has been reported.[539] This technique, involving modification of enzymes bound to a solid phase by means of an immobilized ligand, should have general utility and allow selective active-site labelling of oligomeric enzymes.

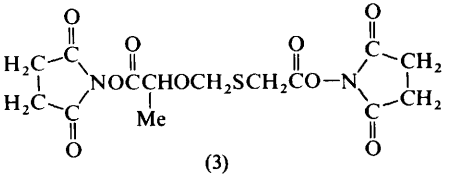
[532] C. H. Moore and P. B. Garland, *Biochem. Soc. Trans.*, 1979, **7**, 945.
[533] R. S. Zukin, *Biochemistry*, 1979, **18**, 2139.
[534] S. Margel, S. Zisblatt, and A. Rembaum, *J. Immunol. Meths.*, 1979, **28**, 341.
[535] D. Sinha and F. Karush, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 554.
[536] Cell Surface Labelling, ed. R. P. Becker and O. Johari, Scanning Electron Microscopy Inc., Chicago, 1979.
[537] M. O. Lively, T. F. Moran, and J. C. Powers, *J. Biol. Chem.*, 1979, **254**, 262.
[538] G. Jaeck and F. W. Berz, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 885.
[539] L. Andersson and K. Mosbach, *Eur. J. Biochem.*, 1979, **94**, 565.

4 Chemical Cross-linking

Several reviews covering this topic have appeared.[540-542]

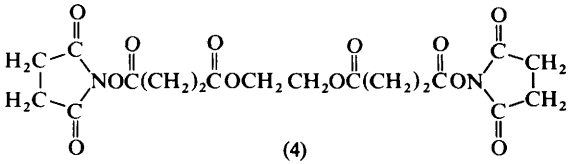
The *N*-hydroxysuccinimide ester continues to be a popular protein-reactive group in the design of cross-linking agents. The synthesis of a range of such di-esters, both cleavable and non-cleavable, has been reported, and used in linking studies with myosin.[543] The new periodate-cleavable reagent described by these authors, *NN'*-bis(succinimidylacetate)tartaramide, has a linking distance of 13 Å and complements the two similar reagents, disuccinimidyl tartarate (6 Å) and *NN'*-bis-(3-succinimidyloxycarbonylpropyl)tartaramide (18 Å), reported last year.[544] The non-cleavable agent, disuccinimidyl suberate, has been used to probe the insulin-responsive glucose-transport system of the rat adipocyte.[545]

Two new cleavable centres have been proposed for situations where the use of disulphide and glycol centres is compromised. A disuccinimidyl reagent containing a monothioacetal group (3) has been synthesized and used to cross-link glycer-



(3)

aldehyde 3-phosphate dehydrogenase.[546, 547] The linkage is cleaved by $HgCl_2$ under mild, neutral conditions and two-dimensional SDS gel electrophoresis can be used in the normal manner. The second reagent, ethyleneglycol bis(succinimidyl succinate) (4), can be cleaved with hydroxylamine (1 M).[548] Haemoglobin and lactate dehydrogenase were reversibly cross-linked with this reagent and trypsin was successfully immobilized on hexamethylenediamine–Sepharose CL-4B.



(4)

A series of *SS'*-polymethylenebis(methanethiosulphonates) of varying chain lengths has been prepared and cross-linking demonstrated with a number of enzymes.[549] These reagents are stable, react readily with thiols, and are reversible by disulphide reduction. Radiolabelled *S,S'*-octamethylbis(methanethiosulphonate) was used to study in detail the molecular structure of glyceraldehyde 3-phosphate dehydrogenase.

[540] H. Tae, *Biochim. Biophys. Acta*, 1979, **559**, 39.
[541] M. Das and C. F. Fox, *Ann. Rev. Biophys. Bioeng.*, 1979, **8**, 165.
[542] H. G. Whittman, *Can. J. Biochem.*, 1979, **57**, 1251.
[543] M. Hill, J. J. Bechet, and A. d'Albis, *FEBS Lett.*, 1979, **102**, 282.
[544] R. J. Smith, R. A. Capaldi, D. Muchmore, and F. Dahlquist, *Biochemistry*, 1978, **17**, 3719.
[545] P. F. Pilch and M. P. Czech, *J. Biol. Chem.*, 1979, **254**, 3375.
[546] D. C. Muchmore and F. W. Dahlquist, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 599.
[547] D. C. Muchmore and F. W. Dahlquist, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 601.
[548] P. M. Abdella, P. K. Smith, and G. P. Royer, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 734.
[549] D. P. Bloxham and R. P. Sharma, *Biochem. J.*, 1979, **181**, 355.

N-(4-carboxycyclohexylmethyl)maleimide *N*-hydroxysuccinimide ester has been synthesized and used to introduce maleimide groups on to glucose oxidase.[550] The modified enzyme was then treated with reduced rabbit IgG or Fab′ to give conjugates which retained substantial enzymic activity. The potential use of such conjugates for immunoenzymometric assay procedures is indicated.

A new bifunctional maleimide, dithiobis-*N*-ethylmaleimide, has been used to cross-link two irradiation-exposed sites, believed to be thiols, of spinach chloroplast thylakoid coupling factor.[551] The uncoupling of photophosphorylation which resulted was reversed by dithiothreitol. The kinetics of *p-NN′*-phenylenebismaleimide and its cysteine adducts have been investigated.[552] Hydrolysis is much more rapid than that found with the monofunctional analogue, *N*-ethylmaleimide, and can limit the extent of cross-linking.

Ethyl bromoacetimidate has been proposed as a short-distance heterobifunctional agent for exploring the micro-environment of active sites containing essential lysine residues.[553] Using ribonuclease as an example, the reagent was first reacted at pH = 9.0 with lysine; on lowering the pH, cross-linking to a neighbourhood histidine occurred.

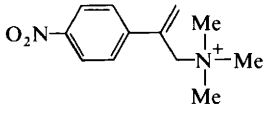
One assumed advantage of the use of bisimidoesters for protein cross-linking is that the charge on the protein is not altered. With α-crystallin, however, it has been demonstrated that additional positive charges are introduced by reaction at pH = 8.0, and it is suggested that these result from side reactions leading to polyamidine formation.[554] As a result, interpretation of cross-linking data, particularly with regard to inter-residue distances and symmetry relations, may be compromised.

The disulphide bond-promoting properties of diazenedicarboxylic acid bisamides have been investigated and the formation of aldolase polymers described.[555] Reaction proceeds *via* the intermediate, RHCONH·N(S-aldolase)CONR, which can be isolated and subsequently used for S—S cross-linking or immobilization onto thiopropyl-Sepharose 6B.

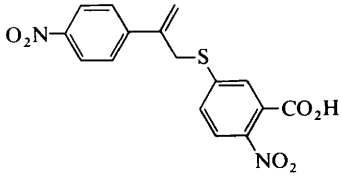
Two aspirin derivatives, bis(3,5-dibromosalicyl)succinate and bis(3,5-bromosalicyl)fumarate, have been shown to be potent acylating agents of intracellular haemoglobin (A or S) *in vitro*.[556] Both reagents cross-link β-chains, probably at the β-cleft. The modified haemoglobins show increased oxygen affinities and reduced gelation or sickling tendencies.

An interesting new approach to protein cross-linking, 'equilibrium-transfer alkylation', has been described.[557] The reagents 2-(nitrophenyl)allyltrimethylammonium iodide (5a) and 2-(*p*-nitrophenyl) allyl-4-nitro-3-carboxyphenyl sulphide (5b) react by Michael addition with protein nucleophiles with elimination of the trimethylammonium or mercaptonitrobenzoate group, respectively. The new double bond formed can then react with a further protein nucleophile. By a series of

[550] S. Yoshitabe, Y. Yamada, E. Ishikawa, and R. Masseyeff, *Eur. J. Biochem.*, 1979, **101**, 395.
[551] J. V. Maroney and R. E. McCarty, *J. Biol. Chem.*, 1979, **254**, 8951.
[552] P. Knight, *Biochem. J.*, 1979, **178**, 191.
[553] J. Diopoh and M. Olomucki, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1257.
[554] R. J. Siezen, *FEBS Lett.*, 1979, **100**, 75.
[555] M. T. Mas, J. Buczylko, and M. Kochman, *Eur. J. Biochem.*, 1979, **100**, 393.
[556] J. A. Walder, R. M. Zangg, R. Y. Walder, J. M. Stiele, and I. M. Klotz, *Biochemistry*, 1979, **18**, 4265.
[557] S. Mitra and R. G. Lauton, *J. Am. Chem. Soc.*, 1979, **101**, 3097.



(5a)



(5b)

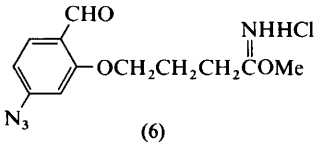
such Michael reactions, the reagent is thus free to roam over the surface of the protein until the most thermodynamically stable cross-link equilibrium is established. At any time, the cross-linking can be 'fixed' by reduction of the nitro-group to the amine. The major advantage of this approach is that conformationally untypical, kinetically determined cross-links are eliminated, and thus more reliable information on the relative proximity of protein nucleophiles can be obtained. Ribonuclease was cross-linked by this method, and links between Lys-7 and Lys-37 and (probably) between Lys-31 and Lys-41 were identified.

5 Photocross-linking

An attempt has been made to characterize chemically the cross-link formed by the u.v. irradiation of protein–nucleic acid complexes.[558] Analysis of the cross-link formed between the gene-5 protein of bacteriophage fd and fd DNA show that thymine and, to a lesser extent, cytosine bases are involved and that the major linkage is between a thymine and Cys-33. The u.v.-induced cross-link between 15S RNA and protein S7 in *E. coli* 30S ribosomal subunits has been precisely located;[559] uridine-1239 is the cross-linked residue.

Photocross-linking has been used to investigate the contact surface of four methionine-accepting tRNAs in complexes with *E. coli* methionine: tRNA ligase; all show a similar cross-linking pattern.[560] The contact surface consists of three regions: (i) the narrow groove of the anticodon stem and its extension into the anticodon loop, (ii) the 3′ terminal residues, and (iii) the 3′ side of the '**T** arm'.

For protein–protein photocross-linking, a new cleavable tri-functional reagent, 4-(6-formyl-3-azido-phenoxy)butyrimidate (6) has been synthesized.[561] The



(6)

imido-group is first used for attachment to amino-groups of a specific protein whose environment is to be investigated. At this point the reagent can be made radioactive with [^{3}H]NaBH$_4$. After photochemical cross-linking, the cross-link can be cleaved with ammonia and the radioactive protein identified. The known complex between ribosomal proteins L7 and L10 was identified by this technique.

[558] P. R. Paradiso, Y. Nakashima, and W. Konigsberg, *J. Biol. Chem.*, 1979, **254**, 4739.
[559] C. Zwieb and R. Brimacombe, *Nucl. Acid. Res.*, 1979, **6**, 1775.
[560] J. J. Rosa, M. D. Rosa, and P. B. Sigler, *Biochemistry*, 1979, **18**, 637.
[561] J. A. Maassen, *Biochemistry*, 1979, **18**, 1289.

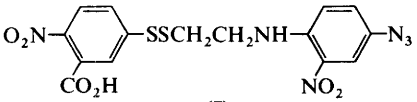
A new cleavable heterobifunctional reagent, *N*-[4-(*p*-azidophenylazo) benzoyl]-3-aminopropyl-*N'*-oxysuccinimide ester has been synthesized and its use for the identification and study of membrane receptors investigated.[562] Specifically, the reagent was used to acylate peanut agglutinin and the derivative then bound to receptors on neuramidase-treated human erythrocyte ghosts. After irradiation, the labelled ghosts were solubilized, immunoprecipitated with anti-agglutinin serum, and analysed by SDS gel electrophoresis before and after dithionite cleavage. Asialoglycoprotein A and a second glycoprotein (apparent molecular weight 5800—6100 dalton) were implicated as agglutinin receptors.

p-Azidophenacyl iodoacetate has been prepared for use as a photocross-linking reagent for enzymes with active-site cysteine residues in mind.[563] The reagent has a hydroxylamine-cleavable ester linkage allowing removal of any reagent which has not photocross-linked to the protein. This facility eliminates some of the ambiguities obtained with *p*-azidophenacyl bromide. Two ^{14}C-labelled reagents, with the label on either side of the ester linkage, were used to study yeast alcohol dehydrogenase.

6 Affinity Labelling

A review covering the affinity labelling of biological systems has recently appeared.[564]

The technique continues to be of great use in receptor investigations. A photolabile derivative of α-bungarotoxin has been prepared by reduction of a single disulphide bridge followed by reaction with the mixed disulphide (7).[565] The



(7)

derivative was used to affinity cross-link to polypeptide components of the *Torpedo californica* acetylcholine receptor. A 40 000 dalton component, to which the toxin is known to bind, was labelled together with a polypeptide of 65 000 dalton which is a major membrane constituent. Photoaffinity labels have been prepared from ouabain and strophanthidin by the reductive amination of *N*-(2-nitro-4-azidophenyl)ethylenediamine to the periodate-oxidized moiety of ouabain and the C-19 aldehyde side chain of strophanthidin.[566, 567] Both labels attached to the 93 000 dalton protein of purified *Electrophorus electricus* $(Na^+ + K^+)$ATPase, indicating that this protein contains both the sugar and the steroid binding site of the digitalis binding centre. In whole membrane preparations, three other proteins (50 000, 45 000, and 12 800 dalton) were labelled. An arylazide enkephalin deriva-

[562] C. L. Jaffe, H. Lis, and N. Sharon, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 402.
[563] S. M. Hixson, S. F. Burroughs, T. M. Caputo, B. B. Crapster, M. V. Daly, A. W. Lourie, and M. L. Wasko, *Arch. Biochem. Biophys.*, 1979, **192**, 296.
[564] V. Chowdry and S. H. Westheimer, *Ann. Rev. Biochem.*, 1979, **48**, 293.
[565] V. Witzemann and M. A. Rafferty, *Biochem. Biophys. Res. Commun.*, 1979, **85**, 623.
[566] T. B. Rogers and M. Lazdunski, *Biochemistry*, 1979, **18**, 135.
[567] T. B. Rogers and M. Lazdunski, *FEBS Lett.*, 1979, **98**, 373.

tive, [D-Ala^2, Met^5]enkephalin-Tyr-*N*-(2-nitro-4-azidophenyl)ethylenediamine, has been synthesized and shown to be an effective photoaffinity label for the enkephalin receptor.[568]

2,3′-Dialdehyde derivatives of ATP and ADP have been shown to act as affinity labels for the ATP and ADP binding site, respectively, of the coupling factor-latent ATPase from *Mycobacterium phlei*.[569] These studies show that the catalytic site for ATP hydrolysis is located in the α-subunit, while ADP binding sites involved in ATP synthesis are present in both α- and β-subunits. The fluorescent ATP photoaffinity label, 8-azido-1,N^6-etheno-ATP, has been used to study the low-affinity binding site of yeast mitochondrial F_1-ATPase;[570] it was located on the β-subunit.

3′-*O*-3-[*N*-(4-Azido-2-nitrophenyl)amino]propionyl-ATP has been used to identify the ATP binding site of myosin.[571] The label was associated with the 25 000 dalton fragment at the *N*-terminus of the heavy chain. No label appeared in the 21 000 dalton fragment containing the two essential thiols.

The synthesis of 8-azido[^{32}P]guanosine 3′,5′-cyclic monophosphate has been described.[572] The reagent has been used as an affinity label for cyclic GMP-dependent protein kinase. *N*-(2,3-Epoxypropyl)-*N*-amidinoglycine(epoxycreatine) has been synthesized to probe the active site of creatine kinase.[573]

A tritiated photosensitive analogue of the classical uncoupler 2,4-dinitrophenol has been synthesized.[574] Photoaffinity labelling of mitochondria by 2-azido-4-nitrophenol showed that 90—95% of the incorporated label was associated with polypeptides and that two in particular (56 000 and 31 000 dalton) were associated with 60% of the activity.

The molecular basis for the observed leucine auxotrophy of *E. coli* cells, when treated with the bacteriostatic 6-amino-7-chloro-5,8-dioxoquinoline, has been elucidated.[575] The quinone reacts with essential thiol groups at the active site of leucyl–tRNA synthetase.

The 3′-OH terminal binding site of DNA-dependent RNA polymerase has been labelled with three affinity analogues: 3′-(bromoacetamido)-3′-deoxy-ATP, 3′-(isothiocyanato)-2,3′-dideoxy-ATP, and 3′-amino-3′-deoxy-ATP; the last analogue was cross-linked with glyoxal.[576] These reagents were built on to the ternary complex of enzyme, poly(dT), and the labelled oligonucleotide, [5′-^{32}P]*p*$(Ap)_{10}$A, using the catalytic competence of the enzyme. The label was located primarily on the β′-subunit.

A photosensitive derivative of phosphatidylcholine, 1-palmitoyl-2-7-(4-azido-2-nitrophenoxy)-[1-^{14}C]heptanoyl-*sn*-glycero-3-phosphocholine, has been synthe-

[568] T. T. Lee, R. E. Williams, and C. F. Fox, *J. Biol. Chem.*, 1979, **254**, 11787.
[569] G. Kumar, V. K. Kalra, and A. F. Brodie, *J. Biol. Chem.*, 1979, **254**, 1964.
[570] R. Gregory, D. Recktenwald, B. Mess, H. J. Schafer, P. Scheurich, and K. Dose, *FEBS Lett.*, 1979, **108**, 253.
[571] L. Szilagyi, M. Baliut, F. A. Streter, and J. Gergely, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 936.
[572] R. L. Geahlen, B. E. Haley, and E. G. Krebs, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2213.
[573] M. A. Marletta and G. L. Kenyan, *J. Biol. Chem.*, 1979, **254**, 1879.
[574] W. G. Hanstein, Y. Hatefi, and H. Kiefer, *Biochemistry*, 1979, **18**, 1019.
[575] K. Wiebauer, A. Ogilvie, and W. Kersten, *J. Biol. Chem.*, 1979, **254**, 327.
[576] V. W. Ambrong and F. Eckstein, *Biochemistry*, 1979, **18**, 5117.

sized in order to study the lipid-binding site of phosphatidylcholine exchange protein.[577] After trypsin and *Staphylococcus aureus* protease digests of the labelled protein, it was shown that the binding site consists, in part, of the cluster of apolar residues -Val-Phe-Met-Tyr-Tyr-Phe-. Two other arylazido phospholipids, one with the label at the non-polar end, the other at the polar end, have been used to label the membrane protein cytochrome *c* oxidase.[578] Subunits I, III, and VII were the principal elements labelled, I predominantly by the 'deep' label, VII predominantly by the 'shallow' label. Similar results were obtained from studies using 5-[^{125}I]iodonaphthyl-1-azide and *S*-(4-azido-2-nitrophenyl)-[^{35}S]thiophenol.[579] In a system where yeast cytochrome *c* oxidase and yeast mitochondria were incorporated into Tween 80 micelles, flash photolysis of these hydrophobic aryl azides caused subunits I, II, III, and VII to be labelled. In addition the thiophenol reagent labelled subunit V.

The use of benzophenone as a photolabile group has been investigated using chymotrypsin and *p*-benzoylphenyl acetate esters.[580] Irradiation of the acyl enzyme gives rise to a complex of uncertain structure. Because the benzophenone moiety is located deep in the hydrophobic pocket of the enzyme, reaction with the solvent is eliminated and therefore labelling efficiency is high.

A new affinity labelling methodology, termed 'chemoaffinity labelling', has been proposed.[581] In this technique, a specific chemical is used to bind reactive groups on the substrate or drug to reactive functions on the protein. The technique has been illustrated with various glycocorticoid derivatives, substituted at the C-21 position with —SH and —NH_2 nucleophiles, and glycocorticoid receptors. Addition of *o*-phthalaldehyde to the steroid–receptor complex leads to formation of a fluorescent isoindole cross-link between receptor and steroid nucleophiles. Energy transfer from tryptophan to isoindole was observed. It remains to be seen how generally applicable this approach will be.

[577] P. Moonen, H. P. Haagsman, L. L. M. Van Deenan, and K. W. A. Wirtz, *Eur. J. Biochem.*, 1979, **99**, 439.
[578] R. Bisson, C. Montecucco, H. Gutweniger, and A. Azzi, *J. Biol. Chem.*, 1979, **254**, 9962.
[579] N. Cerletti and G. Schatz, *J. Biol. Chem.*, 1979, **254**, 7746.
[580] P. Campbell and T. L. Gioannini, *Photochem. Photobiol.*, 1979, **29**, 883.
[581] S. S. Simons, E. B. Thompson, and D. F. Johnson, *Biochemistry*, 1979, **18**, 4915.

Appendix: Proteins Involved in Modifications Discussed in the Text

Protein	*Ref.*	*Protein*	*Ref.*
Acetylcholine receptor	530, 531, 565	Immunoglobulin	535
Agglutinin receptor	562	Insulin	523
Albumin	500, 514, 516, 518, 520, 522, 532, 574	Lactalbumin	500
		Lactate dehydrogenase	548
		Leucyl-tRNA synthetase	575
Alcohol dehydrogenase	539, 563	Lima bean inhibitor	527
Aldolase	555	Lysozyme	522, 523
Antithrombin III	537	Maltose receptor	533
ATPase	566, 567, 569, 570	Methionine tRNA ligase	560
		Membrane proteins	532, 534, 545, 562
Carbonic anhydrase	509		
α-Chymotrypsin	501, 523, 580	Myosin	543
Collagen	537	Ovalbumin	503, 518
Creative kinase	521, 573	Ovomucoid	525
α-Crystallin	554	Papain	507
Cytochrome *c*	518	Parvalbumin	509
Cytochrome *c* oxidase	578, 579	Pepsin	523
Deoxyribonuclease	505, 506	Phosphatidylcholine exchange protein	577
Digitalis receptor	566, 567		
fd Gene 5 protein	558	Plasma proteins	519
Enkephalin receptor	568	Prolactin	529
Fetuin	520	Protein kinase	572
Fibrinogen	523	Pyruvate kinase	511
Glucose oxidase	550	Ribosomes	559, 561
Glucose-6-phosphate oxidase	514	RNA polymerase	516
		Ribonuclease	515, 517, 520, 525, 538, 553, 557
Glucocorticoid receptor	581		
Glutamate dehydrogenase	510		
Glyceraldehyde-3-phosphate dehydrogenase	546, 547, 549	Spectrin	504
		Thermolysin	537
Haemoglobin	548	Thylakoid coupling factor	551
Histone	512	Trypsin	522, 548

PART II: *X*-Ray Studies *by W. D. Mercer*

1 Introduction

The year of 1979 has been one of consolidation as well as advancement for *X*-ray crystallographic studies of amino-acids, peptides, and proteins. Many structures are now entering the final 'tidying-up' processes of structural refinement and substrate-binding studies, while the flow of new structures continues apace. It is unfortunate to note that this increased output has been matched by a decrease in the standards of proof-reading by several journals.

The crystal structures of amino-acids and peptides are tabulated for both the free compounds (Table 1) and their complexes with metal ions (Table 2). Some structures missed in the 1978 review are included and again the use of the Cambridge Crystallographic Data File is most gratefully acknowledged.

Preliminary crystallization reports for proteins are given in Table 3 and show the continuing use of polyethylene glycol (PEG) as a crystallizing agent. Low-

Table 1 *Structure determinations of amino-acids and peptides*

Compound	*Space group*	*Cell dimensions*						*Z*	*R*	*Ref.*
		a/nm	*b*/nm	*c*/nm	α/deg	β/deg	γ/deg			
N-Acetyl-dehydroAla	$P2_1/n$	1.4904	1.0162	0.3943	90	91.70	90	4	0.060	*a*
NN-Diethyl-β-Ala benzene clathrate	*Pbcm*	0.5491	1.3426	1.7766	90	90	90	4	0.086	*b*
S-Carboxymethyl-L-Cys	$P2_1$	1.0290	0.7039	0.5093	90	95.09	90	2	0.021	*c*
(*RR*)-*N*-Acetyl-*S*-(2-nitro-1-phenyl-ethyl)-L-Cys	$P2_1$	0.5485	1.2760	1.1071	90	96.72	90	2	0.033	*d*
L-γ-Carboxy-Glu	$P2_1$	0.5050	0.9600	0.7793	90	99.98	90	2	0.052	*e*
DL-α-Methyl-Glu hemihydrate	$C2/c$	2.1466	0.8233	1.0573	90	119.81	90	8	0.046	*f*
DL-γ-Carboxy-Glu monoammonium salt	$Pna2_1$	0.9880	1.1447	0.7619	90	90	90	4	0.066	*g*
L-PyroGlu-*NN'*-dicyclohexyl-urea	$P2_12_12_1$	1.2416	1.7622	0.8423	90	90	90	4	0.061	*h*
N-(Phosphonomethyl)-Gly	$P2_1/c$	0.8682	0.7973	0.9875	90	105.74	90	4	0.030	*i*
2-Butenyl-Gly.hydrochloride	$P2_1/c$	1.2506	0.6471	1.0826	90	98.91	90	4	0.11	*j*
1,2,3-Triaminoguanidinium nitrate	*Pbcm*	0.8389	1.2684	0.6543	90	90	90	4	0.083 0.051	*k* *l*
L-Histidinium trimesate $\frac{1}{3}$ acetone	$P2_12_12_1$	1.8097	1.3353	0.6737	90	90	90	4	0.057	*m*
Imidazole[n]	$P2_1/c$	0.7569	0.5366	0.9785	90	119.08	90	4	0.026	*o*
5-(3,3-Dimethyl-1-triazemyl)imidazole-4-carboxamide	$P2_1/n$	1.4042	1.0661	1.1914	90	91.49	90	8	0.042	*p*
2-Amino-6-methyl dipyrido[1,2-α : 3′2′-d]imidazole[q]	$C2/c$	1.6579	0.9080	1.5137	90	119.02	90	8	0.04	*r*

As above bromide hydrate[q]	$P1$	1.0659	0.9342	0.7440	105.43	75.59	108.66	2	0.05	*r*
N-Acetyl-DL-Met diethylamide	$P2_1/c$	0.9357	1.9763	0.8256	90	115.15	90	4	0.046	*s*
DL-HomoPro.tetrahydrate	$P2_1/c$	1.3404	0.6510	1.2962	90	108.94	90	4	0.079	*t*
1-Acetylprolinamide	$P2_12_12_1$	0.6680	0.7685	1.5627	90	90	90	4	0.046	*u*
N-Benzyloxycarbonyl-α-amino-isobutyryl-L-Pro-methylamide	$P2_12_12_1$	0.7705	1.1365	2.1904	90	90	90	4	0.054	*v*
Putrescine diphosphate[w]	$P2_1/a$	0.7879	0.9734	0.8126	90	110.19	90	2	0.030	*x*
Sarcosine hydrochloride	$P2_1$	0.9000	0.5930	0.5410	90	96.00	90	2	0.100	*y*
Thienyl-DL-Ser monohydrate	*Pbca*	2.8038	0.9817	0.6536	90	90	90	8	0.078	*z*
DL-*threo*-3,4-Dihydroxyphenyl-Ser	$P2_12_12_1$	0.6046	0.5590	2.5999	90	90	90	4	0.14	*aa*
As above: hydrochloride trihydrate	$P2_1/c$	1.2153	0.7065	1.8779	90	123.15	90	4	0.038	*aa*
Tryptamine: 1-Thyminyl acetic acid[bb]	$P2_1/c$	0.9793	0.6122	2.8160	90	95.46	90	4	0.058	*cc*
3,5-Dinitro-L-Tyr	$P2_12_12_1$	0.7888	2.0193	0.7424	90	90	90	4	0.052	*dd*
3,5-Dinitro-L-Tyr sodium salt	$P1$	0.8271	1.3357	0.6864	110.4	97.0	92.7	2	0.065	*dd*
Tyramine hydrochloride[ee]	*Pbcn*	1.9973	1.0768	0.8246	90	90	90	8	0.053	*ff*
Gly-L-Ala	$P2_12_12_1$	0.9693	0.9524	0.7537	90	90	90	4	0.031	*gg*
Gly-DL-Ala	$P2_1/c$	1.0694	0.7773	0.9174	90	117.94	90	4	0.044	*hh*
Gly-ciliatine	$P2_1/c$	1.0426	0.4930	1.4693	90	94.93	90	4	0.077	*ii*
cyclo(-L-Pro-L-Ala-)	$P2_1$	0.9266	0.6551	0.7267	90	111.40	90	2	0.038	*jj*
cyclo(-L-Pro-D-tert-Leu-)	$P2_12_12$	1.0942	1.6438	0.6452	90	90	90	2	0.046	*kk*
N-Pivaloyl-D-Ala-L-Pro-*N'*-isopropylamide	$P2_12_12_1$	0.8963	1.0433	1.9329	90	90	90	4	0.041	*ll*

Table 1 *cont.*

Compound	*Space group*	*Cell dimensions*								
		a/nm	*b*/nm	*c*/nm	*α*/deg	*β*/deg	*γ*/deg	*Z*	*R*	*Ref.*
N-(t-Butoxy carbonyl)Pro-Leu-benzyl ester	$P2_1$	1.0475	1.7910	0.6303	90	90.213	90	2	0.072	*mm*
As above: ethyl ester	$P2_12_12_1$	0.9917	2.1960	0.9956	90	90	90	4	0.062	*mm*
D-Val-L-Tyr-L-Val dihydrate	$P2_1$	0.9643	0.9964	1.1964	90	98.30	90	2	0.053	*nn*
Methyl-L-Ala-*ON*-dimethyl-L-Tyr-L-Ala[oo]	$P2_1$	1.2581	0.6265	1.3095	90	106.36	90	2	0.048	*pp*
t-Butoxycarbonyl-L-Pro-L-prolyl glycinamide	$P2_1$	1.0243	0.9941	1.0550	90	114.92	90	2	0.041	*qq*
L-Pro-L-Tyr-L-Ile-L-Leu	$P2_1$	0.9840	2.6750	0.5305	90	92.72	90	2	0.062	*rr*
Tyr-Gly-Gly-Phe[ss]	$P2_12_12$	1.2912	2.1730	1.0760	90	90	90	4	0.082	*tt*
Gly-Gly-Phe-Leu[ss]	$P2_1$	0.9110	0.6200	1.9760	90	102.6	90	2	0.073	*tt*
cyclo(-*Gly*-L-Pro-L-Ser-D-Ala-L-Pro-)[uu]	$P2_1$	1.2392	0.9149	1.0428	90	97.3	90	2	0.082	*vv*
cyclo-(L-Leu-L-Phe-Gly-D-Leu-D-Phe-Gly-) dihydrate	$P1^{ww}$ $P1^{xx}$	0.8599 0.8701	1.6285 1.6948	0.6136 0.6172	92.38 93.65	95.85 96.29	87.14 95.78	1 1	0.068 —	*yy* *yy*
cyclo(-Gly-L-Pro-D-Phe-)$_2$[zz]	$P2_1$	1.9694	0.9005	1.0357	90	104.05	90	2	0.099	*aaa*
cyclo(-*Gly*-L-Pro-D-Ala-)$_2$[bbb]	$P2_12_12_1$	0.9389	1.0379	2.2006	90	90	90	4	0.055	*ccc*
Butoxycarbonyl(L-Val-D-Val)$_4$-OMe[ddd]	$P3_221$	1.2760	1.2760	6.3190	90	90	120	6	0.130	*eee*

cyclo-Tri-D-Val-L-(α-hydroxyiso-valeryl)-L-Val-D-(α-hydroxyisovaleryl)-]	$P2_1/n$	2.4995	1.2996	1.1110	90	90	89.20	2	0.121	*fff*

[a] D. Ajo, G. Granozzi, E. Tondello, A. Del Pra, and G. Zanotti, *J. Chem. Soc., Perkin Trans. 2*, 1979, 927. (*b*) M. A. Peterson, H. Hope, and C. P. Nash, *J. Am. Chem. Soc.*, 1979, **101**, 946. (*c*) A. D. Mighell, C. R. Hubbard, J. Harris, J. A. Staffa, and C. Zervos, *Acta Crystallogr.*, 1979, **B35**, 1258. (*d*) W. Winter, G. Heusel, H. Fouad, and G. Jung, *Chem. Ber.*, 1979, **112**, 3171. (*e*) K. A. Satyshur and S. T. Rao, *Acta Crystallogr.*, 1979, **B35**, 2260. (*f*) C. Derricott and J. Trotter, *Acta Crystallogr.*, 1979, **B35**, 2230. (*g*) K. A. Satyshur, S. T. Rao, J. Stenflo, and J. W. Suttle, *Acta Crystallogr.*, 1979, **B35**, 1493. (*h*) F. Bechtel, J. P. Bideau, and M. Cotrait, *Cryst. Struct. Commun.*, 1979, **8**, 815. (*i*) P. Knuuttila and H. Knuuttila, *Acta Chem. Scand.*, 1979, **B33**, 623. (*j*) S. Swaminathan and K. K. Chacko, *Acta Crystallogr.*, 1979, **B35**, 211. (*k*) A. J. Bracoti, *Acta Crystallogr.*, 1979, **B35**, 760. (*l*) C. S. Choi and E. Prince, *Acta Crystallogr.*, 1979, **B35**, 761. (*m*) F. H. Herbstein and M. Kapon, *Acta Crystallogr.*, 1979, **B35**, 1614. (*n*) Neutron study at 103 K. (*o*) R. K. McMullan, J. Epstein, J. R. Ruble, and B. M. Craven, *Acta Crystallogr.*, 1979, **B35**, 688. (*p*) H. C. Freeman and N. D. Hutchinson, *Acta Crystallogr.*, 1979, **B35**, 2051. (*q*) Possible mutagens isolated from charred meat. (*r*) K. Yamaguchi, Y. Iitata, K. Shudo, and T. Okamoto, *Acta Crystallogr.*, 1979, **B35**, 2266. (*s*) A. Aubry, J. Protas, M. T. Cung, and M. Marraud, *Acta Crystallogr.*, 1979, **B35**, 2634. (*t*) S. K. Bhattacharjee and K. K. Chacko, *Acta Crystallogr.*, 1979, **B35**, 396. (*u*) U. Druck, W. Littke, and P. Main, *Acta Crystallogr.*, 1979, **B35**, 253. (*v*) B. V. V. Prasad, N. Shamala, R. Nagaraj, R. Chandrasekavan, and P. Balaram, *Biopolymers*, 1979, **18**, 1635. (*w*) *X*-Ray and neutron studies. (*x*) F. Takusagawa and T. F. Koetzle, *Acta Crystallogr.*, 1979, **B35**, 867. (*y*) S. C. Bhattacharyya and N. N. Saha, *J. Cryst. Mol. Struct.*, 1978, **8**, 105. (*z*) S. Swaminathan and K. K. Chacko, *Acta Crystallogr.*, 1979, **B35**, 208. (*aa*) A. Mostad, C. Rømming, and M. Strømme, *Acta Chem. Scand.*, 1979, **B33**, 219. (*bb*) 1 : 1 complex showing salt bridging. No interactions seen between the indole and pyrimidine rings. (*cc*) T. Ishida, M. Inoue, and K. I. Tomita, *Acta Crystallogr.*, 1979, **B35**, 1642. (*dd*) V. Cody, D. A. Langs, and J. P. Hazel, *Acta Crystallogr.*, 1979, **B35**, 1829. (*ee*) Shows alternating hydrophobic and hydrophilic layers. (*ff*) A. Podder, J. K. Dattagupta, N. N. Saha, and W. Saenger. *Acta Crystallogr.*, 1979, **B35**, 649. (*gg*) A. H.-J. Wang and I. C. Paul, *Cryst. Struct. Commun.*, 1979, **8**, 269. (*hh*) W. F. Paton and I. C. Paul, *Cryst. Struct. Commun.*, 1979, **8**, 275. (*ii*) C. Garrigou-Lagrange, F. Habault, and M. Cotrait, *J. Mol. Struct.*, 1979, **54**, 197. (*jj*) M. Cotrait and F. Leroy, *Cryst. Struct. Commun.*, 1979, **8**, 819. (*kk*) I. Sklenár and J. Jecný, *Acta Crystallogr.*, 1979, **B35**, 513. (*ll*) A. Aubry, J. Protas, G. Boussard, and M. Marraud, *Acta Crystallogr.*, 1979, **B35**, 694. (*mm*) H. Sugino, I. Tanaka, and T. Ashida, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 2855. (*nn*) A. F. Mishnev, Y. Y. Bleidelis, A. E. Shvets, Y. E. Antsans, and G. I. Chipens, *Zh. Struct. Khim.*, 1978, **19**, 847. (*oo*) *cis*-1,2-peptide bond. Structure shows a new type of intramolecular hydrogen bond. (*pp*) R. B. Bates, V. J. Hruby, and G. R. Kriek, *Acta Crystallogr.*, 1979, **B35**, 188. (*qq*) I. Tanaka, T. Ashida, Y. Shimonishi, and M. Kakudo, *Acta Crystallogr.*, 1979, **B35**, 110. (*rr*) M. Cotrait, S. Geoffre, M. Hospital, and G. Precigoux, *Acta Crystallogr.*, 1979, **B35**, 114. (*ss*) Portions of enkephalin. Tyr-Gly-Gly-Phe shows a β-turn structure while Gly-Gly-Phe-Leu shows a bent conformation. (*tt*) T. Prangé and C. Pascard, *Acta Crystallogr.*, 1979, **B35**, 1812. (*uu*) All *trans*-peptides with significant deviations from planarity in three of them. (*vv*) I. L. Karle, *J. Am. Chem. Soc.*, 1979, **101**, 181. (*ww*) At 138 K. (*xx*) At 295 K. (*yy*) M. B. Hossain and D. Van der Helm, *Acta Crystallogr.*, 1979, **B35**, 2781. (*zz*) Contains two type II β-turns without strong 4 → 1 hydrogen bonds. (*aaa*) J. N. Brown and C. H. Yang, *J. Am. Chem. Soc.*, 1979, **101**, 445. (*bbb*) Contains two type II β-turns. Significant deviation from internal two-fold symmetry showing only one transannular 4 → 1 hydrogen bond. (*ccc*) E. C. Kostansek, W. N. Lipscomb, and W. E. Thiessen, *J. Am. Chem. Soc.*, 1979, **101**, 834. (*ddd*) Antiparallel β-double helix as has been proposed for the gramicidin A structure. (*eee*) E. Benedetti, B. Di Blasio, C. Pedone, G. P. Lorenzi, L. Tomasic, and V. Gramlich, *Nature, (London)*. 1979, **282**, 630. (*fff*) A. D. Vasilev, T. A. Shibanova, V. I. Andrianov, V. I. Simonov, A. A. Sanasaryan, V. I. Ivanov, and Y. A. Ovchinnikov, *Bioorg. Khim.*, 1978, **4**, 1157.

Table 2 *Structure determinations of complexes of amino-acids and peptides with metals*

Compound	*Space group*	*Cell dimensions*								
		a/nm	*b*/nm	*c*/nm	α/deg	β/deg	γ/deg	*Z*	*R*	*Ref.*
Bis-[D-β-(2-pyridyl)-α-alaninato] cobalt(III) nitrate hemihydrate	$C222_1$	0.9248	1.8349	2.1697	90	90	90	—	0.062	*a*
β-Alaninium tetrachloroiron(III)	$P2_1/c$	0.6966	1.1994	1.2444	90	91.01	90	4	0.058	*b*
δ-Tris-(L-Asn)-cobalt(III) trihydrate	$P2_12_12_1$	3.9082	0.7523	0.6643	90	90	90	4	0.055	*c*
(Creatinine) phenylmercury(II) nitrate monohydrate	$P2_1/n$	1.0255	1.9838	0.6859	90	97.81	90	4	0.071	*d*
Δ-(*R*)-μ-Cystinato-tetrakis-(ethylenediamine)cobalt (III) tetraperchlorate hexahydrate	$P2_12_12_1$	3.5708	0.8869	0.6847	90	90	90	2	0.072	*e*
Dichloro[(2*S*,*SR*)-*S*-methyl Cys-*S*-oxide]platinum(II) hydrate[f]	$C2$	1.7721	0.5386	1.1781	90	104.8	90	4	0.034	*g*
Calcium di-L-Glu tetrahydrate	$P4_32_12$	0.7562	0.7562	2.9077	90	90	90	4	0.032	*h*
1-Methylethylene-1,2-bis-(*N*-methylcarbamylglycinato)-copper(II) dihydrate	$Pca2_1$	1.3451	0.9166	1.3138	90	90	90	4	0.043	*i*

1-Ethylethylene-1,2-bis-(*N*-methylcarbamylglycinato)-copper(II) dihydrate	$Pca2_1$	1.3745	0.9301	1.3275	90	90	90	4	0.048	*i*
Tetra-μ-(*N*-acetylglycinato)bis-(aquo)copper(II)	$P2_1/c$	0.7288	0.8892	1.9473	90	103.04	90	2	0.036	*j*
Diaquo-bis-(*N*-acetylglycinato)copper(II) dihydrate	$C2/c$	2.1145	0.5046	1.8248	90	127.55	90	4	0.031	*k*
Tetra-μ-Gly-dimolybdenum(II) chloride hydrate	$P\bar{1}$	1.1941	1.2022	1.1614	92.42	93.04	88.94	3	0.047	*l*
As above—trihydrate	$P2_1/c$	0.8350	0.9346	1.4807	90	103.42	90	2	0.038	*l*
Di-μ-Gly-bis-[di-isothiocyanatomolybdenum(II)] monohydrate	$P2_1/n$	2.0877	0.8928	2.4346	90	105.23	90	8	0.073	*m*
Disodium bis-(glycylglycinato)nickel(II) octahydrate	$C2/c$	2.7830	0.6190	1.3930	90	121.92	90	4	0.047	*n*
As above—nonahydrate	$P\bar{1}$	2.1330	0.5770	0.8790	99.6	89.5	90.1	2	0.073	*n*
Hydrogen-bis-(*O*-aminobenzene sulphonylglycinato)silver(I)	$P2_1/n$	0.5730	1.2160	1.3940	90	94.9	90	2	0.102	*o*
cis-Bis-(1-methylimidazole)-bis-(*o*-nitrophenolato)-cobalt(II)	$P2_1/c$	1.3745	0.8010	2.1841	90	114.18	90	4	0.040	*p*
trans-Amine-bis-(L-histidinato)cobalt(III) perchlorate dihydrate	$P2_12_12_1$	1.0712	1.1569	1.6049	90	90	90	4	0.069	*q*

Table 2 cont.

Compound	*Space group*	*Cell dimensions*						*Z*	*R*	*Ref.*
		a/nm	*b*/nm	*c*/nm	α/deg	β/deg	γ/deg			
Bis-(*cyclo*-L-His-L-His)copper(II) perchlorate tetrahydrate	$P3_221$	0.8450	0.8450	4.200	90	90	120	3	0.103	*r*
Hexakis-(imidazole)-nickel(II) diperchlorate	$P2_1/n$	1.6229	0.7217	1.1469	90	90.00	90	2	0.060	*s*
Bis-(L-leucinato)-copper(II)	$P2_1$	0.9725	0.5127	1.4689	90	105.79	90	2	0.032	*t*
Di-μ-L-Leu-bis-[di-isothiocyanato-molybdenum(II)] hydrate	$P2_1$	0.9034	2.3807	1.4489	90	93.75	90	4	0.080	*u*
Bis-(DL-norleucinium)-hexaiodotellurium	$P1$	0.8028	0.8281	1.7163	137.86	90.44	73.60	1	0.046	*v*
(L-Met-glycinato)-copper(II)	$P2_12_12_1$	0.9889	1.1545	0.9337	90	90	90	4	0.024	*w*
D-Pantothenic acid calcium bromide salt	$P2_12_12_1$	0.6821	1.3635	1.4448	90	90	90	4	0.032	*x*
Bis-(L-serinato)-palladium(II)	$P2_12_12_1$	0.8828	0.9705	1.1315	90	90	90	4	0.054	*y*

(*a*) S. R. Ebner, R. A. Jacobson, and R. J. Angelici, *Inorg. Chem.*, 1979, **18**, 765. (*b*) B. Kirkwood and R. D. Willett, *Acta Crystallogr.*, 1979, **B35**, 1883. (*c*) M. Sekizaki, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 403. (*d*) A. J. Canty, N. Chaichit, and B. M. Gatehouse, *Acta Crystallogr.*, 1979, **B35**, 592. (*e*) P. A. Tucker, *Acta Crystallogr.*, 1979, **B35**, 71. (*f*) Platinum ion co-ordinated by amino-acid sulphur and nitrogen atoms. Pt—S bond length of 0.2182 nm is the shortest yet seen. (*g*) L. J. Nicholls and W. A. Freeman, *Acta Crystallogr.*, 1979, **B35**, 2392. (*h*) H. Einspahr and C. E. Bugg, *Acta Crystallogr.*, 1979, **B35**, 316. (*i*) C. K. Prout, D. S. Sanderson, and M. C. Couldwell, *Cryst. Struct. Commun.*, 1979, **8**, 181. (*j*) M. R. Udupa and B. Krebs, *Inorg. Chim. Acta*, 1979, **37**, 1. (*k*) M. R. Udupa and B. Krebs, *Inorg. Chim. Acta*, 1978, **31**, 251. (*l*) A. Bino, F. A. Cotton, and P. E. Fanwick, *Inorg. Chem.*, 1979, **18**, 1719. (*m*) A. Bino and F. A. Cotton, *Inorg. Chem.*, 1979, **18**, 1381. (*n*) H. C. Freeman and J. M. Guss, *Acta Crystallogr.*, 1978, **B34**, 2451. (*q*) P. N. Ray and N. N. Saha, *J. Cryst. Mol. Struct.*, 1978, **8**, 67. (*p*) R. G. Little, *Acta Crystallogr.*, 1979, **B35**, 2398. (*q*) N. Thorup, *Acta Chem. Scand.*, 1979, **A33**, 759. (*r*) F. Hori, Y. Kojima, K. Matsumoto, S. Ooi, and H. Kuroya, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1076. (*s*) G. J. M. Ivarsson and W. Forsling, *Acta Crystallogr.*, 1979, **B35**, 1896. (*t*) T. G. Fawcett, M. Ushay, J. P. Rose, R. A. Lalancette, J. A. Potenza, and H. J. Schugar, *Inorg. Chem.*, 1979, **18**, 327. (*u*) A. Bino and F. A. Cotton, *Inorg. Chem.*, 1979, **18**, 1381. (*v*) P. L. Haridou, H. Jedrzejczak, and S. Szwabski, *Acta Crystallogr.*, 1979, **B35**, 1843. (*w*) J. Dehand, J. Jordanov, F. Keck, A. Mosset, J. J. Bonnet, and J. Galy, *Inorg. Chem.*, 1979, **18**, 1543. (*x*) L. Delucas, H. Einspahr, and C. E. Bugg, *Acta Crystallogr.*, 1979, **B35**, 2724. (*y*) R. S. Vagg, *Acta Crystallogr.*, 1979, **B35**, 341.

Table 3 *Preliminary X-ray data on proteins*

Protein	*Source*	*Space group*	*Cell dimensions*			
			a/nm	*b*/nm	*c*/nm	*β*/deg
$tRNA^{Leu}_{CUG}$	*E. coli*	$P4_22_12$	13.3	13.3	6.6	—
α-Lactalbumin	Baboon milk	$P2_12_12$	3.55	6.91	4.61	—
Exocellular DD-carboxy-peptidase-transpeptidase[c]	*Streptomyces* R61	$P2_12_12_1$	5.11	6.74	10.29	—
Crambin[f]	*Crambe abyssinica*	$P2_1$	4.11	1.87	2.27	90.6
Uteroglobin	—	$P2_12_12$	4.448	3.693	3.234	—
		$P2_1$	4.456	4.606	3.743	120.92
Neurophysin II[i]	Bovine	$P2_12_12_1$	12.16	6.79	6.21	—
Catalase	*Micrococcus luteus*	$P4_22_12$	10.66	10.66	10.63	—
DD-Carboxypeptidase-endopeptidase[j]	*Streptomyces albus G*	$P2_1$	5.11	4.97	3.87	100.6
G.Actin : DNAse I	Chicken gizzard	$P2_12_12_1$	4.20	22.53	7.74	—
G.Actin : DNAse I	*Physarum*	$P2_12_12_1$	4.2	22.1	7.7	—
Cytochrome c_3	*Desulphovibrio vulgaris* Miyazaki F	$P2_12_12_1$	5.41	6.89	3.50	—
Cytochrome c_3	*D. vulgaris* Miyazaki K	$P2_12_12_1$	4.35	4.12	6.29	—
Aspartate aminotransferase	Bovine mitochondria	$P1$	5.56	5.86	7.60	$\alpha = 85.5$ $\beta = 109.1$ $\gamma = 115.6$
Ribosomal protein BL17[q]	*B. stearo-thermophilus*	$P2_12_12$	13.53	3.75	4.89	—
Cytochrome $c_{551.5}$	*Desulphuromonas acetoxidans*	$P2_12_12_1$	4.410	3.755	4.508	—
ω-Amino-acid : pyruvate aminotransferase	*Pseudomonas* sp. F-126	$I2_12_12_1$	12.41	13.79	6.12	—
Ubiquitin	Bovine thymus	$P2_12_12_1$	5.09	4.29	2.90	—
Cro regulatory protein[v]	Phage λ	$R32$	9.19	9.19	2.689	—
Lysozome[x]	*Streptomyces erythraeus*	$P2_12_12_1$	5.001	6.198	6.785	—
β-Lactoglobulin	Buffalo milk	$P6_3$	6.7	6.7	14.2	—
		$P3_121$	5.44	5.44	11.32	—
Adenylosuccinate synthetase	Rabbit muscle	$P4_32_12$ or $P4_12_12$	7.12	7.12	19.48	—
Maltose binding protein	*E. coli*	$P2_1$	5.77	6.51	4.47	100.66
D-Galactose binding protein	*E. coli*	$P2_1$	6.58	3.67	6.14	100.28
Cytochrome oxidase	*Pseudomonas aeruginosa*	$P2_12_12$	12.28	8.72	7.34	—
Ascorbic acid oxidase	Zucchini	$P2_12_12_1$	19.07	12.52	11.23	—
Bacteriophage MS2	—	—	—	—	—	—
2-Fe Ferredoxin	*Halobacterium*	$P6_322$	6.06	6.06	12.78	—

Mol. wt./ dalton *and no. of subunits*	*Mol. wt. of asymmetric unit*	V_m/ nm^3 $dalton^{-1}$	*Precipitant*	pH	*Ref.*
—	—	—	Ammonium sulphate	—	*a*
14 400 (1)	14 400	1.96	Ammonium sulphate	6.8	*b*
38 000 (1)	38 000	2.33	PEG6000[d]	7.6	*e*
5000 (1)	5000	1.74	Ethanol	—	*g*
15 800 (2)	7900	1.68	—	—	*h*
15 800 (2)	15 800	2.08	—	7.0	*h*
10 000 (?)	40 000	3.20	Ammonium sulphate	—	*j*
240 000 (4)	60 000	2.55	Ammonium acetate	5.4	*k*
18 500 (1)	18 500	2.61	PEG6000	8.0	*m*
74 000 (1 + 1)	74 000	2.47	PEG6000	—	*n*
74 000 (1 + 1)	74 000	2.41	PEG6000	—	*n*
14 000 (1)	14 000	2.33	Ammonium sulphate	6—8	*o*
14 000 (1)	14 000	2.01	Ammonium sulphate	6—8	*o*
93 000 (2)	—	—	PEG4000	8—9	*p*
17 000 (1)	17 000	3.65	Ammonium sulphate	—	*r*
9800 (1)	9800	1.90	PEG6000	7.2	*s*
172 000 (4)	43 000	3.04	Ammonium sulphate	7.8	*t*
8500 (1)	8500	1.87	PEG4000	5.6	*u*
15 000 (2)	45 000	2.50	Phosphate	7.5	*w*
18 500 (1)	18 500	2.84	Ammonium sulphate	—	*y*
36 000 (2)	36 000	2.32	Sodium chloride	3.5	*z*
36 000 (2)	18 000	2.69	Ammonium sulphate	8.5	*z*
54 000 (1)	54 000	2.29	Ammonium sulphate	7.0	*aa*
40 500 (1)	40 500	2.04	PEG6000	4.2	*bb*
32 000 (1)	32 000	2.22	PEG6000	4.9	*bb*
126 000 (2)	63 000	3.12	Ammonium sulphate	7.0	*cc*
140 000 (2)	70 000	2.40	Phosphate	7.0	*dd*
—	—	—	PEG6000	—	*ee*
14 000 (1)	14 000	2.42	Phosphate	7.0	*ff*

Table 3 *cont.*

Protein	*Source*	*Space group*	*Cell dimensions*			
			a/nm	*b*/nm	*c*/nm	*β*/deg
Aspartate amino-transferase	Pig mitochondria	*P*1	5.590	5.854	7.703	$\alpha = 85.05$ $\beta = 109.79$ $\gamma = 115.91$
α-Amylase[hh]	Porcine pancreas	$P2_12_12_1$	7.04	11.46	11.81	—
Tyrocidine A	*B. brevis*	*R*32	3.4	3.4	5.0	—
Lysozyme	Embden goose eggs	$P2_1$	3.83	6.57	4.52	116
		*P*1	3.99	4.22	5.79	$\alpha = 98.8$ $\beta = 102.5$ $\gamma = 90.5$
Fc Fragment IgG	Rabbit	$P2_1$[ll]	6.885	7.250	6.040	105.1
		$P2_1$[mm]	8.155	5.565	6.885	105.1
D-Glyceraldehyde-3-phosphate dehydrogenase	Mediterranean lobster	*C*2	12.84	9.99	8.03	113.4
Ferredoxin	*Azobacter vinelandii*	$P4_12_12$ (or $P4_32_12$)	5.53	5.53	9.59	—
		*P*1	4.68	5.87	6.43	$\alpha = 105.8$ $\beta = 82.5$ $\gamma = 110.5$
Glycolate oxidase	Spinach	*I*422	14.81	14.81	13.49	—
		$P4_22_12$	14.54	14.54	10.42	—
Southern bean mosaic virus	—	—	55.2	34.1	55.1	—
Haemoglobin	Sea cucumber	$P2_1$	4.60	4.53	4.09	104.5
		$P4_12_12$ (or $P4_32_12$)	7.70	7.70	6.15	—
Glutamate dehydrogenase	Rat liver	$P6_222$ (or $P6_422$)	10.1	10.1	72.4	—
Bowman–Birk inhibitor[uu]	Soybean seeds	$P6_122$ (or $P6_522$)	9.136	9.136	6.393	—
Dihydrolipoyl transacetylase: dihydrolipoyl dehydrogenase subcomplex[ww]	*E. coli*	$P2_13$[xx]	21.15	—	—	—
Semisynthetic ribonuclease[zz]	Bovine	$P3_221$	6.44	6.44	6.45	—
Azurin	*Alcaligenes denitrificans*	$C222_1$	7.50	7.41	9.95	—
Cytochrome *c'*	*Alcaligenes denitrificans*	$P6_122$ (or $P6_522$)	5.47	5.47	18.5	—
Lysozyme[ccc]	*Cygnus atratus*	$P2_1$	4.62	6.51	3.87	110

Mol. wt./ dalton *and no. of subunits*	*Mol. wt. of asymmetric unit*	V_m/ nm^3 $dalton^{-1}$	*Precipitant*	pH	*Ref.*
93 000 (2)	—	—	PEG4000	7.5	*gg*
50 000 (1)	100 000	2.38	—	—	*ii*
—	—	—	—	—	*jj*
22 000 (1)	44 000	2.30	—	—	*kk*
22 000 (1)	22 000	2.10	Sodium chloride	4.5	*kk*
50 000 (2)	50 000	2.91	Sodium chloride	4.7	*nn*
50 000 (2)	50 000	3.02	Sodium chloride	4.7	*nn*
140 000 (4)	72 000	3.26	Ammonium sulphate	7.0	*oo*
14 500 (1)	14 500	2.53	Ammonium sulphate	8.5	*pp*
14 500 (1)	60 000—70 000	2.2—2.75	Ammonium sulphate	7.4	*pp*
—	—	—	t-Butanol	8.3	*qq*
—	—	—	Ammonium sulphate	8.3	*qq*
—	—	—	—	—	*rr*
17 500 (1)	17 500	2.35	PEG6000	8	*ss*
35 000 (2)	17 500	2.60	PEG6000	7	*ss*
336 000 (6)	168 000	3.19	PEG6000	7.0	*tt*
8000 (1)	16 000	2.40	PEG4000	5.6	*vv*
—	—	2.00	Ammonium sulphate	—	*yy*
13 500 (1)	13 500	—	Ammonium sulphate	5.7	*aaa*
14 000 (1)	28 000	2.47	Ammonium sulphate	6.0	*bbb*
14 000 (1)	14 000	2.85	Ammonium sulphate	8.0	*bbb*
20 500 (1)	20 500	2.67	Sodium chloride	6.5	*ddd*

(*a*) M. Spencer, S. Neidle, and T. A. Jones, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 66. (*b*) R. Aschaffenburg, R. E. Fenna, D. C. Phillips, S. G. Smith, D. H. Buss, R. Jenness, and M. P. Thompson, *J. Mol. Biol.*, 1979, **127**, 135. (*c*) A penicillin-sensitive enzyme. (*d*) Polyethylene glycol. The number refers to the approximate molecular weight. (*e*) J. R. Knox, M. L. DeLucia, N. S. Murthy, J. A. Kelly, P. C. Moews, J.-M. Frère, and J.-M. Ghuysen, *J. Mol. Biol.*, 1979, **127**, 217. (*f*) A plant seed protein from a crucifer. Diffracts strongly to 0.088 nm resolution. (*g*) M. M. Teeter and W. A. Hendrickson, *J. Mol. Biol.*, 1979, **127**, 219. (*h*) J. P. Mornon, R. Bally, F. Fridlansky, and E. Milgrom, *J. Mol. Biol.*, 1979, **127**, 237. (*i*) Complex with the dipeptide L-phenylalanyl-L-tyrosine. (*j*) C. S. Yoo, B.-C. Wang, M. Sax, and E. Breslow, *J. Mol. Biol.*, 1979, **127**, 241. (*k*) A. L. Marie, F. Parak, and W. Hoppe, *J. Mol. Biol.*, 1979, **129**, 675. (*l*) This enzyme shows low sensitivity to penicillin. (*m*) O. Dideberg, J.-M. Frère, and J.-M. Ghuysen, *J. Mol. Biol.*, 1979, **129**, 677. (*n*) H. Sugino, N. Sakabe, K. Sakabe, S. Hatano, F. Oosawa, T. Mikawa, and S. Ebashi, *J. Biochem.*, 1979, **86**, 257. (*o*) S. Bando, Y. Matsuura, N. Tanaka, N. Yasuoka, M. Kakudo, T. Yagi, and H. Inokuchi, *J. Biochem.*, 1979, **86**, 269. (*p*) S. Capasso, A. M. Gargillo, G. Marino, L. Mazzarella, P. Pucci, and G. Sannia, *FEBS Lett.*, 1979, **101**, 351. (*q*) A protein from the 50S ribosomal subunit. (*r*) K. Appelt, J. Dijk, and O. Epp, *FEBS Lett.*, 1979, **103**, 66. (*s*) R. Haser, F. Payan, R. Bache, M. Bruschi, and J. Le Gall, *J. Mol. Biol.*, 1979, **130**, 99. (*t*) Y. Morita, S. Aibara, K. Yonaha, S. Toyama, and K. Soda, *J. Mol. Biol.*, 1979, **130**, 211. (*u*) W. J. Cook, F. L. Suddath, C. E. Bugg, and G. Goldstein, *J. Mol. Biol.*, 1979, **130**, 353. (*v*) A repressor protein binding to phage DNA late in the lytic cycle. (*w*) W. F. Anderson, B. W. Matthews, Y. Takeda, and H. Echols, *J. Mol. Biol.*, 1979, **130**, 507. (*x*) Chemically and mechanistically very different from the hen egg white lysosome family. (*y*) R. Sarma, M. Kakudo, S. Hara, and T. Ikenaka, *J. Mol. Biol.*, 1979, **131**, 409. (*z*) M. Bolognesi, J. Liberatori, R. Oberti, and L. Ungaretti, *J. Mol. Biol.*, 1979, **131**, 411. (*aa*) S. Harada, Y. Matsuura, N. Tanaka, M. Kakudo, Y. Matsuda, H. Ogawa, H. Shiraki, and H. Nakagawa, *J. Mol. Biol.*, 1979, **132**, 729. (*bb*) F. A. Quiocho, W. E. Meador, and J. W. Pflugrath, *J. Mol. Biol.*, 1979, **133**, 181. (*cc*) T. Takano, R. E. Dickerson, S. A. Schichman, and T. E. Meyer, *J. Mol. Biol.*, 1979, **133**, 185. (*dd*) R. Ladenstein, A. Marchesini, and S. Palmieri, *FEBS Lett.*, 1979, **107**, 407. (*ee*) W. Min Jou, A. Raeymaekers, and W. Fiers, *Eur. J. Biochem.*, 1979, **102**, 589. (*ff*) J. L. Sussman, P. Zipori, M. Harel, A. Yonath, and M. M. Werber, *J. Mol. Biol.*, 1979, **134**, 375. (*gg*) E. Eichele, G. C. Ford, and J. N. Jansonius, *J. Mol. Biol.*, 1979, **135**, 513. (*hh*) Also contains information on α-mannosidase. (*ii*) P. M. D. Fitzgerald, P. J. Stankiewicz, S. Campbell-Smith, and A. McPherson, *J. Mol. Biol.*, 1979, **135**, 753. (*jj*) B. Magdoff-Fairchild and J. G. White, *J. Mol. Biol.*, 1979, **135**, 757. (*kk*) M. G. Grütter, K. L. Rine, and B. W. Matthews, *J. Mol. Biol.*, 1979, **135**, 1029. (*ll*) Covalently linked dimeric molecule. (*mm*) Reduced, alkylated, non-covalently linked dimer. (*nn*) R. Aschaffenburg, M. Lewis, D. C. Phillips, E. M. Press, S. G. Smith, B. J. Sutton, and C. W. Mountford, *J. Mol. Biol.*, 1979, **135**, 1033. (*oo*) R. Berni, A. Mozzarelli, G. L. Rossi, M. Bolognesi, and R. Oberti, *J. Biol. Chem.*, 1979, **254**, 8004. (*pp*) C. D. Stout, *J. Biol. Chem.*, 1979, **254**, 3598. (*qq*) Y. Lindquist and C.-I. Brändén, *J. Biol. Chem.*, 1979, **254**, 7403. (*rr*) I. Rayment, J. E. Johnson, and M. G. Rossmann, *J. Biol. Chem.*, 1979, **254**, 7403. (*rr*) I. Rayment, J. E. Johnson, and M. G. Rossmann, *J. Biol. Chem.*, 1979, **254**, 5243. (*ss*) W. M. Carson, T. R. Bowers, G. B. Kitto, and M. L. Hackert, *J. Biol. Chem.*, 1979, **254**, 7400. (*tt*) J. J. Birktoft, F. Miake, L. J. Banaszak, and C. Frieden, *J. Biol. Chem.*, 1979, **254**, 4915. (*uu*) A protease inhibitor with a high cysteine content (as high as 20%). (*vv*) C. H. Wei, S. P. Basu, and J. R. Einstein, *J. Biol. Chem.*, 1979, **254**, 4892. (*ww*) Two of the three enzymes in the pyruvate dehydrogenase complex. (*xx*) The dihydrolipoyl transacetylase, the core enzyme of the pyruvate dehydrogenase complex, consists of 24 subunits with octahedral (432) symmetry. (*yy*) C. C. Fuller, L. J. Reed, R. M. Oliver, and M. L. Hackert, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 431. (*zz*) Residues 1—118 plus the peptide 111—124 bound as a non-covalent complex. (*aaa*) D. M. Sasaki, P. D. Martin, M. S. Doscher, and D. Tsernoglou, *J. Mol. Biol.*, 1979, **135**, 301. (*bbb*) G. E. Norris, B. F. Anderson, E. N. Baker, and S. V. Rumball, *J. Mol. Biol.*, 1979, **135**, 309. (*ccc*) A 'goose-type' lysozyme isolated from the egg white of the black swan. (*ddd*) M. Masakuni, R. J. Simpson, and N. W. Isaacs, *J. Mol. Biol.*, 1979, **135**, 313.

resolution structure determinations are reported in Table 4, while Table 5 covers the results obtained from low-angle *X*-ray and neutron-scattering measurements.

The methods and equipment used in crystallography continue to evolve. An elegant technique for analysing crystallizing conditions has been presented and several papers on the use of the oscillation camera for data collection have appeared. The various strategies for the refinement and comparison of protein structures have been further considered, and several groups have attempted to derive information on the dynamics of protein structure from crystallographic refinements. The increased number of groups using computer graphics systems is accompanied by a decrease in the reports of the hardware of such systems. These developments are all covered in Section 2.

Table 4 *Low-resolution structures*

Protein	*Source*	*Resolution*/nm	*Comments*	*Ref.*
Pyrophosphatase	Baker's yeast	0.6	The molecule is an elongated ellipsoid with semiaxes 2.6 × 2.2 × 4.8 nm. The dimeric molecule is distinctly bilobal and the molecular diad axis has been identified	*a*
β-Lactoglobulin	Cow's milk	0.6	The structures of four crystal forms are presented and compared. The four structures are similar and show features tentatively identified as α-helices and β-pleated sheet	*b*
Aspartate aminotransferase	Chicken mitochondria	0.45	The dimeric molecule has dimensions 10.5 × 6.0 × 5.0 nm consisting of subunits related by a molecular diad axis. Each subunit, 7.0 × 5.0 × 4.0 nm, contains at least 7 helices, one of which is 5 nm long. The coenzyme and substrate binding sites have been identified, the active sites lying close to the intersubunit boundary	*c*
Ferredoxin	*Azotobacter vinelandii*	0.4	The molecule is 3.0 × 2.5 × 3.5 nm in size and contains two iron–sulphur clusters separated by 1.1 nm. Several stretches of polypeptide chain are visible in the map	*d*
Immunoglobulin J539	Mouse	0.45	A mouse galactan binding immunoglobulin Fab. The map was interpreted by computer-fitting of known immunglobulin domains to the electron-density map. The structure is very similar to that of McPC603	*e*

(*a*) V. V. Makhaldiani, E. A. Smirnova, A. A. Voronova, I. P. Kuranova, E. G. Arutyunyan, B. K. Vainshtein, W. Höhne, B. Binwald, and G. Hansen, *Dokl. Biochem.*, 1979, **240**, 200. (*b*) D. W. Green, R. Aschaffenburg, A. Camerman, J. C. Coppola, P. Dunnill, R. M. Simmons, E. S. Komorowski, L. Sawyer, E. M. C. Turner, and K. F. Woods, *J. Mol. Biol.*, 1979, **131**, 375. (*c*) G. Eichele, G. C. Ford, M. Glor, and J. N. Jansonius, *J. Mol. Biol.*, 1979, **133**, 161. (*d*) C. D. Stout, *Nature* (*London*), 1979, **279**, 83. (*e*) M. A. Navia, D. M. Segal, E. A. Padlan, D. R. Davies, N. Rao, S. Rudikoff, and M. Potter, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4071.

Table 5 *Low-angle scattering results*

Protein	*Source*	*Molecular weight*/dalton	R_G/nm	V/nm^3	*Comments*	*Ref.*
Phosphofructokinase	*Dunaliella alina*	480 000	7.725	1067	Model proposes the hexamer to be 15 nm in diameter with the 6 subunits arranged with C_6 symmetry	*a*
Adenosine triphosphatase	*E. coli*	358 000	4.19	739	The molecule appears to be composed of spherical parts bound together by a flexible portion of the polypeptide chain	*b*
Hexokinase B	Yeast	104 000	3.128	—	The results are consistent with the B$_{II}$ dimer structure existing in solution	*c*
Phosphoglycerate kinase	Yeast	40 000	2.334	—	There are measurable decreases in the radius of gyration on the binding of substrate, suggesting a closing of the interdomain cleft	*d*
Aspartate transcarbamylase	*E. coli*	295 000	4.59	—	The molecule appears to swell upon the binding of a transition-state analogue	*e*
Low-density lipoprotein	Rhesus monkey serum	—	—	—	The molecular parameters for the low- and high-temperature forms are presented and models for the structure discussed	*f*
Protein S1	*E. coli* ribosomes	85 000	8.0	140	Results suggest S1 is organized into two distinct subdomains within its elongated overall shape	*g*
5-S RNA-L18-L25	*E. coli* ribosomes	68 000	3.95	—	The increase in the radius of gyration of free 5-S RNA on the binding of L18 + L25 is discussed in terms of possible structural models	*h*
BF_1-ATPase	*E. coli*	315 000	4.63	—	Neutron small-angle scattering study	*i*
Bacteriophage ϕ29	—	—	—	—	Scattering curve can be simulated by a cylinder of 39 nm diameter by 46 nm in height. The protein coat is *ca.* 3 nm thick	*j*
Bacteriophage λ	—	—	—	—	The formation of isometric head shells under a variety of conditions has been studied	*k*

(*a*) H. H. Paradies and W. Vettermann, *Arch. Biochem. Biophys.*, 1979, **194**, 88. (*b*) H. H. Paradies and U. D. Schmidt, *J. Biol. Chem.*, 1979, **254**, 5257. (*c*) R. C. McDonald, D. M. Engelman, and T. A. Steitz, *J. Biol. Chem.*, 1979, **254**, 2942. (*d*) C. A. Pickover, D. B. McKay, D. M. Engelman, and T. A. Steitz, *J. Biol. Chem.*, 1979, **254**, 11323. (*e*) M. C. Moody, P. Vachette, and A. M. Foote, *J. Mol. Biol.*, 1979, **133**, 517. (*f*) V. Luzzati, A. Tardieu, and L. P. Aggerbeck, *J. Mol. Biol.*, 1979, **131**, 435. (*g*) H. Labischinski and A.-R. Subramanian, *Eur. J. Biochem.*, 1979, **95**, 359. (*h*) R. Österberg, *Eur. J. Biochem.*, 1979, **97**, 463. (*i*) M. Satre and G. Zaccai, *FEBS Lett.*, 1979, **102**, 244. (*j*) J. A. Subirana, J. Lloveras, M. Lambardero, and E. Viñuela, *J. Mol. Biol.*, 1979, **128**, 101. (*k*) W. C. Earnshaw, R. W. Hendrix, and J. King, *J. Mol. Biol.*, 1979, **134**, 575.

Sections 3—9 cover the protein structures determined at high resolution. Further studies on the control of phosphorylase and the action of kinases are noteworthy. Phosphofructokinase, one of the few unknown structures in glycolysis, has had its structure determined and pyruvate kinase has been taken to higher resolution. The crystal structures of subtilisin inhibitor, *Streptomyces griseus* proteases A and B, α-lytic protease, pepsin, and *Endothia* protease have been reported.

Other structures of particular note include dihydrofolate reductase complexed with NADPH and inhibitor, the active site of rhodanese, the selenium enzyme glutathione peroxidase, bacterio-chlorophyll, the flavoenzyme *p*-hydroxybenzoate hydroxylase, a DNA-unwinding protein, gene 5 product phage fd, and citrate synthase.

Protein structural analysis and structure prediction continue to improve and refine their methods, the use of computer graphics to produce colour-coded space-filling drawings being particularly useful. The increased number of structures now available at atomic resolution has led to more groups examining the observed conformations of side-chains in proteins.

X-Ray diffraction studies on some viruses, nucleic acids including yeast initiator $tRNA_f^{met}$, muscle, membrane systems, bacterial pili and flagella, and ribosomes are presented here together with studies on naturally occurring and synthetic fibrous proteins.

The traumatic change from Ångstrom units to nanometres has been made this year and will not, it is hoped, be too confusing for the reader.

2 Equipment and Methods

General Crystallography.—Several reviews on protein structure have appeared in a single volume. Fruton [1] has reviewed the early theories on protein structure while Hodgkin [2] has presented a brief history of protein crystallography.

Declercq, Germain, and Woolfson [3] have described two techniques for the solution of complex structures when MULTAN fails and presented examples of their use. Karle [4] has further discussed triplet phase invariants, in this case for centrosymmetric crystals, and Hauptman [5] has considered a probabilistic theory of the three-phase structure semi-invariant in space group $P\bar{1}$.

A statistical method for selecting reflections for Bijvoet-difference measurement in crystals containing anomalous scatterers has been reported [6] and the application of the method to three crystal structures is described. Ino and Minami [7] have presented a method for calculating the intensity of *X*-ray diffraction from small crystalline particles whose boundary is described by a shape function. Flack and Vincent [8] have analysed the intensity errors from imperfectly spherical crystals irradiated with an inhomogeneous *X*-ray beam. Arnberg and his co-workers [9] have

[1] J. S. Fruton, *Ann. N.Y. Acad. Sci.*, 1979, **325**, 1.
[2] D. C. Hodgkin, *Ann. N.Y. Acad. Sci.*, 1979, **325**, 121.
[3] J. P. Declercq, G. Germain, and M. M. Woolfson, *Acta Crystallogr.*, 1979, **A35**, 622.
[4] J. Karle, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2089.
[5] H. Hauptman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4747.
[6] D. Velmurugan, S. Parthasarathy, and V. Parthasarathi, *Acta Crystallogr.*, 1979, **A35**, 463.
[7] T. Ino and N. Minami, *Acta Crystallogr.*, 1979, **A35**, 163.
[8] H. D. Flack and M. G. Vincent, *Acta Crystallogr.*, 1979, **A35**, 795.
[9] L. Arnberg, S. Hovmöller, and S. Westman, *Acta Crystallogr.*, 1979, **A35**, 497.

discussed the significance of 'non-significant' or 'unobserved' reflections and have successfully refined a structure using only previously discarded reflections. The inclusion of the weakest reflections in the refinements was shown to lower the standard deviations and the authors recommend such inclusions as standard practice. Vickovic and Viterbo [10] have also presented a statistical method for estimating the unobserved reflections and have used the method successfully to solve a structure for which more than 60% of the data were classed as too weak to measure.

Main [11] has presented a theoretical comparison of the β-synthesis, the weighted γ'-synthesis, and the weighted $2F_{obs} - F_{calc}$ synthesis. He has shown that the $2F_o—F_c$ synthesis is the one which most closely approaches the true electron density.

Miller and Welberry [12] have described a three-dimensional model of crystal-growth disorder.

Protein Crystallography.—The crystallization of tryptophan tRNA synthetase has been studied by incomplete factorial experiments.[13] The method establishes favourable precipitation procedures within broad limits which are then more exhaustively tested for a limited number of crystallizing factors. The production of large crystals suitable for a structure determination is described in full.

A method for stabilizing crystals grown in methylpentanediol to produce heavy-atom derivatives has been described.[14] Pre-grown cytochrome *c* peroxidase crystals were transferred to polyethylene glycol solutions (12%) in which they could tolerate heavy-atom solutions ten times more concentrated than in the original mother liquor.

A technique using 0.445 nm *X*-radiation generated by a laser-produced plasma source has been reported.[15] The technique is described and its use in structural kinetic studies is considered.

A limited-range step-scan method for rapid collection of diffractometer data has been reported.[16] Data collection is a two-pass process, the first pass using the intense reflections to predict peak positions and peak widths, and these values are used in the second pass to control data collection. Time for collecting data is minimized since only a limited number of steps in the region of the peak maximum are used for collecting counts. Time is further saved by assuming the background intensity is a function of 2θ only and measuring it by scanning between selected reciprocal lattice rows.

Winkler and his co-workers [17] have described the modifications of the standard methods which are essential when using the oscillation method for crystals with an average cell dimension in excess of 25 nm. In particular, it is necessary to correct partially recorded intensities and all the considerations are discussed with the data collection of tomato bushy stunt virus as an example.

[10] I. Vickovic and D. Viterbo. *Acta Crystallogr.*, 1979, **A35**, 500.
[11] P. Main, *Acta Crystallogr.*, 1979, **A35**, 779.
[12] G. H. Miller and T. R. Welberry, *Acta Crystallogr.*, 1979, **A35**, 391.
[13] C. W. Carter, jun. and C. W. Carter, *J. Biol. Chem.*, 1979, **254**, 12219.
[14] B. Eriksson, B. Ersson, P. Kierkegaard, L. O. Larsson, U. Skoglund, M. Ludwig, and T. Yonetani, *J. Mol. Biol.*, 1979, **A35**, 225.
[15] R. D. Frankel and J. M. Forsyth, *Science*, 1979, **204**, 622.
[16] J. C. Hanson, K. D. Watenpaugh, L. Sieker, and L. H. Jensen, *Acta Crystallogr.*, 1979, **A35**, 616.
[17] F. K. Winkler, C. E. Schutt, and S. C. Harrison, *Acta Crystallogr.*, 1979, **A35**, 901.

Wilson and Yeates [18] have described the procedures used in Oxford for processing oscillation camera data and Stuart and Walker [19] have described an empirical method for correcting rotation-camera data for absorption and decay effects. Another method for optimizing absorption corrections has been reported.[20]

Green [21] has considered a probability method for phasing macromolecular isomorphous replacement data. The mathematical techniques used are similar to those employed in the derivation of probability distributions of structure semi-invariants and the one derivative case is considered in detail. Hendrickson [22] has examined the various ways of utilizing phase information obtained from anomalous scattering measurements. He derives exact general expressions and shows how these can be simplified for the special situations that normally apply in practice.

Neutron Diffraction.—The application of a time-of-flight neutron diffractometer designed on the basis of a pulsed neutron source has been described.[23] The use of the machine is discussed and the results of a test experiment are presented. Hewat [24] has reported corrected and simplified absorption corrections for neutron diffraction.

Protein Dynamics by Crystallography.—A preliminary analysis of the temperature factors obtained for the atoms of hen egg white lysozyme after refinement at 0.2 nm has been published.[25] If the contributions to the temperature factors from experimental errors, crystal disorder, and imperfections in the molecular model are neglected, the temperature factors can be used as a measure of the thermal motion of the atoms. The apparent motion is compatible with the pair of molecules that show the strongest interactions in the crystal either moving as a rigid body with libration about a common axis or vibrating in an intramolecular mode with the principal amplitudes radial to this axis. Side-chains exposed to the solvent and certain segments of the main polypeptide chain show greater apparent motion than the rest of the molecule. (See also Section 5.)

An interesting report on sub-nanosecond motions of tryptophan residues studied using a synchrotron as a pulsed-light source has been published.[26] A simulation of the molecular dynamics of pancreatic trypsin inhibitor during a period of 100 ps has also been published [27] and the picosecond dynamics of the tyrosine side chains in the same protein have been analysed.[28]

Protein Refinements.—Schomaker and Marsh [29] have presented a discussion on refinements in space groups of unnecessarily low symmetry while Carruthers [30] has reported a method for rapidly calculating structure factors for a trial structure moved to alternative sites in the cell.

[18] K. Wilson and D. Yeates, *Acta Crystallogr.*, 1979, **A35**, 146.
[19] D. Stuart and N. Walker, *Acta Crystallogr.*, 1979, **A35**, 925.
[20] J. Rigoult, A. Tomas, and C. Guidi-Morosini, *Acta Crystallogr.*, 1979, **A35**, 587.
[21] E. A. Green, *Acta Crystallogr.*, 1979, **A35**, 351.
[22] W. A. Hendrickson, *Acta Crystallogr.*, 1979, **A35**, 245.
[23] A. M. Balagurov, E. Borca, M. Dlouha, Z. Gheorghiu, G. M. Mironova, and V. B. Zlokazov, *Acta Crystallogr.*, 1979, **A35**, 131.
[24] A. W. Hewat, *Acta Crystallogr.*, 1979, **A35**, 248.
[25] M. J. E. Sternberg, D. E. P. Grace, and D. C. Phillips, *J. Mol. Biol.*, 1979, **130**, 231.
[26] I. Munro, I. Pecht, and L. Stryer, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 56.
[27] M. Karplus and J. A. McCammon, *Nature (London)*, 1979, **277**, 578.
[28] J. A. McCammon, P. G. Wolynes, and M. Karplus, *Biochemistry*, 1979, **18**, 927.
[29] V. Schomaker and R. E. Marsh, *Acta Crystallogr.*, 1979, **B35**, 1933.
[30] J. R. Carruthers and D. J. Watkin, *Acta Crystallogr.*, 1979, **A35**, 860.

Bianchi and his co-workers [31] have discussed constrained refinement techniques with reference to three particular structures.

Two procedures to extend and refine phases starting from a medium-sized set of known phases have been reported.[32] Tests of the procedures on structures with 376 and 400 atoms are described.

Hoard and Nordman [33] have reported a fast Gauss–Seidel least-squares refinement procedure for atomic parameter refinements in large asymmetric units. The method combines the advantages of the Gauss-Seidel algorithm with rigid-group constraint and subsidiary parameter elastic restraint capabilities. Considerable increases in computer efficiency have been obtained by determining contributions from one atom to all reflections at each calculation. Application of the procedure to flavodoxin at 0.25 nm is described.

Carruthers and Watkin [34] have presented a weighting scheme for least-squares structure refinement while Carpenter [35] has discussed the errors in co-ordinates, bond lengths, and bond angles calculated from the standard deviations in atom co-ordinates.

Hendrickson [36] has described a simple, efficient, and general method for finding the transformations necessary to superimpose two similar structures. He also presents the formulae necessary to extract the independent rotation variables from the transformation matrix which is obtained. Some examples of its application are reported.

Bethge and Mathews [37] have presented a rotation function study of flavocytochrome b_2 which has allowed the orientation of the molecular 222 symmetry axes to be determined. (See also Section 4.)

The phase extension and refinement of Bence–Jones protein Rhe at 0.19 nm resolution have been published.[38] The method uses a limited subset of the available data and a non-interactive computer graphics system and in the example described included a restrained least-squares refinement in reciprocal space. (See also Section 3.)

The phase angles of the α-chymotrypsin dimer have been extended from 0.28 to 0.18 nm resolution by Fourier phase refinement and electron-density modification using fast Fourier-transform methods.[39] The refinement converged after seven cycles; the average change in phase angle in the last cycle was 3.8°. The average change from the 0.28 nm starting phase set was 28° and the increase in quality of the electron density is discussed. (See also Section 6.)

Chambers and Stroud [40] have compared two independently refined models of bovine trypsin. The comparison shows that the structures are remarkably similar in the well determined regions, the average difference between internal main-chain

[31] R. Bianchi, R. Destro, T. Pilati, and M. Simonetta, *Acta Crystallogr.*, 1979, **A35**, 539.
[32] G. J. Olthof, L. Sint, and H. Schenk, *Acta Crystallogr.*, 1979, **A35**, 941.
[33] L. G. Hoard and C. E. Nordman, *Acta Crystallogr.*, 1979, **A35**, 1010.
[34] J. R. Carruthers and D. J. Watkin, *Acta Crystallogr.*, 1979, **A35**, 698.
[35] G. B. Carpenter, *Acta Crystallogr.*, 1979, **A35**, 248.
[36] W. A. Hendrickson, *Acta Crystallogr.*, 1979, **A35**, 158.
[37] P. H. Bethge and F. S. Mathews, *Acta Crystallogr.*, 1979, **A35**, 412.
[38] W. Furey, jun., B. C. Wang, C. S. Yoo, and M. Sax, *Acta Crystallogr.*, 1979, **A35**, 810.
[39] N. V. Raghavan and A. Tulinsky, *Acta Crystallogr.*, 1979, **B35**, 1776.
[40] J. L. Chambers and R. M. Stroud, *Acta Crystallogr.*, 1979, **B35**, 1861.

atoms being 0.0146 nm. Differences of up to 0.3—0.9 nm are observed for some of the poorly defined external side-chains. (See also Section 6.)

Structure Storage and Display.—Wang and his co-workers [41] have compared the interpretations of the electron-density map of Bence–Jones protien Rhe using an optical comparator and a computerized automated map interpretation program. Both methods found 113 residues although there were notable differences in the assignment of α-carbon positions found by the two methods. Despite being wrong in some of its interpretation, the computerized method seems to have considerable potential after further development.

The Cambridge Crystallographic Data Centre system for the storage, retrieval, analysis, and display of molecular structures has been described.[42] The files cover 25 000 structural study results covering the literature from 1935 onwards. The use of this system has provided invaluable assistance in the preparation of this chapter since 1978.

The use of the system to retrieve and analyse data with respect to intermolecular interactions has also been published.[43]

Miller [44] in an appendix to a paper on lactate dehydrogenase describes the interactive computer graphics system used to investigate the packing of the lactate dehydrogenase molecules in known crystal unit cells. (See Section 7, ref. 109.)

Swanson [45] has described how computer graphics can be used to show three-dimensional structures without restriction to plane sections of electron density or the use of 'cage-contouring'. The core-tracing technique is described in detail and illustrated by means of stereo-diagrams. The more modest outlay necessary to examine relatively large structures is sure to appeal to many potential users of such a system.

3 Immunoglobulins and Lectins

General.—The three-dimensional structure of immunoglobulins has been extensively reviewed by Amzel and Poljak.[46]

Bence–Jones Protein Rhe.—The crystal structure of protein Rhe, a λ-type V_L dimer, has been determined at 0.3 nm resolution.[47] The electron-density map was calculated using phases determined by two isomorphous heavy-atom derivatives and has an average figure of merit of 0.765. The complete polypeptide backbone chain could be traced and showed that protein Rhe contained a large amount of β-pleated sheet and was folded in a similar manner to another λ-type Bence–Jones protein's variable domain. The protein consists of 113 residues and a tentative sequencing was performed. The crystallographic sequence has assisted in the ordering of proteolytically generated fragments. Nine protein strands, some of

[41] B.-C. Wang, C. S. Yoo, W. Furey, jun., and M. Sax, *J. Mol. Biol.*, 1979, **135**, 305.
[42] F. H. Allen, S. Bellard, M. D. Brice, B. A. Cartwright, A. Doubleday, H. Higgs, T. Hummelink, B. G. Hummelink-Peters, O. Kennard, W. D. S. Motherwell, J. R. Rodgers, and D. G. Watson, *Acta Crystallogr.*, 1979, **B35**, 2331.
[43] P. Murray-Rust and W. D. S. Motherwell, *J. Am. Chem. Soc.*, 1979, **101**, 4374.
[44] J. R. Miller, *J. Biol. Chem.*, 1979, **254**, 7620.
[45] S. M. Swanson, *J. Mol. Biol.*, 1979, **129**, 637.
[46] L. M. Amzel and R. Poljak, *Annu. Rev. Biochem.*, 1979, **48**, 961.
[47] B.-C. Wang, C. S. Yoo, and M. Sax, *J. Mol. Biol.*, 1979, **129**, 657.

them in a β-pleated sheet, surround a central hydrophobic core and the three hypervariable regions are found to be located at one end of the ellipsoidal subunit. Two sections of chain protrude from the main body of the subunit.

The Rhe dimer consists of two subunits related by a crystallographic two-fold axis and is formed mainly by contacts between the two protruding regions of chain. The interactions at the dimer interface seem to be mainly hydrophobic in character based on the tentative sequence, but several hydrogen bonds are also involved. The association of the monomers produces a channel-like opening at one end of the dimer, the opening being 1.0 nm deep by 1.0—1.5 nm wide. The residues lining this cavity come from the hypervariable regions.

The monomer association is very different from that seen for protein Rei, the channel-like opening not being seen in Rei nor in any other antibody whose structure is known. As well as differences in the dimer, the monomers show one area of differences of up to 0.7 nm, this being the non-hypervariable loop from residues 37—46. The possible reasons for these differences are considered together with their immunological implications.

The phase extension and refinement of Bence–Jones protein Rhe at 0.19 nm resolution has also been reported.[38] The increased quality of the map has allowed several sequence changes to be made. Unlike protein Rei, there are no indications of disorder at the disulphides and residue Pro-8 is seen to be in the *trans* configuration not *cis* as seen in Rei.

Concanavalin A.—*X*-Ray absorption fine-structure spectroscopy has been used to characterize the binding of zinc and calcium ions to concanavalin A.[48] With just zinc present, the zinc ion is hexa-co-ordinated but when a calcium ion binds to the calcium-specific site this decreases to a co-ordination number of five. The geometries and interconversions of the complexes are discussed.

The crystal structure of demetallized concanavalin A has been determined at 0.32 nm resolution by the molecular-replacement method.[49] Refinement of the initially fitted structure using a constraint–restraint least squares procedure has lowered the *R*-factor from 0.47 to 0.26. The structural changes observed on demetallization are small but significant, the manganese ion ligands showing the greatest effects. The changes seen in this site are most easily explained by changes in charge on removal of the metal. The inability of metal-free concanavalin A to bind calcium ions can also be explained in structural terms.

The results reported disagree with several previously published observations of structural changes on the removal of metal ions.

Some segments of the backbone are also seen to become flexible and this may account for the increased susceptibility of demetallized concanavalin A to proteolysis.

4 Oxygen- and Electron-carrying Proteins

Haem Chemistry.—The synthesis and crystal-structure determination of a six-co-ordinate high-spin iron(III) porphyrin has been reported[50] and shows that the

[48] A. J. Kalb, E. A. Stern, and S. M. Heald, *J. Mol. Biol.*, 1979, **135**, 501.
[49] M. Shoham, A. Yonath, J. L. Sussman, J. Moult, W. Traub, and A. J. Kalb, *J. Mol. Biol.*, 1979, **131**, 137.
[50] W. R. Scheidt, I. A. Cohen, and M. E. Kastner, *Biochemistry*, 1979, **18**, 3546.

large high-spin iron atom is centred in the porphyrin plane. The compound examined provides a model for the geometry of haem in aquomethaemoglobin.

General Chemistry of Haem Proteins.—Perutz[51] has reviewed the structure and function of haemoglobin while Moffat and his co-workers[52] have proposed a structural model for the kinetic behaviour of haemoglobin. Case and Karplus[53] have analysed theoretically the binding of carbon dioxide and oxygen to myoglobin and have attempted to determine the role of the globin matrix on the binding pathway.

Argos and Rossmann[54] have analysed the common structural features of haem-binding proteins by comparing cytochrome c_{551} with cytochrome b_5 and the globin fold. Of the 82 residues located in cytochrome c_{551}, 49 are found to be topologically equivalent to the globin fold with an r.m.s. deviation of 0.35 nm for the α-carbon positions. When compared to cytochrome b_5, 41 are equivalent with an r.m.s. deviation of 0.49 nm. The common fold represents a central haem-binding core corresponding to the central exon of certain globin genes. The haem iron atoms and the inclinations of the haem planes are also seen to be very similar and the haem face is directed in a similar manner in all three proteins. These similarities may represent divergence from a common ancestral protein but could be due to convergent requirements of haem binding. Various tests using other known structures are reported.

Myoglobin.—The structure determinations of metmyoglobin at 250, 275, and 300 K have been described[55] and the subsequent refinement used to obtain data on the dynamics of the protein structure. When the dynamics are examined it is found that the large displacements are virtually independent of temperature and 95 residues have at least two conformational substates. As expected, displacements on the surface of the protein are larger than those in the interior and the general movements are similar to those reported for lysozyme. (See Section 5, ref. 76.) Movements of residues near the haem group are examined and possible approach routes to the iron atom agree with other recent results.[53] The authors stress that this method can yield only spatial information, other techniques, *e.g.* n.m.r., are necessary to obtain temporal information.

Haemoglobin.—The structure of horse azide methaemoglobin compared to methaemoglobin has been examined by difference Fourier analysis at 0.28 nm resolution.[56] The azide ligands are co-ordinated end-on at angles of *ca.* 125° to the haem axis, this binding mode being very similar to that seen for azide binding to free haem. The off-axis binding means that, unlike carbon monoxide or cyanide, the azide perturbs the protein structure less despite its large size. The haem stereochemistry and the position of the iron atom are hardly altered, but on the β-chain helix E is pushed away from the haem upon azide binding. This movement is not so pronounced in the α-chain.

[51] M. F. Perutz, *Annu. Rev. Biochem.*, 1979, **48**, 327.
[52] K. Moffat, J. F. Deatherage, and D. W. Seybert, *Science*, 1979, **206**, 1035.
[53] D. A. Case and M. Karplus, *J. Mol. Biol.*, 1979, **132**, 343.
[54] P. Argos and M. G. Rossmann, *Biochemistry*, 1979, **18**, 4951.
[55] H. Frauenfelder, G. A. Petsko, and D. Tsernoglou, *Nature (London)*, 1979, **280**, 558.
[56] J. F. Deatherage, S. K. Obendorf, and K. Moffat, *J. Mol. Biol.*, 1979, **134**, 419.

Baldwin and Chothia [57] have produced a very detailed analysis of the structural changes related to ligand binding and the allosteric mechanism of haemoglobin. Their analysis has compared human deoxy-, horse met-, and human carbon monoxy-haemoglobin using computer graphics and least-squares fitting methods. The changes seen on going from the deoxy to either of the liganded forms are seen to be very similar and consist of changes in tertiary structure of the $\alpha_1\beta_1$ dimer as well as changes in the packing of $\alpha_1\beta_1$ against $\alpha_2\beta_2$. The detailed changes observed are presented in a series of excellent computer-generated space-filling and skeletal-type diagrams and are discussed in light of other evidence on the possible mechanisms of action. Ten specific differences in structure on the change from the deoxy to the liganded state are identified and related to the functional properties of the protein.

Nitric oxide haemoglobin has been compared to horse methaemoglobin by difference Fourier analysis at 0.28 nm resolution.[58] The nitric oxide ligand binds off the haem axis and at a binding angle of *ca.* 145°. This is very similar to the binding seen in a model compound and suggests that the globin has little effect on the process. As was seen with azide binding [56] there is little structural change within the protein subunits.

Haemoglobin C.—Fitzgerald and Love [59] have determined the structure of two of the crystal forms of deoxy-haemoglobin C by molecular replacement. The haemoglobin C has a lysine residue at postion $\beta6$ instead of a glutamate, and energetically favourable interactions involving this residue are seen in both crystal forms. Since these interactions are intermolecular and obviously stabilize the crystal lattice, it seems this could be the reason for the observed *in vivo* crystallization of the protein.

The structure of the cyanometh form of haemoglobin C has been examined by electron microscopy [60] and by *X*-ray diffraction using the molecular replacement method.[61] The molecular packing in the crystal has been elucidated and the lysine residue at $\beta6$ is seen to be involved in intermolecular crystal contacts.

Haemoglobin A.—Human haemoglobin carbamylated at residue $\alpha1$ valine has been prepared and its crystal structure determined.[62] Since a part of the alkaline Bohr effect has been attributed to the free amino group of this residue its interactions with chloride ions are of particular interest. Studies on human deoxy-haemoglobin, with and without the carbamylated group, show the presence of two anion-binding sites associated with $\alpha1$ valine which are blocked when this group is carbamylated. Carbamylation also removes the Bohr effect and these results suggest that chloride binding to one or both of the anion binding sites is linked to the oxygen and proton affinity of haemoglobin. One site is located between the amino group of $\alpha1$ valine and the hydroxyl group of $\alpha131$ serine on the same protein chain while the other lies between the amino group of $\alpha1$ valine and the guanidinium side-chain of $\alpha141$ arginine on the other α-chain.

[57] J. Baldwin and C. Chothia, *J. Mol. Biol.*, 1979, **129**, 175.
[58] J. F. Deatherage and K. Moffat, *J. Mol. Biol.*, 1979, **134**, 401.
[59] P. M. D. Fitzgerald and W. E. Love, *J. Mol. Biol.*, 1979, **132**, 603.
[60] T. E. Houston, R. L. Girling, E. L. Amma, and T. H. J. Huisman, *Biochim. Biophys. Acta*, 1979, **576**, 497.
[61] R. L. Girling, T. E. Houston, E. L. Amma, and T. H. J. Huismann, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 768.
[62] S. O'Donnell, R. Mandaro, T. M. Schuster, and A. Arnone, *J. Biol. Chem.*, 1979, **254**, 12204.

Haemoglobin S.—A three-dimensional reconstruction of the supramolecular structure of haemoglobin S has been performed [63] and has shown the arrangement of the molecules in the fibres.

Wellems and Josephs [64] have reported a technique for crystallizing haemoglobin S which involves stirring the solution until fibre alignment and fusion occur.

X-Ray diffraction studies on fibres and crystals of deoxygenated haemoglobin S have been described.[65] The packing of the molecules in fibres and crystallites are slightly different, the crystallites being isomorphous with single crystals of the protein. The basic unit of both the fibre and the crystal is a double filament of molecules with an axial repeat of 6.4 nm. The two filaments are held together by contacts between β6 valine in one strand with hydrophobic residues on the other strand and these are likely to be the important interactions *in vivo*.

Sickling-deer Haemoglobin.—The *X*-ray structure of sickling-deer type-III haemoglobin has been determined [66] by the molecular replacement method and refined to an *R*-factor of 0.25. Examination of molecular packing has shown that the molecules pack into linear arrays or fibrils. These fibrils then pack together, with *ca.* 2.7 nm displacement between adjacent fibrils in the fibre, into a hexameric structure with a central solvent cavity.

Haemerythrin.—Studies on the binding of mercuri-iodide ions to haemerythrin have been published [67] and compared to the known structure of haemerythrin. The effects of mercurials on the quaternary structure of the enzyme are discussed.

Erythrocruorin.—The structures of four different liganded states of erythrocruorin have been refined at 0.14 nm resolution.[68] Refinement has been by a constrained process giving *R*-factors better than 0.19 for each structure with positional errors in the co-ordinates better than 0.01 nm. The four structures examined are aquomet-, cyanomet-, deoxy-, and carbon monoxy-erythrocruorin, the largest differences being seen for the deoxy-/cyanomet pair. The observed changes in tertiary structure propagate outwards from the ligand-binding site, helix E which passes on the distal side of the haem group being most affected by ligand binding. Steric hindrance forces the cyanide and carbon monoxide ligands to bind at an angle to the haem axis, the strain on the ligand being partially relieved by the movement of the haem deeper into the haem pocket.

Differences in the positions of the iron atoms are spin dependent but small, and there seems to be no spin dependent changes in the porphyrin stereochemistry. The structure of the porphyrin is determined primarily by the chemical properties of the ligand and its interaction with the haem and the protein. It is these non-covalent interactions which are responsible for the initiation of structural changes on ligand binding.

Cytochrome b_5.—Guiard and Lederer [69] have analysed the amino-acid sequences of yeast flavocytochrome b_2 and liver sulphite oxidase and compared them to the

[63] G. W. Dykes, R. H. Crepeau, and S. J. Edelstein, *J. Mol. Biol.*, 1979, **130**, 451.
[64] T. E. Wellems and R. Josephs, *J. Mol. Biol.*, 1979, **135**, 651.
[65] B. Magdoff-Fairchild and C. C. Chiu, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 223.
[66] R. L. Girling, W. C. Schmidt, jun., T. E. Houston, E. L. Amma, and T. H. J. Huisman, *J. Mol. Biol.*, 1979, **131**, 417.
[67] S. E. Clarke, L. C. Sieker, R. E. Stenkamp, and J. S. Loehr, *Biochemistry*, 1979, **18**, 684.
[68] W. Steigemann and E. Weber, *J. Mol. Biol.*, 1979, **127**, 309.
[69] B. Guiard and F. Lederer, *J. Mol. Biol.*, 1979, **135**, 639.

sequence of cytochrome b_5. The amino-acid alignment is discussed in terms of the known three-dimensional structure of cytochrome b_5, and the possibility that this protein forms a family based on the 'cytochrome b_5 fold' is proposed.

Cytochrome b_{562}.—The structure of *E. coli* cytochrome b_{562} has been determined at 0.25 nm resolution using the single isomorphous replacement method with anomalous scattering information.[70] The average overall figure of merit is 0.90 and the electron density was averaged about the non-crystallographic dyad axis which relates the two molecules in the triclinic unit cell.

The molecule is cylindrical in shape, approximately 2.5 nm in diameter, and 5.0 nm long. It is composed of four helices lying parallel to each other with non-helical loops connecting them. Two of these helices are slightly distorted from perfect α-structure and there is no β-structure at all. The haem group is located at one end of the cylinder and its iron atom is co-ordinated by a methionine and a histidine side chain. The haem presents its propionic acid groups to the solvent while the vinyl groups are buried. One face of the haem is totally buried while the histidine-binding face is half accessible to solvent. The two phenylalanines in the molecule are packed internally near the haem group while the two tyrosines lie on the surface but are also near the haem group. The folding pattern resembles that of haemerythrin and tobacco mosaic virus protein and is totally unlike cytochrome b_5, cytochrome *c*, or the globins. The protein, although showing some features of cytochrome b_5 and cytochrome *c*, seems to represent a completely new class of cytochrome structure.

Cytochrome c_3.—The amino-acid sequence and three-dimensional structure of cytochrome c_3 from *Desulphovibrio desulphuricans* have been determined.[71] The *X*-ray structure was determined at 0.25 nm resolution using three heavy-atom derivatives which gave an average overall figure of merit of 0.76. The molecule is bean-shaped with overall dimensions 4.5 nm × 2.8 nm × 3.2 nm and the four haem groups and their ligands were clearly seen. The four haem groups lie near the surface of the protein with haems 2 and 4 sharing the long groove which divides the molecule into two halves. Each of these halves binds a haem group. The haems are linked to the protein by two thioether bonds, as in cytochrome *c*, while the fifth and sixth ligands are supplied by histidine residues, as in cytochrome b_5. Two of the histidines lie consecutively in the sequence, His-48 and His-49. The haem binding areas of the four groups are all different reflecting the varying degrees of exposure of the haems to the solvent. Sequence alignments for several species of cytochrome c_3 suggest that the structure is characteristic of the cytochrome c_3 group.

Cytochrome *c*.—The structural analysis of bonito heart ferricytochrome *c* at 0.28 nm resolution has been reported.[72] Data were collected for the orthorhombic crystal form and two heavy-atom derivatives were used to determine the phases. The average overall figure of merit is 0.77 and the path of the polypeptide chain was readily followed for the two independent molecules in the asymmetric unit. Comparison of the α-carbon positions for bonito ferricytochrome *c* and ferrocytochrome *c* gave an r.m.s. deviation in position of 0.019 nm but until refinements

[70] F. S. Mathews, P. H. Bethge, and E. W. Czerwinski, *J. Biol. Chem.*, 1979, **254**, 1699.
[71] R. Haser, M. Pierrot, M. Frey, F. Payan, J. P. Astier, M. Bruschi, and J. Le Gall, *Nature (London)*, 1979, **282**, 806.
[72] Y. Matsuura, Y. Hata, T. Yamaguchi, N. Tanaka, and M. Kakudo, *J. Biochem.*, 1979, **85**, 729.

are performed it is not known if this reflects genuine differences in structure. When molecular packing is examined for the two forms, however, several differences in surface residues become apparent. These differences may well be important in the interactions of the different forms of the cytochrome with the oxidase and reductase proteins of the electron-transfer reactions.

Labhardt and Yuen [73] have used *X*-ray absorption fine-structure spectroscopy to examine changes around the iron atom during the reduction of horse heart cytochrome *c*. Their results, accurate to ± 0.0003 nm, show no conformational change in the haem region after reduction.

Flavocytochrome b_2.—A rotation-function study of flavocytochrome b_2 has allowed the orientation of the molecular two-fold axes to be determined.[37] The data used, 1.0—0.55 nm resolution, were collected for a crystal form which has one tetrameric molecule of molecular weight 230 000 dalton as the asymmetric unit. Two possible solutions to the orientation of the molecular 222 axes are obtained but the results favour one of the alternatives more strongly.

Ferredoxin.—A chloroplast-type ferredoxin from *Spirulina platensis* has been crystallized and an electron-density map calculated at 0.28 nm resolution.[74] Phases were determined by the single isomorphous replacement method together with anomalous dispersion data and gave an overall average figure of merit of 0.49. Four of the six cysteine residues are involved in the iron–sulphur clusters which have similar conformations to those seen in bacterial ferredoxins. The protein also shows $>$N—H·····S bonds whose geometries are similar to the bacterial ferredoxins, rubredoxin, and high-potential iron protein.

Rubredoxin.—The structure of rubredoxin has been refined at 0.12 nm resolution to a final *R*-factor of 0.127.[75] The model presented consists of 423 protein atoms and 127 solvent oxygen atoms and only 15 side-chain atoms are not observed in the refined model.

The iron–sulphur atoms have refined well and show iron–sulphur bond lengths ranging from 0.224 nm to 0.233 nm with bond angles ranging from 104° to 114°. Despite the fairly regular main-chain arrangement the molecule shows only a small amount of secondary structure. Several irregular 3_{10} helices occur allowing the chain to change direction through 90°. A number of peptide groups are seen to be significantly non-planar and agree with theoretical predictions. The surface distribution of side-chains is seen to be asymmetric leading to a distinct hydrophobic patch in the vicinity of the iron–sulphur cluster. This asymmetry may well be important in the interaction of rubredoxin with other charged molecules.

5 Lysozyme and Ribonuclease

Lysozyme.—An attempt to analyse the true atomic motion of the atoms in hen egg white and human lysozymes has been reported.[76] Refinements of the two structures

[73] A. Labhardt and C. Yuen, *Nature (London)*, 1979, **277**, 150.
[74] T. Tsukihara, K. Fukuyama, H. Tahara, Y. Katsube, Y. Matsuura, N. Tanaka, M. Kakudo, K. Wada, and H. Matsubara, *J. Biochem.*, 1979, **84**, 1645.
[75] K. D. Watenpaugh, L. C. Sieker, and L. H. Jensen, *J. Mol. Biol.*, 1979, **131**, 509.
[76] P. J. Artymiuk, C. C. F. Blake, D. E. P. Grace, S. J. Oatley, D. C. Phillips, and M. J. E. Sternberg, *Nature (London)*, 1979, **280**, 563.

were carried out by different methodologies. The hen egg white lysozyme was refined at 0.2 nm resolution using only two temperature factors for each residue, one for the four main-chain atoms and the β-carbon and another for the side-chain atoms beyond the β-carbon. The current *R*-factor is 0.22 for 1001 protein atoms and 250 water molecules.

The human lysozyme refinement used a constrained least-squares method and has progressed to an *R*-factor of 0.18 at 0.15 nm resolution using 1026 protein atoms and 130 water molecules. The higher resolution of the human lysozyme data has allowed for individual temperature factors for each atom.

Plots of the mean-square displacement for the main-chain atoms of the two molecules show similar characteristics – a fairly constant background with peaks corresponding to increased displacements at residues 47, 70—73, 101, and 102. Since there are 57 amino-acid substituents and one residue inserted in human lysozyme these parallel curves suggest some particular property of the lysozyme molecule. The observed displacements could be due to rigid-body displacements—the shape of the molecule—or to more complex intramolecular displacements—the structure of the molecule. The preliminary analyses suggest that the displacements must have an intramolecular component superimposed on either a rigid-body motion or a general breathing model. The most significant finding is that the lips of the active-site cleft and the region of the enzyme that undergoes conformational change on ligand binding are included in the regions with the greatest displacements. For this reason it would be interesting to evaluate the effect of substrate-binding on the observed displacements.

X-Ray analyses at 0.25 nm resolution of the crystal structures of lysozyme–gadolinium(II) and lysozyme–gadolinium(III)–*N*-acetyl-D-glucosamine have been presented.[77] There are two main binding sites for gadolinium, 0.36 nm apart, one lying near Glu-35 and the other near Asp-52. In the presence of gadolinium the *N*-acetyl-D-glucosamine binds in a manner similar to that seen for the β-anomer in subsite C and the binding is accompanied by numerous small conformational changes. The structural reasons for not observing a single central binding site are discussed and preliminary crystallographic data on lysozyme co-crystallized with gadolinium salts are presented.

Crystallographic studies on the binding of a bacterial cell wall trisaccharide to hen egg white lysozyme have been published.[78] The trisaccharide, NAM–NAG–NAM, is seen to bind to sites B, C, and D of the lysozyme active site and the electron-density map in these areas is affected by the displacement of *ca.* 10 water molecules. As well as the position of the substrate, a concerted shift of the chain from residue Gly-71 to Leu-75 is seen. This movement is relayed from the active site. Close inspection of the binding of the substrate suggests that the orientation seen is that of an initial Michaelis complex with the enzyme. It is proposed that productive binding requires the additional binding energy of two more hexose units in sites E and F. The binding mode observed is discussed in terms of known chemical facts for the substrate binding.

The crystallization of hen egg white lysozyme at 40 and 50 °C in the presence of

[77] S. J. Perkins, L. N. Johnson, P. A. Machin, and D. C. Phillips, *Biochem. J.*, 1979, **181**, 21.
[78] J. A. Kelly, A. R. Sielecki, B. D. Sykes, and M. N. G. James, *Nature (London)*, 1979, **282**, 875.

N-acetylglucosamine and chitobiose has been described.[79] In the absence of the ligands, lysozyme crystallized in the orthorhombic B crystal form at 40 and 50 °C. Tetragonal type A crystals grown at 20 °C dissolve and transform to type B when put at 40 °C.

In the presence of *N*-acetylglucosamine the temperature transition point is lowered and type B crystals containing no sugar are obtained. Using chitobiose, isomorphous type A crystals were obtained at both 20 and 40 °C. At 50 °C, however, type B crystals were formed, but there seemed to be no sugar binding. Chitobiose-grown type A crystals grown at 20 °C would not transform to the B form when put at 40 °C; temperatures above 40 °C would, however, break the complex. These results suggest a flexibility in the active-site area of lysozyme which becomes more rigid on substrate binding.

Chemical studies on the binding of *N*-acetyl chitotriose to hen egg white lysozyme esterified at Asp-52 have also been reported.[80]

Pincus and Scheraga [81] have carried out energy calculations to investigate the favoured binding mode of oligomers of *N*-acetylglucosamine to the active site of lysozyme. A detailed analysis of the interactions involved is presented and is shown to account for some of the known binding properties of the enzyme.

Examination of the structure of a temperature-sensitive mutant of bacteriophage T4 lysozyme [82] has allowed the molecular basis of thermostability to be examined. The mutation is a conversion of Arg-96 to a histidine residue. The hydrocarbon chain of the arginine residue contributes to the hydrophobic core of the *C*-terminal lobe and the imidazole side-chain may well destabilize this core. Apart from Arg-96 no changes larger than a few hundredths of a nanometre are observed.

Ribonuclease.—Crystalline complexes of ribonuclease-S with substituted purine nucleotides at the 8-position have been examined by *X*-ray diffraction.[83] The complexes with 8-oxo-3′-AMP, 8-bromo-3′-AMP, and 8-thio-3′-AMP have been studied at 0.3 nm resolution. Of these three compounds only 8-oxo-3′-AMP can be bound in the pyrimidine binding-site of the ribonuclease molecule and the structure is close to that seen with pyrimidine nucleotides.

6 Proteolytic Enzymes

Trypsin.—Two independently obtained *X*-ray structures of bovine trypsin have been compared by Chambers and Stroud.[40] The structure of di-isopropylfluorophosphate-inhibited bovine trypsin was refined to an *R*-factor of 0.157 at 0.15 nm resolution while the structure of benzamidine-inhibited enzyme has been refined to an *R*-factor of 0.229 at 0.18 nm resolution. When the structures are compared, most of the differences fall in the 0.0—0.1 nm range, with the maximum differences for atoms found in both electron-density maps being 0.2—0.4 nm. The factors determining the current differences between the two structures are discussed.

[79] J. Berthou, A. Lifchitz, J. Saint-Blancard, and P. Jolles, *FEBS Lett.*, 1979, **108**, 10.
[80] K. Fukae, S. Kuramitsu, and K. Hamaguchi, *J. Biochem.*, 1979, **85**, 141.
[81] M. R. Pincus and H. A. Scheraga, *Macromolecules*, 1979, **12**, 633.
[82] M. G. Grütter, R. B. Hawkes, and B. W. Matthews, *Nature (London)*, 1979, **277**, 667.
[83] A. G. Pavlovskii, N. S. Padyukova, and M. Y. Karpeiskii, *Dokl. Biochem.*, 1978, **242**, 327.

Trypsin Inhibitor.—McLachlan [84] has analysed the structure of soybean trypsin inhibitor (Kunitz) and shown that the molecule consists of three very similar irregular Y-shaped lobes of antiparallel twisted β-sheet. These three lobes are grouped around a central axis and linked by hydrogen bonds to form a six-stranded barrel, the lobes being related by *ca.* 120° rotations around the axis. The region which interacts with trypsin lies on a loop between the first two lobes suggesting that, if the structure has evolved from an unlinked trimer, the inhibitor activity was not originally present. Possible evolutionary pathways are discussed.

Chymotrypsin.—The structure of chymotrypsin has been reported at 0.18 nm resolution, after Fourier phase refinement and extension.[39] Comparison with the 0.28 nm resolution map shows increased clarity at 0.18 nm resolution; peptide carbonyl groups become very clear and hydrogen bonds become visible. The active-site residues are clearly seen and the authors report that a new model is under construction.

McLachlan [85] has analysed the structures of the serine proteinase enzyme family in terms of their domain structure. Improved methods for shape comparison of proteins are described and their application to chymotrypsin is discussed.

Thermolysin.—The binding of L-benzylsuccinic acid, a known inhibitor of carboxypeptidase A, to thermolysin has been examined at 0.23 nm resolution.[86] The inhibitor binds in a manner analogous to that of other dipeptide inhibitors with one carboxyl group becoming a fourth ligand for the zinc atom and the other interacting with Arg-203 and Asn-112. The benzyl group occupies the hydrophobic specificity pocket but is displaced somewhat since the tetrahedral methylene group of the benzyl succinic acid is thought to resemble the presumptive transition state. The results are shown to be consistent with a general base mechanism for both carboxypeptidase A and thermolysin with a glutamic acid residue promoting the attack of a water molecule on the peptide carbonyl carbon.

Subtilisin Inhibitor.—The crystal structures of *Streptomyces* subtilisin inhibitor and its complex with subtilisin BPN′ have been described.[87–89]

The structure of the inhibitor has been determined at 0.26 nm resolution [89] using three isomorphous derivatives and the dimeric molecule is seen to be 3.0 nm × 4.0 nm × 6.5 nm in size. Each subunit has a five-stranded antiparallel β-sheet and two short α-helices; the subunit–subunit interface is formed by a stacking of the two β-sheets. The conformation around the active site seems to be very rigid and is similar to the structure of soybean trypsin inhibitor and almost identical to bovine pancreatic trypsin inhibitor. Apart from the active site there is little structural similarity suggesting convergent evolution has occurred.

The crystal structure of the complex of subtilisin BPN′ with this inhibitor was solved at 0.43 nm resolution.[88] Two subtilisin molecules bind to each dimeric inhibitor molecule; the conformations of the enzyme and the inhibitor are

[84] A. D. McLachlan, *J. Mol. Biol.*, 1979, **133**, 557.
[85] A. D. McLachlan, *J. Mol. Biol.*, 1979, **128**, 49.
[86] M. C. Bolognesi and B. W. Matthews, *J. Biol. Chem.*, 1979, **254**, 634.
[87] Y. Mitsui, Y. Satow, Y. Watanabe, S. Hirono, and Y. Iitaka, *Nature (London)*, 1979, **277**, 447.
[88] S. Hirono, K. T. Nakamura, Y. Iitaka, and Y. Mitsui, *J. Mol. Biol.*, 1979, **131**, 855.
[89] Y. Mitsui, Y. Satow, Y. Watanabe, and Y. Iitaka, *J. Mol. Biol.*, 1979, **131**, 697.

unchanged upon complex formation. The inhibitor binds forming an antiparallel β-sheet, as is also seen for the trypsin–trypsin inhibitor complexes, and binds in a similar way to substrates. The structure confirms that the scissile bond is between Met-73 and Val-74.

The two structure determinations and comparisons of the structures with other proteinase inhibitors have been published.[87]

***Streptomyces griseus* Protease B.**—The structure of protease B from *S. griseus* has been determined at 0.28 nm resolution by the method of isomorphous replacement using five heavy-atom derivatives.[90] The map could be readily interpreted and the active-site residues, Ser-214, Asp-102, His-57, and Ser-195, are seen to be in very similar conformations to pancreatic α-chymotrypsin. Despite only 18% identity of primary structure there is 63% topological equivalence within an r.m.s. deviation of 0.207 nm for these two proteins. The main difference between the two proteins lies in the need for the zymogen form of the enzyme of the multicellular organism to avoid autolysis. Comparison of *S. griseus* protease B with protease A from the same organism shows a 61% identity of sequence and an 85% topological equivalence with an r.m.s. deviation of 0.146 nm. The tertiary structures at the active site are very similar; the differences in the structures are localized at three external loops.

***Streptomyces griseus* Protease A.**—The refinement of the structure of *S. griseus* protease A at 0.18 nm resolution has been reported.[91] The refinement proceeded by a restrained-parameter least-squares procedure to a conventional *R*-factor of 0.139 and refinement included positional and individual thermal parameters for the atoms and positions of the enzyme molecule, overall temperature factors, and occupancies for 175 solvent molecules. A total of 33 refinement cycles has been performed and the deviation of the refined structure from ideal bond lengths is an overall r.m.s. 0.002 nm. The average conformational angle of the peptide bonds is 179.7° for the 180 peptide bonds in the structure. Of the 175 solvent molecules included in the final model, 22, with occupancies in the range 1.00—0.38, are located in the active site or substrate binding area. Only when these solvent molecules were included did the active site take on its final conformation. In this conformation Ser-195 ($\chi_1 = -77°$) lies with its γ-oxygen 0.31 nm away from $N^{\varepsilon 2}$ of His-57, indicating a rather weak interaction. The final structure is also compared with trypsin and the general strategy of refinement discussed.

The binding of a specific tetrapeptide aldehyde compound to *S. griseus* protease A has been examined at 0.28 nm resolution.[92] The inhibitor, *N*-acetylprolylalanyl-prolylalaninal, binds covalently to the side chain of Ser-195. There are five hydrogen bonds between the inhibitor and protein main-chain atoms, three of these bonds giving an approximate antiparallel β-sheet structure. Inhibitor binding leads to a large conformational change for the imidazole group of His-75; this is accomplished by a 94° rotation about C_α—C_β and 26° about C_β—C_γ. The possibility that this complex is a transition-state analogue is discussed.

[90] L. T. J. Delbaere, G. D. Brayer, and M. N. G. James, *Can. J. Biochem.*, 1979, **57**, 135.
[91] A. R. Sielecki, W. A. Hendrickson, C. G. Broughton, L. T. J. Delbaere, G. D. Brayer, and M. N. G. James, *J. Mol. Biol.*, 1979, **134**, 781.
[92] G. D. Brayer, L. T. J. Delbaere, M. N. G. James, C.-A. Bauer, and R. C. Thompson, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 96.

α-Lytic Protease.—The crystal structure of α-lytic protease from *Myxobacter* 495 has been determined at 0.28 nm resolution.[93] The multiple isomorphous replacement method was used employing five heavy-atom derivatives, and the overall figure of merit is 0.83. The electron-density map has been interpreted as a chain of 198 amino-acid residues.

The α-lytic protease has a structural core similar to that of the pancreatic serine proteases; 108 α-carbon positions are topologically equivalent to porcine elastase, within 0.2 nm, and its tertiary structure is closely similar to the *Streptomyces griseus* proteases. In particular α-lytic protease, like the *S. griseus* enzymes, has an exposed amino-terminus lying in the solvent, a shortened uranyl loop (residues 65—84), a major deletion of residues in the autolysis loop (residues 144—155), and an altered conformation of the methionine loop (residues 168—182). The α-lytic protease also shows a buried arginine residue, Arg-138, forming an ion pair to Asp-194.

The conformations of the active-site residues, Ser-214, Asp-102, His-57, and Ser-195, seem to be similar to those seen in the other serine proteases with Asp-102 lying in a highly polar environment and a weak interaction between His-57 and Ser-195. The altered specificity of α-lytic protease has also been studied and the size of the specificity pocket is seen to be diminished by a residue on an inserted loop, not just a simple non-conservative change. Such a change also occurs with a methionine residue replacing a valine and these changes together determine the specificity for small hydrophobic side-chains. Portions of the polypeptide chain known to be important in substrate binding other than at the specificity site are seen to be conserved and the stereochemistry of substrate binding is discussed.

Acid Proteases.—*General.* Structural analysis of endothiapepsin has been extended [94] to show that each of the two structurally equivalent lobes, related by the dyad axis through the active centre, contains two similar halves related by a further local dyad axis. In one of these lobes 22 pairs of α-carbon atoms are topologically equivalent with an r.m.s. deviation of 0.192 nm, while in the other lobe 17 pairs are equivalent with an r.m.s. deviation of 0.231 nm. Either convergent evolution or gene quadruplication may have occurred.

Similar studies on porcine pepsin and the related acid proteases have been reported by Andreeva and Gustchina.[95] They show close similarities in the sequences of some topologically equivalent areas even though, in general, sequence homology is low. The implications of this structural repeat in evolutionary terms is considered.

Pepsin. The structure of porcine pepsin at higher resolution (0.27 nm) has been described.[96] The structure determination has been hindered by the necessity to produce heavy-atom derivatives of the crystals at pH = 2 and the fact that heavy-atom labelled inhibitors destroyed isomorphism. The molecule is ellipsoidal in shape, 5.5 nm × 4.5 nm × 3.2 nm, and shows a clearly defined cleft. The secondary structure consists mostly of β-structure although there are one short helix and

[93] G. D. Brayer, L. T. J. Delbaere, and M. N. G. James, *J. Mol. Biol.*, 1979, **131**, 743.
[94] T. L. Blundell, B. T. Sewell, and A. D. McLachlan, *Biochim. Biophys. Acta*, 1979, **580**, 24.
[95] N. S. Andreeva and A. E. Gustchina, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 32.
[96] N. S. Andreeva, A. A. Fedorov, A. E. Gustchina, R. R. Riskulov, N. E. Shutskever, and M. G. Safro, *Mol. Biol.*, 1978, **12**, 704.

three spiral twists. The structure is described in detail and compared to other known acid proteases.

Endothiapepsin. The structure of endothiapepsin strongly cross-linked with glutaraldehyde has been determined at 0.245 nm resolution and compared to the native structure.[97] The cross-links are clearly seen, with lengths corresponding to five or ten carbon-atom chains, and seem to involve all sterically available $\geq N-H$ and $-NH_2$ groups. The only significant changes in conformation occur in the first seven residues as a result of the *N*-terminus being cross-linked to residue 283 of a neighbouring molecule and residues 2 and 6 being involved in an intramolecular cross-link. The results indicate that strong cross-linking preserves the morphology of biological specimens at the molecular level.

Cross-linked crystals have also been shown to bind 1,2-epoxy-3-(*p*-nitrophenoxy)propane, a specific inhibitor of the acid proteases. The inhibitor binds at the active-site aspartate residue (Asp-220) with another inhibitor molecule covalently bound to Asp-33. These two aspartate residues are involved in a hydrogen bond in the native enzyme and only small conformational changes occur on inhibitor binding.

7 Glycolytic Enzymes

Phosphorylase.—Fletterick and his co-workers have published[98] computer-generated van der Waals contour drawings of the surface of the phosphorylase *a* molecule. The computer system allows for colour coding of acidic and basic residues, bound ligands, or conformational changes. Two faces of the molecule can be distinguished structurally and functionally. A concave catalytic face contains the glycogen storage sites on its periphery with the entrances to the glucose-1-phosphate binding sites of the active centres, adjacent to the pyridoxal phosphate moieties, near its centre. Just outside the active site is a binding site for negative effectors such as caffeine. On the side of the dimer opposite to the catalytic face is found a convex control face containing binding sites for the allosteric activator AMP. Close to these sites are the phosphorylated serine residues, Ser-14, hydrogen bonded to Arg-69 of the same subunit and Arg-43 of the symmetry-related subunit.

The surface topography of the glucose-stabilized T-state and the changes which occur during the allosteric transitions induced by AMP and substrates have been analysed using this system. The two conformational states, taut and relaxed, exhibit different structural arrangements of the binding sites for the negative effector, caffeine, and the positive effector, AMP. Linkage between the various sites can be demonstrated by observed conformational changes in the surface topography. It is also seen that since the control face is on the opposite side to the catalytic face the interconverting enzymes can bind to and act on the phosphorylase molecule while it is bound to glycogen. Although the Ser-14 residues are 4 nm apart this distance could be readily bridged by a multimeric kinase or the large molecular weight form of the phosphatase.

The calculation of the 0.25 nm resolution electron-density map from which the surface topography drawings were generated has been reported.[99] Five heavy-atom

[97] C.-H. Wong, T. J. Lee, T.-Y. Lee, T.-H. Lu, and I.-H. Yang, *Biochemistry*, 1979, **18**, 1638.
[98] R. J. Fletterick, S. Sprang, and N. B. Madsen, *Can. J. Biochem.*, 1979, **57**, 789.
[99] S. Sprang and R. J. Fletterick, *J. Mol. Biol.*, 1979, **131**, 523.

derivatives were used in the determination of the phases and allowed the interpretation of both main-chain and side-chain density, giving an unambiguous correlation with the known primary structure. Two modifications of the interpretation at 0.3 nm resolution have been necessary.

The enzyme is clearly organized into two domains both with an α/β packing topology, and the active site lies in a crevice at the interface of the two domains. α-D-Glucose is bound at this site and lies 0.6 nm away from the pyridoxal phosphate coenzyme which is covalently bound through the ε-amino group of Lys-679. The larger *N*-terminal domain is folded into two subdomains; one is the glycogen storage lobe and the other contains Ser-14 phosphate and the AMP effector site.

The binding of the allosteric activator, AMP, and the inhibitor, ATP, to phosphorylase has been described at 0.3 nm resolution.[100] Site N, the allosteric effector site, lies close to the subunit–subunit interface while site I, the nucleoside inhibitor site, blocks the entrance to the active-site cleft. AMP in the effector site makes several readily defined interactions and IMP appears to bind in the same manner. At 0.3 nm resolution no well defined conformational changes of the protein are observed, although there are indications of a disturbance of the crystal lattice. This suggests that the crystal lattice forces prevent the allosteric response of the enzyme in the crystalline state.

Hexokinase.—Small-angle *X*-ray scattering measurements from solutions of yeast hexokinase B have allowed estimates of the radii of gyration of the enzyme with and without bound sugar substrates.[101] The radius of gyration decreases by 0.095 nm on binding glucose and by 0.125 nm on binding glucose-6-phosphate. These decreases are consistent with a closing of the slit between the two lobes on sugar binding.

These changes have been further analysed by computer-drawn space-filling models of the two forms of hexokinase.[102] Similar bilobal structures are seen for adenylate kinase and phosphoglycerate kinase, and it is suggested that closure of the cleft is common to all kinase mechanisms. Preliminary results for pyruvate kinase and phosphofructokinase suggest that they too may function by movements of two domains about a flexible hinge region.

6-Phosphogluconate Dehydrogenase.—The binding of $NADP^+$, NADPH, NADPH plus 6-phosphogluconate, nicotinamide–8-bromoadenine dinucleotide phosphate, and 3-iodopyridine–adenine dinucleotide phosphate to 6-phosphogluconate dehydrogenase has been studied at 0.6 nm resolution.[103] The molecules are seen to bind in a similar position in a cleft in the enzyme, distant from the dimer interface. The orientation of the coenzyme has been determined from the $NADP^+$: iodo-analogue difference density and the ternary complex has tentatively shown the substrate binding site. The bromo-analogue gives no clear indication of the bromine atom and the analogue is seen to bind deeper in the cleft than $NADP^+$. The clearest defined difference density is that for NADPH and model-building

[100] L. N. Johnson, E. A. Stura, K. S. Wilson, M. S. P. Sansom, and I. T. Weber, *J. Mol. Biol.*, 1979, **134**, 639.

[101] R. C. McDonald, T. A. Steitz, and D. M. Engelman, *Biochemistry*, 1979, **18**, 338.

[102] C. M. Anderson, F. H. Zucker, and T. A. Steitz, *Science*, 1979, **204**, 375.

[103] M. A. Abdallah, M. J. Adams, I. G. Archibald, J.-F. Biellmann, J. R. Helliwell, and S. E. Jenkins, *Eur. J. Biochem.*, 1979, **98**, 121.

using an interactive graphics system suggests a preferred NADPH conformation which is significantly different from the NAD^+ conformation in the lactate dehydrogenase ternary complex.

Phosphofructokinase.—The crystal structure of phosphofructokinase from *Bacillus stearothermophilus* has been determined at 0.24 nm resolution.[104] The enzyme, a tetramer of molecular weight 136 000 dalton, is not inhibited by ATP or citrate nor is it activated by AMP. The electron-density map, calculated using phases obtained from three heavy-atom derivatives, could be interpreted almost completely; the helices and β-sheet regions are particularly clear. The side-chain density agreed well with the known sequence and carbonyl groups could be seen in the internal parts of the structure. A few surface loops are less clearly defined.

Each subunit is composed of two domains, each of which has a central β-sheet sandwiched between α-helices. The topology of these structures is unique. The first domain has seven strands of β-sheet, the second domain only four, and the two sheets point towards a deep cleft which forms the active site. Each subunit forms close contacts with only two of the other three subunits in the tetramer and the tetramer shows a 0.7 nm diameter solvent-filled channel running through the molecule along the y-axis.

Three binding sites have been identified. Site A binds fructose-6-phosphate or phosphate when fructose-6-phosphate is washed out. The phosphate group is bound by amino-acid side-chains from two subunits. The fructose 1-hydroxyl group lies close to the ATP γ-phosphate; the ATP molecule is bound in site B. The ATP molecule is bound to domain 1 with the γ-phosphate near the domain boundary. Site C, the ADP effector site, is bound in a cleft between subunits related by the x-dyad. The β-phosphate group is bound by three arginine residues, two from one subunit, and one from the other subunit. The diphosphate moiety points down into a cleft explaining the inactivity of AMP and ATP and the lack of specificity for the base. The stereochemistry of the three sites, all of which bind phosphorylated substrates, are compared and shown to have significantly different chemical properties.

Phosphoglycerate Kinase.—The fitting of sequenced peptides to a high-resolution electron-density map of horse muscle phosphoglycerate kinase has yielded the complete three-dimensional structure of the enzyme.[104a] The 0.25 nm resolution electron-density map was interpreted by fitting the sequences of the 14 cyanogen bromide peptides to the density. The subunit consists of 416 residues and is folded into two separate globular domains. The two domains consist almost entirely of the *N*-terminal and *C*-terminal halves of the chain. There are only two links between the two domains.

The *C*-terminal domain secondary structure is arranged with the same topology as the NAD-binding domain of the dehydrogenases. The binding of Mg–ATP at 0.3 nm resolution and Mn–ATP at 0.25 nm resolution have been studied and the binding site has been located on the *C*-terminal domain. The adenine ring is almost completely buried in a narrow slot with the ribose group located in a shallow

[104] P. R. Evans and P. J. Hudson, *Nature (London)*, 1979, **279**, 500.

[104a] R. D. Banks, C. C. F. Blake, P. R. Evans, R. Haser, D. W. Rice, G. W. Hardy, M. Merrett, and A. W. Phillips, *Nature (London)*, 1979, **279**, 773.

depression on the surface. The α-phosphate group is bound to a lysine side-chain but the β- and γ-phosphates do not interact with any side-chains. The area of the γ-phosphate, where the phosphoglycerate would be expected to bind, is exposed to solvent and is devoid of functional groups. The nearest functional groups are 1 nm away, suggesting three possible explanations. The most likely explanation is that the 3-phosphoglycerate binds at a site on the *N*-terminal domain in a position opposed to the ATP-binding site. The assignment of this site as the substrate binding site agrees with the observed n.m.r. 'map' of the active site. The 3-phosphoglycerate site is 1 nm away from the γ-phosphate of the ATP and this gap must therefore be closed to allow catalysis. The suggestion is that the PGK molecule bends about the hinge between the domains, bringing the two substrate molecules together. This mechanism has also been proposed for hexokinase and other kinases.[102]

Pyruvate Kinase.—The crystal structure of cat muscle pyruvate kinase has been extended from 0.31 nm resolution to 0.26 nm resolution,[105] the increased resolution enabling a detailed chain tracing to be performed for two out of the three domains in each of the four subunits. Domain A is composed of an eight-stranded cylindrical parallel β-pleated sheet with an outer co-axial cylinder of eight α-helices. The helices connect adjacent strands of the β-pleated sheet. Domain B is made up of a closed antiparallel β-sheet structure while domain C is a five-stranded β-sheet of which four strands are parallel. Again, β-strand connections are by α-helical segments.

Since the sequence of pyruvate kinase is largely unknown only the backbone atoms of the chain could be positioned with certainty. Carbonyl oxygens were visible in many areas allowing the polypeptide chain direction to be determined but the course of the chain in domain B is less certain than that traced in domains A and C. Of the 550 residues expected from the molecular weight, 432 are present in the molecule as currently interpreted.

There is a very close similarity in the structures of triosephosphate isomerase and domain A of pyruvate kinase; these similarities extend to the location of residues thought to be involved in the active site of each enzyme. The interpretation of domain C shows that it has some similarity to the dehydrogenase nucleotide-binding fold.

A non-active-site binding pocket for ADP has been found but since the muscle enzyme is not believed to have any allosteric control properties the inference is that this is a vestigial effector binding site. The liver enzyme is subject to such allosteric control. Detailed comparisons of the pyruvate kinase molecule with the proposed homologous proteins are presented in a series of admirably clear diagrams.

Alcohol Dehydrogenase.—The complex of horse liver alcohol dehydrogenase with NADH and the inhibitor dimethyl sulphoxide has been studied at 0.45 nm resolution in a study independent of the apo-enzyme structure determination.[106] Comparison of the two structures has allowed the coenzyme binding site to be examined. The ADP part of the coenzyme interacts in a similar way to the ADP–ribose bound to apo-enzyme. The nicotinamide ring is close to the zinc atom and the carboxamide group is hydrogen-bonded to a threonine side-chain. Electron density connected to the active-site zinc atom is interpreted as the dimethyl

[105] D. I. Stuart, M. Levine, H. Muirhead, and D. K. Stammers, *J. Mol. Biol.*, 1979, **134**, 109.
[106] H. Eklund and C.-I. Brändén, *J. Biol. Chem.*, 1979, **254**, 3458.

sulphoxide inhibitor. It is also seen that the catalytic domains rotate relative to the monomer–monomer interface core region. One important functional result of this observed rotation is a closing of the clefts in the subunits, causing the active-centre zinc and its surroundings to become shielded from the solution. This could well be important in the mechanism of the enzyme.

The binding of Cibacron Blue F3GA to liver alcohol dehydrogenase has been reported.[107] Three-dimensional data to 0.37 nm resolution were collected and a difference electron-density map calculated. The dye binds in the ADP–ribose binding site in an essentially extended conformation. The anthraquinone ring, ring D, binds in the same place as the adenine ring of the coenzyme while ring B lies in the same position as the pyrophosphate moiety in the ADP–ribose structure. Ring A, however, binds *ca.* 1 nm away from the nicotinamide binding site, lying in a cleft between the domains.

The amino-acid substitutions in two functional mutants of yeast alcohol dehydrogenase have been determined [108] and their positions in the structure of the enzyme discussed.

Lactate Dehydrogenase.—The crystal structure of mouse testicular lactate dehydrogenase, isoenzyme C_4, has been determined at 0.29 nm resolution [109] and a tentative amino-acid sequence determined.[110] The structure was solved [109] by the molecular-replacement method and the overall folding pattern was very similar to LDH structures previously observed. However, there was a notable difference in the area of the flexible polypeptide loop, residues 99—112. The density for this loop was very diffuse and could not be traced confidently. Detailed analysis, using a molecular-graphics system,[44] shows that the molecules must have subunits in the closed ternary-complex-type conformation despite the fact that there is no evidence of bound substrate or coenzyme. An amino-acid substitution at position 30, arginine instead of glycine, shows the guanidinium group to be within 0.3 nm of the adenine ribose 2′ and 3′ hydroxyl groups and could explain the observations of a lower catalytic turnover rate and the ability to use NADPH as a reductant.

The tentative sequencing of the mouse testicular LDH C_4 [110] has led to an amino-acid composition which agrees well with chemical analysis. The necessary changes in amino-acid sequence were, by and large, conservative and confined to external portions of the molecule. Residues in the Q and P subunit-contact regions were particularly well conserved. The minimum base changes per codon values were similar between the C and H isoenzymes and the C and M isoenzymes.

8 Hormones

Insulin.—Rhombohedral pig insulin crystals in the zinc-bound state have been examined by electron diffraction after thin-sectioning.[111] The crystals in the embedded state were trigonal with cell dimensions of $a = 7.4$ nm and $c = 3.1$ nm as measured from the diffraction patterns. These values compare with $a = 8.2$ nm

[107] J.-F. Biellmann, J.-P. Samama, C.-I. Brändén, and H. Eklund, *Eur. J. Biochem.*, 1979, **102**, 107.
[108] C. Wills and H. Jörnvall, *Nature (London)*, 1979, **279**, 734.
[109] W. D. L. Musick and M. G. Rossmann, *J. Biol. Chem.*, 1979, **254**, 7611.
[110] W. D. L. Musick and M. G. Rossmann, *J. Biol. Chem.*, 1979, **254**, 7621.
[111] R. H. Lange, J. Blödorn, G. Magdowski, and H. J. Trampisch, *J. Ultrastruct. Res.*, 1979, **68**, 81.

and $c = 3.3$ nm for wet unfixed crystals and suggest that electron-microscope studies of intracellular protein crystals may lead to reductions in cell lengths of *ca.* 10% when compared with the hydrated state.

The refinement at 0.15 nm of the crystal structure of pig-2Zn-insulin has allowed detailed comparison of the two independent molecules in the 2Zn-dimer.[112] The refinements have been performed by Fourier and fast-Fourier least-squares methods and have allowed much more accurate positioning of the atoms. The monomer structures of the two molecules are very similar except for the A-chain *N*-terminal residues A1—A6; the difference is a 40° rotation around ϕ of the A5—A6 peptide bond. The refined structure has also been analysed in terms of dimer and hexamer formation. These structural results show that the insulin molecule is deformable and it seems probable that interaction with its receptor will lead to conformational changes in the insulin molecule. The important insulin residues identified chemically all lie on the surface of the molecule; some of them are involved in dimer formation. It is possible that the insulin molecule undergoes a conformational change before or during the binding process and structural evidence consistent with this belief is discussed.

The crystal structure of hagfish insulin at 0.31 nm resolution has been reported.[113] The hagfish is the most primitive vertebrate extant and the most primitive animal whose insulin has been characterized. The asymmetric unit of the crystal form used is an insulin monomer, the two subunits of the dimer being related by an exact two-fold crystal axis. Three-dimensional data for the native and three isomorphous heavy-atom derivatives were collected on a diffractometer. Despite a low average figure of merit the electron-density map was clear except for some surface residue side-chains. Initial interpretation was accomplished by superimposing the known pig insulin structure and the two structures were seen to correspond closely. Observed structural differences involve both main-chain and, more frequently, side-chain atoms. The most striking structural match is seen in two largely invariant regions, B9—B19 and B23—B26, which contain the residues buried by dimer formation. Only the *N*- and *C*-termini of the B-chain backbone differ greatly between the pig and hagfish molecules, despite their different aggregation states and 19 changes in a sequence of 51 residues. However, there are striking differences in their biological activities which seem unlikely to be due to the dimer-forming residues. It is most probable that hagfish insulin is incapable of undergoing the subtle conformational changes necessary before or during receptor binding.

9 Other Globular Proteins

L-Arabinose Binding Protein.—The sugar binding site of L-arabinose binding protein has been located using the substrate analogue 6-bromo-6-deoxy-D-galactose.[114] The binding site is situated deep in a cleft formed by the asymmetric contributions of both of the two similar domains. Early attempts to locate the arabinose binding site suggested that the native structure had been solved with the

[112] E. J. Dodson, G. G. Dodson, D. C. Hodgkin, and C. D. Reynolds, *Can. J. Biochem.*, 1979, **57**, 469.
[113] J. F. Cutfield, S. M. Cutfield, E. J. Dodson, G. G. Dodson, S. F. Emdin, and C. D. Reynolds, *J. Mol. Biol.*, 1979, **132**, 85.
[114] M. E. Newcomer, D. M. Miller, tert., and F. A. Quiocho, *J. Biol. Chem.*, 1979, **254**, 7529.

sugar *in situ*. A difference Fourier map at 0.35 nm resolution of crystals soaked in the analogue showed a peak corresponding to the $—CH_2Br$ moiety of the analogue; this peak overlaps some unexplained electron density seen in the native map. The $—CH_2Br$ moiety is seen to lie *ca.* 0.65 nm from the reactive single cysteine residue, Cys-64, while the sugar ring lies 0.45 nm from this residue. Preliminary assignment of the other binding residues has been made.

The interaction of L-arabinose binding protein with Cibacron Blue 3G-A has shown [115] that the dye is not suitable as a definite probe for the dinucleotide folding pattern and a possible explanation for the wide occurrence of the folding pattern has been presented.

Dihydrofolate Reductase.—The binding of the coenzyme, NADPH, to *Lactobacillus casei* dihydrofolate reductase has been examined at 0.25 nm resolution.[116] The molecule is seen to bind in an extended conformation with several dihedral angles, especially in the adenine mononucleotide portion, differing from their minimum-energy conformations. The adenine ring is bound in an apparently non-specific hydrophobic cleft while the ribose–phosphate portions are involved in numerous specific hydrogen-bonded and charge–charge interactions. The catalytically important nicotinamide ring is bound in an intricately formed cavity, one wall of which includes the pyrazine ring of the methotrexate inhibitor molecule. Comparison with the *E. coli* enzyme–inhibitor structure has allowed tentative assignment of structural changes induced by coenzyme binding which involve two extended loops of polypeptide chain. No overall structural homology is seen when the dihydrofolate reductase dinucleotide binding domain is compared to four dehydrogenase structures.

Rhodanese.—*X*-Ray studies on the active site of bovine liver rhodanese at 0.25 nm resolution have been reported.[117] The active site is identified as a depression between the two domains and in sulphur-substituted enzyme the electron density at Cys-247, the essential cysteine, is seen to correspond to that of a persulphide group which interacts *via* hydrogen bonds with several peptide —NH and side-chain —OH groups. The active-site pocket has one hydrophilic and one hydrophobic face, neither of which seems to interact with the persulphide group.

Treatment with cyanide removes the extra sulphur atom with only minor conformational changes in the active site. A mechanism in which Cys-247 acts as the essential nucleophile with the positive charges on Arg-186 and Lys-249 acting as 'electrophilic assistants' is proposed. The sulphur-substituted enzyme and the transition state are stabilized by several hydrogen bonds.

Glutathione Peroxidase.—The three-dimensional structure of bovine erythrocyte glutathione peroxidase has been solved at 0.28 nm resolution by the multiple isomorphous replacement method.[118] The tetrameric enzyme, molecular weight 84 000 dalton, consists of four identical monomers, of 178 residues, each of which contains a selenium atom. The molecule has close, if not exact, 222 symmetry.

The shape of each monomer is nearly spherical with a radius of *ca.* 1.9 nm and the

[115] R. S. Beissner, F. A. Quiocho, and F. B. Rudolph, *J. Mol. Biol.*, 1979, **134**, 847.
[116] D. A. Matthews, R. A. Alden, S. T. Freer, N.-H. Xuong, and J. Kraut, *J. Biol. Chem.*, 1979, **254**, 4144.
[117] J. H. Ploegman, G. Drent, K. H. Kalk, and W. G. J. Hol, *J. Mol. Biol.*, 1979, **127**, 149.
[118] R. Ladenstein, O. Epp, K. Bartels, A. Jones, R. Huber, and A. Wendel, *J. Mol. Biol.*, 1979, **134**, 199.

electron-density map is good enough to allow a tentative sequence to be determined. The subunit is built up of a central core of two parallel and two antiparallel strands of β-pleated sheet surrounded by four α-helices; one of the helices runs anti-parallel to the β-strands to give the now familiar βαβ structure. The catalytically active selenium atom is located near the first turn of the first helix of the secondary structure and is situated in a depression on the surface of the molecule which is built up from residues of two subunits. The vicinity of the selenocysteine residue, Cys-35, shows an accumulation of aromatic residues and together with His-68 may be involved in catalysis. A difference Fourier synthesis between oxidized and substrate-reduced enzyme showed a single peak at the selenium site which is interpreted as showing the loss of one or more selenium-bound oxygen atoms when the enzyme becomes reduced. This is consistent with the two-electron redox cycle involving a reversible transition of the active-site selenium from selenic acid to seleninic acid.

A comparison with thioredoxin-S_2 reveals large areas of structural resemblance with striking similarities being seen in the positioning of the active-site selenium atom, the S—S bridge of thioredoxin-S_2, and the persulphide group of rhodanese with respect to the secondary structural elements.

Bacteriochlorophyll.—The three-dimensional structure of a bacteriochlorophyll *a*-containing protein from a green photosynthetic bacterium has been determined at 0.28 nm resolution.[119] Four isomorphous derivatives were used and a final figure of merit of 0.74 was obtained. A detailed description of the data-collection procedure is given.

The bacteriochlorophyll *a*-protein is seen to consist of three identical subunits which are tightly packed around a three-fold symmetry axis. Each subunit is composed of seven chlorophyll molecules contained in a protein shell; the shell consists of a 15-strand β-sheet which wraps the chlorophyll molecules away from the aqueous surroundings. There are extensive contacts between the phytyl side-chains of the seven chlorophyll molecules in each subunit; these hydrocarbon chains form a central hydrophobic core. There are also extensive contacts between the protein and the chlorophyll head-groups and phytyl tails. By contrast there is little interaction between the head groups. Five of the seven magnesium atoms have a histidine side-chain as a fifth ligand, one has a peptide carbonyl oxygen, and another a water molecule.

The overall folding leads to a space-filling centre of each subunit; there are few, if any, solvent molecules within the subunits. Intersubunit contacts are extensive and made up in part of helices and irregular loops. The open end of the subunit structure is occluded by a symmetry-related subunit and so excludes solvent entry. Folding of the protein is presumed to be a co-operative process requiring both the protein and the chlorophyll core.

The spectral properties and the *in vivo* organization of both the chlorophyll and the protein are considered.

***p*-Hydroxybenzoate Hydroxylase.**—The structure of *p*-hydroxybenzoate hydroxylase complexed with its substrate has been determined at 0.25 nm resolution.[120]

[119] B. W. Matthews, R. E. Fenna, M. C. Bolognesi, and M. F. Schmid, *J. Mol. Biol.*, 1979, **131**, 259.
[120] R. K. Wierenga, R. J. De Jong, K. H. Kalk, W. G. J. Hol, and J. Drenth, *J. Mol. Biol.*, 1979, **131**, 55.

The enzymatic reaction involves, as well as substrate, oxygen, NADPH as electron donor, and FAD as an electron-transferring group. The enzyme is a monomer of molecular weight 43 000 dalton and was isolated from *Pseudomonas fluorescens*. Data collection was by oscillation photography and three isomorphous derivatives were used giving an overall figure of merit of 0.74.

The molecular boundary was easily defined and the FAD pyrophosphate group was the highest feature in the electron-density map. The chain could be clearly followed in most areas, even on some exposed large-loop regions, and a continuous chain of 391 residues has been fitted to the map.

The molecule is shaped like a rectangular prism with overall dimensions 7.0 nm × 5.0 nm × 4.5 nm with a groove that divides the molecule into two lobes. This groove provides access to the active site. One helix protrudes from the surface of the molecule and is involved in intermolecular contacts across a crystal two-fold axis. This suggests that the enzyme may be a dimer in solution as there are extensive contacts across this interface.

The secondary structure consists of four β-sheets and eight helices, totalling 42% of the residues. The sheets vary from three to seven strands; one is a six-stranded parallel sheet. The molecule can be divided into three domains, I—III, and side-chains from all three domains contribute to the active site which is assumed to be located near the isoalloxazine ring of the FAD molecule. The binding of FAD is clearly seen with the AMP moiety bound at a $\beta\alpha\beta$ unit analogous to the nucleotide binding in dehydrogenases. A pocket near this isoalloxazine binding site is identified as the binding site for the nicotinamide ring of NADPH. The substrate molecule would then lie on the opposite side of the isoalloxazine ring from the NADPH; the two substrates have reached the active site *via* different routes.

The binding of substrate analogues and NADP and a comparison with other nucleotide binding proteins are also given together with a proposal for the catalytic mechanism of the enzyme.

Gene 5 Product–Phage fd.—The gene 5 product, a DNA unwinding protein, has been crystallized and its structure determined by the multiple isomorphous replacement method at 0.23 nm resolution.[121] The essentially globular monomer appears to consist of three secondary structural elements, a twisted three-stranded antiparallel β-pleated sheet and two distinct antiparallel β-loops. A long groove 3 nm in length is formed by the underside of the β-sheet and one of the loops, and on the basis of chemical assignment of important functional residues this is identified as the DNA-binding site. Residues in this groove could intercalate with, or stack upon, the DNA bases. The protein dimerizes by close interactions of two of the β-loops across a molecular dyad axis and in so doing buries many of the hydrophobic residues. Each of the secondary structural elements appears to have a specific functional responsibility and it seems that the protein is the minimum possible size to fulfil its structural role.

Aspartate Carbamoyltransferase.—The binding sites for CTP, CDP, 5-bromo-CTP, and ATP on aspartate carbamoyltransferase have been located on 0.3 nm resolution electron-density maps.[122] The activator, ATP, binds in the *anti-*

[121] A. McPherson, F. A. Jurnak, A. H. J. Wang, I. Molineux, and A. Rich, *J. Mol. Biol.*, 1979, **134**, 379.
[122] R. B. Honzatko, H. L. Monaco, and W. N. Lipscomb, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5105.

conformation while the inhibitor, 5-bromo-CTP, binds in the *syn*-conformation; both compounds bind in the same region of the enzyme.

Binding of CDP and CTP shows that the γ-phosphate is important in the binding of allosteric effectors to the enzyme. Unfortunately the electron density is not clear near the proposed γ-phosphate binding site but does include a portion of polypeptide chain containing Arg-55 and Lys-56 which are potential phosphate ligands. These structural results are related in detail to biochemical studies.

Erabutoxin.—The atomic co-ordinates of erabutoxin determined from a 0.25 nm resolution electron-density map have been published.[123] The molecule consists of three principal lobes formed by the five strands of the antiparallel β-structure. The molecule is open, only one strand thick in most areas, but is highly involved in intramolecular interactions. The authors also discuss the homologous distribution of side-chains with reference to the long- and short-sequence neurotoxins.

Transferrin.—An electron-density map of rabbit plasma transferrin at 0.6 nm resolution has been obtained [124] which shows the bilobal nature of the molecule. The molecular boundary is well defined and shows the molecule to be *ca.* 9.5 nm × 6.0 nm × 5.0 nm, consisting of two roughly equal spherical lobes. It is thought that the two lobes may correspond to the two halves of the polypeptide chain which sequencing studies have shown may be the product of a gene-doubling process. Higher-resolution studies will be necessary to confirm this.

Carbonic Anhydrase B.—The water structure around the 27 active-site amino-acid residues of human carbonic anhydrase B has been studied in a Monte Carlo simulation.[125] The results suggest that the zinc ion is at least five- if not six-co-ordinated and the water structure is ordered up to 1 nm away from the zinc ion.

Citrate Synthase.—The crystal structure of pig heart citrate synthase at 0.35 nm resolution has been reported.[126] Chain tracing reveals 355 residues of the expected 430; the unseen 80 residues are *N*-terminal and are disordered in the crystal. The dimeric molecule is located on a crystallographic two-fold axis and is 7.5 nm × 6.0 nm × 9.0 nm in size. The secondary structure shows a preponderance of α-helix but no β-structure. Each monomer consists of two closely associated domains. One of these is the *C*-terminal 55 residues which fold into three antiparallel helices, while the other 300 residues are folded into 12 helices, some of which are completely internal. Preliminary substrate and inhibitor-binding studies are presented and future work on the crystal structure is outlined.

10 Protein Conformation and Structure Prediction

Analysis of Protein Structures.—Computer simulation calculations on liquid water, aqueous solutions, and biological interfaces have been reported [127] in an approach in which many-body forces are specifically taken into account through molecular

[123] M. R. Kimball, A. Sato, J. S. Richardson, L. S. Rosen, and B. W. Low, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 950.
[124] B. Gorinsky, C. Horsburgh, P. F. Lindley, D. S. Moss, M. Parkar, and J. L. Watson, *Nature (London)*, 1979, **281**, 157.
[125] E. Clementi, G. Corongiu, B. Jönsson, and S. Romano, *FEBS Lett.*, 1979, **100**, 313.
[126] G. Wiegand, D. Kukla, H. Scholze, T. A. Jones, and R. Huber, *Eur. J. Biochem.*, 1979, **93**, 41.
[127] P. Barnes, J. L. Finney, J. D. Nicholas, and J. E. Quinn, *Nature (London)*, 1979, **282**, 459.

polarization. The drawbacks of other methods and the advantages of this approach are discussed in detail.

Hildebrand [128] has presented facts which disagree with the existence of a hydrophobic effect while Pauling [129] has described a simple theory of diamagnetic anisotropy which has allowed the calculation of the molar diamagnetic anisotropy of the peptide group.

Argos and his co-workers [130] have considered the factors which influence thermostability in proteins. Amino-acid sequences for mesophilic and thermophilic ferredoxin, glyceraldehyde-3-phosphate dehydrogenase, and lactate dehydrogenase have been compared and it has been shown that Gly, Ser, Ser, Lys, and Asp in mesophiles are generally substituted by Ala, Ala, Thr, Arg, and Glu in thermophiles. These changes suggest that thermostability can be conferred by the addition of many small changes throughout the molecule without significant change in the backbone conformation. The overall effect of the changes is primarily to increase internal hydrophobicity while decreasing external hydrophobicity. They also favour helix-stabilizing residues in helices. The amino-acid change in a thermosensitive phage T4 lysozyme has also been discussed.[82]

Sprang and his co-workers [131] have considered the solvent accessibility properties of the phosphorylase *a* dimer and other multidomain, multisubunit proteins. Predictions of the accessible surface seem to be fairly accurate for smaller proteins but err increasingly with increase in molecular weight; phosphorylase is predicted very badly. Janin [132] has re-assessed his equation for the prediction of accessible surface areas of proteins, and Gates [133] has also reconsidered this problem in terms of area–volume relationships and the shapes of the protein molecules.

Vinogradov [134] has analysed hydrogen bond lengths between protonated amine groups and the oxygen atom of an acceptor group for bonds with angles greater than 150°.

Bhat and his co-workers [135] have analysed the side-chain conformations of amino-acids in 23 proteins and have shown that the population distribution of side-chains in the various allowed regions of conformational space can be largely explained on the basis of simple steric considerations. They have also analysed the population distributions of the conformational angles of groups of amino-acid residues. Gelin and Karplus [136] have calculated the side-chain torsinal potentials for bovine pancreatic trypsin inhibitor. The potentials are analysed to define the relative roles and importance of contributions from the dipeptide backbone, the protein, and the crystalline environment of solvent and other protein molecules.

Lifson and Sander [137] have analysed the amino-acid sequences of known β-strands and have shown that parallel and antiparallel strands differ in amino-acid

[128] J. H. Hildebrand, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 194.
[129] L. Pauling, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2293.
[130] P. Argos, M. G. Rossmann, U. M. Grau, H. Zuber, G. Frank, and J. D. Tratschin, *Biochemistry*, 1979, **18**, 5698.
[131] S. Sprang, D. Yang, and R. J. Fletterick, *Nature* (*London*), 1979, **280**, 333.
[132] J. Janin, *Nature* (*London*), 1979, **277**, 491.
[133] R. E. Gates, *J. Mol. Biol.*, 1979, **127**, 345.
[134] S. N. Vinogradov, *Biopolymers*, 1979, **18**, 1559.
[135] T. N. Bhat, V. Sasisekharan, and M. Vijayan, *Int. J. Pept. Protein Res.*, 1979, **13**, 170.
[136] B. R. Gelin and M. Karplus, *Biochemistry*, 1979, **18**, 1256.
[137] S. Lifson and C. Sander, *Nature* (*London*), 1979, **282**, 109.

content. Some of the amino-acid secondary-structure preferences calculated include some of the largest values reported so far for any substructure and their results also suggest a way towards improving prediction of secondary structure.

The *X*-ray structure of α-cyclodextrin has revealed interactions with water molecules which lead to a network of circular hydrogen bonds.[138] Such interactions had been predicted from quantum chemical considerations but this is the first observation of such structures with independent water molecules.

Efimov[139] has considered the packing of α-helices in proteins. The predictive method he derives has successfully predicted helix packing arrangements for five proteins and is analogous to a similar approach derived by the same author for the packing of β-structures.

Stereochemically satisfactory four-fold helical structures for polyglycine, polyproline, and glycine–proline co-polymers have been described[140] on the basis of model-building studies.

A proposed δ-helical structure for the isolated headpiece of the lac repressor protein has been described.[141] The proposed 4.3_{14} helix allows for stacking of the three tyrosine residues predicted from n.m.r. results and produces a structure suitable for interaction with the DNA molecule.

Richardson[142] has shown that with just two short local reconnections of the 2-keto-3-deoxy-6-phosphogluconate aldolase molecule it can be interpreted as an eight-stranded β-barrel. The size, shape, topology, and handedness of connection match those of triose phosphate isomerase and the first domain of pyruvate kinase. The new tracing requires the crossing of gaps in the electron-density map but such jumps have been seen in maps at this resolution (0.35 nm).

Weatherford and Salemme[143] have analysed the conformational properties of parallel β-pleated sheets and they suggest that it is the preference for non-planar peptide bond distortions which generates the β-sheet twist. This preference generates local left-handed helical character to the polypeptide chains and this naturally produces right-twisted β-sheets with optimal hydrogen-bond geometry. The underlying distortions result from the tetrahedral distortion of the peptide nitrogen atoms.

Rose[144] has reported an automatic procedure for the identification of domains in globular proteins from the *X*-ray-derived co-ordinates. Using this procedure he has shown that domains can be iteratively decomposed into subdomains leading to a hierarchy of molecular structure. The method allows the production of computer-generated space-filling colour-coded views of the structural domains. The 22 proteins studied have shown the whole spectrum of domain size.

Prediction of Protein Structure.—Maxfield and Scheraga[145] have produced a simplified version of their method for structure prediction. An increase in the size of

[138] W. Saenger, *Nature (London)*, 1979, **279**, 343.
[139] A. V. Efimov, *J. Mol. Biol.*, 1979, **134**, 23.
[140] V. Sasisekharan and V. N. Balaji, *Macromolecules*, 1979, **12**, 28.
[141] R. Chandrasekaran, T. S. Jardetzky, and O. Jardetzky, *FEBS Lett.*, 1979, **101**, 11.
[142] J. S. Richardson, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 285.
[143] D. W. Weatherford and F. R. Salemme, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 19.
[144] G. D. Rose, *J. Mol. Biol.*, 1979, **134**, 447.
[145] F. R. Maxfield and H. A. Scheraga, *Biochemistry*, 1979, **18**, 697.

the data base from 3681 to 5082 residues led to only a 1—2% increase in accuracy while the use of homologous sequences gave an increase of 6—8% in accuracy. Since it will require a large increase in the data base to reduce statistical errors significantly, the use of homologous sequences appears to be a promising way of improving predictions.

Robson and Osguthorpe [146] have proposed a new model and parameters for the fully automatic simulation of protein folding. The parameters were obtained and refined by empirical observations of known proteins and, with pancreatic trypsin inhibitor as an example, have led to a model with an r.m.s. fit of 0.6 nm and good secondary structure. Changes during the simulated folding show that α-helices and extended chain predicted at the outset, or formed early in the simulation, are conserved. Certain residues are important as flexible hinge points to bring the secondary structural elements together in the tertiary packing. In general, these hinge points are not glycine residues, but glycine residues are strong candidates for reverse-turn regions. The authors point out that reverse turns are locally determined and likely to be fairly stable, unlike hinge points which are determined by tertiary interactions.

A computer program designed to simulate the folding of a peptide chain consisting solely of helical segments and connecting links of known lengths has been described.[147] Possible interaction sites are calculated using probable changes in solvent contact area as a guide. The helices are then paired according to the list of potential sites and several filtering criteria applied. With myoglobin this led to an acceptable structure list of 121 possible foldings which could be reduced to 20 by slight changes in the parameters. One of these structures closely resembles the α-helical disposition in myoglobin.

Ptitsyn and his co-workers [148] have proposed a folding mechanism for all β-proteins based on simple and physically reasonable assumptions. The folding pathway is assumed to depend only on the total number of β-strands and on the position of the initiating complex in the protein chain. Depending on the number of β-strands, the possible topologies reduce from large numbers (10^2—10^8) to a few.

Rashin and Yudman [149] have considered the prediction of protein quaternary structure. They have assumed that protein self-assembly should include two main steps to be effective. These are a rough recognition process in which interactions do not demand any detailed fitting of subunit surfaces followed by a fitting process of these possible sites, which leads to the most stable structure. The first process has been successfully carried out for six proteins and simulation of the second process has been successful for two proteins. The methods and results are described in detail.

Morgan, Miller, and McAdon [150] have discussed the concept of molecular complementarity and presented a theorem which describes the symmetry required of self-complementary surfaces. A detailed observation shows that the surface of chymotrypsin and concanavalin A have this symmetry while the α-chain of human deoxyhaemoglobin does not.

[146] B. Robson and D. J. Osguthorpe, *J. Mol. Biol.*, 1979, **132**, 19.
[147] F. E. Cohen, T. J. Richmond, and F. M. Richards, *J. Mol. Biol.*, 1979, **132**, 275.
[148] O. B. Ptitsyn, A. V. Finkelstein, and P. Falk, *FEBS Lett.*, 1979, **101**, 1.
[149] A. A. Rashin and B. H. Yudman, *FEBS Lett.*, 1979, **101**, 6.
[150] R. S. Morgan, S. L. Miller, and J. M. McAdon. *J. Mol. Biol.*, 1979, **127**, 31.

Delbaere, Brayer, and James [151] have compared the predicted model of α-lytic protease with the structure determined by *X*-rays. The structure of the bacterial serine protease is compared to that of other known serine proteases and has allowed correct prediction of the three topologically equivalent strands in domain I of α-lytic protease and four of the six strands in domain II. The authors suggest that predictive methods are useful for detecting possible conformational similarities, and thus homologies, but are less useful in predicting the structural detail of substrate binding sites and catalytically important residues.

Austen [152] has predicted secondary structures for the amino-terminal extension sequences of secreted proteins. Twenty-one proteins are considered and their amino-acid sequences and predicted secondary structures are compared. The possibility that these *N*-terminal signal peptides could function by helping to form a tunnel for the secreted protein to traverse the membrane is discussed.

11 Viruses

The binding of oligonucleotides to disks of tobacco mosaic virus at 0.5 nm resolution has been reported.[153] Crystals of the TMV disks were soaked in adenylyladenylyl–guanosine (AAG) or in the hexanucleotide fraction of a ribonuclease digest of yeast RNA. At high concentrations the crystals were disrupted, most probably due to normal nucleation of assembly effects, but at low concentrations the crystals remained intact and difference Fourier maps have been calculated. Several movements of helices are seen and the left radial helix stiffens so that it becomes visible from 4 nm to 3 nm radius. A feature in the map at 4 nm radius and just below the right radial helix has been tentatively identified as the nucleotide. The amino-acids which may be involved in the binding have been examined and include a conserved glycine. Of all the nine residues which make close contacts, seven are totally conserved.

The structures of polyoma virion and capsid crystal structures have been compared.[154] The complete virus particles and the empty capsids crystallize isomorphously and show the virus particles to be *ca.* 49 nm in diameter, agreeing well with the value obtained from electron microscopy. The structures and possible strategies for three-dimensional data collection are also discussed.

A structural change of the PF1 filamentous bacterial virus induced by temperature has been studied by *X*-ray diffraction.[155] Although local protein interactions alter by only a few hundredths of a nanometre, the cumulative effect at the end of the 2000 nm long filament is a shortening of 100 nm and a rotation of 15 turns. The observed phase transition has suggested a model for virion assembly in the bacterial membrane. Highly ordered fibres of PF1 have been produced by drying concentrated virus solutions in a strong magnetic field.[156] *X*-Ray studies have shown a substantial improvement in the alignment of the phage parallel to the fibre axis.

[151] L. T. J. Delbaere, G. D. Brayer, and M. N. G. James, *Nature* (*London*), 1979, **279**, 165.
[152] B. M. Austen, *FEBS Lett.*, 1979, **103**, 308.
[153] J. Graham and P. J. G. Butler, *Eur. J. Biochem.*, 1979, **93**, 333.
[154] K. W. Adolph, D. L. D. Caspar, C. J. Hollingshead, E. E. Lattman, W. C. Phillips, and W. T. Murakami, *Science*, 1979, **203**, 1117.
[155] C. Nave, A. G. Fowler, S. Malsey, D. A. Marvin, and E. J. Wachtel, *Nature* (*London*), 1979, **281**, 232.
[156] J. Torbet and G. Maret, *J. Mol. Biol.*, 1979, **134**, 843.

A neutron-scattering study of the solution structures of bacteriophages PF1 and fd has been reported.[157] The results suggest that the phages have similar structures and also confirm that PF1 undergoes a reversible temperature-induced structural change in solution between 16 and 6 °C.

The preparation and examination of crystalline sheets derived from head proteins dissociated from T-even bacteriophages have been published.[158] An *X*-ray and electron-diffraction study of the distal half of the tail fibre of bacteriophage T4 has also been reported.[159]

12 Nucleic Acids

tRNA.—The crystal structure of a eukaryotic initiator transfer-RNA, $tRNA_f^{met}$, has been determined at 0.45 nm resolution.[160] Despite over 80% of the crystal volume being solvent it was difficult to determine the molecular boundary until the inner reflections ($d > 1.4$ nm) were included. Four labelled residues aided the interpretation of the electron-density map and a refinement to 0.6 nm has been performed. In contrast to the known $tRNA^{Phe}$ structures the anticodon arm is seen to be poorly localized and it seems that the junction of the D and anticodon arms may serve as a hinge point to allow alterations of the structure during function. Intermolecular contacts are made by the anticodon arms and the flexibility of these arms may be the cause of the crystals being poorly ordered. Refinement with a more flexible model has reduced the *R*-factor to 0.25 for all the data to 0.4 nm resolution.

DNA and DNA-binding Compounds.— The crystal structure of putrescine diphosphate has been reported [161] and shows the interaction between the amine groups of the putrescine and the phosphate groups. The structure serves as a model for understanding amine–nucleic acid interactions.

Several papers on the structure of DNA have been published.[162-166] The structure of a left-handed double helical DNA fragment has been determined at atomic resolution [162] and the proposed side-by-side model of DNA has been analysed critically in structural terms.[163] The ethanol-induced transition of DNA has been shown to be from the B-form to the A-form as the ethanol concentration is increased.[164] The positions of caesium ions in crystalline B-DNA have been determined [165] and the involvement of magnesium and lithium salts in the structure of phage T2 DNA has been investigated.[166]

Several papers have appeared on DNA–drug interactions.[167-171] These include

[157] J. Torbet, *FEBS Lett.*, 1979, **108**, 61.
[158] U. Aebi, R. Van den Broek, P. R. Smith, B. ten Heggeler, J. Dubochet, V. V. Mesyanzhinov, A. Tsugita, and J. Kistler, *J. Mol. Biol.*, 1979, **130**, 255.
[159] W. C. Earnshaw, E. B. Goldberg, and R. A. Crowther, *J. Mol. Biol.*, 1979, **132**, 101.
[160] R. W. Schevitz, A. D. Podjarny, N. Krishnamachari, J. J. Hughes, P. B. Sigler, and J. L. Sussman, *Nature (London)*, 1979, **278**, 188.
[161] N. H. Woo, N. C. Seeman, and A. Rich, *Biopolymers*, 1979, **18**, 539.
[162] A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. van der Marel, and A. Rich, *Nature (London)*, 1979, **282**, 680.
[163] R. J. Greenall, W. J. Pigram, and W. Fuller, *Nature (London)*, 1979, **282**, 880.
[164] S. B. Zimmerman and B. H. Pfeiffer, *J. Mol. Biol.*, 1979, **135**, 1023.
[165] I. Y. Skuratovskii, L. I. Volkova, K. A. Kapitonova, and V. N. Bartenev, *J. Mol. Biol.*, 1979, **134**, 369.
[166] I. Y. Skuratovskii and V. N. Bartenev, *Mol. Biol.*, 1979, **12**, 1052.
[167] T. D. Sakore, B. S. Reddy, and H. M. Sobell, *J. Mol. Biol.*, 1979, **135**, 763.

crystalline complexes with 9-amino-acridine,[167] acridine orange,[168] proflavine,[168] ellipticine,[169] 3,5,6,8-tetramethyl-*N*-methyl phenanthrolinium,[169] proflavine,[170] and anthramycin.[171]

The higher-order structures of chicken erythrocyte chromatin in solution have been studied by neutron scattering.[172] These structures have been examined under various conditions of ionic strength and the proposed models for the core particles are discussed.

13 Muscle

The three-dimensional structure of tropomysin filaments has been determined by *X*-ray crystallography.[173] Data to 2 nm resolution have been collected and an electron-density map at this resolution has been calculated. The molecule is seen to wind along a line parallel to the body diagonal of the unit cell and is 41.03 nm long. Using data collected from mercury-labelled crystals, it has been possible to locate the position of the single cysteine residue in each chain. The head-to-tail overlap regions have been identified and the molecules seem to show a non-helical globular overlap region. The filament also appears to be slightly kinked in several places along its length. The relevance of the observed structure to the role of tropomyosin in muscle regulation is discussed.

Low-angle *X*-ray diffraction patterns from crab leg muscle have been recorded[174] both in the living resting state and in rigor. Possible explanations for the observed diffraction patterns are presented. Equatorial *X*-ray diffraction patterns have been recorded from frog sartorius muscle in the resting state and during isometric contractions.[175] By observing the relative changes in intensity of the reflections it has been possible to show that these changes can be used as a sensitive measure of the number of cross-bridges formed in the isometric state.

Using a fast *X*-ray diffraction technique the movement of the myosin heads during a heart beat have been studied.[176] The change in the ratio of the intensities of the 1,0 and 1,1 reflections closely followed the development of tension in the muscle.

X-Ray diffraction data have been presented[177] which suggest how the myosin rods form the thick filaments of crustacean muscles by additional supercoiling of the α-helical coiled coil rods. The positions of cross-bridges can be determined from those reflections which weaken in rigor and have been used to determine both the rotational and the screw symmetry of the filaments from fast and slow muscle. The author suggests that natural transformations of this structure may describe the myosin backbone in many other animals.

[168] B. S. Reddy, T. P. Seshadri, T. D. Sakore, and H. M. Sobell, *J. Mol. Biol.*, 1979, **135**, 787.
[169] S. C. Jain, K. K. Bhandary, and H. M. Sobell, *J. Mol. Biol.*, 1979, **135**, 813.
[170] H. M. Berman, W. Stallings, H. L. Carrell, J. P. Glusker, S. Neidle, G. Taylor, and A. Achari, *Biopolymers*, 1979, **18**, 2405.
[171] L. H. Hurley and R. Petrusek, *Nature (London)*, 1979, **282**, 529.
[172] P. Suau, E. M. Bradbury, and J. P. Baldwin, *Eur. J. Biochem.*, 1979, **97**, 593.
[173] G. N. Phillips, jun., E. E. Lattman, P. Cummins, K. Y. Lee, and C. Cohen, *Nature (London)*, 1979, **278**, 413.
[174] Y. Maeda, I. Matsubara, and N. Yagi, *J. Mol. Biol.*, 1979, **127**, 191.
[175] L. C. Yu, J. E. Hartt, and R. J. Podolsky, *J. Mol. Biol.*, 1979, **132**, 53.
[176] I. Matsubara, N. Yagi, and M. Endoh, *Nature (London)*, 1979, **278**, 474.
[177] J. S. Wray, *Nature (London)*, 1979, **277**, 37.

X-Ray diffraction studies have also been used to follow the binding of glycolytic enzymes to rabbit psoas muscle.[178] The changes observed in the diffraction pattern are consistent with the binding of fructose biphosphate aldolase to the thin filaments of the muscle, the extent of the change being consistent with the increase in the mass of the thin filaments produced by enzyme binding. Similar effects were seen with a mixture of aldolase and glyceraldehyde 3-phosphate dehydrogenase.

14 Membranes and Membrane-bound Proteins

The crystal structure of lecithin dihydrate has been reported [179] as have small-angle *X*-ray scattering studies on the structure of dimyristoylphosphatidylcholine vesicles and their complexes with apolipoprotein C-III.[180] *X*-Ray studies on membranes isolated from *Salmonella typhimurium* have been published.[181]

Three papers on the effects of anaesthetics on the structures of membranes have been published.[182-184] Treatment of the membrane with n-pentane is shown to increase the thickness of the myelin period lattice from 17 to 18 nm.[183] An *X*-ray and neutron diffraction study on the effects of nitrous oxide, halothane, and cyclopropane on lecithin–cholesterol bilayers has been reported.[184] The results of this study suggest that the lipid bilayer is not the primary site of action of general anaesthetics.

The changes in bilayer structure induced by surface charges have been examined by *X*-ray diffraction.[185] Increasing the surface charge gives a decrease in the bilayer thickness whereas the hydrocarbon-chain packing remains unaltered. These results are interpreted in terms of a tilt of the chains caused by the surface charges on the polar heads.

Partial amino-acid sequences for bacteriorhodopsin have been determined and related to the known *X*-ray structure of the protein.[186] The location of retinal within the membrane has been determined by neutron diffraction using membranes reconstituted with protonated or deuteriated retinal.[187] A distinct scattering density difference between the two preparations was observed and shows that the β-ionone ring portion of the retinal is situated centrally in the membrane. Neutron diffraction has also been used to study the areas of hydration within the purple membrane.[188] The results exclude the possibility of passive transport occurring *via* a bulk water channel in the protein.

Electron microscopy and image reconstruction of crystals of ubiquinone : cytochrome *c* reductase membrane crystals from *Neurospora crassa* has been reported.[189] The results suggest that the reductase is a rod-like enzyme

[178] M. Stewart, D. J. Morten, and F. M. Clarke, *Biochem. J.*, 1979, **183**, 663.
[179] R. H. Pearson and I. Pascher, *Nature (London)*, 1979, **281**, 499.
[180] P. Laggner, A. M. Gotto, jun., and J. D. Morrisett, *Biochemisty*, 1979, **18**, 164.
[181] T. Ueki, T. Mitsui, and H. Nikaido, *J. Biochem.*, 1979, **85**, 173.
[182] G. L. Turner and E. Oldfield, *Nature (London)*, 1979, **277**, 669.
[183] R. Padron, L. Mateu, and J. Requena, *Biochim. Biophys. Acta*, 1979, **552**, 535.
[184] N. P. Franks and W. R. Lieb, *J. Mol. Biol.*, 1979, **133**, 469.
[185] F. Jähnig, K. Harlos, H. Vogel, and H. Eibl, *Biochemistry*, 1979, **18**, 1459.
[186] J. E. Walker, A. F. Carne, and H. W. Schmitt, *Nature (London)*, 1979, **278**, 653.
[187] G. I. King, W. Stoeckenius, H. L. Crespi, and B. P. Schoenborn, *J. Mol. Biol.*, 1979, **130**, 395.
[188] G. Zaccai and D. J. Gilmore, *J. Mol. Biol.*, 1979, **132**, 181.
[189] P. Wingfield, T. Arad, K. Leonard, and H. Weiss, *Nature (London)*, 1979, **280**, 695.

which spans the membrane with its elongated axis perpendicular to the plane of the membrane.

Similar techniques have been used to study the structure of cytochrome *c* oxidase in deoxycholate-derived two-dimensional crystals.[190]

Two neutron diffraction studies on phosphatidylcholine model membranes have been reported.[191,192] In the first paper[191] the conformation in the head-group region was examined by selective deuteriation. The data clearly show that the zwitterionic phosphocholine group lies almost parallel to the membrane surface in both the gel and the liquid state. In the second paper [192] selective deuteriation of the hydrocarbon chains was used. In the gel phase the data confirm a model with chains in an all-*trans* configuration and tilted with respect to the bilayer normal.

Image reconstruction and electron microscopy has been used to examine the structure of tubulin in zinc-induced sheets.[193] The data show the presence of two classes of morphological units, confirming the representation of tubulin as an $\alpha\beta$-heterodimer. The more extensive contacts between subunits of a dimer when compared to inter-dimer contacts can also be seen. In another study [194] the structure of zinc-induced tubulin filaments was compared with native hydrated microtubules. The data suggest that the three-dimensional model of zinc-tubulin protofilaments is a good first approximation to the low-resolution structure of assembled tubulin.

15 Other Biological Structures

Bacterial Pili and Flagella.—*X*-Ray diffraction and electron microscope studies on the F sex pili of *E. coli* have been reported.[195] The pilus structure is deduced to consist of four co-axial helices of pitch 12.8 nm with the pilin subunits elongated and overlapping along the axis of these helices. A molecular model of the structure at low resolution is proposed.

A three-dimensional image reconstruction of straight flagella from a mutant of *Salmonella typhimurium* has been compared with the known *X*-ray data.[196] The straight-to-helical transitions of bacterial flagella have also been studied by *X*-ray diffraction [197] and the results seem to prove the predicted presence of two classes of straight flagella forms.

Ribosomes.—The attachment of ribosomes to membranes in lizard oocytes and chick embryos has been investigated by electron microscopic methods.[198] The crystalline ribosomes are found to bind to membranes *via* a single unique site on the large subunit. It was also possible to dissociate selectively the small subunits from the complexes and so estimate the positions of these subunits over the membrane surface.

[190] S. D. Fuller, R. A. Capaldi, and R. Henderson, *J. Mol. Biol.*, 1979, **134**, 305.
[191] G. Büldt, H. U. Gally, J. Seelig, and G. Zaccai, *J. Mol. Biol.*, 1979, **134**, 673.
[192] G. Zaccai, G. Büldt, A. Seelig, and J. Seelig, *J. Mol. Biol.*, 1979, **134**, 693.
[193] L. K. Tamm, R. H. Crepeau, and S. J. Edelstein, *J. Mol. Biol.*, 1979, **130**, 473.
[194] L. A. Amos and T. S. Baker, *Nature (London)*, 1979, **279**, 607.
[195] W. Folkhard, K. R. Leonard, S. Malsey, D. A. Marvin, J. Dubochet, A. Engel, M. Achtman, and R. Helmuth, *J. Mol. Biol.*, 1979, **130**, 145.
[196] Y. Shirakihara and T. Wakabayashi, *J. Mol. Biol.*, 1979, **131**, 485.
[197] R. Kamiya, S. Asakura, K. Wakabayashi, and K. Namba, *J. Mol. Biol.*, 1979, **131**, 725.
[198] P. N. T. Unwin, *J. Mol. Biol.*, 1979, **132**, 69.

The internal structure of *E. coli* ribosomes has been examined by neutron and *X*-ray scattering.[199] The data confirm that the RNA is localized predominantly within the particle with the protein located predominantly on the periphery.

The production of ordered helical ribbons of small ribosomal subunits has been reported.[200] Electron micrographs of these ribbons diffract to 6 nm resolution and the optical diffraction patterns suggest that adjacent subunits are related by a two-fold screw axis parallel to the ribbon axis with a helical repeat distance of 22 nm.

Naturally Occurring Polypeptides.—The crystal structure of the potassium complex of gramicidin A has been determined [201] and shows that there are two cation-binding sites per antibiotic channel. Potassium ion binding induces similar conformational changes to those seen with caesium ions. The crystal structure of naturally occurring antamanide in the uncomplexed form has been determined [202] and compared to the known structures of biologically active synthetic forms of the compound. The crystal structure of meso-valinomycin has also been reported [203] and its structure compared to other known forms of valinomycin.

Various Other Structures.—Gap junctions containing regular hexagonal arrays of connexions have been isolated from rat liver and studied to a resolution of 1.8 nm by electron microscopy and image reconstruction.[204] The results seem to show the existence of two configurations of the connexon subunits. A model for peptide folding based on the incipient 3_{10} helix observed for pivaloylprolylprolyl-*N*-methylalanine has been proposed [205] and a double-stranded β-helix with anti-parallel chains has been observed for oligo(-L-valyl-D-valyl-).[206]

X-Ray diffraction studies on operculin, the glycine-rich protein present as the major component of the gastropod shell-sealing plate, have been published [207] and lead to the conclusion that the operculae contain anti-parallel-chain pleated sheets. The crystal structure of hyper-modified nucleoside Q which is found in the wobbling position of t-RNA has been determined [208] as has been the structure of methoxatin,[209] the coenzyme isolated from the primary alcohol dehydrogenases of methylotrophic bacteria. The three-dimensional conformations of two prostaglandins have been determined and compared.[210] An electron-microscope and image-reconstruction study [211] of rat preputial gland β-glucuronidase suggests an approximately triangular molecular outline in projection and is taken to indicate a tetrahedral arrangement of the four subunits in the molecule.

[199] I. N. Serdyuk, A. K. Grenader, and G. Zaccai, *J. Mol. Biol.*, 1979, **135**, 691.
[200] M. W. Clark, M. Hammons, J. A. Langer, and J. A. Lake, *J. Mol. Biol.*, 1979, **135**, 507.
[201] R. E. Koeppe II, J. M. Berg, K. O. Hodgson, and L. Stryer, *Nature (London)*, 1979, **279**, 723.
[202] I. L. Karle, T. Wieland, D. Schermer, and H. C. J. Ottenheym, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1532.
[203] V. Z. Pletnev, N. M. Galitskii, V. T. Ivanov, and Y. A. Ovchinnikov, *Biopolymers*, 1979, **18**, 2145.
[204] G. Zampighi and P. N. T. Unwin, *J. Mol. Biol.*, 1979, **135**, 451.
[205] Y. V. Venkatachalapathi and P. Balaram, *Nature (London)*, 1979, **281**, 83.
[206] E. Benedetti, B. Di Blasio, C. Pedone, G. P. Lorenzi, L. Tomasic, and V. Gramlich, *Nature (London)*, 1979, **282**, 630.
[207] S. Hunt, R. D. B. Fraser, T. P. MacRae, and E. Suzuki, *J. Mol. Biol.*, 1979, **129**, 149.
[208] S. Yokoyama, T. Miyazawa, Y. Iitaka, Z. Yamaizumi, H. Kasai, and S. Nishimura, *Nature (London)*, 1979, **282**, 107.
[209] S. A. Salisbury, H. S. Forrest, W. B. T. Cruse, and O. Kennard, *Nature (London)*, 1979, **280**, 843.
[210] G. T. De Titta, D. A. Langs, and J. W. Edmonds, *Biochemistry*, 1979, **18**, 3387.
[211] M. R. Dickson, M. Stewart, D. E. Hawley, and C. A. Marsh, *Biochem. J.*, 1979, **181**, 1.

16 Fibrous Proteins and Polysaccharides

Collagen.—The wide-angle *X*-ray diffraction pattern of air-dried lens capsule collagen has been reported [212] and shows that the lens capsule collagen has the same structure as tendon collagen. *X*-Ray diffraction has also been used to compare the structure of mammalian collagen with elastoidin fibre structures.[213] A model for the structure of collagen in kangaroo tail tendon has been proposed, based on quantitative *X*-ray diffraction data.[214] New data have been collected on the crystalline structure of collagen fibrils in tendon [215] and a proposal of quasi-hexagonal molecular packing in collagen fibrils has been presented.[216] Structural studies on calcified, partly calcified, and uncalcified turkey leg tendons have been published.[217] The uncalcified tendon shows a diffraction pattern very similar to that of rat tail tendon and a discussion of the mechanism of calcification is presented. The glutaraldehyde-induced stress state of collagen has been examined using synchrotron radiation;[218] the observed variation in the 67 nm period is undetected with the aid of conventional *X*-ray sources. The binding of phosphotungstic acid to rat tail collagen has been examined using conventional and synchrotron *X*-radiation.[219] Two specific reaction steps, dependent on the steric arrangement of the collagen fibres, are observed.

Chitin.—The preparation of spun chitin fibres and their diffraction patterns have been reported.[220]

17 Synthetic Polypeptides

Structural studies on some poly(α-amino-acids) with hydrophobic side chains have been reported.[221] Poly(L-valine) has two structural forms; one is an α-helical conformation which is unfavourable. The other conformation is a β-pleated sheet as is the observed structure of poly(L-isoleucine). Poly(L-phenylalanine) exists as a mixture of ω- and α-helix.

The analysis of the structure of poly(4-hydroxy-L-proline) shows that it is a left-handed helical structure with intrachain hydrogen bonds.[222] The solid-state conformation of *N*- and *C*-terminally blocked heptamethylcysteine has been reported [223] as have the structures of two derivatives of hepta-D-alanine.[224] The conformation of the A-B-A-type block co-polymer with poly(γ-benzyl-L-glutamate) as the A-component and polybutadiene as the B-component has been published.[225]

[212] N. Roveri, A. Ripamonti, A. Bigi, D. Volpin, and M. G. Giro, *Biochim. Biophys. Acta*, 1979, **576**, 404.
[213] R. J. Chandross and R. S. Bear, *J. Mol. Biol.*, 1979, **130**, 215.
[214] R. D. B. Fraser, T. P. MacRae, and E. Suzuki, *J. Mol. Biol.*, 1979, **129**, 463.
[215] R. D. B. Fraser and T. P. MacRae, *J. Mol. Biol.*, 1979, **127**, 129.
[216] D. J. S. Hulmes and A. Miller, *Nature (London)*, 1979, **282**, 878.
[217] C. Berthet-Colominas, A. Miller, and S. W. White, *J. Mol. Biol.*, 1979, **134**, 431.
[218] R. Jonak, H. Nemetschek-Gansler, T. Nemetschek, H. Riedl, J. Bordas, and M. Koch, *J. Mol. Biol.*, 1979, **130**, 511.
[219] T. Nemetschek, H. Riedl, and R. Jonak, *J. Mol. Biol.*, 1979, **133**, 67.
[220] S. Tokura, N. Nishi, and J. Noguchi, *Polymer J.*, 1979, **11**, 781.
[221] O. Yamashita, T. Yamane, T. Ashida, S. Yamashita, and T. Yamashita, *Polymer J.*, 1979, **11**, 763.
[222] S. K. Brahmachari, M. Bansal, V. S. Ananthanarayanan, and V. Sasisekharan, *Macromolecules*, 1979, **12**, 23.
[223] P. Spadon, D. R. Rueda, and A. Del Pra, *Macromolecules*, 1979, **12**, 1121.
[224] D. R. Rueda, P. Spadon, G. M. Bonora, and G. Zanotti, *Int. J. Pept. Protein Res.*, 1979, **13**, 374.
[225] A. Nakajima, T. Hayashi, K. Kugo, and K. Shinoda, *Macromolecules*, 1979, **12**, 840.

PART III: Conformation and Interaction of Peptides and Proteins in Solution *edited by R. H. Pain, with contributions by B. Adams, A. Benson, T. Brittain, F. E. Cohen, R. Devonshire, D. P. E. Dickson, H. W. E. Rattle, R. M. Stephens, M. J. E. Sternberg, and E. J. Wood.*

Introductory Remarks

The year of 1979 has seen the publication of several important books concerned with the conformation of proteins. Schulz and Schirmer [1] provide an extended survey of protein structures as revealed by crystallographic studies. The energetic, dynamic, and evolutionary factors underlying the emergence of these structures are discussed in a stimulating and revealing fashion. Lest anyone should think that the hydrophobic interaction has been buried in the text books, two new monographs by Tanford [2] and Ben-Naim [3] show that this important phenomenon is still central to an understanding of proteins and membranes and still a lively and sometimes controversial topic of discussion. Following closely on Lapanje's monograph on protein denaturation [4] reported in 1979, these books provide an up to date source of reference for the scientist interested in the fundamentals of protein structure in solution.

One of the interesting developments apparent from the literature for 1979 is in the field of protein dynamics. Further theoretical and experimental studies reported this year confirm the general picture of globular proteins as imperfectly packed, internally mobile structures, but go on to study this motion at a much higher degree of resolution. References to such work will be found in Sections 1, 2, and 4. Several of the techniques used in this work are reviewed, together with other techniques used in studying protein conformation and interactions, in a recent volume of Methods in Enzymology.[5] This is an area which is likely to prove basic to our ultimate understanding of enzyme activity and its control by ligand interaction.

1 Theoretical Aspects of Protein Conformation

Contributed by F. E. Cohen and M. J. E. Sternberg

This year, Schulz and Schirmer have published a textbook that provides a comprehensive review, to the beginning of 1978, of work on the analysis and prediction of protein structure.[1] In this article we aim to highlight the major areas of current interest in theoretical work on protein conformation.

Potential-energy Functions.—The significance of any theoretical energy calculation is obviously linked to the accuracy of the potential function. Thus, considerable effort has been devoted to the development of improved potential functions.[6-15]

[1] G. E. Schulz and R. H. Schirmer, 'Principles of Protein Structure', Springer-Verlag, Heidelberg, 1979.
[2] C. Tanford, 'The Hydrophobic Effect', 2nd edn., Wiley, New York, 1980.
[3] A. Ben-Naim, 'Hydrophobic Interactions', Plenum, New York, 1980.
[4] S. Lapanje, 'Physicochemical Aspects of Protein Denaturation', Wiley, New York, 1978.
[5] C. H. W. Hirs and S. N. Timasheff, *Methods Enzymol.*, 1979, Vol. 61.
[6] A. T. Hagler, P. Dauber, and S. Lifson, *J. Am. Chem. Soc.*, 1979, **101**, 5110.
[7] A. T. Hagler, S. Lifson, and P. Dauber, *J. Am. Chem. Soc.*, 1979, **101**, 5122.
[8] A. T. Hagler, P. Dauber, and S. Lifson, *J. Am. Chem. Soc.*, 1979, **101**, 5131.
[9] B. R. Gelin and M. Karplus, *Biochemistry*, 1979, **18**, 1256.
[10] A. S. Kolaskar and D. Prashanth, *Int. J. Pept. Protein Res.*, 1979, **14**, 88.

These studies are conveniently divided into two groups, intra-protein[6-11] and protein–water interactions.[14-18]

In a series of papers, Hagler and his co-workers[6-8] have fitted standard potential energy functional forms to crystallographic data available on small molecules,[6] established a bench mark for assessing the quality of potential functions,[7] and studied the results of calculations on a series of carboxylic acids with two different force fields.[8] The technique of fitting potential function parameters to crystallographic data has been used in studies of torsional potentials[9,10] and of hydrogen bonds.[11] Hillier and Robson[12] have sought to extend the quality of representations of the dipeptide energy surface through *ab initio* quantum mechanical methods. All of these methods rely on pairwise additivity of interactions, and this assumption is questioned by the work of Finney and his co-workers[13] on the contribution of non-additive electrostatic energies to the energy of an ensemble of water molecules.

Since the effect of water on protein structure is significant,[14-19] gas-phase calculations of protein folding will never reproduce the structures observed crystallographically. Discussions of the nature of the protein–water interface all conclude that the effect is substantial[14-17] but disagree as to how this effect can best be modelled.[18,19] Clearly, much more work in this area is required.

Energy Calculations on Polypeptides and Proteins.— Energy calculations on short polypeptides have been used to model the short-range interactions in proteins[20-24] as well as to explore the conformations of biologically interesting oligopeptides.[25-29] Occasionally, these calculations have led to new insights into the properties of peptides.[30-33] Detailed energy calculations have been used to explain observed properties of proteins.[34-37]

[11] S. N. Vinogradov, *Int. J. Pept. Protein Res.*, 1979, **14**, 281.
[12] I. H. Hillier and B. Robson, *J. Theor. Biol.*, 1979, **76**, 83.
[13] P. Barnes, J. L. Finney, J. D. Nicholas, and J. E. Quinn, *Nature (London)*, 1979, **282**, 459.
[14] J. H. Hildebrand, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 194.
[15] C. Tanford, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4175.
[16] P.-H. Yang and J. A. Rupley, *Biochemistry*, 1979, **18**, 2654.
[17] C.-H. Yang, J. N. Brown, and K. D. Kopple, *Int. J. Pept. Protein Res.*, 1979, **14**, 12.
[18] F. Vovelle and M. Ptak, *Int. J. Peptide Protein Res.*, 1979, **13**, 435.
[19] Z. I. Hodes, G. Nemethy, and H. A. Scheraga, *Biopolymers*, 1979, **18**, 1611.
[20] A. T. Hagler, P. S. Stern, R. Sharon, J. H. Becker, and F. Naider, *J. Am. Chem. Soc.*, 1979, **101**, 6842.
[21] M. V. Volkenstein, I. B. Golovanov, and V. M. Sobolev, *Mol. Biol.*, 1979, **13**, 633.
[22] D. Peters and J. Peters, *J. Mol. Struct.*, 1979, **53**, 103.
[23] Z. I. Hodes, G. Nemethy, and H. A. Scheraga, *Biopolymers*, 1979, **18**, 1611.
[24] R. P. Sheridan, R. H. Lee, N. Peters, and L. C. Allen, *Biopolymers*, 1979, **18**, 2451.
[25] F. A. Gorin, T. M. Balasubramanian, C. D. Barry, and G. R. Marshall, *J. Supramol. Struct.*, 1978, **9**, 27.
[26] R. Virudachalam and V. S. R. Rao, *Biopolymers*, 1979, **18**, 571.
[27] V. Madison, *Biopolymers*, 1978, **17**, 1601.
[28] E. M. Popov and G. M. Lipkind, *Mol. Biol.*, 1979, **13**, 363.
[29] G. V. Nikiforovich, V. I. Leonova, S. G. Galaktionov, and G. I. Chipens, *Int. J. Pept. Protein Res.*, 1979, **13**, 36.
[30] S. Suhai, T. C. Collins, and J. Ladik, *Biopolymers*, 1979, **18**, 899.
[31] L. Pauling, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2293.
[32] N. Saenger, *Nature (London)*, 1979, **279**, 343.
[33] T. Kakitani and H. Kakitani, *Biophys. Struct. Mech.*, 1979, **5**, 55.
[34] D. A. Case, B. H. Huynh, and M. Karplus, *J. Am. Chem. Soc.*, 1979, **101**, 4433.
[35] E. Clementi, G. Corongiu, B. Jonsson, and S. Romano, *FEBS Lett.*, 1979, **100**, 313.
[36] J. B. Matthews, G. I. H. Hanania, and F. R. N. Gurd, *Biochemistry*, 1979, **18**, 1919.
[37] J. B. Matthews, G. I. Hanania, and F. R. N. Gurd, *Biochemistry*, 1979, **18**, 1928.

Hagler *et al.*[20] have added a vibrational component to their potential energy function to study the properties of some hexapeptides. Thus, entropic contributions were considered in detail for the first time. Although enthalpic terms favoured certain conformations, entropic effects occasionally reversed these preferences. These calculations were in good agreement with a variety of physicochemical measurements. Hodes *et al.*[23] have developed an approximation for modelling the solvent contribution to the energy of various dipeptide conformations. Even for dipeptides, minimum-energy conformations are altered when the solvent is included. Quantum mechanical calculations of hydrogen bond strength by Sheridan *et al.*[24, 25] show positive co-operativity. Thus, the energy of stabilization for α-helices and β-sheets due to hydrogen bonds is 25% stronger than the sum of these bond energies in isolation.

With the improvements in representations of the potential energy surface and of algorithms to search conformation space, a variety of oligopeptides have been studied in detail.[20,25-29] These include the enkephalins,[25] peptidoglycans,[26] gramicidin A,[28] and oxytocin.[29] Calculations on the structure of gramicidin A suggest that it may form a π-helix. This unusual structural feature has been invoked to explain how this peptide could act as a proton or cation pump.[28] Other calculations have suggested that peptides could act as semiconductors mediating charge transfer between proteins and small acceptor molecules like glyoxal[30] or that the torsional potential of certain amino-acids might explain visual excitation.[33]

In several calculations the structure of a protein is fixed and the environment is varied.[34-37] Case *et al.*[34] have performed detailed quantum mechanical calculations on the energy states of the haem ring in oxy- and carboxy-haemoglobin to explain Mössbauer data. Clementi *et al.*[36] used Monte Carlo techniques to simulate water structure in the active cleft of carbonic anhydrase. They concluded that the essential Zn^{2+} atom was penta- or hexa-co-ordinated. In an attempt to explain the Bohr effect and the titration behaviour of haemoglobin, Matthew *et al.*[36, 37] have studied the charge distribution of the α- and β-chains. The charge distribution is significantly different from that for myoglobin, and the implications for the pK_a values of various ionizable groups are discussed.

Analysis and Prediction of Secondary Structure.—The work on the secondary structure of proteins has led to a more detailed understanding of observed structures[38-43] as well as the development and application of methods of prediction.[44-54]

In 1973, Chothia[38] observed that in globular proteins the β-strands, when viewed along the chain, have a right-handed twist of *ca.* 20° per peptide unit. He

[38] C. Chothia, *J. Mol. Biol.*, 1973, **75**, 295.
[39] D. W. Weatherford and F. R. Salemme, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 19.
[40] K. Raghavendra and V. Sasisekharan, *Int. J. Pept. Protein Res.*, 1979, **14**, 326.
[41] S. Lifson and C. Sander, *Nature (London)*, 1979, **282**, 109.
[42] M. Bansal, S. K. Brahmachari, and V. Sasisekharan, *Macromolecules*, 1979, **12**, 19.
[43] V. Sasisekharan and V. N. Balaji, *Macromolecules*, 1979, **12**, 128.
[44] I. D. Kuntz, G. M. Crippen, and P. A. Kollman, *Biopolymers*, 1979, **18**, 939.
[45] P. Y. Chou and G. D. Fasman, *Annu. Rev. Biochem.*, 1978, **47**, 251.
[46] F. R. Maxfield and H. A. Scheraga, *Biochemistry*, 1979, **18**, 697.
[47] E. V. Barkovsky and V. A. Bandarin, *Bioorg. Khim.*, 1979, **5**, 24.
[48] G. D. Rose, *Nature (London)*, 1978, **272**, 586.

attributed this phenomenon to entropy as the right-handed twisted structure has more accessible conformational states than the left-handed or the straight chain. Weatherford and Salemme[39] have used computerized model-building studies to suggest that the twist of the β-sheet results from distortions in the peptide bond. They note that in peptide crystal structures the amide nitrogen has a partial double-bond character and the peptide ω-angle is typically $-175°$ rather than the planar angle of $-180°$. Sheet structures with optimum hydrogen bonding built with these distortions are found to have a right-handed twist. An alternative explanation for the twist is proposed by Raghavendra and Sasisekharan[40] who performed classical energy calculations. They included both intrachain and interchain interactions in antiparallel β-sheet and found that right-handed twisted structures were energetically more favourable. The importance of side chains on sheet structure has been considered by Lifson and Sander.[41] They analysed the conformational preferences for residue types in parallel and antiparallel arrangements of β-strands. There is a marked distinction in preferences that may prove useful in improving empirical methods of secondary-structure prediction.

Work has continued on the improvement of methods of secondary-structure prediction.[44-47] Kuntz and his co-workers[44] have developed a scheme, similar to that of Rose,[48] to predict chain reversals based on the hydrophobicity of the residues that can be brought together by a hairpin turn. The existing prediction algorithms have been applied to several proteins[49-54] including pituitary hormones,[49] β-lactamases,[50] the ribosomal protein L7/L12,[51] and calcium-binding proteins.[52]

Analysis of Tertiary and Quaternary Structure.—The trend that we noted last year has continued. Most of the examinations of tertiary and quaternary structure of proteins consider the accessibility to solvent[55-58] and the packing of residues.[59-67] The studies of chain topology[68-71] are often directed towards the probable evolution of a protein.

[49] M. D. Jibson and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, **14**, 113.
[50] P. C. Moews and J. R. Knox, *Int. J. Pept. Protein Res.*, 1979, **13**, 385.
[51] C. A. Luer and K.-P. Wong, *Biochemistry*, 1979, **18**, 2019.
[52] H. P. Vogt, W. Strassburger, A. Wollmer, J. Fleischhauer, B. Bullard, and D. Mercola, *J. Theor. Biol.*, 1979, **76**, 297.
[53] P. Y. Chou and G. D. Fasman, *Biophys. J.*, 1979, **26**, 385.
[54] M. Merle, G. Lefevre, and G. Milhaud, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 455.
[55] J. Janin, *Nature (London)*, 1979, **277**, 491.
[56] S. Sprang, D. Yang, and R. J. Fletterick, *Nature (London)*, 1979, **280**, 333.
[57] R. J. P. Williams, *Biochem. Soc. Trans.*, 1979, **7**, 481.
[58] R. E. Gates, *J. Mol. Biol.*, 1979, **127**, 345.
[59] G. M. Crippen and I. D. Kuntz, *Int. J. Pept. Protein Res.*, 1978, **12**, 47.
[60] I. D. Kuntz and G. M. Crippen, *Int. J. Pept. Protein Res.*, 1979, **13**, 223.
[61] M. Prabhakaran and P. K. Ponnuswamy, *J. Theor. Biol.*, 1979, **80**, 485.
[62] W. R. Krigbaum and A. Komoriya, *Biochim. Biophys. Acta*, 1979, **576**, 204.
[63] W. R. Krigbaum and A. Komoriya, *Biochim. Biophys. Acta*, 1979, **576**, 229.
[64] T. N. Bhat, V. Sasisekharan, and M. Vijayan, *Int. J. Pept. Protein Res.*, 1979, **13**, 170.
[65] A. V. Efimov, *J. Mol. Biol.*, 1979, **134**, 23.
[66] R. S. Morgan, S. L. Miller, and J. M. McAdon, *J. Mol. Biol.*, 1979, **127**, 31.
[67] A. A. Rashin and B. H. Yudman, *Mol. Biol.*, 1979, **13**, 698.
[68] G. D. Rose, *J. Mol. Biol.*, 1979, **134**, 447.
[69] T. L. Blundell, B. T. Sewell, and A. D. McLachlan, *Biochim. Biophys. Acta*, 1979, **580**, 24.
[70] N. S. Andreeva and A. E. Gustchina, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 32.
[71] A. D. McLachlan, *J. Mol. Biol.*, 1979, **128**, 49.

In 1971, Lee and Richards [72] introduced the notion of the surface area of an atom that is accessible to the solvent. This concept was used to quantify the extent to which non-polar side chains are buried and confirms that nearly all polar atoms that do not form hydrogen bonds are exposed to the solvent. Chothia noted [73] that for monomeric proteins of molecular weight 6000—35 000 dalton the total accessible surface area is proportional to (molecular weight)$^{2/3}$. Janin[55] examined 22 proteins and found that the number of buried residues (n_B) is related to the total number of residues (n) by $n^{1/3} - n_B{}^{1/3} =$ constant. This relationship is explained by a simple geometric argument. However, in a similar analysis, Sprang *et al.*[56] have added oligomeric proteins including, for the first time, phosphorylase which has a molecular weight of 97 400 dalton. They found that neither Chothia's [73] nor Janin's[55] relationship between accessibility and molecular weight applies to the phosphorylase monomer. This failure was attributed to the large multidomain structure of the enzyme. Williams [57] has pointed out that as proteins become more mobile the ratio of hydrophobic to charged amino-acids in their sequences decreases.

The packing density of globular proteins has been investigated by Crippen and Kuntz.[59, 60] They find considerable variation in the interior density. The low-density regions are primarily associated with clusters of non-polar side chains, whereas high density arises from the protein backbone adopting a regular secondary structure. Other workers have considered the radial distribution of residue types with regard to various chemical and conformational properties.[61-63] Bhat *et al.*[64] surveyed the observed conformation of side chains. Efimov [65] has noted that the allowed side-chain conformations are restricted when pairs of α-helices pack, owing to the requirement of the close packing of hydrophobic residues.

Monod *et al.* [74] emphasized that when a protein forms a dimer with isologous association the binding sites are identical for each member of the dimer. Morgan *et al.*[66] have developed an algorithm to establish whether part of the surface of a protein has the correct shape for isologous dimerization. They find that the surfaces of chymotrypsin and concanavalin A have the required symmetry, whereas that of the α-chain of human deoxyhaemoglobin does not, and hence does not dimerize. Rashin *et al.*[67] also consider the requirements for isologous molecular association.

In contrast to the above investigations which emphasize the location and conformation of individual residues, some workers have examined the relative positions of segments of the main chain. Rose [68] has developed a computer algorithm to dissect proteins into domains—'separable, continuous chain regions of the polypeptide chain'. All the proteins studied are formed from such domains. The smallest domains are α-helices and β-strands whilst the larger units can be decomposed hierarchically into these limiting components. McLachlan and others [69] find structural evidence that gene quadruplication could have occurred during the evolution of the serine and acid proteases.

Prediction of Tertiary Structure.—A variety of topics have been considered: the

[72] B. K. Lee and F. M. Richards, *J. Mol. Biol.*, 1971, **55**, 379.
[73] C. Chothia, *J. Mol. Biol.*, 1976, **105**, 1.
[74] J. Monod, J. Wyman, and J. P. Changeux, *J. Mol. Biol.*, 1965, **12**, 88.

evaluation of the success of a simulation,[75-77] the development of new methods of prediction,[78-87] and the investigation of the folding pathway.[88-91]

The success of a folding simulation is often quantified by the r.m.s. deviation between the predicted and the native structures. Cohen and Sternberg [75] point out that two measures of this deviation have been reported. One is based on superimposing the two structures so as to minimize the sum of squares of the distances between atoms made equivalent. The other is derived from a comparison of intramolecular distances. This leads to confusion as the first measure is 1.3 times the second. They also obtained a statistical measure of the success of a simulation. A series of compact, self-avoiding, random C^{α} structures was generated for different protein chains. For pancreatic trypsin inhibitor and for myoglobin, the mean and standard deviation of the r.m.s. deviation between the random and the crystal structures were 11.2 ± 1.4 Å and 16.2 ± 1.4 Å, respectively. Many previous simulations were found to be not much better than random predictions. Schulz [76] has performed similar random-walk studies to assess the evolutionary significance of a particular r.m.s. deviation between two structures.

Robson and Osguthorpe [78] have developed a new model for simplified energy calculations on proteins. The two backbone dihedral angles (ϕ, ψ) are represented by a single variable which samples most of the allowed conformations. Their simulation of folding begins with each residue assigned to the conformation indicated by prediction of secondary structure. The minimum-energy conformation of pancreatic trypsin inhibitor had an r.m.s. deviation from the native of 8 Å.

Because of the difficulties with energy calculations, several workers have tried to pack α-helices and β-strands into the native fold using simpler models. Cohen *et al.*[79] have developed a computer algorithm to pack α-helices in myoglobin. Over 10^8 trial structures were first generated by docking together all combinations of predicted hydrophobic patches on the surfaces of the native α-helices. However only 20 folds were found which did not violate the steric and chain-connectivity constraints. The additional use of two distance constraints on haem binding further restricted the number of structures to two.[80] The r.m.s. deviation between one of these structures and the native was 4.3 Å (see Figure 1).

Ptitsyn and his co-workers [81-83] have used models for the folding pathway of β-sheets to predict probable folds for the antiparallel and the parallel structures. For β-sheet sandwiches, such as those that occur in immunoglobulin domains, the

[75] F. E. Cohen and M. J. E. Sternberg, *J. Mol. Biol.*, 1980, **138**, 321.
[76] G. E. Schulz, *J. Mol. Biol.*, 1980, **138**, 335.
[77] L. T. J. Delbaere, G. D. Brayer, and M. N. G. James, *Nature (London)*, 1979, **279**, 165.
[78] B. Robson and D. J. Osguthorpe, *J. Mol. Biol.*, 1979, **132**, 19.
[79] F. E. Cohen, T. J. Richmond, and F. M. Richards, *J. Mol. Biol.*, 1979, **132**, 275.
[80] F. E. Cohen and M. J. E. Sternberg, *J. Mol. Biol.*, 1980, **137**, 9.
[81] O. B. Ptitsyn, A. V. Finkelstein, and P. Falk, *FEBS Lett.*, 1979, **101**, 1.
[82] O. B. Ptitsyn and A. V. Finkelstein, *Biofizika*, 1979, **24**, 27.
[83] A. V. Finkelstein, O. B. Ptitsyn, and P. Bendzko, *Biofizika*, 1979, **24**, 21.
[84] B. Gutte, M. Daumigen, and E. Wittschieber, *Nature (London)*, 1979, **281**, 650.
[85] J. S. Richardson, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 285.
[86] G. M. Crippen, *Int. J. Pept. Protein Res.*, 1979, **13**, 320.
[87] N. S. Goel and M. Ycas, *J. Theor. Biol.*, 1979, **77**, 253.
[88] M. Karplus and D. L. Weaver, *Biopolymers*, 1979, **18**, 1421.
[89] N. Go and H. Taketomi, *Int. J. Pept. Protein Res.*, 1979, **13**, 235.
[90] N. Go and H. Taketomi, *Int. J. Pept. Protein Res.*, 1979, **13**, 447.
[91] M. I. Kanehisa and T. Y. Tsong, *Biopolymers*, 1979, **18**, 1375.

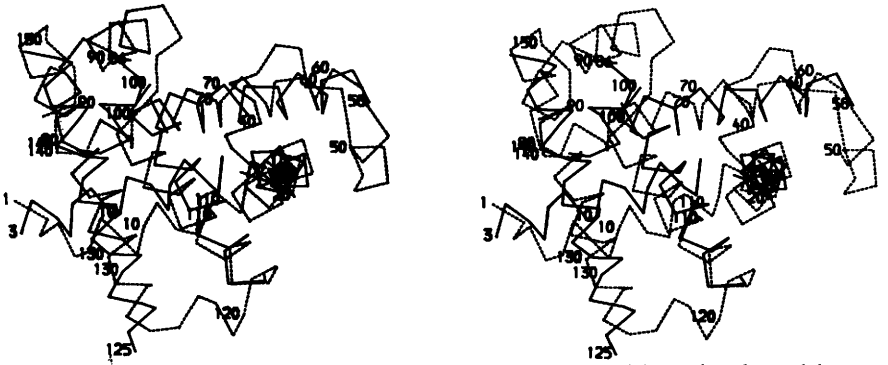


Figure 1 *The packing of helices in myoglobin. The super-imposition of the predicted myoglobin structure I97 seen here in solid lines on the crystallographically determined α-carbon co-ordinates connected by dotted lines. The r.m.s. deviation between these two structures is 4.5 Å. The other acceptable structure, I88, has an r.m.s. deviation of 0.3 Å from I97 and an r.m.s. deviation of 4.5 Å from the native*[80, 81]

requirement for a Greek-key and restrictions on connections reduces the number of allowed folds from between 10^2 and 10^8 to only a few. Gutte *et al.*[84] have used secondary-structure prediction and model building to suggest the sequence of a 34-residue polypeptide which would have nucleic acid binding activity. They synthesized this polypeptide and it showed strong interaction with single-stranded DNA.

Crippen and his co-workers[44, 86] have explored the use of distance geometry to predict protein structure. A series of structures is generated from a list of allowed inter-residue distances. For pancreatic trypsin inhibitor, the inclusion of hydrophobic contacts, turn positions, and disulphide-bridge information leads to generated structures in which the mean r.m.s. deviation from the native is *ca.* 9 Å.

Several workers have considered the mechanism of protein folding. Karplus and Weaver[88] detail their diffusion–collision–coalescence model. Go and Taketomi[89, 90] have continued work on Monte Carlo simulations on a two-dimensional lattice.

Protein Dynamics.—Distinct from the problem of the prediction of protein structure is the question of dynamic motion about the equilibrium position. The theoretical approach to this problem, the calculation of molecular dynamics,[92–99] attempts to solve the classical equations of motion defined by an empirical potential function for all non-hydrogen atoms and integrate these equations as a function of time. An experimental approach[100–102] has been to analyse the crystallographic

[92] P. J. Rossky and M. Karplus, *J. Am. Chem. Soc.*, 1979, **101**, 1913.
[93] R. H. Levy and M. Karplus, *Biopolymers*, 1979, **18**, 2465.
[94] W. L. Peticolas, *Biopolymers*, 1979, **18**, 747.
[95] M. Karplus and J. A. McCammon, *Nature (London)*, 1979, **277**, 578.
[96] J. A. McCammon, P. G. Wolynes, and M. Karplus, *Biochemistry*, 1979, **18**, 922.
[97] T. Ooi, K. Nishikawa, M. Oobatake, and H. A. Scheraga, *Biochim. Biophys. Acta*, 1978, **536**, 390.
[98] D. A. Case and M. Karplus, *J. Mol. Biol.*, 1979, **132**, 343.
[99] J. A. McCammon and M. Karplus, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3585.
[100] M. J. E. Sternberg, D. E. P. Grace, and D. C. Phillips, *J. Mol. Biol.*, 1979, **130**, 231.
[101] P. J. Artymiuk, C. C. F. Blake, D. E. P. Grace, S. J. Oatley, D. C. Phillips, and M. J. E. Sternberg, *Nature (London)*, 1979, **280**, 563.
[102] H. Frauenfelder, G. A. Petsko, and D. Tsernoglou, *Nature (London)*, 1979, **280**, 558.

temperature factors of a refined protein structure and express these as molecular motions. Detailed n.m.r. measurements of proteins have also been useful.[103-106]

Karplus and others have studied the vibrational modes of protein substructures[92-94] as well as whole proteins.[95-97] Work on α-helices has shown that thermal fluctuations could account for ϕ and ψ variations of 12 and 15°[93] and that fluctuations in helix length are small[94] and related to the character of hydrogen bonds.[93] Studies of the fluctuations of amino-acid residues in pancreatic trypsin inhibitor have revealed a mean-square positional displacement of 0.75 Å.[95] These include the rotation of tyrosine rings by as much as 30°.[96, 97] Complete rotational freedom is prevented by the matrix-damping effects of the protein.[96] The kinetics of ligand binding have also been studied by this method.[98, 99]

Experimental confirmation of these predicted modes of motion has come from two sources.[100-107] Phillips and others[100-102] have used the individual atomic temperature factors obtained from refined crystal structures to examine thermal fluctuations in proteins. An analysis of hen egg white[100] and human[101] lysozymes which crystallize in different space groups has produced a consistent picture of conformational variations possibly due to the high frequency modes of motion for lysozyme. A crystallographic study of myoglobin at a variety of temperatures[102] has produced a similar view of protein mobility. Mobility is inversely related to the distance from the centroid and is larger for amino-acid side chains than for the polypeptide backbone. This is consistent with the n.m.r. results of Wüthrich and his co-workers on PTI.[103-107] In a novel procedure, Richards[108] has attempted to explain n.m.r. measurements for hydrogen exchange and spectroscopic measurements of fluorescence quenching by studying volume fluctuations. These calculations describe the effect of packing defects which are commonly observed in protein structures.[109]

Conclusion.—We consider that one exciting aspect of work during this year is the variety of different techniques that have been developed to predict protein structure from sequence. A combination of secondary-structure prediction,[44-47] computerized model-building,[79-83] distance geometry,[44, 86] and simplified energy calculations[78] might well enable one to predict the approximate fold of many globular proteins.

2 Stability and Mechanism of Folding of Globular Proteins

Contributed by B. Adams

Stability.—Lesk and Chothia[110] examine how the different amino-acid sequences of the globins determine similar structures. They compare the atomic structure of nine globins, in which the sequence homology is as low as 16%. The principal structural determinants are apparently 59 residues involved in α-helix–α-helix and

[103] G. Wagner and K. Wüthrich, *J. Mol. Biol.*, 1979, **134**, 75.
[104] A. Dubs, G. Wagner, and K. Wüthrich, *Biochim. Biophys. Acta*, 1979, **557**, 177.
[105] R. Richarz, P. Sehr, G. Wagner, and K. Wüthrich, *J. Mol. Biol.*, 1979, **130**, 19.
[106] G. Wagner and K. Wüthrich, *J. Mol. Biol.*, 1979, **130**, 31.
[107] K. Wüthrich and G. Wagner, *J. Mol. Biol.*, 1979, **130**, 1.
[108] F. M. Richards, *Carlsberg Res. Commun.*, 1979, **44**, 47.
[109] F. M. Richards, *J. Mol. Biol.*, 1974, **82**, 1.
[110] A. M. Lesk and C. Chothia, *J. Mol. Biol.*, 1980, **136**, 225.

α-helix–haem packings. Buried side-chain residues were kept non-polar but not necessarily the same size; the mean variation in size of amino-acids at a given site is 56 Å^3. There is also a shift in relative helix orientation. These shifts are coupled to maintain the geometry of the haem-building site.

Studies on nuclease A and B show that the refolding is apparently identical for both proteins.[111] Nuclease B has an extra 19 residues on the amino-terminal end, and the folding is apparently thermodynamically and kinetically independent of these residues.

Argos *et al.*[112] compare the sequences of thermophilic and mesophilic proteins. Their results indicate that thermal stability is increased by small changes throughout the molecule. These changes increase the internal and decrease the external hydrophobicity and increase the proportion of helix-forming residues in helices.

By modifying lysine residues of cytochrome *c* and examining the stability against denaturation Schejter *et al.*[113] were able to quantify the effect of electrostatic forces on the stability of cytochrome *c*. A value of 5.6 kcal mol^{-1} is obtained for the stability of unmodified cytochrome *c*.

Roy *et al.*[114] examined the role of hydrogen bonding on the stability of oxytocin. They synthesized analogues of the hormone in which residues involved in hydrogen bonding were replaced by other amino-acids incapable of forming hydrogen bonds. These analogues invoked a biological response, but with lower potency than the native hormone. This indicates that hydrogen bonds do not constitute a conformational constraint essential for hormone activity.

The effect of the γ-turn in the stabilization of bradykinin has been investigated.[115] Changes in the c.d. spectrum were used to probe the overall conformation and ^{13}C n.m.r. the γ-turn, as temperature was increased. The γ-turn persisted at temperatures above which a pronounced change in the c.d. spectrum had taken place. The γ-turn is therefore apparently not involved in the thermal stability of this peptide.

Conformational Dynamics.—The overall picture of protein fluidity indicates rather restricted motion in the centre and freer motion towards the outside, especially near the active site. The motion is probably essential in both function and regulation of a protein, as suggested by Huber.[116, 129]

Information on protein dynamics has been gained from protein crystallography. Using lysozyme crystals, motion has been apparently detected.[117, 118] The motion is greater towards the outside, especially at the active site, and lower in the centre of the protein. Only weak evidence was found for the proposed hinge bending, but this is probably due to the restraints of the crystal lattice. It must be remembered that,

[111] A. Davis, G. R. Parr, and H. Taniuchi, *Biochem. Biophys. Acta*, 1979, **578**, 505.
[112] P. Argos, M. G. Rossmann, U. M. Gran, H. Zuber, G. Frank, and J. D. Tratschin, *Biochemistry*, 1979, **18**, 5698.
[113] A. Schejter, M. Zuckerman, and I. Aviram, *J. Biol. Chem.*, 1979, **254**, 7042.
[114] J. Roy, U. Roy, D. C. Gozin, and I. L. Schwartz, *Proc. Natl. Adcad. Sci. USA*, 1979, **76**, 3309.
[115] J. R. Cann, R. E. London, J. M. Stewart, and N. A. Matwigoff, *Int. J. Pept. Protein Res.*, 1979, **14**, 388.
[116] R. Huber, *Trends Biochem. Sci.*, 1979, **4**, 271.
[117] M. J. E. Sternberg, D. E. P. Grace, and D. C. Phillips, *J. Mol. Biol.*, 1979, **130**, 231.
[118] P. J. Artymiuk, C. C. F. Blake, D. E. P. Grace, S. J. Oatley, D. C. Phillips, and M. J. E. Sternberg, *Nature (London)*, 1979, **280**, 563.

using this technique, it is impossible to differentiate between thermal motion and static disorder.

Another group, using metmyoglobin were able to get round this problem by carrying out *X*-ray diffraction at four temperatures between 220 and 300 K.[119] This gives the spatial distribution of thermal motion and showed a condensed, fairly rigid core and a semi-liquid structure towards the outside.

Hydrogen-exchange kinetics have also been used.[120] This shows slow exchange of proton in the protein core. This is explained in terms of small structural motion or fluctuation between two states.

Time-resolved fluorescence polarization of tryptophan has been studied with proteins containing only one tryptophan residue by the use of synchrotron radiation.[121] Varying degrees of motions were seen ranging from no internal motion to subnanosecond fluctuations. Functionally significant changes in conformation can take place in the picosecond time scale. Another group working on haemoglobin A have conjugated cystein-93 with 1,5-AEDANS, a fluorescent probe.[122] The decay of fluorescence anisotropy indicated internal degrees of rotational freedom.

Proton relaxation parameters were used to detect motion in angiotensin pentapeptide.[123] This indicated that side-chain movement was different from that of the backbone. N.m.r. was also used to view internal motion in the myosin S1 fragment.[124] Much of this motion was lost on association with actin.

Theoretical support for internal motion comes from Efimov[125] who, working with the packing of α-helices in globular proteins, predicts rotational isomers of helical regions.

There is also evidence for proteins undergoing large changes in overall structure. Stopped-flow fluorescence measurements indicate two conformational states for acetylcholinesterase;[126] the change occurs after binding by bisquaternary ammonium ligands. Also phosphoglycerate kinase is believed to have a hinge-bending mechanism of catalysis;[127, 128] the change in conformation is induced by substrate binding.

Effect of Ligands.—The influence of co-enzyme on the reactivation of yeast glyceraldehyde-3-phosphate dehydrogenase has been investigated.[130] NADH and NAD^+ both increased the rate of reactivation, which involved both the refolding of the monomers and the association of these monomers into tetramers.

The effect of strong ligands on trypsinogen has also been studied.[131] On binding

[119] H. Frauenfelder, G. A. Petsko, and D. Tsernoglou, *Nature (London)*, 1979, **280**, 558.
[120] C. K. Woodward and B. D. Hilton, *Annu. Rev. Biophys. Bioeng.*, 1979, **8**, 99.
[121] I. Munro, I. Pecht, and L. Stryer, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 56.
[122] E. Bucci, C. Fronticelli, K. Flanigan, J. Perlman, and R. F. Steiner, *Biopolymers*, 1979, **18**, 1261.
[123] N. Niccolai, M. P. Miler, and W. A. Gibbons, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 157.
[124] S. Highsmith, K. Alasaka, M. Konrad, R. Goody, K. Halmer, N. Wade-Jardetzky, and O. Jardetzky, *Biochemistry*, 1979, **18**, 4238.
[125] A. V. Efimov, *J. Mol. Biol.*, 1979, **134**, 23.
[126] M. B. Bolger and P. Taylor, *Biochemistry*, 1979, **18**, 3622.
[127] R. D. Banks, C. C. F. Blake, P. R. Evans, R. Haser, D. W. Rice, G. W. Hardy, M. Mernet, and A. W. Phillips, *Nature (London)*, 1979, **279**, 773.
[128] C. A. Pickover, D. B. McKay, D. M. Engelman, and T. A. Steitz, *J. Biol. Chem.*, 1979, **254**, 11323.
[129] R. Huber, *Nature (London)*, 1979, **280**, 538.
[130] H. Krebs, R. Rudolph, and R. Jaenicke, *Eur. J. Biochem.*, 1979, **100**, 359.
[131] W. Bode, *J. Mol. Biol.*, 1979, **127**, 357.

to the dipeptide Ile-Val, β-guanidinobenzoate-trypsinogen takes up a trypsin-like conformation as observed from its c.d. spectrum.

The effect of certain penicillin substrates on penicillinase conformation and activity has been reviewed.[132]

Solvent Effects on Denaturation and the Denatured State.—Ahmad and Salahuddin have studied the effect of pH on the reversible unfolding of ovalbumin by guanidine hydrochloride.[133] The lower the pH the greater is the unfolding by a given concentration of guanidine hydrochloride. The unfolded forms are apparently viscometrically identical whatever the pH and are believed to be totally structureless.

In a paper by Ahmad and Bigelow [134] the effect of urea and salt denaturants on ribonuclease A is investigated. On the addition of salt to ribonuclease A a co-operative unfolding transition occurs. If urea is then added, a second co-operative transition takes place to a second, apparently less structured conformation. At low urea concentrations the salt transition occurs at a lower salt concentration. At high urea concentrations, but not high enough to cause denaturation by itself, salt causes a urea-type transition. Low salt concentration stabilizes the protein against urea, but higher salt concentration destabilizes it.

Jirgenson showed the existence of more than one denatured state for leuco-agglutinin.[135] Acidification induces the formation of large amounts of β-sheet. Sodium dodecyl sulphate causes the formation of an α-helix; this effect is enhanced by lower pH. Edta was shown to enhance denaturation, indicating that calcium ions help to maintain conformation.

Protein Fragments and Complementation.—Elongation factor G,[136] *E. coli* tryptophan synthetase,[137] thermolysin,[138] thioredoxin,[139] and phosphoglycerate kinase [140] have been cleaved into peptides corresponding to structural domains. These peptides have all been shown to be capable of reforming their native structure. The isolated domains of elongation factor G showed slight destabilization to denaturation. Peptides of thioredoxin have been shown to be capable of complementation to give a dimer with 1—2% of the native activity.

Labhardt and Baldwin have continued work on the refolding of ribonuclease S. They showed a fast refolding step when the S-peptide and S-protein join and a slower refolding step following the re-association.[141] This apparently rules out the possibility of a rate-limiting nucleation event followed by rapid propagation of the native structure and indicates that the S-peptide may serve to stabilize a folding intermediate. In a second paper they characterized by kinetic studies a stable

[132] R. H. Pain and R. Virden in 'Beta-Lactamases', ed. J. M. T. Hamilton-Miller and J. T. Smith, Academic Press, London, 1980, p. 170.
[133] F. Ahmad and A. Salahuddin, *Biochem. Biophys. Acta*, 1979, **576**, 333.
[134] F. Ahmad and C. C. Bigelow, *J. Mol. Biol.*, 1979, **131**, 607.
[135] B. Jirgenson, *Biochem. Biophys. Acta*, 1979, **577**, 307.
[136] Y. B. Alakov, O. A. Stengrevics, V. V. Filimonov, and S. Y. Venyaminov, *Eur. J. Biochem.*, 1979, **99**, 585.
[137] M. E. Goldberg and A. Hogberg-Raiband, *J. Biol. Chem.*, 1979, **254**, 7752.
[138] C. Vita, A. Fontana, J. R. Seeman, and I. M. Chaiken, *Biochemistry*, 1979, **18**, 3023.
[139] I. Slaby and A. Holmgren, *Biochemistry*, 1979, **18**, 5584.
[140] B. Adams, R. J. Burgess, E. A. Carrey, I. R. Mackintosh, C. Mitchinson, R. M. Thomas, and R. H. Pain in 'Protein Folding', ed. R. Jaenicke, Springer-Verlag, Heidelberg, 1980, p. 447.
[141] A. M. Labhardt and R. L. Baldwin, *J. Mol. Biol.*, 1979, **135**, 231.

folding intermediate of the S-protein at pH = 1.7.[142] This intermediate combines faster with the S-peptide than does the fully denatured S-protein.

Synthetic Proteins which Fold.—The problem of specifically joining two large peptides has been overcome in the production of synthetic cytochrome *c* from natural and synthetic peptides.[143-146] Peptides from the cyanogen bromide cleavage of cytochrome *c* were separated and the ε-amino groups were protected. The *N*-terminal amino-groups were then modified with *N*-hydroxysuccinimidyl-*t*-butyloxycarbonyl-L-methionate. These peptides were then condensed with peptides, either natural or synthetic, with an azide derivative at their *C*-terminus. Cytochrome *c* produced in this manner was apparently identical in structure and function to natural cytochrome *c*.

A 34-amino-acid peptide that binds single-stranded DNA and CMP (and cleaves RNA next to C) has been synthesized.[147] The choice of primary structure was based on the secondary-structure prediction of Chou and Fasman to give a $\beta\beta\alpha$ structure and designed to fold on the anticodon of $tRNA^{Phe}$ (GAA). The peptide was, however, designed to bind guanine rather than cytosine.

Identification of Folding Intermediates.—Intermediates in the refolding of reduced ribonuclease A have been studied after trapping by rapid alkylation with iodoacetate.[148] Intermediates containing 1—4 disulphide bonds had no enzymatic or native antigenic activity. This suggested a two-state folding process where the intermediates are rapidly interconvertible, relatively unstable microstates of the unfolded protein. This is in agreement with a ^{13}C n.m.r. study of the thermal unfolding of this protein [149] which describes the reaction as being loosely two-state, but showing evidence of minor intermediates.

Schmid and Baldwin [150] investigated the refolding of ribonuclease A by studying the protection of amide protons against exchange. The evidence indicated that an intermediate exists in which more than one-third of the protons are protected against exchange. This is probably due to hydrogen bonding, because water exclusion, as seen by tyrosine absorbance, occurs later in the folding process. Intermediates have also been seen by lowering the pH [151] and in a photochemical surface-labelling study.[152] These results indicate a sequential folding process with kinetically observable intermediates.

Reduced ribonuclease was studied by an immunological determination of the folding of ribonuclease during air oxidation.[153] The protein was shown to refold region by region, starting with a central region. This is in agreement with a

[142] A. M. Labhardt and R. L. Baldwin, *J. Mol. Biol.*, 1979, **135**, 245.
[143] D. L. Ledden, P. T. Nix, and P. K. Warme, *Biochem. Biophys. Acta*, 1979, **578**, 401.
[144] P. T. Nix and P. K. Warme, *Biochem. Biophys. Acta*, 1979, **578**, 413.
[145] P. J. Boon, G. I. Tesser, and R. J. F. Nisard, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 61.
[146] C. J. A. Wallace and R. E. Offord, *Biochem. J.*, 1979, **179**, 169.
[147] B. Gutte, M. Däumigen, and E. Wittschieber, *Nature (London)*, 1979, **281**, 650.
[148] T. E. Creighton, *J. Mol. Biol.*, 1979, **129**, 411.
[149] O. W. Howarth, *Biochem. Biophys. Acta*, 1979, **576**, 163.
[150] F. X. Schmid and R. L. Baldwin, *J. Mol. Biol.*, 1979, **135**, 199.
[151] P. J. Hagerman, F. X. Schmid, and R. L. Baldwin, *Biochemistry*, 1979, **18**, 293.
[152] R. R. Matheson and H. A. Scheraga, *Biochemistry*, 1979, **18**, 2437.
[153] L. G. Chavez and H. A. Scheraga, *Biochemistry*, 1977, **16**, 1849.

theoretical pathway for the refolding derived from a contact map computed from crystallographic studies.[154]

A stable intermediate in the unfolding of diazoacetylglycine ethyl ester–pepsin by urea has been characterized.[155] This intermediate can be studied in 8M-urea even at pH = 4.3 and 25 °C. It had a higher viscosity and lower secondary structure than the native protein.

A paper by Light and Odozynski suggests the existence of more than one refolding pathway for bovine trypsinogen.[156] The refolding was studied by binding to pancreatic trypsin inhibitor Sepharose. Certain disulphides were specifically modified and the rest were reduced. It was shown that protein with one disulphide modified refolds slower than does unmodified trypsinogen. This suggested the presence of a second slower refolding pathway.

There is also evidence for intermediates in the refolding of penicillinase.[140, 157] In the second of these two papers urea gradient gels are used to examine intermediates. The unfolding is apparently two-state, with no detectable intermediates. The refolding, however, is not two-state. A rapid formation of state H (characterized in ref.140) is seen at intermediate urea concentrations. At low urea concentrations a second intermediate I is seen. This is more compact than H, but not quite as compact as the native protein.

Theories of Folding Pathways.—*Nucleation.* The peptide pivaloyl-Pro-Pro-Ala-NHMe was synthesized and shown by n.m.r. to have a 3_{10} helical conformation in solution.[158] It is suggested that, as the transition 3_{10} helix to α-helix is very easy, a similar peptide could act as a nucleation centre in a protein.

Proline Isomerization. In this theory it is suggested that the slow-refolding forms of unfolded proteins contain non-native proline isomers; the slow refolding is due to the slow kinetics of proline isomerization. The slow phase of reactivation has been investigated for eleven proteins, containing between 6 and 28 proline residues.[159] They all show first-order kinetics with half-times ranging from 0.15 to 12.1 min at 25 °C and increasing with increasing numbers of proline residues. The reactivation kinetics were independent of solvent viscosity and gave an activation energy of 19 kcal mole^{-1}. The rate of interconversion of the two forms of unfolded ribonuclease A has been shown to be independent of guanidinium chloride concentrations.[160] Both these results are consistent with proline isomerization but appear to be inconsistent with the cluster model (see below).

A ^{13}C n.m.r. study on the steric effects of proline isomerization in proline-containing oligopeptides has been carried out.[161] This revealed that the isomerization affects the chemical shifts of neighbouring residues and also that the position of the isomerization equilibrium of a proline residue depends on neighbouring residues.

[154] G. Nemethy and H. A. Sheraga, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6050.
[155] F. Ahmad and P. McPhie, *Can. J. Biochemistry*, 1979, **57**, 1090.
[156] A. Light and T. W. Odorzynski, *J. Biol. Chem.*, 1979, **254**, 9162.
[157] T. E. Creighton and R. H. Pain, *J. Mol. Biol.*, 1980, **137**, 431.
[158] Y. V. Venkatachalapathi and P. Balaram, *Nature (London)*, 1979, **281**, 83.
[159] E. Stellwagen, *J. Mol. Biol.*, 1979, **135**, 217.
[160] F. X. Schmid and R. L. Baldwin, *J. Mol. Biol.*, 1979, **133**, 285.
[161] R. Deslauriers, J. M. Becker, A. S. Steinfeld, and F. Naider, *Biopolymers*, 1979, **18**, 523.

At 0 °C, refolding, as measured by 2'-CMP binding, occurs 100 times faster than proline isomerization.[162] Assuming the proline-isomerization theory, this suggests either that folding increases the rate of proline isomerization or that folding can occur before proline isomerization. The former is analogous to a finding that folding can increase the rate of disulphide bond formation.[163]

The role of proline in protein folding has been reviewed by Freedman.[164] It is suggested that some non-native proline isomers are permissive, that is, they allow the formation of the native protein conformation even when they are in the non-native isomer. It would appear that, although proline isomerization may be a rate-limiting step in the refolding of many proteins, not all the proline residues are equally important.

Urea-gradient gels have been used to examine the refolding kinetics for several proteins.[165] The presence of slow- and fast-refolding states for unfolded ribonuclease, chymotrypsin, and α-chymotrypsinogen was shown. The refolding of the slow-folding states at 2 °C was faster than would be expected for *cis–trans* isomerism. This supports the theory that the incorrect proline isomer may not completely block refolding. Urea-gradient gels have shown little kinetic heterogeneity in the refolding of penicillinase,[157] a proline-containing protein.

Cluster Model. In the cluster model it is proposed that a protein in the process of folding initially forms small hydrophobic clusters. These clusters then come together to give the native protein structure.[166]

In three papers Wüthrich and his co-workers have used ^{1}H n.m.r. to investigate the conformation of the basic pancreatic trypsin inhibitor (BPTI). In the first paper they made individual assignments of amide proton resonances.[167]

After reduction of the disulphide bond at Cys-14 to Cys-38 in BPTI they examined the effects of adding charged groups, cleavage of the Lys-13—Ala-16 bond, and removal of Ala-16 and Arg-17.[168, 169] They showed a destabilizing effect per charged group of 1—2 kJ mole^{-1}. They also showed the changes were manifested in the amide exchange but not in the ^{1}H n.m.r. of the aromatic rings. Previous papers[170–172] indicated that the proton-exchange rate was promoted by structural fluctuation. They concluded that this was because the aromatic residues are buried in hydrophobic clusters whereas the amide groups are not.

Theoretical support for hydrophobic clusters comes from two papers: firstly, the suggestion that the microstability depends on the saturation of the protein structure with non-polar contacts,[173] secondly, the observation that non-polar side chains tend to occur in clusters.[174]

[162] K. H. Cook, F. X. Schmid, and R. L. Baldwin, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6157.
[163] D. E. Isenman, D. Lancet, and I. Pecht, *Biochemistry*, 1979, **18**, 3327.
[164] R. Freedman, *Nature (London)*, 1979, **279**, 756.
[165] T. E. Creighton, *J. Mol. Biol.*, 1980, **137**, 61.
[166] G. Wagner and K. Wüthrich, *Nature (London)*, 1978, **275**, 247.
[167] A. Dubz, G. Wagner, and K. Wüthrich, *Biochem. Biophys. Acta*, 1979, **577**, 177.
[168] G. Wagner, H. Tschesche, and K. Wüthrich, *Eur. J. Biochem.*, 1979, **95**, 239.
[169] G. Wagner, A. J. Kalb, and K. Wüthrich, *Eur. J. Biochem.*, 1979, **95**, 247.
[170] R. Richarz, P. Sehr, G. Wagner, and K. Wüthrich, *J. Mol. Biol.*, 1979, **130**, 19.
[171] G. Wagner and K. Wüthrich, *J. Mol. Biol.*, 1979, **130**, 31.
[172] G. Wagner and K. Wüthrich, *J. Mol. Biol*, 1979, **134**, 75.
[173] P. L. Privalov and T. N. Tsakova, *Nature (London)*, 1979, **280**, 693.
[174] W. R. Krigbaum and A. Komoriya, *Biochem. Biophys. Acta*, 1979, **576**, 204.

Rose discusses the idea that globular proteins are organized in a hierarchical manner, with domains and sub-domains [175] (see also ref. 1). It is suggested that this could be reflected in a folding process of hierarchical condensation; the pathway of folding is described as a tree of local folding interactions. This is also suggested in a paper by Karplus and Weaver,[176] who suggest as well that the microdomains could be α-helices, β-strands, or hydrophobic clusters.

Kanchisa and Tsong examine the dynamics of the cluster model of protein folding.[177] They predict that the rate-limiting step would be the formation of a critical-size cluster. This would be followed by rapid growth and then a second slow step, the merging of preformed cluster. However, both these slow steps could be expected to be dependent on the viscosity of the solvent, and this is not the case for many proteins.[159] But the cluster model should not be ruled out, as short-range interactions involved in forming the initial clusters or the shuffling of a relatively condensed protein in the merging of performed clusters would be independent of solvent viscosity.

Protein Folding *in vivo*.—Bergman and Kuehl describe the formation of disulphide bonds in nascent immunoglobulin polypeptides. Interchain bonds are often formed before completion of the heavy chains.[178] Intrachain disulphides in the *N*-terminal segment of the light chain are formed on nascent light chains.[179] This supports the theory that proteins fold, as they are made, from the *N*-termini. They suggest the *N*-terminal end of a protein has longer to fold than the rest of a protein so that *in vitro* studies on protein folding are not necessarily accurate models for *in vivo* protein folding.

However, it must be remembered that there is considerable evidence for separate domains folding independently.[136–140] The immunoglobulin light chain consists of two domains each having one disulphide bond. Once the *N*-terminal half of the protein is synthesized it can fold as a domain, including disulphide bond formation, but not necessarily from the *N*-terminal end.

3 Immunological Probes of Protein Conformation

Contributed by A. Benson

Introduction.—When used as immunogens under favourable circumstances, proteins elicit antibodies against antigenic sites on the surface exposed to the environment. This is important to protein chemists interested in conformational probes, since it is a specific recognition process between two complex molecules, occurring with great precision.[180] Rather than the use of a mixed specificity antibody population which is inevitably raised against any protein antigen, a more recent approach is to use monoclonal antibodies from mouse cell hybridomas elicited with a native protein.[181] The antigenic sites on the protein are not necessarily unique to that protein, and may also occur on a chemically modified

[175] G. D. Rose, *J. Mol. Biol.*, 1979, **134**, 447.
[176] M. Karplus and D. L. Weaver, *Biopolymers*, 1979, **18**, 1421.
[177] M. I. Kanchisa and T. Y. Tsong, *Biopolymers*, 1979, **18**, 1375.
[178] L. W. Bergman and W. M. Kuehl, *J. Biol. Chem.*, 1979, **254**, 5690.
[179] L. W. Bergman and W. M. Kuehl, *J. Biol. Chem.*, 1979, **254**, 8869.
[180] J. D. Capra and A. B. Edmundson, *Sci. Amer.*, 1977, **236**, 50.
[181] G. Kohler and C. Milstein, *Nature (London)*, 1975, **256**, 495.

protein or a related protein from another organ or organism.[182] In the case of a protein molecule having several immunogenic determinants, a mixed specificity population of antibodies, each bivalent, is elicited. These cross-link between identical sites on different molecules, thus forming a three-dimensional lattice, or precipitate. Utilizing this, precipitin reactions, such as double-diffusion in agar gels, with more sensitive radioimmunoassay and micro-complement fixation, are used to measure the extent of antigen–antibody binding.

Changes and Similarities in Protein Conformation.—The interaction of the antigen-combining site on the antibody with the antigenic sites on the protein depends heavily on the correct arrangement of the amino-acid side chains and thus on the backbone conformation in the area of interaction.[180] Chemical modification of the side chains of both cod allergen M [183] and Timothy grass pollen [184] degraded the immunochemical reaction against antibody specific to the native states.

The individual antigenic sites of lysozyme [185] and myoglobin,[186] both of known three-dimensional structure, have been studied extensively, while five antigenic sites have been localized for serum albumin.[187] These antigenic sites are found on the surface of the intact molecule, commonly at exposed sites, although radically different in structural terms. In myoglobin the site is composed of groups close together along the primary sequence[188] and depends mainly on the secondary structure, while in lysozyme the site is composed of groups from widely separated regions[189] and is dependent mainly upon tertiary folding, and also on conformational constraints, such as disulphide bridges.[190]

Antibodies can be produced against small fragments of zymogens, showing antigenic sites detectable on them when in isolation, but these fragments, when present in the intact zymogen, may be immunologically undetectable. In human prothrombin, antibodies directed against the activation fragment F_2 react minimally with the intact zymogen [191] owing to the masking of the fragment and the presence of a critical disulphide bridge. Antibodies against the native format of the γ-carboxyglutamic acid-rich region of bovine prothrombin have indicated a metal-induced conformational change,[192] and a sub-population of antibodies specific for antigenic determinants in the metal–prothrombin binary complex in metal-stabilized prothrombin loses binding ability in the absence of metal ions.[193] In the case of cod allergen M, the polymerization of the allergen reduces strongly the immunoreactivity with antibodies to the native state;[183] this indicates masking of antigenic determinants in the polymer.

It is possible that, while a change in antigenicity is a sensitive indication of

[182] M. J. Crumpton in 'The Antigens', ed. M. Sela, Academic Press, New York, 1974, Vol. 2, p. 1.
[183] J. Apold and S. Elsayed, *Mol. Immunol.*, 1979, **16**, 559.
[184] A. Malley, D. Begley, and A. Forsham, *Mol. Immunol.*, 1979, **16**, 929.
[185] M. Z. Atassi, *Immunochemistry*, 1978, **15**, 909.
[186] M. Z. Atassi in 'Immunochemistry of Proteins', ed. M. Z. Atassi, Plenum Press, New York, 1977, Vol. 2, p. 77.
[187] M. Z. Atassi, S. Sakata, and A. L. Kazim, *Biochem. J.*, 1979, **179**, 327.
[188] M. Z. Atassi, *Immunochemistry*, 1975, **12**, 423.
[189] M. Z. Atassi and C.-L. Lee, *Biochem. J.*, 1978, **171**, 419.
[190] M. Z. Atassi, C.-L. Lee, and R. C. Pai, *Biochim. Biophys. Acta*, 1976, **427**, 745.
[191] H. K. Lau, J. S. Rosenberg, D. L. Beeler, and R. D. Rosenberg, *J. Biol. Chem.*, 1979, **254**, 8751.
[192] B. Furie, K. L. Provost, R. A. Blanchard, and B. C. Furie, *J. Biol. Chem.*, 1978, **253**, 8980.
[193] B. Furie and B. C. Furie, *J. Biol. Chem.*, 1979, **254**, 9766.

surface-group rearrangements, even a considerable alteration in conformation may not be reflected in an antigenic change. The bovine serum fragment comprising the last one-third of the albumin molecule was modified at four of its eight arginine residues to give considerable conformational changes as shown by circular dichroism. However, no changes in immunochemical reactivity relative to the unmodified form were observed.[194]

Molecular Evolution.—Immunochemical cross-reactivity is a commonly used technique to estimate similarities between related proteins. However, it is subject to the restriction mentioned before that the surface features of a protein are subject to far less stringent evolutionary controls than are those of conformation-determining residues, except for general polar for polar substitution rules.[195] However, relationships based on the immune cross-reaction between proteins and enzymes, with the same function in different organisms, have been shown, such as β-galactosidases from different bacterial origins,[196] tryptophanyl-tRNA synthetase,[197] and between seed lectins of different origins.[198] These observations indicate the evolutionary stability of certain structural features, and may indicate a common evolutionary precursor. Closely related species may demonstrate an exclusive immune cross-reactivity, such as in anti-human kininogen antibodies reacting only with monkey serum kininogen,[199] whereas the immunological relationships of human and primary parotid secretion proline rich proteins suggest a common precursor molecule.[200]

With enzymes found in the same cell or organism, and with the same function, immune cross-reactivity can show protein relationships, but similar function and immune cross-reaction are not interdependent. Monoamine oxidases from different organs are immunologically identical, but differ functionally;[201] liver enzyme catalyses a higher ratio of β-phenylethylamine to 5-hydroxytryptamine oxidation, whereas the placental enzyme has a high ratio of 5-hydroxytryptamine to benzylamine oxidation. Conversely, three types of 3-deoxy-D-arabinoheptulosonate 7-phosphate synthetase, which catalyse the same reaction in a single cell, demonstrate little or no cross-reactivity.[202] Immunochemically different forms of enzymes found in different tissues have been studied. Esterases from rat liver microsomes cross-react with kidney and lung enzymes, but not with those of heart or brain.[203] Pig kidney diamine oxidase antibody shows no reaction with plasma diamine oxidase,[204] and amino-L-histidine hydrolase from mouse olfactory mucosa, kidney, and uterus is immunologically distinct from that of heart or liver.[205] Care must be taken when drawing conclusions of this type if the protein is

[194] A. L. Kazim, A. F. S. A. Habeeb, and M. Z. Atassi, *Mol. Immunol.*, 1979, **16**, 457.
[195] N. Arnheim in 'The Antigens', ed. M. Sela, Academic Press, New York, 1973, Vol. 1, p. 378.
[196] N. Guiso, J.-C. Hubert, and A. Ullman, *Mol. Immunol.*, 1979, **16**, 409.
[197] V. L. Sheinker, S. F. Berestein, A. M. Mazo, N. S. Ambartsumyan, O. V. Rokhlin, O. O. Favora, and L. L. Kesseler, *Eur. J. Biochem.*, 1979, **97**, 540.
[198] J. Howard, J. Kindinger, and L. M. Shannon, *Arch. Biochim. Biophys.*, 1979, **192**, 457.
[199] W. Sakamoto and O. Nishikaze, *J. Biochem.*, 1979, **86**, 1549.
[200] E. E. Kousvelari and F. G. Oppenheim, *Biochim. Biophys. Acta*, 1979, **578**, 76.
[201] S. M. Russel, J. Davey, and R. J. Mayer, *Biochem. J.*, 1979, **181**, 15.
[202] R. J. McCandless and K. M. Herman, *J. Biol. Chem.*, 1979, **254**, 3761.
[203] J. Anner and M. Raftell, *Mol. Immunol.*, 1979, **16**, 797.
[204] A. Oratore, P. Guerrier, A. Ballini, B. Mandori, and A. F. Agro, *FEBS Lett.*, 1979, **104**, 154.
[205] F. L. Margolis, M. Grillo, C. E. Brown, T. H. Williams, R. G. Pitcher, and G. L. Elgar, *Biochim. Biophys. Acta*, 1979, **570**, 311.

extracted by the use of detergents which may differentially modify the antigenic activity of otherwise similar structures.

Antibody Binding Effects.—A useful comparison for antigen–antibody binding is the 'lock and key' model proposed previously for enzyme–substrate recognition. However, this is increasingly treated with caution.[180] High-affinity antibody against a protein can shift the equilibrium between antigenically different conformations by binding preferentially to one of the conformations, by 'induced fit' of antigenic determinants, or by stabilizing the conformation.[182]

Examples of change induced by specific antibodies are (i) the increased affinity of oxygen for haemoglobin,[206] where antibody combines with the carboxyhaemoglobin to stabilize the liganded conformation and increase the oxygen affinity, (ii) the slow reversal of hapten-induced inhibition of malate dehydrogenase by hapten-specific antibody,[207] (iii) the increased specific binding of insulin to receptors in the presence of divalent anti-insulin antibody,[208] and (iv) the activation of liver phosphorylase to increased binding of glucose 1-phosphate by attachment of specific antibody to the NH_2 terminal region.[209] Inhibition of enzymes, from the interaction with antibody, may be a result of induced conformational change, as in phenylalanyl-tRNA synthetase,[210] where antibody against any of the subunits affects the function of other subunits; this may also be the case with the antibody inhibition of tryptophanyl-tRNA synthetase.[197] Inhibition of enzyme activity, when used in cross-reaction tests, can show differences in enzymes. Marked differences in cytochrome P-450 activity of chemically induced microsomes, compared to normal non-treated control microsomes, were shown by cross-inhibition tests,[211] whereas antibodies which inhibit the actin-activated Mg-ATPase activity of myosin II do not inhibit myosin IA,[212] thus indicating conformational differences. Steric hindrance exerted by binding of antibody can be reflected in substrate specificity, as many enzymes active against low molecular weight substrates are not significantly inhibited by specific antibody, although activity towards high molecular weight substrates may be decreased.[213] Glycogen phosphorylase *b*, when complexed with antibody, demonstrates a lowered glycogen affinity, while glucose 1-phosphate affinity was unaffected.[214] The same antibody concentration inhibits metabolism of 7-ethoxycoumarine rather than benzphetamine by cytochrome P-450.[215]

Activating antibodies, which 'lock' the enzyme β-lactamase into a particular conformation, operate in two types of stimulating phenomena: first, they directly increase the activity of the enzyme against a substrate, such as benzylpenicillin, by four times and, second, they relieve deactivation brought about by a substrate

[206] J. Dean and A. N. Schechter, *J. Biol. Chem.*, 1979, **254**, 9185.
[207] E. F. Ullman, R. A. Yoshida, J. I. Blakemore, E. Maggio, and R. Leute, *Biochim. Biophys. Acta*, 1979, **567**, 66.
[208] Y. Schechter, K. J. Chang, S. Jacobs, and P. Cuatrecasas, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2720.
[209] A. M. Janski and D. J. Graves, *J. Biol. Chem.*, 1979, **254**, 1644.
[210] H. Hennecke, I. Walter, and H. Franz, *Hoppe-Seyler's Z. Physiol. Chem.*, 1977, **356**, 197.
[211] L. S. Kaminsky, M. J. Fasco, and F. P. Guengerich, *J. Biol. Chem.*, 1979, **254**, 9657.
[212] H. Gadasi and E. D. Kom, *J. Biol. Chem.*, 1979, **254**, 8095.
[213] B. Cinader in 'Antibodies to Biologically Active Molecules', ed. B. Cinader, Pergamon Press, Oxford, 1967, p. 88.
[214] S. J. Tzartos and A. E. Evangelopolous, *Eur. J. Biochem.*, 1977, **74**, 233.
[215] C. J. Serabjit-Singh, C. R. Wolf, and R. M. Philpot, *J. Biol. Chem.*, 1979, **254**, 9657.

such as methicillin (reviewed in ref. 216). At high ratios of antiserum to rabbit AMP deaminase, inhibition was partly reversed; this is interpreted as a differential binding of inhibiting and activating antibody.[217]

Conformational Equilibria.—The attainment of native-like three-dimensional structure by isolated protein fragments, in spite of the general requirements of overall sequence, has been shown by immunochemical evidence. The reduced fragments of albumin were shown to re-form native-like structures,[218] and fragment 13—105 of hen egg white lysozyme was shown to regenerate native-like structures.[219] The cyanogen bromide fragments FI (when Ca-stabilized) and FII of thermolysin both have the propensity to form native-like local conformations and form sufficient stabilizing interactions to maintain the structure at equilibrium.[220] Polypeptide fragments from cod allergen M, one analogous to two domains, the other to one domain in the intact molecule, neutralize a large proportion of antibodies against the native state; this indicates structural homology between fragments and native molecule.[221]

The use of antibody against folded and unfolded protein structure has been used to examine conformational properties. Creighton, Kalef, and Avon [222] have developed a method for the immunochemical analysis of the conformational properties of intermediates trapped in the unfolding of bovine pancreatic trypsin inhibitor. The interactions of each of the trapped intermediates, all with disulphide bonds frozen, with antibodies specific for the native folded or reduced unfolded protein, were used to determine the probability of the various segments of the polypeptides adopting either configuration. They concluded that no segment of the polypeptide chain adopts the stable native form until the entire chain is able to do so simultaneously.

4 Nuclear Magnetic Resonance

Contributed by H. W. E. Rattle

With the volume of protein n.m.r. literature continuing to rise steeply, it is a pleasure to be able to report no decline in the quality of the work described. General reviews are relatively few, although one may be found in Methods in Biochemical Analysis,[223] and a volume of collected reviews [224] may be of interest: it contains critical and pleasantly practical articles on n.m.r. of serine proteases and of model compounds in the study of haem proteins, among others.

Methods.—An instrumental method of promise is the so-called two-dimensional *J*-resolved spectroscopy. This is simply described,[225] as are methods of performing

[216] R. H. Pain and R. Virden in 'Beta-Lactamases', ed. J. M. T. Hamilton-Miller and J. T. Smith, Academic Press, New York, 1979, p. 141.
[217] D. J. Selby and O. P. Chilson, *Mol. Immunol.*, 1979, **16**, 631.
[218] J. M. Teale and D. C. Benjamin, *J. Biol. Chem.*, 1977, **252**, 4521.
[219] E. R. Johnson, W. L. Anderson, D. B. Wetlaufer, G.-Li Lee, and M. Z. Atassi, *J. Biol. Chem.*, 1978, **253**, 3408.
[220] C. Vita, A. Fontana, J. R. Seeman, and I. M. Chaiken, *Biochemistry*, 1979, **18**, 3023.
[221] J. Apold and S. Elsayed, *Mol. Immunol.*, 1979, **16**, 205.
[222] T. E. Creighton, E. Kalef, and R. Arnon, *J. Mol. Biol.*, 1978, **123**, 129.
[223] I. D. Campbell and C. M. Dobson, *Methods Biochem. Anal.*, 1979, **25**, 1.
[224] 'Biological Applications of Magnetic Resonance', ed. R. G. Shulman, Academic Press, New York, 1979.
[225] K. Wüthrich, K. Nagayama, and R. Ernst, *Trends Biochem. Sci.*, 1979, **4**, N178.

selective spin decoupling in the *J*-resolved mode.[226] We have long been used to acronyms for n.m.r. techniques, such as DEFT and WEFT; the trend takes a turn to the permissive with SECSY—spin-echo correlated spectroscopy—which yields two-dimensional homonuclear correlated spectra with manifest connectivities between spin-coupled nuclei and should prove useful for the study of biopolymers.[227] Pulse-sequence methods have also been used in a comparative study of spin-echo decays for protons of various globular proteins in solution [228] and in measuring spin–lattice decay of small molecules in solutions containing proteins.[229] The conformations of small molecules bound to proteins may also be investigated by using transfer of saturation effects—irradiation at the frequency of a given proton in the bound ligand gives an intensity change in the signal of a different proton in the excess free ligand *via* a nuclear Overhauser effect between the two protons while the ligand is still bound. The protons must be within *ca.* 4 Å of each other for this to occur, and structural information is thus made available.[230] Two more familiar experimental approaches, proton-relaxation enhancement [231] and spin-labelling,[232] have been critically surveyed and reviewed. Newly developed labelling techniques include the reductive methylation of free NH_2 groups using sodium cyanoborohydride, which methylates only free lysines and the terminal amino-group of the protein, and may be used to introduce methyl groups labelled with ^{13}C, ^{14}C, ^{2}H, or ^{3}H into the molecule.[233] It is pointed out [234] that the ^{13}C signal from methionine sulphoxide is 22.6 p.p.m. downfield of that from methionine, thus providing a means for determining the extent of methionine oxidation in intact protein. Other considerations of more general interest to n.m.r. in biochemistry include a theoretical calculation of heteronuclear vicinal coupling constants in peptides and their angular dependence,[235] the choice of coupling-constant parameters for conformational calculations,[236] and an outline of the use of amide-proton titration shifts for conformational studies of polypeptides, especially those involving hydrogen bonding.[237] A considerable mass of chemical-shift and spin–spin splitting data for all the common amino-acids, determined using specially synthesized tetrapeptides,[238] will be of great value to many who use ^{1}H n.m.r. on proteins. Deuterium-isotope effects on ^{13}C chemical shifts of amino-acids are considered,[239] while a general theory of internal librations and rotations in

[226] K. Nagayama, P. Bachmann, R. R. Ernst, and K. Wüthrich, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 218.
[227] K. Nagayama, K. Wüthrich, and R. R. Ernst, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 305.
[228] S. I. Aksenov and A. V. Filatov, *Biofizika*, 1979, **24**, 602.
[229] D. L. Rabenstein, T. Nakashima, and G. Bigam, *J. Magn. Reson.*, 1979, **34**, 669.
[230] J. P. Allbrand, B. Birdsall, J. Feeney, G. C. K. Roberts, and A. S. V Burgen, *Int. J. Biol. Macromol.*, 1979, **1**, 37.
[231] D. R. Burton, S. Forsen, K. Gunnar, and R. A. Dwek, *Progr. Nucl. Magn. Reson. Spectrosc.*, 1979, **13**, 1.
[232] J. S. Hyde, H. M. Swartz, and W. E. Antholine, *Spin Labelling*, 1979, **2**, 71.
[233] N. Jentoft and D. J. Dearborn, *J. Biol. Chem.*, 1979, **254**, 4359.
[234] J. S. Cohen, J. Yariv, A. J. Kalb, L. Jacobson, and Y. Schechter, *J. Biochem. Biophys. Methods*, 1979, **1**, 145.
[235] P. Mohanakrishnan and K. R. K. Easwaran, *Biopolymers*, 1979, **18**, 1769.
[236] R. B. Martin, *J. Phys. Chem.*, 1979, **83**, 2404.
[237] A. Bundi and K. Wüthrich, *Biopolymers*, 1979, **18**, 299.
[238] A. Bundi and K. Wüthrich, *Biopolymers*, 1979, **18**, 285.
[239] J. J. Led and S. B. Petersen, *J. Magn. Reson.*, 1979, **33**, 603.

molecules has been developed and applied to the field, temperature, and concentration dependence of the ^{13}C spin–lattice relaxation and nuclear Overhauser enhancements for a number of polymers and peptides.[240] The activation energy for most of the observed motions is 20—30 kJ mole^{-1}.

Amino-acids and Synthetic Peptides.—Perhaps not surprisingly, the amino-acid which attracts most attention is proline. The ring conformations of this molecule have been studied using lanthanide-induced shifts[241] and the effects of ring-puckering on ^{13}C relaxation times investigated,[242] while the approximate conformation of the related *cis*-D-allo-hydroxyproline has been reinvestigated.[243] Evidence is presented for two different *trans* conformers in poly(L-proline) and poly(γ-hydroxy-L-proline) and for *cis–trans* isomerism in the polymer,[244] a conclusion supported by work on proline-containing tripeptides.[245] In inert solvents such peptides form various β-turns depending on sequence.[246] The triple helix formed by the collagen model peptide (Pro-Pro-Gly)$_{10}$ may be unfolded at higher temperatures with large resultant changes in the ^{13}C spectrum.[247] When proline is incorporated into cyclic peptides, it is a major determinant of conformation; studies published recently report a boat conformation for *cyclo*-(L-Pro$_2$-D-Pro),[248] the ^{15}N resonance investigation of the known β- and γ-turns in *cyclo*-(Gly-Pro-Gly-D-Ala-Pro),[249] the agreement between crystalline and solution conformations (determined by ^{13}C n.m.r.) for *cyclo*-(Pro)$_3$,[250] an all-*trans* conformation containing a type II β-turn for *cyclo*-(Gly-Pro-Ser-D-Ala-Pro) in inert solvents,[251] and the side-chain conformations in *cyclo*-(Asp-Pro) determined by ^{13}C—^{13}C vicinal coupling constants.[252] The pH dependence of ^{13}C chemical shifts between diastereoisomeric linear peptides, including some proline-containing ones, was found to be small,[253] which contrasts significantly with previous findings on *cyclo*-(Pro-Leu) and *cyclo*-(Pro-D-Leu), where major differences were observed between the isomers in the more highly constrained structures.

Several papers published in 1979 report investigations into aromatic amino-acid side chains as factors in molecular recognition. Specific recognition of single-stranded nucleic acids by tyrosine-containing peptides is claimed[254] while phenylalanine and tryptophan are bound specifically to β-NAD$^+$.[255] A small chemotactic peptide, formyl-L-Met-L-Leu-L-Phe, is shown to take up a β-conformation which is

[240] O. W. Howarth, *J. Chem. Soc., Faraday Trans. 2*, 1979, **75**, 863.
[241] D. F. DeTar and N. P. Luthra, *J. Org. Chem.*, 1979, **44**, 3299.
[242] R. E. London, *J. Am. Chem. Soc.*, 1978, **100**, 2678.
[243] M. J. O. Anteunis, P. Cjypers, and M. Budesinsky, *Bull. Soc. Chim. Belg.*, 1979, **88**, 147.
[244] D. S. Clark, J. J. Dechter, and L. Mandelkern, *Macromolecules*, 1979, **12**, 626.
[245] R. Deslauriers, J. M. Becker, A. S. Steinfeld, and F. Naider, *Biopolymers*, 1979, **18**, 523.
[246] G. Boussard, M. Marraud, and A. Aubry, *Biopolymers*, 1979, **18**, 1297.
[247] R. Di Blasi and A. S. Verdini, *Biopolymers*, 1979, **18**, 735.
[248] J. W. Bats, A. Friedrich, H. Fuess, H. Kessler, W. Maestle, and M. Rothe, *Angew. Chem.*, 1979, **91**, 573.
[249] K. L. Williamson, L. G. Pease, and J. D. Roberts, *J. Am. Chem. Soc.*, 1979, **101**, 714.
[250] J. Vicar, K. Blaha, F. Piriou, P. Fromageot, and S. Fermandjian, *Biopolymers*, 1979, **18**, 1021.
[251] L. G. Pease, C. H. Niu, and G. Zimmermann, *J. Am. Chem. Soc.*, 1979, **101**, 184.
[252] F. Piriou, F. Toma, J. Savrda, and S. Fermandjian, *Tetrahedron*, 1979, **35**, 441.
[253] R. Deslauriers, I. C. P. Smith, R. L. Somorjai, E. Ralston, R. C. Orlowski, R. W. Walter, *Int. J. Pept. Protein Res.*, 1979, **13**, 473.
[254] R. Mayer, F. Toulme, and T. H. C. Montenay-Garestier, *J. Biol. Chem.*, 1979, **254**, 75.
[255] K. J. Neurohr and H. H. Mantsch, *Can. J. Chem.*, 1979, **57**, 2297.

presumably important to its recognition by the receptor;[256] the side-chain orientations of tryptophan in Trp-Gly peptides have also been discussed.[257] Other small peptides whose conformations have been investigated by n.m.r. include *cyclo*-(L-Cys-L-Cys),[258] peptides containing α-aminoisobutyric acid,[259] and arginyl-glutamic acid dipeptides.[260] Side chain–backbone hydrogen bonds in peptides containing glutamic acid[261] and racemization of lysyldipeptides have been studied.[262] Racemization and stereospecificity are also major concerns in a series of ^{15}N studies of peptides containing glycine and L- and D-alanine.[263–266] With ^{13}C and ^{1}H resonance so firmly established now, ^{15}N is our one remaining new probe into peptide backbones.

Synthetic polypeptides of higher molecular weight attract far less attention than they once did, but their ability to form simple α- and β-structures still makes them valuable. Those interested may find conformational and/or relaxation measurements of poly(L-lysine),[267] poly(γ-ethyl-*N*-methyl-L-glutamate),[268] poly-(Lys^{50}Tyr^{50}) and -(Lys^{50}Phe^{50}),[269] poly(D-glutamic acid),[270] and poly(γ-benzyl-L-glutamate), poly(benzyl-L-aspartate), and poly(L-alanine).[271] Liquid crystals of poly(γ-benzyl glutamate) are orientated with the helical polymer rods parallel to the magnetic field applied in the n.m.r. spectrometer.[272] The age-old dispute over n.m.r.-determined helix contents in synthetic polypeptides in solution rolls on, with theoretical calculations of conformational life-times[273] and a report (which this reviewer finds hard to believe) that poly(L-alanine) is helical in solution in trifluoroacetic acid.[274]

Turning to research more directly biochemical in content, we find a report on zinc and cobalt binding to glycyl-L-tyrosine, pseudo-substrate for the Zn-containing enzyme carboxypeptidase A,[275] and another on experiments which complete the determination of the stereospecificity of the biosynthesis of valine from pyruvate.[276] Magnesium-25 resonance is applied to studies of magnesium binding to bovine prothrombin fragment I[277] and combined with calcium-43 resonance and

[256] E. L. Becker, H. E. Bleich, A. R. Day, R. J. Freer, J. A. Glasel, and J. Visintainer, *Biochemistry*, 1979, **18**, 4656.
[257] P. Skrabal, V. Rizzo, A. Baici, F. Bangerter, and P. L. Luisi, *Biopolymers*, 1979, **18**, 995.
[258] S. Capasso, L. Mazzarella, and T. Tancredi, *Biopolymers*, 1979, **18**, 1555.
[259] R. Nagaraj, N. Shamala, and P. Balaram, *J. Am. Chem. Soc.*, 1979, **101**, 16.
[260] G. Lancelot, R. Mayer, and H. Claude, *J. Am. Chem. Soc.*, 1979, **101**, 1569.
[261] R. Mayer, G. Lancelot, and G. Spach, *Biopolymers*, 1979, **18**, 1293.
[262] N. L. Benoiton, K. Kuroda, and F. M. F. Chen, *Int. J. Pept. Protein Res.*, 1979, **13**, 403.
[263] H. R. Kricheldorf, *Makromol. Chem.*, 1979, **180**, 147.
[264] H. R. Kricheldorf and W. E. Hull, *Makromol. Chem.*, 1979, **180**, 1707.
[265] H. R. Kricheldorf and W. E. Hull, *Makromol. Chem.*, 1979, **180**, 1715.
[266] H. R. Kricheldorf and W. E. Hull, *Makromol. Chem.*, 1979, **180**, 161.
[267] L. Mester, B. Kraska, J. Crisba, and M. Mester, 'Asymmetry Carbohydrates', ed. R. E. Harmon, Dekka, New York, 1979, p. 149.
[268] A. Cosani, M. Terbojevich, M. Palumbo, E. Peggion, and M. Goodman, *Macromolecules*, 1979, **12**, 875.
[269] H. Saito, T. Ohki, M. Kodama, and C. Nagata, *Biopolymers*, 1979, **18**, 1065.
[270] T. Hiraoki and K. Hikichi, *Polymer J.*, 1979, **11**, 299.
[271] H. Pivcova, D. Doskocilova, and E. M. Bradbury, *Polymer*, 1979, **20**, 139.
[272] E. T. Samulski, *J. Phys. (Paris), Colloq.*, 1979, (3), 471.
[273] K. Ishiwari and A. Nakajima, *Polymer J.*, 1979, **11**, 95.
[274] H. R. Kricheldorf, *Makromol. Chem.*, 1979, **180**, 2387.
[275] D. W. Appleton, T. P. A. Kruck, and B. Sarkar, *J. Inorg. Biochem.*, 1979, **10**, 1.
[276] S. R. Sylvester and C. M. Stevens, *Biochemistry*, 1979, **18**, 4529.
[277] P. Robertson, K. A. Koehler, and R. G. Hiskey, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 265.

other techniques[278] to demonstrate that divalent cation binding induces the formation of helical structure in the fragment, with a positive co-operativity of Ca^{2+} binding. Spectral studies of peptides with fibrinogen-like reactivity indicate some structural features which may be important for binding to thrombin.[279] A long series of papers on repeat hexapeptides of tropoelastin is extended by conformational studies of a cyclic version of the hexapeptide.[280]

Hormones and Small Natural Peptides.—Histidine titrations of the two-subunit glycopeptide pituitary hormone lutropin have been reported by two groups, with conclusions which differ somewhat between bovine and equine[281] and porcine[282] lutropin. In the latter case it is reported that an abnormally titrating histidine ($pK = 3.3$) is histidine α-87, while the former workers assign an abnormal behaviour to histidine α-83, with a rather more normal $pK \simeq 5.4$ given to α-87. Both agree that subunit association causes significant changes in the internal structure of the protein. Two structural investigations on *C*-terminal fragments (from dipeptide to hexapeptide) of somatostatin[283-284] demonstrate that folded and extended structures are both found in aqueous solution; presumably the studies (and the peptides) will be extended until the full 14-residue peptide structure is found, although the disulphide bridge found in the intact molecule will presumably dictate the final resultant conformation. The conformations of the closely related nonapeptide hormones, arginine, vasopressin, and oxytocin, have been compared by means of the synthesis of specifically deuteriated analogues and spectroscopy at 360 MHz. The backbone of the cyclic moiety and the side chains of Tyr-2, Asp-5, and the cystyl residues are similar.[285] The same workers also discuss comparisons of these hormones with the very similar isotocin, mesotocin, glumitocin, and aspartocin hormones.[286] The amide hydrogen exchange rates of oxytocin and vasopressin indicate a highly mobile dynamic equilibrium between folded and highly solvated conformations.[287] Isotropic motion of the tocin ring was detected by ^{15}N n.m.r. in a study which also used the acyclic tripeptide of oxytocin to assign the resonances of all the nitrogen atoms in the molecule.[288] A potent competitive inhibitor of oxytocin, [1-penicillamine,2-leucine] oxytocin, has been synthesized and characterized by n.m.r.[289] Synthetic oxytocin, labelled at specific points to a level of 90% with ^{13}C, has been used to investigate the interaction between the

[278] H. C. Marsh, P. Robertson, M. E. Scott, K. A. Koehler, and R. G. Hiskey, *J. Biol. Chem.*, 1979, **254**, 10268.
[279] I. D. Rae and H. A. Scheraga, *Int. J. Pept. Protein. Res.*, 1979, **13**, 304.
[280] M. Abu Khaled, H. Sugano, and D. W. Urry, *Biochim. Biophys. Acta*, 1979, **577**, 273.
[281] F. F. Brown, T. F. Parsons, D. S. Sigman, and J. G. Pierce, *J. Biol. Chem.*, 1979, **254**, 4335.
[282] G. Maghuin-Rogister, J. Degelaen, and G. C. K. Roberts, *Eur. J. Biochem.*, 1979, **96**, 59.
[283] K. Hallenga, G. Van Binst, M. Knappenberg, J. Brison, A. Michel, and J. Dirkx, *Biochem. Biophys. Acta*, 1979, **577**, 82.
[284] M. Knappenberg, J. Brison, J. Dirkx, K. Hallenga, P. Deschrijver, and G. Van Binst, *Biochim. Biophys. Acta*, 1979, **580**, 266.
[285] H. R. Wyssbrod, A. J. Fischmann, D. H. Live, V. J. Hruby, N. S. Agarwal, and D. S. Upson, *J. Am. Chem. Soc.*, 1979, **101**, 4037.
[286] V. J. Hruby, K. K. Deb, A. F. Spatola, D. A. Upson, and D. Yamamoto, *J. Am. Chem. Soc.*, 1979, **101**, 202.
[287] N. R. Krishna, D. H. Huang, J. D. Glickson, R. Rowan, tert., and W. Roderich, *Biophys. J.*, 1979, **26**, 345.
[288] D. H. Live, H. R. Wyssbrod, A. J. Fischmann, W. C. Agosta, C. H. Bradley, and D. Cowburn, *J. Am. Chem. Soc.*, 1979, **101**, 474.
[289] V. J. Hruby, K. K. Deb, D. M. Yamamoto, M. E. Hadley, and W. Y. Chan, *J. Med. Chem.*, 1979, **22**, 7.

hormone and bovine neurophysins as functions of pH and temperature;[290] an increase of 100-fold in dissociation rate constant at low pH was attributed to the breaking of a single salt link between the two molecules. Relaxation measurements of interactions between ^{13}C-labelled oxytocin and its diastereoisomer [1-hemi-D-cystine] oxytocin[291] and neurophysin reveal that the modified hormone does not interact with neurophysin. Spin-labelling measurements on neurophysin I[292] show its *N*-terminus to be *ca.* 14 Å from residue 3 of hormones bound to its strong interaction site. Moving from pituitary to pancreas, a 270 MHz study of the titration of insulin with Zn^{2+} shows that the hexamer conformation is fixed by the time one Zn^{2+} ion is added per hexamer, although the native form is found with two ions.[293]

A number of research groups have been taking an interest in the important brain pentapeptides, the enkephalins. Carbon-13 enrichment in Leu-enkephalin[294] improved the assignment of carbonyl resonances, while 270 MHz ^{1}H n.m.r. studies of met-enkephalin showed some conformational changes associated with titration of the ionizable groups of the molecule.[295] Selective deuteriation of the phenylalanine side chain facilitated measurement of its conformation in various solvents,[296] and a paper from the same laboratory[297] on the molecular association and conformation of met-enkephalin and its cationic form, met-enkephalinamide, presents evidence that a folded form found in dimethyl sulphoxide solution is not present in aqueous media. Paramagnetic-probe techniques have been used to compare the conformation of met-enkephalin with other, more flexible peptides, and also to provide an outline structure for the much larger molecule, β-endorphin, of which enkephalin forms a part.[297] The binding of enkephalin to model membranes showed a K_d for binding to phosphatidylserine of 5×10^{-1} M, but no evidence from molecular motion studies of binding to egg lecithin or cerebroside sulphate.[299] Other relaxation measurements[300] using enkephalin selectively deuteriated on the tyrosine ring showed that this ring is rigidly fixed with respect to the peptide chain compared with overall molecular tumbling. Internal-motion studies of fragments of the octapeptide angiotensin[301] and an isomerization from all-*s-trans* to partially *s-cis* in the His–Pro link in angiotensin and thyroliberin analogues are described.[302] A note of warning is sounded by some theoretical calculations[303] of conformations of angiotensin and enkephalin which show that extended conformations are more likely to be present in the acid conditions often

[290] M. Blumenstein, V. J. Hruby, and V. Viswanatha, *Biochemistry*, 1979, **18**, 3552.
[291] R. Deslauriers, I. C. P. Smith, G. L. Stahl, and R. Walter, *Int. J. Pept. Protein Res.*, 1979, **13**, 78.
[292] S. T. Lord and E. Breslow, *Int. J. Pept. Protein Res.*, 1979, **13**, 71.
[293] K. L. Williamson and R. J. Williams, *Biochemistry*, 1979, **18**, 5965.
[294] E. R. Stimson, Y. C. Meinwald, and H. A. Scheraga, *Biochemistry*, 1979, **18**, 1661.
[295] L. Zetta, F. Cabassi, R. Tomatis, and M. Guarneri, *Eur. J. Biochem.*, 1979, **95**, 367.
[296] J. Kobayashi, U. Nagai, T. Higashijima, and T. Miyazawa, *Biochim. Biophys. Acta*, 1979, **577**, 195.
[297] T. Higashijima, J. Kobayashi, U. Nagal, and T. Miyazawa, *Eur. J. Biochem.*, 1979, **97**, 43.
[298] B. A. Levine, D. L. Rabenstein, D. Smyth, and R. J. P. Williams, *Biochim. Biophys. Acta*, 1979, **579**, 279.
[299] H. C. Jarell, P. Tancrede, R. Deslauriers, and I. C. P. Smith, *Am. Chem. Soc. Symp. Ser.*, 1979, **103**, 159.
[300] H. E. Bleich, A. R. Day, R. Freer, and J. A. Glasel, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1146.
[301] N. Neri, P. Maria, and W. A. Gibbons, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 157.
[302] M. Liakopoulou-Kyriakides and R. E. Galardy, *Biochemistry*, 1979, **18**, 1952.
[303] S. Premilat and B. Maigret, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 534.

used for n.m.r. than at neutral pH. Titration studies of the nonapeptide hormone bradykinin [304] indicate that ionization of the terminal carboxyl group propagates structural effects through most of the molecule; the functional role of Ser-6 in the conformation and activity of bradykinin has been probed by ^{13}C spectroscopy.[305] Turning for a moment to smaller intracellular peptides, the molecular dynamics of the tripeptide-reducing agent glutathione (Glu-Cys-Gly) is reported.[306] The binding of carnosine (α-Ala-His) (found in muscle and also considered to have a role in olfaction) to bovine serum albumin [307] and its complexation with copper ions [308] are described. The tetrapeptide tuftsin (Thr-Lys-Pro-Arg) has been compared with a pentapeptide inhibitor.[309]

One of the most intensively studied mammalian proteins must be the bovine basic pancreatic trypsin inhibitor which binds to trypsin with $K_f = 10^{12}$M. A new series of papers deals at some length with ring-current effects on its conformation-dependent n.m.r. shifts,[310] with a conformational effect of cleaving the disulphide bridge between cystines 14 and 38 [311] and other covalent bonds in the same region of the molecule,[312] and the assignments,[313] exchange rates,[314] exchange kinetics,[315] and structural interpretation [316] of the slowly exchanging backbone amide protons of the molecule. All the presently known individual proton resonance assignments of this very stable protein have been presented.[317] Similar proton-exchange studies for bovine pancreatic trypsin inhibitor,[318] porcine pancreatic secretory trypsin inhibitor,[319] and the subtilisin inhibitor produced by *Streptomyces* are reported.[320] Carbon-13 n.m.r. studies of the binding of soybean trypsin inhibitor to trypsin showed that the complexes involve almost exclusively non-covalent binding.[321]

Another much studied molecule, the cyclic decapeptide antibiotic gramicidin S, is again represented in the literature by papers which apply sophisticated n.m.r. methods. Quantitative nuclear Overhauser measurements are combined with T_1 values to calculate interproton distances [322] consistent with a βII-turn/β-sheet

[304] K. Lintner, S. Fermandijan, S. St. Pierre, and D. Regoli, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 803.
[305] R. E. London, J. M. Steward, R. Williams, J. R. Cann, and N. A. Matwiyoff, *J. Am. Chem. Soc.*, 1979, **101**, 2455.
[306] A. A. Isab and D. L. Rabenstein, *FEBS Lett.*, 1979, **106**, 325.
[307] C. E. Brown, F. L. Margolis, T. H. Williams, R. G. Pitcher, and G. J. Elgar, *Arch. Biochem. Biophys.*, 1979, **193**, 529.
[308] R. E. Viola, C. R. Hartzell, and J. J. Villafranca, *J. Inorg. Biochem.*, 1979, **10**, 281.
[309] M. Blumenstein, P. P. Layne, and V. A. Najjar, *Biochemistry*, 1979, **18**, 5247.
[310] S. J. Perkins and K. Wüthrich, *Biochim. Biophys. Acta*, 1979, **576**, 409.
[311] G. Wagner, A. Kalb, and K. Wüthrich, *Eur. J. Biochem.*, 1979, **95**, 249.
[312] H. Tschesche and K. Wüthrich, *Eur. J. Biochem.*, 1979, **95**, 239.
[313] A. Dubs, G. Wagner, and K. Wüthrich, *Biochim. Biophys. Acta*, 1979, **577**, 177.
[314] G. Wagner and K. Wüthrich, *J. Mol. Biol.*, 1979, **130**, 31.
[315] R. Richarz, P. Sehr, G. Wagner, and K. Wüthrich, *J. Mol. Biol.*, 1979, **130**, 19.
[316] G. Wagner and K. Wüthrich, *J. Mol. Biol.*, 1979, **134**, 75.
[317] K. Wüthrich and G. Wagner, *J. Mol. Biol.*, 1979, **130**, 1.
[318] B. D. Hilton and C. K. Woodward, *Biochemistry*, 1979, **18**, 5834.
[319] A. De Marco, E. Menegatti, and M. Guaneri, *Eur. J. Biochem.*, 1979, **102**, 185.
[320] K. Akasaka, S. Fujii, and H. Hatano, *Koen Yoshishu Tanpakushitsu Kozo Toronkai 30th*, 1979, 45.
[321] M. W. Hunkapiller, M. D. Forgac, E. Ho Yu, and J. H. Richards, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 25.
[322] S. Jones, R. Claude, C. T. Sikakana, S. Hehir, K. Mei-Chang, and W. A. Gibbons, *Biophys. J.*, 1978, **24**, 815.

conformation, while side-chain conformations were probed by the application of difference scalar decoupling to specifically deuteriated gramicidin S molecules.[323] Further NOE measurements reveal patterns of internal spin diffusion compatible with the accepted solution conformation of gramicidin S.[324] The property of forming ion channels through membranes exhibited by gramicidin A is investigated by workers from several laboratories. An *N*-terminal–*N*-terminal helical dimer is proposed as the conformation for the channel in phosphatidylcholine vesicles,[325] while an LD-ribbon conformation for the antibiotic is favoured for the isolated linear molecules in dimethyl sulphoxide.[326] The effects of heat on the aqueous gramicidin A–lysolecithin system include a change in the ^{23}Na n.m.r. line-width corresponding to an activation energy of 6.8 kcal mol^{-1}, similar to the ion-transport activation energy;[327, 328] detailed relaxation and quadrupole splitting studies of deuterium in specifically labelled lipids associated with gramicidin A were used to characterize partially motion and hydrocarbon chain order of the lipid molecules in contact with the proteins.[329] Sodium ion interaction with gramicidins is investigated by ^{23}Na resonance for both gramicidin A[330] and gramicidin D.[331] An analogue of valinomycin, another ion-transport antibiotic, is used to demonstrate that its structure consists of six alternating βII-turns forming an octahedral cavity for the cation location.[332] A complete assignment of the ^{1}H spectrum of tyrocidine A is offered in ref. 333; other studies include two on virginiamycin[334, 335] and one on the cyclic tetradepsipeptide phytotoxin produced by the fungus *Alternaria mali*.[336]

Enzymes.—*Oxidoreductases.* A correlation of ^{35}Cl quadrupole relaxation results on the binding of coenzymes, coenzyme fragments, and inhibitors to horse liver alcohol dehydrogenase with *X*-ray crystallographic data[337] suggests that Arg-47 and -271 are involved in the binding of anion to the enzyme. Strong support is given[338] to a functional link between glyceraldehyde-3- phosphate dehydrogenase and membrane ($Na^+ + K^+$) ATPase at the cytoplasmic face of red cell vesicles, while photochemically induced dynamic nuclear polarization has been used to resolve specific resonances in the former protein.[339] Spin-label studies[340] on the geometry of bound substrate, coenzyme, and activator on bovine liver glutamate dehydrogenase show that NADP binds in an open conformation consistent with

[323] K. Mei-Chang, C. R. Jones, T. H. Mahn, P. R. Miller, L. J. F. Leslie, and W. A. Gibbons, *J. Biol. Chem.*, 1979, **254**, 10301.
[324] A. A. Bothner-By and P. E. Johner, *Biophys. J.*, 1978, **24**, 779.
[325] E. R. Blout, J. S. Morrow, and W. Veatch, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4230.
[326] F. Heitz, G. Spach, P. D. Cary, and C. Crane-Robinson, *Eur. J. Biochem.*, 1979, **93**, 135.
[327] D. W. Urry, A. Spisni, and M. Abu Khaled, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 940.
[328] A. Spisni, M. A. Khaled, and D. W. Urry, *FEBS Lett.*, 1979, **102**, 321.
[392] D. Rice and E. Oldfield, *Biochemistry*, 1979, **18**, 3272.
[330] A. Cornelis and P. Laszlo, *Biochemistry*, 1979, **18**, 2004.
[331] H. Monoi and H. Uedaira, *Biophys. J.*, 1979, **25**, 535.
[332] D. G. Davis and B. F. Gisin, *J. Am. Soc.*, 1979, **101**, 3755.
[333] M. Kuo and W. A. Gibbons, *J. Biol. Chem.*, 1979, **254**, 6278.
[334] S. Callens, E. A. Roland, and M. J. O. Anteunis, *Biochim. Biophys. Acta*, 1979, **577**, 324.
[335] R. E. A. Callens and M. J. O. Anteunis, *Biochim. Biophys. Acta*, 1979, **577**, 337.
[336] T. Higashijima, T. Inubushi, T. Ueno, and T. Miyazawa, *FEBS Lett.*, 1979, **105**, 337.
[337] M. Zeppezauer, T. Bull, R. Einarsson, J. E. Norne, and B. Lindman, *Biochemistry*, 1979, **18**, 3407.
[338] E. T. Fossel and A. K. Solomon, *Biochim. Biophys. Acta*, 1979, **553**, 142.
[339] R. M. Scheek, R. Kaptein, and J. W. Verhoeven, *FEBS Lett.*, 1979, **107**, 288.
[340] A. Zantema, M. J. De Smet, and G. T. Robillard, *Eur. J. Biochem.*, 1979, **96**, 465.

the conformation in other dehydrogenases. ^{13}C labelling of lecithin in either the polar or hydrophobic moiety[341] shows that D-β-hydroxybutyrate apodehydrogenase, a lipid-requiring enzyme, causes a reduction in rotational motion in the polar head-group but an increase in motion in the hydrophobic region. Thermodynamic parameters for the binding of ^{19}F to the Cu^{2+} site of galactose oxidase[342] and the properties of copper(II) ions in benzylamine oxidase[343] are reported. Both e.p.r. and ^{19}F n.m.r. have been applied to the determination of the number and type of iron–sulphur centres in succinate dehydrogenase;[344] the results indicate two dimeric (Fe_2S_2) and one tetrameric (Fe_4S_4) centre per molecule of histidyl flavin. Another flavoprotein, the dehydrogenating flavodoxin from *Azotobacter vinelandii*, shows, from ^{31}P resonance, the presence of stoicheiometric quantities of covalently bound phosphorus which may be of catalytic significance.[345]

The role of dihydrofolate reductase as the site of action of the anticancer agents aminopterin and methotrexate make it of obvious interest. Structural studies of the enzyme itself include ^{13}C labelling at arginines, methionines, and tryptophans, coupled with the use of spin-labelled ligands,[346] and a report that two of the four tryptophans undergo slow exchange between alternative stable conformations, being locked into a single conformation upon binding of 3′,5′-dichloromethotrexate.[347] Published n.m.r. spectra of dihydrofolate reductase–methotrexate–NADPH complexes are interpreted with the aid of crystallographic data.[348] Transfer of saturation and difference-spectroscopic experiments on the binding of trimethoprim to dihydrofolate reductase lead to the proposal of a model for the structure of the complex.[349] Of the five histidines in dihydrofolate reductase, three showed no change in pK value and chemical shift on binary complexation of the enzyme with various inhibitors, whereas one was quite different in the folate complex and another in the trimethoprim complex.[350]

A number of workers have reported on studies of the enzyme bovine superoxide dismutase (erythrocuprein). Water co-ordination at copper atoms,[351] zinc binding,[352] and fluoride-ion binding are reported.[353] Selective deuteriation of histidine residues reveals that the deuteriation rates of C-2 hydrogen atoms are dramatically reduced if these residues are co-ordinating metal ions;[354] this conclusion is borne

[341] S. Fleischer, J. O. McIntyre, W. Stoeffel, and B. D. Tunggal, *Biochemistry*, 1979, **18**, 2420.
[343] R. J. Kurland and B. J. Marwedel, *J. Phys. Chem.*, 1979, **83**, 1422.
[343] R. Barker, N. Boden, G. Cayley, S. C. Charlton, R. Henson, M. C. Holmes, I. D. Kelly and P. F. Knowles, *Biochem. J.*, 1979, **177**, 289.
[344] C. J. Coles, R. H. Holm, D. M. Kurtz, jun., W. H. Orme-Johnson, J. Rawlings, T. P. Singer, and G. B. Wong, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3805.
[345] D. E. Edmondson and T. L. James, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3786.
[346] L. Cocco, R. L. Blakeley, R. E. London, J. P. Groff, T. E. Walker, and N. A. Matwiyoff, *Dev. Biochem.*, 1979, **4**, 383.
[347] R. E. London, J. P. Groff, and R. L. Blakley, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 779.
[348] D. A. Matthews, *Biochemistry*, 1979, **18**, 1602.
[349] P. J. Cayley, J. P. Albrand, J. Feeney, G. C. K. Roberts, E. A. Piper, and A. S. V. Burgen, *Biochemistry*, 1979, **18**, 3886.
[350] M. Poe, K. Hoogsteen, and D. A. Matthews, *J. Biol. Chem.*, 1979, **245**, 8143.
[351] N. Boden, M. C. Holmes, and P. F. Knowles, *Biochem. J.*, 1979, **177**, 303.
[352] A. E. G. Cass, H. A. O. Hill, J. V. Bannister, and W. H. Bannister, *Biochem. J.*, 1979, **177**, 477.
[353] P. Viglino, A. Rigo, R. Stevanato, G. A. Ranieri, G. Rotilio, and L. Calabrese, *J. Magn. Reson.*, 1979, **34**, 265.
[354] A. E. G. Cass, H. A. O. Hill, W. H. Bannister, and J. V. Bannister, *Biochem. Soc. Trans.*, 1979, **7**, 716.

out by solvent exchange and nuclear Overhauser measurements.[355] Interactions of long-chain aldehydes with luciferase are considered.[356]

Transferases. ^{31}P Resonance observations on dUMP show that its phosphate pK_a falls from 6.25 to <5 in the presence of thymidilate synthase.[357] The same nucleus was used to investigate the ionization of pyridoxal 5'-phosphate in native and succinylated rabbit muscle glycogen phosphorylase[358] and the paramagnetic effect of chromium–ADP on the ^{31}P nucleus of glucose 6-phosphate when both are bound at the active site of hexokinase.[359] The Cr^{3+}—P distance is 6.6 Å, precluding a direct co-ordination reaction between the two. Paramagnetic probes also find a use in a series of three papers on bovine heart protein kinase; manganese ions and cobalt-labelled tetramine–ATP show that the enzyme is specific for the Δ-stereoisomer of the metal–ATP complex [360] and permit the spatial relationship of the two paramagnetic centres [361] to be determined, as well as the detection of various divalent cation inhibitory and activating sites.[362] A titration of the complex between ATP and rabbit muscle pyruvate kinase, using the ^{31}P resonance of the β-phosphorus of ATP as the parameter, indicates a metal-binding site on the enzyme in addition to that on the ATP at the catalytic site.[363] Involvement of His-32 in the biological activity of α-lactalbumin, which as a regulatory protein converts a transferase to lactose synthetase, has been studied.[364]

The phosphocarrier protein HPr of the phosphoenolpyruvate-dependent phosphotransferase system of *Staphylococcus aureus* has three tyrosines; the magnetic resonance signals have been assigned by nitration followed by tryptic digestion.[365] The analogous protein from *E. coli* shows some compositional heterogeneity; evidence is presented [366] that one tyrosine is present in only half the molecules[367] and that the *E. coli* HPr molecule can exist in two conformations. Paramagnetic-probe measurements of substrate and metal-ion binding to carbamate kinase [368] demonstrate that carbamate binds from 4—8 Å from the metal, thus it is near to the metal–nucleotide substrate site. Truncated driven nuclear Overhauser effects in creatine kinase indicate that the ADP interacts with aromatic residues in the enzyme.[369]

Hydrolases. The new technique of photochemically induced dynamic nuclear polarization (photo-CIDNP) has been applied to bovine pancreatic phospholipase A_2, revealing a pH-dependent conformational change in which deprotonation of

[355] J. D. Stoesz, D. P. Malinowski, and A. G. Redfield, *Biochemistry*, 1979, **18**, 4669.
[356] T. S. Viswanathan, M. R. Campling, and R. J. Cushley, *Biochemistry*, 1979, **18**, 2504.
[357] M. J. Beckage, M. Blumenstein, and R. L. Kisliuk, *Biochem. Biophys. Acta*, 1979, **571**, 157.
[358] M. Hoerl, K. Feldmann, K. D. Schnackertz, and E. J. M. Helmreich, *Biochemistry*, 1979, **18**, 2457.
[359] R. L. Peterson and R. K. Gupta, *Biophys. J.*, 1979, **27**, 1.
[360] J. Granot, A. S. Mildvan, E. M. Brown, H. Kondo, H. N. Bramson, and E. T. Kaiser, *FEBS Lett.*, 1979, **103**, 265.
[361] J. Granot, H. Kondo, R. N. Armstrong, A. S. Mildvan, and E. T. Kaiser, *Biochemistry*, 1979, **18**, 2338.
[362] R. N. Armstrong, H. Kondo, J. Granot, E. T. Kaiser, and A. S. Mildvan, *Biochemistry*, 1979, **18**, 1230.
[363] B. D. N. Rao, F. J. Kayne, and M. Cohn, *J. Biol. Chem.*, 1979, **254**, 2689.
[364] J. P. Prieels, J. E. Bell, M. Schlinder, F. J. Castellino, and R. L. Hill, *Biochemistry*, 1979, **18**, 1771.
[365] U. Schmidt-Adergan, P. Roesch, F. Rainer, and W. Hengstenberg, *Eur. J. Biochem.*, 1979, **96**, 43.
[366] F. F. Roossien, G. Dooyewaard, and G. T. Robillard, *Biochemistry*, 1979, **18**, 5793.
[367] G. Dooijewaard, F. F. Roossien, and G. T. Robillard, *Biochemistry*, 1979, **18**, 2996.
[368] R. P. Pillai, M. Marshall, and J. J. Villafranca, *Arch. Biochem. Biophys.*, 1980, **199**, 21.
[369] M. Vasak, K. Nagayama, K. Wüthrich, M. L. Mertens, and J. H. R. Kaegi, *Biochemistry*, 1979, **18**, 5050.

the *N*-terminal alanine NH_3^+ disrupts a salt bridge, causing the *N*-terminus to leave the interior of the protein.[370] The same protein has also been investigated *via* histidine titrations[371] and in terms of its phospholipid activation.[372] Alkaline phosphatase, a two-subunit zinc metalloenzyme in which activity is retained when the zinc is replaced by cadmium, has revealed through ^{31}P saturation transfer of bound phosphate the first unequivocal evidence for direct metal–phosphate interaction[373] and also indications[374] that metal ions in the environment may act as allosteric activators of the enzyme in addition to the essential role of the two catalytic Zn^{2+} ions. A similar conclusion is drawn through ^{31}P resonance studies (n.m.r. and e.p.r.) of manganese–alkaline phosphatase,[375] while a ^{35}Cl-based study[376] leads to the proposal of the existence of two forms of the enzyme with different Zn^{2+} binding properties.

The modest size of ribonuclease A makes it a continuing favourite for the application of new experimental techniques and for very detailed study. The aromatic and histidine resonances of the protein have been assigned with the help of photo-CIDNP, leading to a number of conclusions on conformational transitions and inhibitor binding.[377, 378] A proposed interaction between Asp-14 and His-48 was confirmed by ^{13}C spectroscopy,[379] and an interesting new technique, of rotating-frame spin–lattice relaxation in an off-resonance rotating frame, showed that there is a previously unreported intermediate stage in the unfolding of RNase A by guanidinium chloride.[380] The thermal unfolding of the enzyme has been followed by ^{13}C resonance.[381] Probes into the RNase A structure have been applied in the form of Cu^{2+} ions[382] and hexacyano-chromate,[383] which may have applications in mapping the active sites of other enzymes, as may the technique of reductively methylating lysyl residues with ^{13}C-formaldehyde and sodium cyanoborohydride.[384] The ability of ribonuclease cleaved at residue 20 to retain its activity as RNase S has been exploited,[385] in reconstituting active enzyme with a fully synthetic, ^{13}C-labelled, *N*-terminal peptide 1—15, followed by detailed observations of the pH and temperature dependence of the environments of the labelled sites. Porcine pancreatic ribonuclease, a glycoprotein, has been studied by histidine titration before and after removal of 80% of the attached carbohyd-

[370] E. H. J. M. Jansen, G. J. M. Van Scharrenburg, A. J. Slotboom, and G. H. De Haas, *J. Am. Chem. Soc.*, 1979, **101**, 7397.
[371] A. Aguiar, G. H. De Haas, E. H. J. M. Eugene, A. J. Slotboom, and R. J. P. Williams, *Eur. J. Biochem.*, 1979, **100**, 511.
[372] M. F. Roberts, M. Adamich, R. J. Robson, and E. A. Dennis, *Biochemistry*, 1979, **18**, 3301.
[373] J. D. Otvos, J. R. Alger, J. E. Coleman, and I. M. Armitage, *J. Biol. Chem.*, 1979, **254**, 1778.
[374] J. D. Otvos, I. M. Armitage, J. F. Chlebowski, and J. E. Coleman, *J. Biol. Chem.*, 1979, **254**, 4707.
[375] R. E. Weinger, J. F. Chlebowski, P. H. Haffner, and J. E. Coleman, *J. Biol. Chem.*, 1979, **254**, 9739.
[376] J. E. Norne, H. Szajn, H. Csopak, P. Reinarsson, and B. Lindman, *Arch. Biochem. Biophys.*, 1979, **196**, 552.
[377] B. G. J. M. Bolscher, J. A. Lenstra, and R. Kaptein, *J. Magn. Reson.*, 1979, **35**, 163.
[378] J. A. Lenstra, B. G. J. M. Bolscher, S. Stob, J. J. Beintema, and R. Kaptein, *Eur. J. Biochem.*, 1979, **98**, 385.
[379] J. Santoro, H. P. Juretschke, and H. Rueterjans, *Biochim. Biophys. Acta*, 1979, **578**, 346.
[380] T. L. James and S. P. Sawan, *J. Am. Chem. Soc.*, 1979, **101**, 7050.
[381] O. W. Howarth, *Biochim. Biophys. Acta*, 1979, **576**, 163.
[382] L. Sportelli and V. Viti, *Biochim. Biophys. Acta*, 1979, **580**, 100.
[383] F. Inagaki, K. Watanabe, and T. Miyazawa, *J. Biochem. (Tokyo)*, 1979, **86**, 591.
[384] J. E. Jentoft, N. Jentoft, T. A. Gerken, and D. G. Dearborn, *J. Biol. Chem.*, 1979, **254**, 4366.
[385] C. Niu, S. Matsuura, H. Shindo, and J. S. Cohen, *J. Biol. Chem.*, 1979, **254**, 3788.

rate;[386] the titration curves were identical within experimental error, indicating that the heterosaccharides project away from the surface of the protein into the solution environment.

The indole NH resonances of the tryptophanyl residues of hen egg white lysozyme have been re-assigned by the use of paramagnetic ion probes at the active site.[387] Natural-abundance ^{13}C measurements have been made of lysozyme carboxyl groups in relation to the binding environment for *N*-acetylglucosamine[388] and of the effect of carboxymethylation of His-15;[389] deuteriation of the same residues was used to show[390] that lysozyme is extensively dimerized at pH = 7.5.

Incubation of [^{13}C]benzylpenicillin with *Streptomyces* R61 DD–carboxypeptidase causes the formation of a complex which appears to be a penicilloyl-enzyme[391] with no non-covalently bound penicillin present. Binding of Gly-Tyr dipeptide to manganese-substituted carboxypeptidase A in solution is apparently different from its binding to Zn–carboxypeptidase A in the crystal form, as indicated by relaxation measurements.[392] Labelling of α-chymotrypsin with fluorine yields a lot of information about molecular motion *via* selective Overhauser effects[393] and T_1 and T_2 measurements.[394, 395] Cross-saturation measurements reveal that although *N*-acetyl-L-phenylalaninal exists predominantly in its hydrated form in aqueous solution, it is the aldehyde and not the hydrate which is the effective inhibitor of chymotrypsin.[396] A series of serine proteases, modified *via* di-isopropyl phosphorylation of Ser-195 (chymotrypsinogen numbering system), were used to derive p*K* values for shift of the label phosphorus ^{31}P resonance which correspond to the p*K* values of His-57;[397] histidyl and tyrosyl titration measurements for the serine protease *subtilisin Novo* are reported,[398] as is direct evidence for non-productive enzyme–inhibitor complexes in the interaction of trifluoroacetyl peptide chloromethyl ketones with porcine pancreatic elastase.[399] Lithium-7 resonance and paramagnetic probes were used[400] to show that the separations between Gd^{3+} and enzyme-bound Li^+ ions are 7.0 and 9.1 Å, setting an upper limit on the spacing between Ca^{2+} sites on the enzyme at 16.1 Å.

Lyases. The effect of carbonic anhydrase on the CO_2–HCO_3 equilibrium has been the subject of two studies. In one,[401] it is shown that the maximal exchange rates are

[386] F. C. Wang and C. H. W. Hirs, *J. Biol. Chem.*, 1979, **254**, 1090.
[387] R. E. Lenkinski, J. L. Dallas, and J. D. Glickson, *J. Am. Chem. Soc.*, 1979, **101**, 3071.
[388] H. Hashizume, *Rep. Fac. Sci., Shizuoka Univ.*, 1979, **13**, 43.
[389] W. J. Goux and A. Allerhand, *J. Biol. Chem.*, 1979, **254**, 2210.
[390] J. B. Wooten and J. S. Cohen, *Biochemistry*, 1979, **18**, 4188.
[391] J. Degelaen, J. Feeney, G. C. K. Roberts, A. S. V. Burgen, J. M. Frere, and J. M. Ghuysen, *FEBS Lett.*, 1979, **98**, 53.
[392] A. Nakano, M. Tasumi, K. Fujiwara, K. Fuwa, and T. Moyazawa, *J. Biochem. (Tokyo)*, 1979, **85**, 1001.
[393] J. T. Gerig, K. F. S. Luk, and D. C. Roe, *J. Am. Chem. Soc.*, 1979, **101**, 7698.
[394] J. T. Gerig, B. A. Halley, D. T. Loehr, and J. A. Reimer, *Org. Magn. Reson.*, 1979, **12**, 352.
[395] J. T. Gerig, D. T. Loehr, K. F. S. Luk, and D. C. Roe, *J. Am. Chem. Soc.*, 1979, **101**, 7482.
[396] R. Chen, D. G. Gorenstein, W. P. Kennedy, G. Lowe, D. Nurse, and R. M. Schulz, *Biochemistry*, 1979, **18**, 921.
[397] M. A. Porubcan, W. M. Westler, I. B. Ibanez, and J. L. Markley, *Biochemistry*, 1979, **18**, 4108.
[398] S. Omar, M. F. Brown, P. Silver, and T. Schleich, *Biochem. Biophys. Acta*, 1979, **578**, 261.
[399] J. L. Dimicoli, A. Renaud, P. Lestienne, and J. G. Bieth, *J. Biol. Chem.*, 1979, **254**, 5208.
[400] E. M. Stephens and C. M. Grisham, *Biochemistry*, 1979, **18**, 4876.
[401] I. Simonsson, B. H. Jonsson, and S. Lindskog, *Eur. J. Biochem.*, 1979, **93**, 409.

greater than the maximal turnover rates, and a proposal is made that the interconversion mechanism is nucleophilic, such as a reversible reaction of Zn-co-*ordinated* OH^- with CO_2; the other [402] confirms that CO_2 *per se* does not bind at the metal. Solvent-water relaxation in carbonic anhydrase solutions exhibits diamagnetic as well as paramagnetic effects, with different pH dependence, when the enzyme Zn^{2+} is replaced by Co^{2+} [403] and a water molecule bound to the paramagnetic centre is demonstrated.[404] Hydrated trichloroacetaldehyde in carbonic anhydrase solutions also binds to the metal ion.[405] Carbon dioxide activation of ribulosephosphate carboxylase [406] involves formation of a carbamate between the CO_2 and an enzyme NH_2 group. The pyridoxal phosphate binding site of D-serine dehydratase was investigated by ^{31}P resonance [407] with the result that fixation of the dianionic phosphate, probably *via* a salt bridge with an arginine residue, is essential to the transaldimination step of the catalytic mechanism.

Isomerases and Ligases. Assignment and titrations of low-field resonances in the 360 MHz spectrum of porcine pancreatic colipase [408, 409] indicate that two distant regions of the covalent structure are brought together to form a hydrophobic-aromatic site which may be involved in the binding of bile salt micelles. The interaction of porcine colipase with taurodeoxycholate supports this conclusion;[410] all tyrosine residues appear to be affected by the binding. Complexes between triosephosphate isomerase and substrates and inhibitors, studied by ^{31}P resonance,[411] indicate that the di-anion form of the ligand binds more effectively; a similar conclusion is drawn for the binding of pyridoxal phosphate and α-glucan phosphorylases.[412] Paramagnetic probe experiments on the binding sites of phosphoribosylpyrophosphate synthetase and *E. coli* glutamine synthetase are reported.[413, 414]

Haem Proteins.—Model-compound studies as aids to the interpretation of haem-protein spectra are reviewed; [224] other model studies of temperature-dependent spin-state equilibrium in an azide-iron(III) haem–octapeptide complex [415] and of high-spin iron(III) natural porphyrin derivatives [416] have also been made.

Cytochrome *c* oxidase, a member of the cytochrome *a* group in which the haem prosthetic group contains a formyl side chain, is found in association with lipids, and the interaction has been studied by ^1H n.m.r. of sonicated vesicles containing

[402] I. Bertini, E. Borghi, and C. Luchinat, *J. Am. Chem. Soc.*, 1979, **101**, 7069.
[403] J. W. Wells, S. I. Kandel, and S. H. Koenig, *Biochemistry*, 1979, **18**, 1989.
[404] I. Bertini, G. Canti, C. Luchinat, and A. Scozzafava, *Inorg. Chim. Acta*, 1979, **36**, 9.
[405] I. Bertini, E. Borghi, G. Canti, and C. Luchinat, *J. Inorg. Biochem.*, 1979, **11**, 49.
[406] M. H. O'Leary, R. J. Jaworski, and F. C. Hartman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 673.
[407] K. D. Schnackerz, K. Feldmann, and W. E. Hull, *Biochemistry*, 1979, **18**, 1536.
[408] P. Canopmo and P. J. Cozzone, *Biochimie*, 1979, **61**, 343.
[409] P. Canioni and P. J. Cozzone, *FEBS Lett.*, 1979, **97**, 353.
[410] T. Wieloch, B. Borgstrom, K. Falk, and S. Forsen, *Biochemistry*, 1979, **18**, 1622.
[411] I. D. Campbell, R. B. Jones, P. A. Kiener, and S. G. Waley, *Biochem. J.*, 1979, **179**, 607.
[412] D. Palm, K. H. Schaechtele, K. Feldmann, and E. J. M. Helmreich, *FEBS Lett.*, 1979, **101**, 403.
[413] T. M. Li, R. L. Switzer, and A. S. Mildvan, *Arch. Biochem. Biophys.*, 1979, **193**, 1.
[414] P. B. Chock, J. J. Villafranca, S. G. Rhee, G. A. Ubom, and E. R. Stadtman, Proc. Symp. N.M.R. Biochem., ed. S. J. Opella and P. Lu, Dekka, New York, 1979, p. 405.
[415] Y. Huang and R. J. Kassner, *J. Am. Chem. Soc.*, 1979, **101**, 5807.
[416] D. L. Budd, G. N. La Mar, K. C. Langry, K. M. Smith, and R. Nayyir-Mazhir, *J. Am. Chem. Soc.*, 1979, **101**, 609.

the protein and phosphatidylcholine, indicating that lipid molecules are immobilized close to the protein;[417] specific deuteriation of the phospholipid followed by quadrupole-echo Fourier transform spectroscopy indicates that a simple two-site exchange model may be sufficient to explain the interaction.[418] The effects of cholate on the cytochrome oxidase–phospholipid interaction are reported.[419] Proteins of the cytochrome *b* group are defined as containing a protohaem or related haem group not covalently bound to protein; the group includes cytochrome P-450, which is reported[420] to have one or two protons exchanging between a binding site on a haem ligand and bulk water. The β-phenethylmercaptan-protohaem–CO complex is proposed as a simple model for iron(II) cytochrome P-450,[421] and studies of related mono-oxygenases from rat, rabbit, and other organisms conclude that increased enzyme specificity goes along with a closing of the haem cleft.[422]

The cytochromes *c*, in which the prosthetic group is covalently linked to the protein, have been studied using several approaches. The location of a Cr^{3+} ion in a 1 : 1 chromium–cytochrome *c* complex has been found to be on the surface of the protein, *ca.* 14 Å away from the iron atom, and near His-26 of the protein.[423] Some localized conformational flexibility in an amino-acid side chain making contact with the haem group has been observed.[424] Chloride ions are found to have a binding site on cytochrome *c*;[425] other studies and resonance assignments are reported for *Rhodospirillium rubrum* cytochrome c_2,[426] *Pseudomonas aeruginosa* cytochrome c_{551},[427] and *Crithidia oncopelti* cytochrome *c* 557.[428]

Analysis of the nuclear Overhauser enhancements and T_1 values for a large number of aliphatic ^{13}C resonances in the spectrum of sperm whale myoglobin reveal considerable motional freedom; in view of the tight packing of the myoglobin molecule, their motions may well be concerted.[429] Motional studies of ethyl isocyanide bound to myoglobin and haemoglobin,[430] and titration of histidines of deoxy- and oxy-myoglobins and their comparison with the met and azide forms of the protein are reported.[431] A comparison of the n.m.r. spectra of a large number of diamagnetic myoglobin derivatives with that of a complex between the chlorophyll chromophore and apomyoglobin confirms the location of the chromophore in what is claimed to be the first well defined one-to-one complex between the chlorophyll chromophore and a protein.[432]

[417] K. E. Falk and B. Karlsson, *FEBS Lett.*, 1979, **98**, 25.
[418] S. Y. Kang, H. S. Gutowsky, J. C. Hshung, R. Jacobs, T. E. King, D. Rice, and E. Oldfield, *Biochemistry*, 1979, **18**, 3257.
[419] D. Rice, J. C. Hsung, T. E. King, and E. Oldfield, *Biochemistry*, 1979, **18**, 5885.
[420] S. B. Philson, P. G. Debrunner, P. G. Schmidt, and I. C. Gunsalus, *J. Biol. Chem.*, 1979, **254**, 10173.
[421] A. P. Berzinis and T. G. Traylor, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 229.
[422] S. Maricic, B. Benko, and G. Butschak, *Acta Bio. Med. Ger.*, 1979, **38**, 217.
[423] R. L. Peterson and R. K. Gupta, *FEBS Lett.*, 1979, **107**, 427.
[424] P. D. Burns and G. N. La Mar, *J. Am. Chem. Soc.*, 1979, **101**, 5844.
[425] T. Andersson, E. Thulin, and S. Forsen, *Biochemistry*, 1979, **18**, 2487.
[426] G. M. Smith, *Biochemistry*, 1979, **18**, 1628.
[427] Y. H. Chao, R. Bersohn, and P. Aisen, *Biochemistry*, 1979, **18**, 774.
[428] R. M. Keller, D. Picot, and K. Wüthrich, *Biochim. Biophys. Acta*, 1979, **580**, 259.
[429] R. J. Wittebort, T. M. Rothgeb, A. Szabo, and F. R. N. Gurd, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1059.
[430] J. G. Gilman, *Biochemistry*, 1979, **18**, 2273.
[431] J. P. Ohms, H. Hagenmaier, M. B. Hayes, and J. S. Cohen, *Biochemistry*, 1979, **18**, 1599.
[432] S. G. Boxer and K. A. Wright, *J. Am. Chem. Soc.*, 1979, **101**, 6791.

The mechanism of the co-operative oxygenation of haemoglobin is of continuing interest; it is claimed in two publications on its ^{1}H n.m.r. that simple two-state allosteric models are not adequate.[433, 434] Quaternary structure in haemoglobin is studied by ^{1}H n.m.r. and its tendency to self-association by proton relaxation methods.[437] The greatly enhanced propensity of reduced haemoglobin S (β6 Glu → Val) to associate, with the consequent 'sickling' of the red corpuscles, has been studied by double-resonance methods which enable a quantitative estimation of the amount of polymerized haemoglobin S present in cells to be made;[438, 439] off-resonance rotating-frame spin–lattice relaxation measurements[440] indicate a tendency of Hb-S to aggregate even in the oxygenated state. Various ligands have been studied in association with haemoglobin, notably 2,3-bisphosphoglycerate,[441] dichloromethane,[442] and benzene and toluene.[443] Association of anions with the special plant haemoglobin involved in nitrogen fixation, leghaemoglobin, is reported.[444, 445] Last on our list of haem proteins are the horseradish peroxidase molecules; it is suggested[446] that one Ca^{2+} ion is essential to activity of the protein and that it functions by maintaining protein structure in the haem vicinity. Indications that the haem pocket is buried come from the lack of lability of the proximal histidine in horseradish peroxidase compared with that in myoglobin;[447] the molecule also appears to have a specific sterically restrictive active site for the binding of a variety of aromatic donor molecules such as *p*-cresol, indolepropionic acid, and benzhydroxamic acid.[448]

Other Proteins.—A detailed study of the blue copper protein azurin from *Pseudomonas aeruginosa* is reported,[449] including a number of assignments and detailed comparisons between oxidized and reduced states of the native enzyme and derivatives substituted with mercury and cobalt. From the same group come similar studies of stellacyanins from *Rhus vernicifera*.[450, 451] The binding of sodium and calcium[452] and imidazole, potassium cyanide, and fluoride ions[453] to haemocyanin molecules are also reported, as are oxidation/reduction effects on the 360 MHz ^{1}H n.m.r. spectrum of neurocuprein.[454]

[433] G. Viggiano, N. T. Ho, and C. Ho, *Biochemistry*, 1979, **18**, 5238.
[434] G. Viggiano and C. Ho, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3673.
[435] S. Neya and I. Morishima, *J. Biol. Chem.*, 1979, **254**, 9107.
[436] T. Huang, *J. Biol. Chem.*, 1979, **254**, 11467.
[437] J. Brnjas-Kraljevic, G. Pifat, and S. Maricic, *Physiol. Chem. Phys.*, 1979, **11**, 371.
[438] J. W. H. Sutherland, W. Egan, A. N. Schechter, and D. A. Torchia, *Biochemistry*, 1979, **18**, 1797.
[439] C. T. Noguchi, D. A. Torchia, and A. N. Schechter, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4936.
[440] T. L. James, R. Matthews, and G. B. Matson, *Biopolymers*, 1979, **18**, 1763.
[441] R. K. Gupta, J. L. Benovic, and Z. B. Rose, *J. Biol. Chem.*, 1979, **254**, 8250.
[442] M. R. Harkey, W. L. Settle, T. L. James, and A. J. Trevor, *Mol. Pharmacol.*, 1979, **16**, 1065.
[443] R. F. Novak, M. Dershwitz, and F. C. Novak, *Mol. Pharmacol.*, 1979, **16**, 1046.
[444] J. Trewhella, P. E. Wright, and C. A. Appleby, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 713.
[445] J. Trewhella, P. E. Wright, and C. A. Appleby, *Nature (London)*, 1979, **280**, 87.
[446] S. Ogawa, Y. Shiro, and I. Morishima, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 674.
[447] G. N. La Mar and J. S. De Ropp, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 36.
[448] I. Morishima and S. Ogawa, *J. Biol. Chem.*, 1979, **254**, 2814.
[449] H. A. O. Hill and B. E. Smith, *J. Inorg. Biochem.*, 1979, **11**, 79.
[450] H. Hill, O. Allen, and W. Lee, *Biochem. Soc. Trans.*, 1979, **7**, 733.
[451] H. A. O. Hill and W. K. Lee, *J. Inorg. Biochem.*, 1979, **11**, 101.
[452] J. A. Norne, H. Gustavsson, S. Forsen, E. Chiancone, H. A. Kuiper, and E. Antonini, *Eur. J. Biochem.*, 1979, **98**, 591.
[453] J. T. Chen, S. T. Shen, C. S. Chung, H. Chang, S. M. Wang, and N. C. Li, *Biochemistry*, 1979, **18**, 3097.
[454] V. S. Gasparov, *Biol. Zh. Arm.*, 1979, **32**, 369.

Conformational studies in solution of protein snake venom toxins have been assisted by selective acetylation and trifluoroacetylation of neurotoxin III from *Naja naja oxiana*; a comparison of conclusions with the known crystal structure of the closely related sea snake neurotoxins was possible.[455] Even without labelling, some low-field resonance assignments have been possible in the 360 MHz spectrum of cobrotoxin.[456]

Detailed study of the *in situ* conformation of membrane proteins is considerably hindered by the complexity and line-width of the lipid spectrum. Despite this problem it has been possible to obtain well resolved superimposed spectra of melittin in lysophosphatidylcholine vesicles;[457] much more detailed information should, however, be obtainable by the use of fully deuteriated micelles, and details of their synthesis and preliminary data on melittin structure in such micelles are presented.[458, 459] Deuteriated phospholipids are also used in investigations of the interaction of sarcoplasmic reticulum ATPase with various lipid systems.[460] Another approach, combining n.m.r. with o.r.d., and c.d. measurements, has been tested on a small peptide in phospholipid vesicles.[461] Carbon-13 labelling is applied to the lipid–apoprotein interactions in high-density human serum lipoprotein,[462] and in conjunction with the shift reagent $DyCl_3$ in the investigation of the operation of phosphatidylcholine exchange protein.[463] The membrane-associated glycoprotein glycophorin, which has a molecular weight of 31 000 dalton, shows sharp protein resonances from its carbohydrate chains. The significance of flexible carbohydrate chains in membranes is discussed in relation to cell-surface chemistry;[464] segmental motion in the peptidoglycan pentaglycine chain in *Staphylococcus* cell walls is investigated by ^{13}C- and ^{15}N-labelling studies.[465] Motional frequencies of carbohydrate chains in glycoproteins are the main results of ^{2}H[466] and ^{13}C studies[467]—the latter associated with natural abundance ^{13}C chemical shift measurements on some glycosylated amino-acids.[468]

Turning now to proteins associated with muscle, we find a study of molecular motions in myosin[469] which shows that there is a region of high mobility in the subfragment I region of the molecule, constituting *ca.* 22% of its structure. Titration with actin quenched the motion. The magnesium-ion-dependent ATPase reaction of myosin[470] and the interaction of water with myosin during dehydration

[455] V. I. Tsetlin, A. S. Arseniev, Y. N. Utkin, A. Z. Gurevich, L. B. Senyavina, V. F. Bystrov, V. T. Ivanov, and Y. A. Ovchinnikov, *Eur. J. Biochem.*, 1979, **94**, 337.
[456] C. H. Fung, C. C. Chang, and R. K. Gupta, *Biochemistry*, 1979, **18**, 457.
[457] J. De Bony, J. Dufourcq, and B. Clin, *Biochim. Biophys. Acta*, 1979, **552**, 531.
[458] L. R. Brown, *Biochim. Biophys. Acta*, 1979, **557**, 135.
[459] J. Lauterwein, C. Boesch, L. R. Brown, and K. Wüthrich, *Biochim. Biophys. Acta*, 1979, **556**, 224.
[460] W. Stoffel, K. Salm, and B. Tunggal, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 523.
[461] B. De Kruijff, A. M. H. P. Van den Besselaar, H. van den Bosch, and L. L. M. Van Deenen, *Biochim. Biophys. Acta*, 1979, **555**, 181.
[462] M. R. Egmond, R. J. P. Williams, E. J. Welsh, and D. A. Rees, *Eur. J. Biochem.*, 1979, **97**, 73.
[463] A. Lapidot and C. S. Irving, *Biochemistry*, 1979, **18**, 1788.
[464] M. A. Bernstein, L. D. Hall, and W. E. Hull, *J. Am. Chem. Soc.*, 1979, **101**,
[465] K. Dill and A. Allerhand, *J. Biol. Chem.*, 1979, **254**, 4524.
[466] K. Dill and A. Allerhand, *FEBS Lett.*, 1979, **107**, 26.
[467] S. Highsmith, K. Akasaka, M. Konrad, R. Goody, K. Holmes, N. Wade-Jardetzky, and O. Jardetzky, *Biochemistry*, 1979, **18**, 4238.
[468] D. I. Levitskii, A. F. Rusak, N. L. Kashtanova, E. D. Vorontsov, V. P. Evdakov, and B. F. Poglazov, *Biokhimiya*, 1979, **44**, 1721.
[469] N. Hiroshi and T. Yasui, *Agric. Biol. Chem.*, 1979, **43**, 89.
[470] S. Forsen, E. Thulin, and H. Lilja, *FEBS Lett.*, 1979, **104**, 123.

have been studied.[471] The action of muscle is heavily dependent on calcium-binding proteins; the calcium-binding properties of the C subunit of troponin have been investigated by ^{113}Cd resonance[472] and the relative compactness of the binding sites of troponin C and of parvalbumin by lanthanide shift methods.[473] Cation-dependent conformational states of two proteins with large sequence homologies with troponin C, calmodulin,[474] and brain calcium-ion-dependent regulator protein[475] seem to show regions of very similar tertiary structure to those previously reported for the troponin subunit. Parvalbumins, another group of calcium-binding proteins found particularly in the muscle of fish, have been subjected to a number of studies mostly based on substitution of a perturbing or reporting metal ion for calcium; reporters include ^{23}Na,[476,477] ^{25}Mg, and ^{113}Cd.[478] Primary and secondary sites of parvalbumins have been investigated using gadolinium(III), yielding dissociation constants,[479] and a number of assignments in the ^{13}C spectrum also follow lanthanide substitution.[480] The influence of calcium binding on the ^{1}H spectrum of parvalbumins has been followed by two groups of workers;[481,482] a model structure for the binding of calcium to the vitamin-D-dependent intestinal calcium-binding protein is presented.[483]

With the discovery that the 'core' histone proteins H2A, H2B, H3, and H4 are found in an octameric complex in chromatin, interest in the behaviour of isolated histone molecules is lessening; one study of self-aggregation in 'core' histones is reported.[484] Histone H1 appears to interact independently with DNA; some ^{13}C measurements on its structured segment lead to the claim that this segment comprises residues 41—89, some thirty residues shorter than found by earlier workers,[485] and the effect of phosphorylation at Ser-37 is shown, in agreement with earlier work, to induce the removal of the central and *N*-terminal regions of the protein from a DNA complex.[486] Some model studies which may well prove useful in future investigations of histones involve the formation of hydrogen bonds between nucleic acid bases and amides[487] and involve work on the exchangeable protons of arginine, oligoarginine, and the arginine-rich histone tetramer.[488] Protamines, which are very arginine-rich, are apparently in an extended confor-

[471] L. Lee, B. D. Sykes, and E. R. Birnbaum, *FEBS Lett.*, 1979, **98**, 169.
[472] K. B. Seamon, *Biochemistry*, 1980, **19**, 207.
[473] K. Seamon, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1256.
[474] J. Parello, P. Reimarsson, E. Thulin, and B. Lindman, *FEBS Lett.*, 1979, **100**, 153.
[475] C. Gerday, J. Grandjean, and P. Laszlo, *FEBS Lett.*, 1979, **105**, 384.
[476] A. Cave, J. Parello, T. Drakenberg, E. Thulin, and B. Lindman, *FEBS Lett.*, 1979, **100**, 148.
[477] A. Cave, M. F. Daures, J. Parello, A. Saint-Yves, and R. Sempere, *Biochimie*, 1979, **61**, 755.
[478] D. J. Nelson, A. D. Theoharides, A. C. Nieburgs, R. K. Murray, F. Gonzales-Fernandez, and D. S. Brenner, *Int. J. Quantum Chem.*, 1979, **16**, 159.
[479] W. J. Birsdall, B. A. Levine, R. J. P. Williams, J. G. Damaille, J. Haiech, and J. F. Pechere, *Biochimie*, 1979, **61**, 741.
[480] D. M. Rice, M. D. Meadows, A. O. Schienman, G. M. Goni, J. C. Gomez, M. A. Moscarello, D. Chapman, and E. Oldfield, *Biochemistry*, 1979, **18**, 5892.
[481] B. A. Wallace and E. R. Blout, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1775.
[482] A. Cave, M. Pages, P. Morin, and C. M. Dobson, *Biochimie*, 1979, **61**, 607.
[483] W. J. Birdsall, B. A. Levine, R. J. P. Williams, C. S. Fullmer, and R. H. Wasserman, *Biochem. Soc. Trans.*, 1979, **7**, 702.
[484] T. Tancredi, P. A. Temussi, G. Di Pascale, and C. Fournier, *Eur. J. Biochem.*, 1979, **100**, 219.
[485] T. Tancredi and P. A. Temussi, *Biopolymers*, 1979, **18**, 1.
[486] M. P. Kirpichnikov, A. V. Kurochkin, B. O. Glotov, and E. S. Severin, *Stud. Biophys.*, 1978, **72**, 171.
[487] G. Lancelot and C. Helene, *Nucleic Acids Res.*, 1979, **6**, 1063.
[488] L. Klevan and D. M. Crothers, *Biopolymers*, 1979, **18**, 1029.

mation in aqueous solutions, but their ^{13}C spectra indicate some partial folding in the presence of phosphate.[489] Considerable regions of tertiary structure are also reported for the ribosomal proteins S4,[490] S15,[491] and S1.[492]

A continuing series of methods for the difficult task of mapping the combining sites of antibodies is extended this year by ^{31}P probes[493] and paramagnetic probes[494] applied to the nitrophenyl-binding mouse myeloma protein M315. A specifically mercuriated inter-heavy-chain disulphide bond was used[495] to produce effects on the ^{35}Cl resonance of solvent chloride anion, which changed on hapten binding, an effect attributed to the alteration of steric relations within the hinge region. Bence–Jones proteins have been studied by titration of the two constant-domain histidines 189 and 198, revealing that κ-type and λ-type Bence–Jones proteins have basically similar conformations in the constant region, but with the latter type less compact than the former.[496]

Any light on the mechanisms of specific molecular recognition is welcome; lectins and periplasmic proteins are useful subjects for investigations of this type. Solvent relaxation dispersion has been used to probe the interaction between concanavalin A and mono- and oligo-saccharides,[497,498] indicating a single saccharide residue binding site, with all the saccharides which bind producing the same protein conformational transition. Binding of branched-chain amino-acids to the branched-chain-specific periplasmic protein[499] and of histidines to the histidine-binding proteins J of *Salmonella typhimurium*[500] are also reported.

The massive task of investigating by n.m.r. the lac-repressor of *E. coli* continues with the report this year of a possible region of δ-helix (a left-handed 4.3_{14} helix) in the structure[501] and indications of enhanced mobility in the headpiece of the protein.[502] The albumins remain useful subjects for protein structure experiments; magnetic relaxation of the denaturation of ovalbumin are reported,[503] as are the internal motions of human serum albumin[504] and the interactions between bovine plasma albumin and sodium dodecyl sulphate[505] and between monovalent and divalent ions and bovine serum albumin.[506] Gold–phosphine drugs interact with albumin.[507]

[489] G. M. Bonotora, L. Ferrara, L. Paolillo, C. Toniolo, and E. Trivellone, *Eur. J. Biochem.*, 1979, **93**, 13.
[490] J. Littlechild, C. A. Morrison, and E. M. Bradbury, *FEBS Lett.*, 1979, **104**, 90.
[491] Z. V. Gogiya, S. Y. Venyaminov, V. N. Bushuev, I. N. Serdyuk, V. I. Lim, and A. S. Spirin, *FEBS Lett.*, 1979, **105**, 63.
[492] P. B. Moore and M. Laughrea, *Nucleic Acids Res.*, 1979, **6**, 2355.
[493] S. K. Dower and R. A. Dwek, *Biochemistry*, 1979, **18**, 3668.
[494] S. K. Dower and R. A. Dwek, *Int. J. Biol. Macromol.*, 1979, **1**, 119.
[495] S. Vuk-Pavlovia, D. E. Isenman, G. A. Elgavish, A. Gafni, A. Licht, and I. Pecht, *Biochemistry*, 1979, **18**, 1125.
[496] Y. Arata and A. Shimizu, *Biochemistry*, 1979, **18**, 2513.
[497] C. F. Brewer and R. D. Brown, *Am. Chem. Soc. Symp. Ser.*, 1979, **88**, 27.
[498] C. F. Brewer and R. D. Brown, *Biochemistry*, 1979, **18**, 2555.
[499] A. M. Chauvet-Monges, J. P. Monti, A. Crevat, C. Gaudin, J. C. Sari, J. P. Sari, and J. P. Belaich, *J. Chim. Phys. Phys. Chim. Biol.*, 1979, **76**, 714.
[500] B. A. Manuck and C. Ho. *Biochemistry*, 1979, **18**, 566.
[501] R. Chandrasekaran, T. S. Jardetzky, and O. Jardetzky, *FEBS Lett.*, 1979, **101**, 11.
[502] N. Wade-Jardetzky, R. P. Bray, W. W. Conover, O. Jardetzky, N. Geisler, and K. Weber, *J. Mol. Biol.*, 1979, **128**, 259.
[503] B. Blicharska and M. Rydzy, *Acta Phys. Pol. A.*, 1979, **A56**, 439.
[504] S. I. Aksenov and A. V. Filatov, *Biofizika*, 1979, **24**, 324.
[505] Y. Inoue, S. Sase, R. Chujo, S. Nagaoka, and M. Sogami, *Biopolymers*, 1979, **18**, 373.
[506] B. Lubas, M. Soltysik-Rasek, and I. Lesniewska, *Biochemistry*, 1979, **18**, 4943.
[507] N. A. Malik and P. J. Sadler, *Biochem. Soc. Trans.*, 1979, **7**, 731.

Turning to structural proteins, measurements of the collagen backbone show rapid re-orientation of the helix backbone in the fibrils;[508] a model for collagen hydration is supported.[509] The structural proteins of enamel matrix appear to fall into two classes, one of which exhibits rapid mobility and accounts for some two-thirds of the enamel matrix.[510] Mobility transitions in solid amorphous silk fibroin [511] and individual ^{13}C resonances, detected in solid major coat protein from phage fd are reported.[512] Labelled fluorotyrosyl residues of M_{13} coat protein are apparently buried in the hydrophobic portion of the membrane bilayer in phospholipid vesicles [513] since they are not accessible to solvent. Molecular mobility studies of TMV coat protein are reported.[514] Finally, the involvement of histidine residues in the substrate binding of the protein elongation factor Tu from an extreme thermophile, *Thermus thermophilus*, is described [515] and ^{113}Cd evidence for very much stronger metal binding sites in the cysteine-rich metal binding protein metallothionein is presented.[516]

5 Infrared and Raman Spectroscopy

Contributed by R. M. Stephens

Model Compounds.—Through the use of Raman and i.r. spectroscopy, the mode of incorporation of glycine, a gelatin inhibitor, in a complex antiperspirant material containing zirconium and aluminium hydroxychlorides was determined. Most of the vibrational modes of glycine were affected and the results indicated that the glycine existed as a dipolar ion co-ordinated to metal ions through the carboxyl group and hydrogen bonded to various constituents through the amino-group. This binding could well contribute to the gelatin-inhibiting property of the antiperspirant by interfering with the extended OH and oxy bridging that commonly exists in solution.[517] Reaction dynamics and properties of melanoidins formed in the reaction of glyceraldehyde with glycine have been followed. After prolonged incubation the water-soluble fraction contained 45—50% of the initial amount of glycine as well as small amounts of glyceraldehyde. The level of glycine incorporation into the insoluble fraction implied a glyceraldehyde : glycine ratio of 2 : 1 in the polymer. Although the spectra were poorly defined no cyclic structures were detected.[518] The i.r. spectra of dibutylglycine and glycine in various solvents, as well as in the vapour phase, have shown that in the gas phase monomeric molecules exist in the neutral form whereas in solution the structure depended on the solvent.[519] The structures of tri-n-butyltin derivatives of L-cysteine, *N*-acetyl-L-

[508] L. W. Jelinski and D. A. Torchia, *J. Mol. Biol.*, 1979, **133**, 45.
[509] Y. G. Buishvili and G. M. Mrevlishvili, *Biofizika*, 1979, **24**, 336.
[510] J. D. Termine, D. A. Torchia, and K. M. Conn, *J. Dent. Res.*, 1979, **58**, 773.
[511] M. Nagura and H. Ishikawa, *Polym. J.*, 1979, **11**, 159.
[512] S. J. Opella, M. H. Frey, and T. A. Cross, *J. Am. Chem. Soc.*, 1979, **101**, 5856.
[513] D. S. Hagen, J. H. Weiner, and B. D. Sykes, *Biochemistry*, 1979, **18**, 2007.
[514] J. L. De Wit, N. C. M. Alma-Zeestraten, M. A. Hemmings, and T. J. Schaafsma, *Biochemistry*, 1979, **18**, 3973.
[515] A. Nakano, T. Miyazawa, S. Nakamura, and Y. Kaziro, *Arch. Biochem. Biochem. Biophys.*, 1979, **196**, 233.
[516] J. D. Otvos and I. M. Armitage, *J. Am. Chem. Soc.*, 1979, **101**, 7734.
[517] R. P. Oertel and R. M. Rusch, *Appl. Spectrosc.*, 1979, **33**, 114.
[518] L. P. Korzhova and Yu. A. Romakov, *Prikl. Biokhim. Mikrobiol.*, 1979, **15**, 585.
[519] G. S. Denisov, I. G. Rumynskaya, and V. M. Shraiber, *Zh. Prikl. Spectrosk.*, 1979, **31**, 275.

cysteine, and α-glutamylcysteinylglycine have been determined from their ^1H and ^{13}C n.m.r., i.r., and Mössbauer spectra. There is evidence that the NH_2 is involved in hydrogen bonding but not the C=O.[520]

The amide I, II, III, V, and ν(NH) regions of the i.r. and Raman spectra of Me_3CO_2C—X—OMe and some deuteriated samples in the solid state have shown that parallel β-sheet structures could be formed when X = Phe and a mixture of parallel and antiparallel extended chains when X = Val or Ile. The conformations present were dependent upon chain length.[521] Conformational analysis of polyethyleneglycol-bound alanine and valine homo-L-oligopeptides indicated that peptides with several amino-acids (n = 5—8) assumed a β-structure in the solid state. At low concentrations in $CDCl_3$ the Ala_4 peptide had a much higher content of intramolecular hydrogen bonds than the Val_4 peptides. At higher concentrations, the extent of peptide association was determined by chain length, solvent, and side-chain interactions. Preliminary results on the relationship between reactivity of polymer-bound growing peptide chains during step-wise synthesis and peptide conformation were given.[522] The synthesis and characterization of tetra-γ-benzyl L-glutamates have shown that all the tetrapeptides have a β-structure of antiparallel chains in the solid state and a σ-conformation in CF_3CH_2OH and dioxan.[523] *X*-Ray and i.r. data obtained from *N*-t-amyloxycarbonyl-L-proline showed that in the solid state the compound is held together through intermolecular urethane hydrogen bonds and that the tertiary amide bond had a *cis* configuration and was not folded into an oxy-C_7 peptide conformation. In cyclohexane non-associated and associated species were found, as well as a small amount of the oxy-C_7 form.[524] The conformations of *N*-t-butyloxycarbonyl-L-prolylsarcosine and *N*-t-butyloxycarbonylsarcosylsarcosine in the solid state and in solution have been analysed using i.r. and *X*-ray diffraction. In the solid state, a *cis*-t-urethane bond and an intermolecular hydrogen bond between the OH of the COOH and the amide oxygen characterized the molecular and crystal structure. In solution the formation of intramolecular hydrogen bonds was negligible even in a solvent of low polarity and high dilution.[525] Far-i.r. spectroscopy of the oligopeptides of the type Nps-(L-Ala)$_n$-(L-Leu)$_m$-(L-Ala)$_n$-OEL when Nps = $2\text{-}O_2NC_6H_4S$ has shown that terminal tripeptide units in the α-helical oligopeptides were not incorporated into the internal helical structures but act as supports for these structures.[526] A study of the structure of some amino-acids and acid amides of the thiophene series has shown that both intramolecular and intermolecular hydrogen bonding is present.[527]

The i.r. spectra of model compounds AcNHCHMeCONHMe and $AcNHCH_2CONHMe$ have been calculated in order to obtain the necessary

[520] G. Domazetis, R. J. Magee, and B. D. James, *J. Organomet. Chem.*, 1979, **173**, 357.
[521] M. H. Baron, C. de Loze, C. Toniolo, and G. D. Fasman, *Biopolymers*, 1979, **18**, 411.
[522] G. M. Bonora, M. Palumbo, C. Toniolo, and M. Mutter, *Makromol. Chem.*, 1979, **180**, 1293.
[523] K. Okahashi and S. Ideha, *Int. J. Pept. Protein Res.*, 1979, **13**, 462.
[524] E. Benedetti, A. Ciajolo, B. D. Blasio, V. Parone, C. Pedone, C. Toniolo, and G. M. Bonora, *Int. J. Pept. Protein Res.*, 1979, **14**, 130.
[525] E. Benedetti, A. Ciajolo, B. D. Blasio, V. Pavone, C. Pedone, C. Toniolo, and G. M. Bonora, *Macromolecules*, 1979, **12**, 438.
[526] R. Katakai, *J. Chem. Soc., Chem. Commun.*, 1978, **16**, 692.
[527] L. V. Khazhakyan, I. P. Badalyants, A. P. Mkrtchyan, and A. S. Noravyan, *Arm. Khim Zh.*, 1978, **31**, 516.

parameters for the theoretical analysis of polymer spectra and for the determination of the sensitivity of i.r. spectra to isomerism. The comparison of dipeptides with different amino-acid residues led to conclusions about the effect of side-chains on the skeletal oscillation of peptide groups.[528] The dipeptide L-alanylglycine was cyclized with ε-aminocaproic acid and used as a model for the β-bend conformation. Spectral data suggested that a type III β-bend existed in the alanylglycine moiety, with an NH · · · OC hydrogen bond in the ε-aminocaproic acid portion of the molecule. In contrast, an open-chain analogue existed in solution but with a significant amount of type II β-bend structure present.[529] Normal vibrational calculations were made for a type I β-turn of MeCO-$(Ala)_4$-NHMe and a type II β-turn of MeCO-$(Ala)_2$-Gly-Ala-NHMe. The force field was refined for β-sheet and α-helical structures. A calculation was also done for MeOCO-Gly-$(Ala)_2$-Gly-OMe which was an appropriate model for two tetrapeptides for which i.r. data was available. The agreement between observed and calculated frequencies was good. The most important result from the calculations was the prediction of bands near 1690 cm^{-1}. Thus bands observed in proteins near 1690 cm^{-1} should be associated with the presence of β-turns as well as of β-sheets. A band near 1665 cm^{-1} is characteristic of the type II turns.[530]

I.r. and n.m.r. data showed that the favoured conformations of R^1CO-Pro-X-NHR^2 and R^1CO-X-Pro-NHR^2 (R^1 = Me_3C, Me_2CH; X = Gly, Ala, D-Ala, $MeNCH_2CO$; R^2 = Me, $CHMe_2$) in solution depended on both the primary structure and the chirality. In inert solvents Pro-D-Ala and Pro-Gly peptides had βII-folds whereas D-Ala-Pro and Pro-Ala peptides preferred the βII-turn and βI-fold, respectively. Pro-Ser and X-Pro peptides exhibited *cis–trans* isomerism about the middle tertiary amide. In DMSO the βII-folds were not destabilized unlike the βI-conformation. All these conformations except for βII-folding for D-Ala-Pro peptides vanished in water.[531] The i.r. spectra of polyglycine with β and spiral structures, as well as their *C*- and *N*-deuteriated derivatives, were calculated using the force constants obtained from the spectra of Ac-Gly-NHMe and satisfactory agreement was obtained between the calculated and experimentally determined spectra.[532] Rotational and translational vibrations in glycine were analysed by the GF matrix method and force constants associated with the hydrogen bonds were obtained.[533] Polarized i.r. spectra obtained for the *a–b* and *a–c* crystallographic axes of alanine single crystals were used to determine transition-moment directions and to evaluate the intensity components of each band along the *a*-, *b*-, and *c*-axes.[534] From the polarized Raman spectra of α-glycine-*c*-[$^2H_2$] and DL-alanine-αβ-[$^2H_4$] crystals splittings due to the non-bonded interactions were observed for the CD_2 bending mode of glycine D-[$^2H_2$] and the CD_3 asymmetric deformation and torsional modes of DL-alanine-α,β-[^2H]. These factor group splittings and their change on *N*-deuteriation were explained by a

[528] S. A. Stepanyan, L. A. Gribov, and E. M. Popov, *Izv. Timiryazevsk. SkH. Akad.*, 1979, 149.
[529] R. Deslauriers, S. J. Leach, F. R. Maxfield, E. Minasian, J. R. McQuie, Y. C. Meinwald, G. Nemethy, M. S. Pottle, and I. D. Rae, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2512.
[530] J. Bandekar and S. Krimm, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 774.
[531] G. Boussard, M. Marraud, and A. Aubry, *Biopolymers*, 1979, **18**, 1297.
[532] S. A. Stepanyan and L. A. Gribov, *Dokl. Akad. Nauk. SSSR*, 1979, **246**, 1418.
[533] J. Herranz, P. Gomez-Sal, and J. L. Nieto, *An. Quim.*, 1978, **74**, 1366.
[534] M. Castineira and J. Herranz, *An. Quim.*, 1979, **75**, 250.

normal co-ordinate analysis with an intermolecular potential of the exp-6 type for the non-bonded interactions. Good agreement was obtained between the observed and calculated lattice frequencies on addition of the N—O non-bonded interaction potential, the hydrogen bond stretching terms, and the Coulomb interaction terms to the intermolecular potential.[535]

Polypeptides.—A new conformation of poly L-phenylalanine has been produced by polymerization of L-phenylalanine *N*-carboxy anhydride using a $BuNH_2$ catalyst in MeCN in the presence of poly(ethylene oxide). The unusual CO form was thermally stable and the crystallinity increased with heat treatment.[536] I.r. and c.d. studies of the peptide chains of the ABA block co-polymers consisting of poly (γ-benzyl L-glutamate) as the A-component and polybutadiene as the B-component have shown that an α-helical conformation is present in solution. In the solid state the polybutadiene chains are in random-coil conformation and form cylindrical domains embedded in the peptide matrix phase, which has an α-helical conformation.[537] Polarized i.r. and *X*-ray diffraction of stretched films of poly(α-isobutyl-L-aspartate) indicated that the conformation in the solid state was a cross-β form. In solution there was evidence that some β-structure was present but it was very unstable.[538] A series of oligopeptides with alternate L-alanyl and L-leucyl residues has been prepared by stepwise synthesis of *p*-nitrophenylsulphenyl *N*-carboxy α-amino-acid anhydrides for lower oligopeptides and fragment condensation with *N*-hydroxysuccinimide and DCC for higher oligopeptides. Conformational assignments made on the basis of i.r. and *X*-ray crystallography showed that α-helical conformations were found in deca- and higher peptides whereas β-structures formed at the heptapeptide level.[539]

Poly(Ala-Lac) (I), where Lac = L-lactic acid residue, poly(Ala-Ala-Lac) (II), and poly(Ala-Ala-Ala-Lac-Lac) of molecular weight 30 000—80 000 dalton were prepared by the matrix-controlled polymerization of the appropriate $H\text{-}Ala_m\text{-}Lac_n\text{-}OC_6Cl_5$ ($m = 1, 2$, $n = 1$; $m = 3$, $n = 2$) on a celite matrix. By c.d. and i.r. spectroscopy (I) was *ca.* 50% ordered in $CHCl_3$ while (II) was helical in $CHCl_3$ and random in CF_3CH_2OH. Poly(Ala-Ala-Lac) undergoes a helix-coil transition on heating in $CHCl_3$.[540] Polypeptides $(Lys\text{-}X\text{-}Ala)_n$ in which X represents residues of alanine, norvaline, valine, or leucine have been synthesized *via* the polymerization of 1-hydroxysuccinimidyl esters of appropriate tripeptides; spectra of these complexes with DNA have been obtained. Alanine- and norvaline-containing polypeptides revealed mostly random-coil conformation at low ionic strength; the content of α-helix increases with increasing NaCl molarity and pH. The valine-containing polypeptide assumes a random-coil conformation independently of ionic strength, but in alkaline medium and in the solid state the β-conformation is formed. The predominant conformation of the leucine-containing polymer in low and medium ionic strengths was α-helix. The behaviour of (Lys-Leu-Ala) was

[535] K. Machida, A. Kagayama, and Y. Saito, *J. Raman. Spectrosc.*, 1979, **8**, 133.
[536] T. Komoto, M. Kodaka, and T. Kawai, *Makromol. Chem.*, 1979, **180**, 819.
[537] A. Nakajima, T. Hayashi, K. Kugo, and K. Shinoda, *Macromolecules*, 1979, **12**, 840.
[538] H. Yuki, Y. Marubayashi, Y. Taketani, T. Tsubota, and Y. Marubayashi, *J. Polym. Sci. Polym. Chem. Ed.*, 1978, **16**, 2237.
[539] R. Katakai, *J. Chem. Soc., Perkin Trans. 1*, 1979, **4**, 905.
[540] M. Goodman, *J. Polym. Sci. Poly. Symp.*, 1978, 62nd Int. Symp. Macromol., 173.

anomalous with respect to properties of the complexes with DNA.[541] The conformation and conformational transitions of poly(His-Ala-Glu) have been investigated using i.r., n.m.r., and c.d. spectroscopy. In water the polypeptide had a disordered conformation. On titrating the side-chain imidazole and carboxyl groups the overall shape of the macromolecule altered, while the backbone conformation changed from one disordered state to another, but never assumed a regular form. In aqueous MeOH and aqueous trifluoroethanol, transitions from a disordered state to the α-helix conformation were observed on increasing the alcohol content of the system.[542]

Extended phonon-dispersion curves for polyglycine, polyalanine, and polyproline were used to analyse the vibrational spectra of H-X_n-OH (X = Gly, n = 2—6;[528] X = Ala, n = 1—6; X = Pro, n = 2—6, 8). When X = Gly, n = 5, or X = Ala, n = 6, the polymers have linear zig-conformations, whereas when X = Gly, n = 6, helical conformations were present; these were similar to those of polyglycine II. When X = Pro, $n > 4$, the conformations were similar to the right-handed three-fold helical structure of polyproline II.[543] Glycine *N*-carboxyanhydride has been polymerized using Et_3N initiation in dioxan at 25 °C for 90 h, and the i.r. spectra in the region 4000—50 cm^{-1} have been extensively analysed for this polymer which had a β-conformation.[544]

Proteins and Natural Peptides.—*Retina Components.* Outer segments of the retinal rod from frog which contain α-helical segments of rhodopsin have been oriented in a magnetic field. The segments were preferentially aligned perpendicular to the plane of the β-disc membrane. It was concluded that the α-helix portion of the protein has an average orientation in the transmembrane direction. Furthermore i.r. spectra of rods in ^2H_2O Ringers solution exhibited two distinct peptide amino-group absorption bands: the unexchanged N—H band, which is dichroic, and the proton-exchanged N—^{2}H, which is non-dichroic. This implies that the oriented part of the protein is in the lipid bilayer, supporting a model for rhodopsin with a hydrophobic core containing partially oriented α-helices with hydrophilic ends consisting of unoriented polypeptide.[545] The amine-I absorption band of the peptide C=O group in rhodopsin has been resolved with a curve synthesizer into components attributable to α-helix, random-coil, and β-pleated sheet conformations. When a suspension of rod outer segments was heated, a gradual conversion of α-helix to random coil was observed up to 55 °C; further heating promoted formation of β-structure. In the dark, it was found that rhodopsin had an enthalpy of denaturation of 119 kJ at 75 °C and bleached samples denatured at 70 °C with an enthalpy of 178 kJ.[546] Resonance Raman spectroscopy has been used to study chemically modified retinal analogues involving chain substitutions, ring substitutions, or Schiff-base linkages. In addition, retinal fragments and fully deuteriated retinals were investigated and i.r. spectra of the four isomers of retinal obtained.

[541] S. Stokrova, K. Zimmermann, J. Sponar, and K. Blaha, *Collect. Czech. Chem. Commun.*, 1978, **43**, 2341.
[542] J. H. Goren, M. Fridkin, E. Katchalski-Katzir, and N. Lotan, *Biopolymers*, 1979, **18**, 981.
[543] M. K. Gupta and V. D. Gupta, *Indian J. Phys.*, 1977, **51**(B), 462.
[544] K. Horitsu, *Tokyo Kasei Daigaku Kenkyu Kiyo*, 1978, **18**, 21.
[545] H. R. Saibil and M. Chabre, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4405.
[546] V. L. Shnyrov, A. L. Berman, and Yu. A. Lazarev, *Biofizika*, 1979, **24**, 752.

Low-frequency resonance Raman spectra were also reported for all of the isomers of retinal, for the protonated and unprotonated Schiff bases of *trans*-retinal, for β-ionone, and for *trans*-3-dehydroretinal. Band assignments were made to specific vibrational motions, which have led to a detailed understanding of the spectral features observed in the resonance Raman spectra of the retinylidene chromophore in rhodopsin and bacteriorhodopsin.[547] A hydrophobic, lipid, and pigment-free polypeptide from the chromophore membrane of *Rhodospirillum rubrum* has been cast from solutions as a monolayer on an air–water interface. Multi-layers built up on Ge plates at different surface pressures were subject to attenuated total reflection i.r. spectroscopy. In all cases the amide-I and -II absorption bands were typical of α-helical and random-coil conformations.[548]

Cytochromes. An i.r. study of carbon monoxide bonding to iron(II) cytochrome P-450 was undertaken to probe the spatial relation between the O_2 and substrate binding sites and to determine whether the presence of a unique axial ligand bound *trans* to the CO of FeCO could be responsible for the red-shifted Soret absorbance band at 450 nm. The data indicated that (i) the FeCO bonding in each of the cytochromes P-450 was similar to that observed in the case of the iron(II)–carbonyl complexes of Hb(HbCO) and myoglobin (MbCO), (ii) in the presence of *d*-camphor the haem-carbonyl of the bacterial cytochrome exhibited non-linear bonding, whereas in its absence both non-linear and linear bonding existed giving evidence for a close spatial relation between the haem–carbonyl and *d*-camphor binding site, (iii) FeCO bonding in cytochrome P-420 was nearly the same as that of denatured HbCO and MbCO, suggesting linear haem-carbonyl bonding and a nitrogenous *trans* axial ligand, (iv) the haem-carbonyl of the liver microsomal cytochrome sensed a less ordered nearest-neighbour environment than in the bacterial cytochrome, perhaps partially the result of their having larger and more flexible substrate-binding sites, (v) identification of the axial ligand bound *trans* to the carbonyl was not possible.[549] Changes produced in the amide-I region of the i.r. spectrum of cytochrome *c* have been studied from films cast from different solvents. The positions of the amide-I and amide-II peaks in solution showed that the molecule had more order in a non-polar solvent. Mixtures of cytochrome *c* with phosphatidylcholine in non-polar solvents indicated the formation of a lipoprotein complex held together by hydrogen bonds between the peptide NH groups of the protein and the carbonyl and phosphate groups of the lipid. A small amount of β-structure was also detected in the complex.[550]

Alamethicin. The 3500—3250 cm^{-1} region of the i.r. spectra of *N*-terminal fragment peptides of alamethicin was studied in dilute solution in $CHCl_3$ and CCl_4. The free-NH stretching frequency (*ca.* 3435 cm^{-1}) was almost constant in intensity, whereas the hydrogen-bonded NH stretching frequency (*ca.* 3350 cm^{-1}) increased in intensity with increasing length of the peptide chain. It was postulated that a B_{10} helical structure is present in the *N*-terminal position of alamethicin, and

[547] R. E. Cookingham, A. Lewis, and A. T. Lemley, *Biochemistry*, 1978, **17**, 4699.
[548] F. Kopp. P. A. Cuendet, K. Muehlethaler, and H. Zuber, *Biochim. Biophys. Acta*, 1979, **553**, 438.
[549] D. H. O'Keefe, R. E. Ebel, J. A. Peterson, J. C. Maxwell, and W. S. Caughey, *Biochemistry*, 1978, **17**, 5845.
[550] V. M. Klimovich, *Mol. Biol. (Kiev)*, 1978, **21**, 32.

Aib-Pro, Pro-X, and Aib-X sequences (Aib = α-aminoisobutyrate, X = any amino-acid) can be accommodated at the corners of type III β-turns.[551]

Silk Fibroin. Silk fibroin films in the random-coil and β-form conformations have been immersed in water at temperatures of 2—130 °C and conformational changes followed using i.r. spectroscopy, *X*-ray diffraction, and differential scanning calorimetry. On treatment with water below 60 °C the random-coil conformation was converted to the β-form and above 70 °C to mixtures of the α- and β-conformations. The β-form content increased as the immersion temperature was raised. The amount of β-form was not affected by immersion in water over the temperature range studied.[552]

Flavoproteins. Riboflavin–apoprotein interactions as studied using i.r. and Raman spectroscopy indicated an irregularly formed β-sheet disordered structure for the apoprotein from hen egg white and a higher content of ordered structure in the flavoprotein as compared with the apoprotein. Intermolecular interaction of the isoalloxazine ring of riboflavin with the apoprotein NH of histidine was indicated. Raman studies of the apoprotein indicate a low content of α-structure and presence of β-structure. Analysis of the visible absorption spectra of a complex of the apoprotein with 2,3-dimethyl-5-ribitylpseudoindophenazine indicated hydrophobic interaction, and Raman spectra showed that hydrophobic interactions influenced changes in the conformation of the apoprotein ligand.[553]

Lysozyme. The far-i.r. absorption spectrum of lysozyme was measured at room and liquid-nitrogen temperatures. Dried layers of single crystals of tetragonal lysozyme chloride with a diameter of 100—300 μm were grown on a silicon plate. Such single-crystal samples had the following advantages in obtaining far-i.r. spectra: (i) surface scattering was reduced, (ii) the protein molecules were closely packed, and (iii) air-drying of the crystals reduced the number of water molecules without considerable change in the original configuration. The spectrum obtained consisted of a strong background absorption and a number of absorption peaks that were poorly defined when the sample was in the lyophilized form. These peaks were ascribed to various delocalized vibrations of the main and side chains.[554]

Haemoglobin. Fourier-transform i.r. spectra of the vibrational absorption bands from each of the cysteine SH groups in haemoglobin have been identified and studied using aqueous solutions of isolated α-chains from human, pig, horse, and cow haemoglobins. The vibrational absorption band frequency of α-104 cysteine SH was highly sensitive to change in the α-chain tertiary structure and the quaternary structure of the tetramer was modulated by the haem-ligand complex. The stretching vibration of the co-ordinated dioxygen was split into two bands at 1156 cm^{-1} by Fermi resonance with the Fe—O vibrational overtone. This strong vibrational coupling was associated with a polarized covalent Fe—O bond.[555] The

[551] C. P. Rao, R. Nagaraj, C. N. R. Rao, and P. Balaram, *FEBS Lett.*, 1979, **100**, 244.
[552] J. Magoshi, M. Mizuide, Y. Magoshi, K. Takahashi, M. Kulo, and S. Nakamura, *J. Polym. Sci. Polym. Phys. Ed.*, 1979, **17**, 515.
[553] Z. Zak, I. Malkiewicz, and G. Pytasz, 'Flavins Flavoproteins: Physicochemical Properties and Function', 1977, 39.
[554] M. Ataka and S. Tanaka, *Biopolymers*, 1979, **18**, 507.
[555] J. O. Alben, G. H. Bose, and P. P. Moh, Biochemical and Clinical Aspects Hemoglobin Abnorm. (Proc. Symp.), 1978, 607.

effects of pH upon the carbon monoxide stretching frequency of CO complexes of soybean legoglobins *a*, c_1 and c_2 sperm whale myoglobin, and human HbA have been reported. The results were consistent with a model in which protonation of the distal histidine permitted protein-free haem FeCO geometry in legoglobin–CO complexes but not in myoglobin or Hb–CO complexes. Thus the pockets of legoglobins appear to be more flexible than the haem pockets of myoglobin and Hb. The effect of pH on the spectra of O_2 complexes has also been reported.[556]

Protein Interactions. Data from amino-acid analysis, SH group determinations, and i.r. spectra in a study of the effects of viral infection on tobacco chloroplasts indicated that membrane proteins of susceptible varieties underwent conformational changes caused by disruption of hydrogen bonds, changes in tendency to aggregation, and metabolic disturbances in the infected cells.[557] Fourier-transform i.r. and laser Raman spectroscopy have been used to study the effects of dodecyl pyridinium bromide on the conformation of Hb myoglobin, bovine serum albumin, RNAase, ovalbumin, lysozyme, trypsin, and β-lactoglobulin in aqueous solution. Addition of the cationic detergent caused a decrease in the α-helix conformation in highly helical proteins. At low detergent concentrations, stabilization of the β-sheet conformation was also observed with H2B and H3. In contrast histone H1, which complexes was studied using i.r. linear dichroism. H1–, H2A–, and H4–DNA complexes were studied at different histone : DNA ratios and various relative humidities. The dichroic ratio enabled the determination of the secondary structure of DNA in the complexes. The B → A transition in DNA was inhibited for 19 or 26 base pairs of DNA mol^{-1} of H2A or H4. The stabilization of DNA in a B-conformation was also observed with H2B and H3. In contrast histone H1, which does not occur in the core of the nucleosomes, permitted DNA in H1–DNA complexes to adopt an A-conformation at low humidities for H1 : DNA ratios $< 0.6 : 1$.[559]

6 Circular Dichroism

Contributed by T. Brittain

General.—*Reviews.* Reviews have appeared discussing the calculation of protein c.d. in the near-u.v.[560] and instrumentation and protein spectra in the vacuum-u.v. region.[561] Conformational analysis of proteins and polypeptides has been reviewed,[562] together with the applications of MO theory to chiroptic phenomena.[563]

Instrumental. The first experimental measurement of photoselected fluorescence-detected c.d. has been reported, which used measurement of the difference in fluorescence intensity for left- and right-circularly polarized light.[564] An apparatus

[556] W. H. Fuchsman and C. A. Appleby, *Biochemistry*, 1979, **18**, 1309.
[557] M. E. Ladygina, V. P. Grishkova, and N. V. Aleshina, *Biokhimiya*, 1979, **44**, 1635.
[558] Z. Wasylewski, *Acta Biochim. Pol.*, 1979, **26**, 205.
[559] E. Taillandier, J. Taboury, J. Liquirer, M. C. Gadenne, M. Champagne, and J. Brahms, *Biopolymers*, 1979, **18**, 1877.
[560] P. C. Kahn, *Methods Enzymol.*, 1979, **61**, 339.
[561] W. C. Johnson, *Annu. Rev. Phys. Chem.*, 1978, **29**, 93.
[562] Y. H. Kim, *Pollimo*, 1979, **3**, 89.
[563] G. Snatzke, *Angew. Chem.*, 1979, **91**, 380.
[564] E. W. Lobenstine and D. H. Turner, *J. Am. Chem. Soc.*, 1979, **101**, 2205.

has been described, which allows measurement of c.d. spectra in both the near-u.v. and vacuum-u.v. without the need to purge with nitrogen.[565]

Theory and Analysis. Papers have appeared dealing with the interpretation and analysis of c.d. of dipeptides. The interpretation of the spectra of cyclic dimers of amino-acids has been reported,[566] together with a mathematical procedure, based on the theory of Hilbert spaces, for the evaluation of c.d. spectra of the diastereoisomeric cyclic dipeptides *cyclo*-(L-Pro-L-X) (X = Gly, Ala, Val, Leu, t-Leu; *cis* series) and *cyclo*-(L-Pro-D-X) (X = Ala, Val, Leu, t-Leu; *trans* series). The relation between the calculated subspectra and the structural characteristic was also discussed.[567] Calculations have been performed using the theory of vibronic coupling to the c.d. spectra of disymmetric dimers and have shown that the intensity and energy distribution in the bands are sensitive to changes of the force constants.[568]

The c.d. of molecules, which are large in comparison to the wavelength of light, has been considered and explicit expressions have been obtained for the c.d. of an exciton dimer and a free particle on a helix.[569] The calculated c.d. of an α-helix and of a coiled coil of two α-helices has been compared with experimental results for tropomyosin and maleyated tropomyosin.[570]

A mathematical expression has been derived to express the mean residue ellipticity $[\theta]$ at any wavelength for a protein in aqueous solution. Using parameters obtained from *X*-ray data this function yields good to excellent estimates of helical content.[571]

An analysis of the Jahn–Teller, pseudo-Jahn–Teller, and c.d. of $(E + A)^* e$ systems has been performed[572] as well as that of forbidden transitions induced by lattice vibrations.[573]

A general method for the direct measurement of differential absorption intensities by using a Fourier-transform spectrometer has been described. An extension of the Grosjean–Legrand polarization-modulation technique to Fourier-transform interferometry has led to specific expressions for the measurement of c.d.[574]

The possibility of measuring c.d. by determining the Stokes parameters of a light beam has been described, using a theoretical analysis based on Mueller calculus.[575]

De Voe's classical coupled-dipole oscillator model for molecular optical properties has been extended to the case where the individual oscillators have complex polarizabilities with Lorentzian band shapes. Simplification is achieved for a system in which a subset of the oscillators have natural frequencies so high that their polarizabilities may be regarded as non-dispersive. For this case the order of the eigenvalue problem is reduced to the number of dispersive oscillators. The

[565] G. Brahms and S. Brahms, *Fr. Demande* 2, 1978, **362**, 388.
[566] R. L. Bowman, *Diss. Abstr. Int. B.*, 1979, **39**, 3805.
[567] P. Pancoska, I. Fric, and K. Blaha, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1296.
[568] S. Czarniecki, *Acta Phys. Pol. A.*, 1979, **55**, 733.
[569] I. Tinoco, *Int. J. Quantum Chem.*, 1979, **16**, 111.
[570] T. Kontani, *Juzen, Igakki, Zasshi*, 1978, **87**, 524.
[571] C. T. Chang, C. S. Wu, and J. T. Yang, *Anal. Biochem.*, 1978, **91**, 13.
[572] M. Z. Zgierski and M. Pawlikowski, *J. Chem. Phys.*, 1979, **70**, 3444.
[573] L. M. Kushkulei and B. S. Tsukerblat, *Fiz. Tverd. Tela*, 1979, **21**, 2254.
[574] L. A. Nafie and M. Diem, *Appl. Spectrosc.*, 1979, **33**, 130.
[575] A. J. Wojtowicz and H. J. Lozykowski, *Acta Phys. Pol. A*, 1978, **54**, 867.

normal-mode method has been illustrated by calculations for a 12-residue right-handed α-helix. The predicted absorption and c.d. spectra are similar to those predicted using Gaussian band-shapes but with a 40-fold reduction in computation time.[576]

It has been shown that Fano-type interference between a discrete transition and a continuous transition can lead to natural c.d. line-shapes which deviate significantly from a single superposition.[577]

An interpretation of the vibrational c.d. of Ala, H-Ala-Ala-OH, H-Ala-Ala-Ala-OH, H-Gly-Ala-OH, and H-Ala-Gly-OH has been published.[578]

Weak c.d. can be induced in electric-dipole allowed transitions of achiral species by orientationally uncorrelated interactions with chiral species. This dispersion-induced c.d. can give useful information about states which are outside the spectroscopically accessible energy range.[579]

For the larval haemoglobin III of the insect *Chironomus thummi thummi* the rotational strength of the Soret and Q-bands has been calculated with a monopole–monopole and a dipole–dipole approximation applied to the atomic co-ordinates of the *X*-ray structure obtained at 2.5 and 1.4 Å. This recalculation using higher-resolution data corroborates the negativity of the rotational strength. The most important contributions are found to arise from the previously neglected peptide bonds. The influence of the backbone in this haemoglobin is rather strong and a substantial contribution is also obtained for the perturbation by one of the propionic acid side chains of the haem.[580]

Small Molecules, Model Compounds, and Synthetic Polymers.—*Amino-acids and Derivatives.* As in previous years most of the studies reported in this section deal with research on heavy metal–amino-acid complexes. The complex of palladium(II) with *NN'*-diacetyl-L-cystine-bis(methylamide) in aqueous solution has been studied as a model for the interaction of the L-cystine-residue disulphide bridge with divalent metals. The interaction of palladium with this ligand is facilitated by the ionization of the peptide NH groups, which leads to a chirality change of the disulphide chromophore, especially at neutral or alkaline pH.[581]

Square-planar complexes of [ML] type where M = Pd^{2+}, or Cu^{2+} for L = *NN'*-bis-(L-alanyl)propane-1,3-diaminate anion and M = Ni^{2+} or Cu^{2+} for L = *NN'*-bis-(L-alanyl)ethane-1,2-diaminate anion have been studied by c.d. in aqueous ethanol and DMF. The results were discussed in comparison with those of the corresponding *cis* and *trans* complexes. Assignment of the component *d–d* bands for the copper complexes was made by comparison with palladium and nickel analogues.[582]

Monochelate compounds of platinum(II) of the type $M[PtZCl_2]$ (M = Cs, K; HZ = L- or D-Ala, L-Val, L-Pro, L-hydroxy-Pro, D-allohydroxy-Pro, *N*-methyl-

[576] J. Applequist, K. R. Sundberg, M. L. Olson, and L. C. Weiss, *J. Chem. Phys.*, 1979, **70**, 1240.
[577] A. E. Hansen, *Chem. Phys. Lett.*, 1978, **57**, 588.
[578] M. Diem, P. J. Gotkin, J. M. Kupfer, and L. A. Nafie, *J. Am. Chem. Soc.*, 1978, **100**, 5644.
[579] B. Norden and P. E. Schipper, *Chem. Scr.*, 1977, **12**, 72.
[580] W. Strassburger, A. Wollmer, H. Thiele, J. Fleischbauer, W. Steigermann, and W. Weber, *Z. Naturforsch., Teil C*, 1978, **33**, 908.
[581] H. Lam-Thanh, K. Lintner, M. Monnot, F. Piriou, and S. Fermandjian, *J. Chim. Phys. Chim. Biol.*, 1978, **75**, 755.
[582] T. Komorita and Y. Shimura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1832.

L-hydroxy-Pro, *N*-methyl-L-Pro) have been prepared and their c.d. spectra measured. The hydroxyprolyl and D-allohydroxyprolyl complexes were assigned the absolute configurations *S* and *R*, respectively. The effects on the optical activity of the complexes of the γ-asymmetric C atom of the hydroxyprolyl complexes and also the substitution of H^+ on the donor N atom of L-proline and L-hydroxyproline by D or Me groups were studied.[583]

A comparison between the c.d. spectra of the powder form and a solution of bis-L-Tyr-Cu^{2+} and the single-crystal electronic spectra has been made and explained in terms of the bonding pattern within the complex.[584] In contradiction to previously published reports the 1 : 1 complex between Cu^{2+} and L-Arg has been shown to possess c.d. activity in the visible region of the spectrum.[585]

Five uns-*cis-mer* and two uns-*cis-fac* complexes of (trimethylenediamine-*NN'*-diacetato)(L-aminocarboxylato)cobalt(III), where L-aminocarboxylato denotes L-Ala, L-Val, L-Pro, L-Asp, or L-Glu, have been characterized using c.d. Each of the uns-*cis-mer* and uns-*cis-fac* isomers showed characteristic c.d. behaviour in the second absorption band.[586] The c.d. spectrum of all-*cis*-[Co(L-His)(D-His)]$^+$ has been reported.[587] The complex [Co(dien)(H_2O)(NaL)](ClO_4)$_2$ [dien = diethylenetriamine, H_2L = (*S*)-Glu] is bidentate with a free $-(CH_2)_2COONa$ group. This behaviour has been explained on the basis of both internal entropy loss accompanying seven-membered-ring formation and glutamic acid steric and conformational considerations.[588]

The solvent effects of *p*-dioxan, cyclohexane, CCl_4, CH_2Cl_2, $ClCH_2CH_2Cl$, EtOH, DMF, and DMSO on the c.d. of EtN-acetylphenylalaninate are apparent in non-linear plots of signal intensity against solvent dielectric constant.[589]

It has been proposed that the c.d. spectra of *N*-DNP derivatives may be used in a general method of determining the absolute configuration of abnormal amino-acids. To this end the c.d. of *N*-DNP-Me amino-acids has been analysed and new rules concerning the chiroptical properties of these derivatives proposed.[590]

Dipeptides and Oligopeptides. The interaction of the anions Cl^-, Br^-, I^-, SCN^-, and ClO_4^- with poly-(L-Arg), poly-(L-homo-Arg), and poly-(L-α-amino-γ-guanidinobutyric acid) has been studied using c.d. The ions I^-, ClO_4^-, and SCN^- induce a transition from coil to helix. Various thermodynamic parameters for the transition have been derived and the results compared with similar data for poly-(L-Lys).[591]

Poly-(L-Arg) complexes with poly-(L-Glu) exist as α-helices, whilst in complexes with poly-(L-Asp) the latter exists as a random structure. In the poly-(L-Arg)–poly-

[583] O. P. Slyudkin, I. A. Selezneva, M. A. Kerchentsev, and L. M. Volshtein, *Zh. Neorg. Khim.*, 1979, **24**, 999.
[584] D. H. Brown, N. G. Mangrio, and W. E. Smith, *Spectrochim. Acta*, 1979, **35**, 123.
[585] R. D. Gillard and P. O'Brien, *J. Inorg. Nucl. Chem.*, 1978, **40**, 1671.
[585] M. Okabayashi, K. Igi, and J. Hidaka, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 753.
[587] S. Bagger and H. P. Jensen, *Acta Chem. Scand. Ser. A*, 1978, **32**, 659.
[588] F. Jursik, B. Hayek, and M. S. Abdel-Moez, *Inorg. Chim. Acta*, 1979, **33**, 123.
[589] T. Komyama and M. Miwa, *Seikei Daigaku Kogakubu Kogaku Hokoku*, 1979, **27**, 1913.
[590] U. Nagai, M. Kawai, M. Katsumi, Y. Kani, R. Ueki, and A. Tanaka, *Tennen. Yuki. Kogobutsu. Toronkai,. Koen. Yoshishu*, 1978, 80.
[591] M. Miyazaki, M. Yoneyama, and S. Sugai, *Polymer*, 1978, **19**, 995.

(L-Glu) complex the poly-(L-Glu) undergoes a transformation at 65 °C into a β-sheet structure.[592]

C.d. studies have shown that poly-(L-dopa) copolymers with L-Glu in Me_3PO_4 possess a right-handed helix structure.[593]

It has been reported that Ac-Aib-Pro-Aib-Ala-Aib-Ala-Glu-Aib-Val-Aib-Gly-OPOE [Aib = $HNCMe_2CO$; POE = $(CH_2CH_2O)_nH$] has been synthesized by a stepwise Merrifield-type procedure. A c.d. study of the intermediates produced in the synthesis has shown that α-helix formation begins at the nonapeptide stage for the Boc-peptide polymers and at the decapeptide stage for the *N*-protonated peptide polymers. A random-coil → α-helix transition in the Boc-derivative Boc-Ala(Aib-Ala)$_n$-OPOE occurs at the octapeptide, whereas in the deblocked derivative this occurs at the nonapeptide level.[594]

Polypeptides. The c.d. of poly-Ala in 95.2% H_2SO_4 shows a single negative band at 195 nm consistent with an expanded random coil whilst poly-Val, poly-Leu, and poly-iso-Leu exhibit a negative band at 197 nm. A weaker positive band at 220 nm, due to interaction of electronic transitions of protonated peptide groups, suggests the presence of short conformationally regular sequences.[595]

It has been shown that dodecyl ammonium chloride induces a coil → helix transition in fully ionized poly-(L-Glu); the concentration of detergent necessary to produce the transition is independent of temperature. However, the c.d. of fully ionized poly-(L-Asp) is scarcely affected by the detergent. It is suggested that these results do not imply different interactions between the detergent and Asp and Glu residues, as the Glu residues are known to have a higher helix-forming tendency.[596]

The visible-u.v. absorption of poly-(L-Glu)–Cu^{II} solutions is pH dependent, but does not parallel the helix → coil transition observed in c.d. studies. The extrinsic c.d. bands of bound Cu^{II} were detected in the three absorption band regions and depend on pH. The optical dissymmetry factor indicated that the 700 and 250 nm bands of bound Cu^{II} are associated with multiple c.d. bands of opposite sign. The intrinsic c.d. showed that the Cu^{II} and pH-induced helices of the poly(Glu)–Cu^{II} complex are alike regarding the polymer backbone.[597]

C.d. has been used to show that, in CF_3CH_2OH, Me_3CO_2C-(L-iso-Leu)$_6$-OMe forms soluble molecular aggregates in which the peptide chain assumes the β-conformation.[598] The study of H-(iso-Leu)$_n$-OMe (n = 1—6) in 95.2% H_2SO_4 has led to a possible explanation for the long wavelength positive band in the c.d. spectrum as being due to the reciprocal effect of π–π^* electronic transitions.[599]

The conformational properties of polyethyleneglycol-bound *N*-t-butyloxycarbonyl-terminated homo-oligomers of L-Ala and L-Val have been investigated in alcoholic solution. The L-Ala oligomers exist in right-handed α-helical, β, and statistical-coil conformations, which depend on chain length, solvent

[592] K. Mita, S. Ichimura, and M. Zama, *Biopolymers*, 1978, **17**, 2783.
[593] H. Yamamoto and T. Hayakawa, *Polymer*, 1978, **19**, 1115.
[594] W. Mayr, R. Oekonomopulos, and G. Jung, *Biopolymers*, 1979, **18**, 425.
[595] G. P. Lorenzi, V. Rizzo, F. Thoresen, and L. Tomasic, *Macromolecules*, 1979, **12**, 870.
[596] W. L. Mattice, R. W. McCord, and P. M. Shippey, *Biopolymers*, 1979, **18**, 723.
[597] K. Yamaoka and T. Masuyima, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1286.
[598] U. Widmer, G. P. Lorenzi, and P. Pino, *Biopolymers*, 1979, **18**, 239.
[599] G. P. Lorenzi and L. Tamasic, *Makromol. Chem.*, 1979, **180**, 1103.

polarity, temperature, and the presence or absence of *N*-blocking groups. The β-structure of the L-Val oligomers are more stable than those of the corresponding L-Ala oligomers.[600] In aqueous solution L-Ala and L-Val oligomers bound to polyethyleneglycol may adopt β or statistical-coil conformations depending on the conditions. The β-structure of the L-Val oligomers are more stable than those of the L-Ala as in the ethanolic solution case. The origin of the positive dichroism at 215 nm in the L-Ala oligomers and the effect of monofunctional and bifunctional polyethyleneglycol on peptide conformations were discussed.[601] Studies have also appeared on the properties of homo-oligomers of L-Met bound to polyethyleneglycol in high-polarity solvents such as alcohols, halogenoalcohols, and water. The Met peptides exist in right-handed α-helical, β, and statistical-coil conformations depending on chain length and solvent conditions.[602] Boc-Val-Met$_n$-OMe (Boc = Me_3CO_2C, n = 1—6) is disordered in hexafluoropropanediol sesquihydrate and $P(O)(OMe)_3$ but is helical in CF_3CH_2OH at the heptamer. It was shown that a central Val substituted in the cases of n = 5 or 6 did not disrupt the conformation of the peptide, but the substitution of a Gly did. The effect of the Gly residue was also found to be a function of its position within the chain.[603]

The residue ellipticity at 200 nm of poly{Cys[$(CH_2)_nCO_2H$]} (n = 1) is twice as large as that when n = 2 for the β-conformation present in aqueous solution. However, the β-conformation of the n = 2 peptide was more stable towards electrostatic repulsion.[604]

The statistical accuracy in measurements of circular dichroism has been tested. One hundred c.d. spectra of *cyclo*-(Gly-Phe-Leu-Gly-Leu-Phe) in MeCN at ten concentrations have shown that the accuracy and reproducibility depends on concentration and wavelength. The maximum relative error of intensity varies from 5 to 12% when going from 274 to 196 nm.[605]

The c.d. spectrum of *cyclo*-(L-Pro-Sar-Sar)$_2$ (Sar = sarcosine) has been obtained under various conditions.[606]

A complete recording of the n–π^* and π^0–π^* Cotton effects, down to 170 nm, has been made for *cyclo*-(Ala-Gly-Gly-Ala-Gly-Gly), *cyclo*-(Ala-Ala-Gly-Ala-Gly-Gly), *cyclo*-(Ala-Gly-Ala-Ala-Gly-Gly), *cyclo*-(Ala-Ala-D-Ala-D-Ala-Ala-D-Ala), and *cyclo*-(Ala-D-Ala-D-Ala-Gly-*S*-Ala-Ala). The relative influence of the L- and D-Ala residues is discussed. Assuming the cyclohexapeptides were hydrogen-bonded pleated sheets, dominant changes in the c.d. resulted from a transition of the overall backbone conformation from one close to C_1 symmetry to one closer to C_2 symmetry. The influence of the L- and D-residues within sheet structure was related to an amide-sector rule.[607] In connection with this study an estimation has been made of the confidence intervals for positive and negative c.d. bands of *cyclo*-(Gly-Leu-Gly)$_2$. Chi-square testing to normality showed a 5% error for positive bands and a 10% error for negative bands at maximum values of molar ellipticities.[608]

[600] G. M. Bonora, C. Toniolo, and M. Mutter, *Polymer*, 1978, **19**, 1382.
[601] C. Toniolo, G. M. Bonora, and M. Mutter, *J. Am. Chem. Soc.*, 1979, **101**, 450.
[602] C. Toniolo, G. M. Bonora, S. Salardi, and M. Mutter, *Macromolecules*, 1979, **12**, 620.
[603] F. Naider, J. M. Becker, A. Ribeiro, and M. Goodman, *Biopolymers*, 1978, **17**, 2213.
[604] S. Ikeda, A. Fukutome, T. Imae, and T. Yoshida, *Biopolymers*, 1979, **18**, 335.
[605] P. Pancoska, I. Fric, and K. Blaha, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1312.
[606] Y. Imanishi and S. Kimura, *Jerusalem Symp. Quantum Chem. Biochem.*, 1978, **11**, 273.
[607] M. Iseli, G. Wagniere, J. G. Brahms, and S. Brahms, *Helv. Chim. Acta*, 1979, **62**, 921.
[608] M. Sekulic-Ivankovic, *Teh. Fiz.*, 1978, **19**, 17.

Interest has recently been shown in the c.d. of the β-turn. The vacuum-u.v. c.d. of an isolated 4 → 1 H-bonded β-turn has been reported in *N*-acetyl-Pro-Gly-Leu-OH. The observed spectrum is in good agreement with theoretical predictions with a strong negative band at 180 nm and a large ratio $[\theta]_{201}/[\theta]_{225}$. These features should be useful in distinguishing between β-turn and β-structure.[609] The β-turn has also been studied in cyclic hexapeptides. One series contained L-Pro-D-Phe in the classical type III β-turn geometry whereas a second series contained D-Phe-L-Pro in the closely related geometry of the gramicidin S turn. No significant Phe contribution in the 195—240 nm region is observed in either of the two hydrogenated series. In spite of the close geometric similarity of the β-turns in these two series the c.d. spectra are quite distinct. Comparison with other data has suggested that it may not be possible to characterize the contributions of all β-turns to the c.d. spectra of proteins by using a single model curve.[610]

The c.d. bands at 222 nm of the copolymers of β-benzyl-Asp with β-*m*- or β-*p*-(phenylazo)benzyl-Asp in $ClCH_2CH_2Cl$ are characteristic of left-handed α-helical conformations, but irradiation of copolymers with >50% azo residues leads to conformational changes. Photoisomerization induces significant instability in the left-handed α-helix of the *meta*-copolymer and a reversal of the sense of the helix for the *para*-copolymer. The conformational change of the *para*-derivative with 59% azo residues occurs abruptly when the azo configuration is >50% *cis*. It has been suggested on the basis of these data that these compounds might serve as models for rhodopsin.[611]

Measurements carried out on poly(*OO'*-di-Me-L-DOPA) show that it possesses a right-handed helix in $CHCl_3$, $ClCH_2CH_2OH$, or dioxan, whilst its copolymer with >50% Glu shows a c.d. peak at 285 nm suggestive of stacking of the aromatic side chains. In CF_3CO_2H or $(CF_3)_2CO$ it is present as a random coil.[612]

Data obtained from c.d. measurements have shown that $(Ala\text{-}Hyp\text{-}Gly)_n$ has collagen-like properties in contrast to $(Ala\text{-}Pro\text{-}Gly)_n$. It appears that the presence of hydroxyproline contributes to collagen-helix stability independent of the presence of an adjacent Pro.[613]

Polypeptides $(Lys\text{-}A\text{-}Ala)_n$ (X = Ala, norVal, Val, and Leu) show mainly random-coil conformation when X = norVal or Ala at low ionic strength; helical content increases with increasing NaCl concentration. The Val-containing polypeptide is a random coil independent of ionic strength but in alkaline solution becomes β. The Leu-containing peptide is predominantly α-helix. Anomalous c.d. spectra could not be fitted by a linear combination of α, β, and random coil.[614]

A number of Pd^{II} and Pt^{II} complexes of amino-acids have been characterized and shown to possess very different c.d. properties from those shown by dipeptide complexes.[615]

[609] S. K. Brahmachari, V. S. Ananthanarayanan, S. Brahms, J. G. Brahms, R. S. Rapaka, and R. S. Bhatnagar, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 605.
[610] C. A. Bush, S. K. Sarkar, and K. A. Kopple, *Biochemistry*, 1978, **17**, 4951.
[611] A. Ueno, J. Anzai, T. Osa, and Y. Kadoma, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 549.
[612] H. Yamamoto and T. Hayakawa, *Polymer*, 1978, **19**, 963.
[613] N. V. Rao and E. Adams, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 654.
[614] S. Stokrowa, K. Zimmermann, J. Sponar, and K. Blaha, *Collect. Czech. Chem. Commun.*, 1978, **43**, 2341.
[615] E. A. Sullivan, *Can. J. Chem.*, 1979, **57**, 62.

The c.d. spectra of Langmuir–Blodgett monolayers and collapsed films of poly-(γ-Me-L-Glu) (PMG) and poly-(L-Ala) (P) collected from the air–water interface of a Langmuir film balance have been obtained. The spectra of the films in the α-helical conformation were consistent with the optical properties expected of polypeptides with the helix axis perpendicular to the direction of propagation of light. PMG gives spectra characteristic of either α- or β-conformation depending on the method of preparation.[616]

Proteins.—*Non-chromophoric Proteins.* Within the limits of experimental error (1—2%) it has been found that the secondary structure of lysozyme in adsorption layers of n-octane, CCl_4, and hydrophobicized quartz does not differ from that in solution, being *ca.* 30% α-helix and *ca.* 10% β-structure.[617]

The origins of the c.d. bands of lima bean proteinase inhibitor have been deduced from a study of acetylation–deacetylation of the sole Tyr residue in the protein and by analogy with Bowman–Birk soybean proteinase inhibitor. A broad negative band centred at 280 nm arises from the largest wavelength transition of cystinyl side chains. The second cystinyl transition gives rise to a positive band at 247 nm. Tyrosine transitions have been identified as a small negative band at 280 nm and a positive shoulder at 232 nm.[618]

The activators Asp, Cys, and edta do not alter the basic conformation of swine muscle glyceraldehyde phosphate dehydrogenase, but do somewhat change the local environment in the region of Tyr and Trp residues. Asp and edta decrease the c.d. activities approximately two-fold and some shifts in maxima were observed on binding. In the near-u.v. region Cys slightly increased optical activity and strongly reduced the trough at 263 nm. All the effectors reduced the magnitude of the positive c.d. band at 300 nm. The decreased activity in the region 305—420 nm suggests the activators bind in the vicinity of the active site.[619]

Unlike the far-u.v. c.d., the near-u.v. c.d. of concanavalin A exhibits a marked time dependence following Ca^{2+} binding. The spectroscopic effects observed in the near-u.v. c.d., which are associated with (i) binding of metals, (ii) Ca^{2+}-induced time dependence, (iii) binding of α-Me-D-mannoside, are all modulated by a symmetric broad 277 nm transition. A hypothetical structural model, which envisages a sensitive Tyr reporter has been proposed to account for these observations.[620] The pleated-sheet content of concanavalin A has been estimated at 41%, whilst wheat germ lectin with a weak negative band at 195 nm appears to contain no pleated sheet. The lectins of *Sophora japonica, Dolichos biflorus,* and *Banderiea simplicifolia* contain large amounts of β-structure as evidenced by their high activity at 195 nm.[621]

Bovine cardiac myosin and its subfraction S1 have a similar system of Cotton effects, attributed to Trp and Tyr residues, which is perturbed on ligand, ATP, and

[616] D. G. Cornell, *J. Colloid Interface Sci.*, 1979, **70**, 167.
[617] M. M. Sorokin, V. N. Izmailova, G. P. Yampolakaya, and A. I. Dolinnyi, *Biofizika*, 1979, **24**, 775.
[618] E. Kay and B. Gumbiner, *J. Biol. Chem.*, 1979, **254**, 7643.
[619] V. Ivanova, N. Tomova, and B. Atanasov, *Symp. Pap.—I.U.P.A.C. Int. Symp. Chem. Nat. Prod.*, 1978, **1**, 21.
[620] A. D. Cardin, W. D. Behnke, and F. Mandel, *J. Biol. Chem.*, 1979, **254**, 8877.
[621] B. Jirgensons, *Makromol. Chem.*, 1979, **180**, 2049.

pyrophosphate binding. Both proteins show the presence of two independent binding sites with constants 10^6—$10^7\,M^{-1}$.[622]

The neurotoxic venom of the Bulgarian viper presents a c.d. spectrum quite similar to a mixture of its two components, a neurotoxic phospholipase A_2 and a non-toxic protein (NC). However, the α-helical content of the venom is higher than that in the two separate components (*ca.* 20%), probably owing to an organization of part of the polypeptide chains from the random coil.[623]

Ribosomal protein S15 shows a high content of secondary structure with 78% α-helix. On the basis of this and other data a theoretical stereochemical analysis has led to a model for the overall protein structure.[624]

The properties of [Leu^5]-enkephalin and some of its analogues have been studied in order to identify any correlation with addictive properties. A Phe^4/cyclohexyl Ala replacement alters the near-u.v. c.d. of the H-bonded Tyr. In the peptide region only [Leu^5]-enkephalin and its cyclohexyl Ala derivative show a positive dichroism, which is maintained in 8 M urea. However, no clear relation was found between solution conformation and biological activity. A type II β-turn, with residues in positions 2 and 3 at the corners, is suggested for the conformation of enkephalin bound to the receptors involved in dependence phenomena.[625]

The c.d. spectra of purified rat liver arginase indicated the presence of 52—56% α-helix, 5—11% β-pleated structure, and 37—39% random coil. The addition of Mn^{2+} did not affect the total conformation of the protein as judged by the far-u.v. c.d. but did increase the molar ellipticity at 285 nm in a manner proportional to the concentration of Mn^{2+}. This increase in asymmetry during activation by Mn^{2+} is seen as reflecting some conformational changes in the enzyme.[626]

Pea and spinach ribulose diphosphate carboxylases, when activated by $MgCl_2$ and $NaHCO_3$, show a marked shift in their c.d. spectra at 275 nm. This result has been interpreted as a conformational change accompanying enzyme activation.[627]

The rate of denaturation of glutamate dehydrogenase by 0—5 M urea has been determined by measuring molar ellipticity as a function of time. In these studies it has been found that substrates such as glutamate or 2-oxoglutarate or cofactors such as NAD^+ or NADH do not stabilize the enzyme. However, the enzyme activity was reversibly inhibited at urea concentrations which had no effect on the measured c.d. spectra.[628] Other studies on glutamate dehydrogenase have used c.d. to follow NADH binding. The existence of two distinct Cotton effects has been associated with two separate binding sites per subunit. C.d. titration of the high-affinity site showed significant dependence of optical activity of the bound coenzyme on the state of protein association, the molar ellipticity of NADH decreasing with increasing protein polymerization. Binding of NADH to the low-

[622] S. J. Smith, *FEBS Lett.*, 1979, **105**, 197.
[623] B. Chorbanov, B. Atanasov, B. Aleksiev, and T. Bukolova-Orlova, *Symp. Pap.—I.U.P.A.C. Int. Symp. Chem. Nat. Prod.*, 1978, **1**, 107.
[624] Z. V. Gogiya, S. Venyaminov, V. N. Bushuev, I. N. Serdynk, V. I. Lim, and A. S. Spirin, *FEBS Lett.*, 1979, **105**, 63.
[625] B. Filippi, P. Giusti, L. Cima, G. Borin, F. Richelli, and F. Marchiori, *Int. J. Pept. Protein Res.*, 1979, **14**, 34.
[626] G. Muszynska and E. Ber, *Int. J. Biochem.*, 1978, **9**, 757.
[627] A. E. Grebanier, D. Champagne, and H. Roy, *Biochemistry*, 1978, **17**, 5150.
[628] L. V. Karaboshyan, N. G. Ekizyan, and S. G. Movesyan, *Biol. Zh. Arm.*, 1978, **31**, 572.

affinity sites in the presence of GTP leads to an inversion of the spectrum, which is not related to the polymerization of the protein.[629]

C.d. studies of porcine heart lactate dehydrogenase with the competitive inhibitors oxalate and oxamate show no effect of the added molecules. Formation of binary complexes of the protein with the coenzymes NAD^+ or NADH induces marked conformational change. Further changes were observed on the addition of oxalate or oxamate to the NADH complexes. These changes are discussed in relation to the mechanism of action of this enzyme.[630]

It has been found that bis-(*S*-carboxymethylated-31,32) bovine seminal RNAase has a far-u.v. c.d. spectrum quite similar to that of bovine pancreatic RNAase A with a minimum at 209 nm and a shoulder at 220 nm. In contrast the native seminal RNAase shows a single negative trough at 215 nm, reminiscent of β-structure, and the absence of a positive peak at 242 nm. The previous assignment of the 242 nm band to exposed Tyr, together with the data obtained for the dissociation of the native dimer into monomeric bis-(*S*-carboxymethylated-31,32), suggests this process leads to the exposure of Tyr. This suggestion is borne out by fluorescence studies. It has further been proposed that the dimeric structure of the native protein protects it towards denaturing agents.[631] The far-u.v. c.d. of the 7P RNAase from *Bacillus intermedius* has also been reported recently. The spectra obtained have been resolved into two bands in the 205–240 nm region. The band at 214 nm is attributed to electronic transitions of the peptide groups and the 230 nm band to Trp residues. The backbone structure has been determined to be 6% α-helix and 20% β-structure. Although the protein contains no disulphide bonds it is stable at pH = 2.5—11.5, but can be denatured by guanidine-HCl at 2.3 M in a process consistent with a two-state model.[632]

Below 240 nm c.d. studies indicate that the ordered structure of *Chalaropsis* lysozyme (21% α-helix, 63% β-sheet, 16% random coil) is quite different from that of hen egg white lysozyme (22% α-helix, 18% β-sheet, 60% random coil) and possesses the highest β-content thus far reported for a globular protein. Above 240 nm the spectrum of *Chalaropsis* lysozyme differs from that for the hen egg white protein with negative bands at 293 nm and 277 nm and a positive band at 247 nm. Denaturation of the two proteins, however, is quite similar, occurring at *ca.* 2.7 M guanidine-HCl. The *Chalaropsis* protein is stable at pH = 2.7—7.3; at pH = 8.0 the c.d. spectrum resembles that induced by guanidine-HCl denaturation.[633] A comparison between the c.d. of hen and turkey lysozymes has also appeared, in which titration of the proteins has allowed an assignment of the p*K* values of Tyr-3 and Tyr-23 in the turkey protein as 11.9 and 9.8, respectively.[634]

The far-u.v. c.d. of the inhibitor (BTCI) exhibits a deep negative band at 201 nm and a small positive band at 218 nm. Reduction with dithioerythritol slightly blue-shifts and markedly increases the negative band and a negative shoulder appears between 215 nm and 229 nm. These c.d. findings are very similar to those found for

[629] L. Zeiri and E. Reisler, *Biopolymers*, 1979, **18**, 2289.
[630] S. S. Chen, *Proc. Malays. Biochem. Soc. Conf.*, 1978, **5**, 139.
[631] C. Grandi, G. D'Alessio, and A. Fontana, *Biochemistry*, 1979, **18**, 3413.
[632] I. A. Bolotina, S. M. Dodkin, V. Lugauskas, A. Z. Khandanyau, and I. B. Leshchinskaya, *Bioorg. Khim.*, 1979, **5**, 203.
[633] J. J. Chang, L. A. Holladay, and J. H. Hash, *J. Biol. Chem.*, 1979, **254**, 7772.
[634] S. Kuramitsu and K. Hamaguchi, *J. Biochem.* (*Tokyo*), 1979, **85**, 443.

proteins in constrained unordered conformation, including other protease inhibitors of soybean and lima bean. In the near-u.v. c.d. bands at 245 nm and 278 nm are assigned mainly to S—S groups. A positive band at 235 nm was assigned to protonated Tyr.[635] The protease inhibitor from *Phaseolus* has also been studied and shown to possess only a small quantity of α-helix (4—7%), whereas the related protein from potato shows no α-helix. However, both proteins have relatively high β-content, 25% for the bean and 30% for the potato protein. Comparison with other protease inhibitors and detergent denaturation studies lead to the conclusion that the basic structural elements of the potato inhibitor are bends and loops stabilized by hydrophobic interactions.[636] Further c.d. studies on the trypsin–chymotrypsin inhibitors from soybean and chickpea have shown that they possess little α-helix, and only very small amounts of β-structure. Neither protein showed evidence of buried Tyr and comparison with other related proteins suggested that previous interpretations of the region (230—250 nm) may be incomplete or incorrect.[637] A report has also appeared concerned with the human serum protease inhibitor α_1-antitrypsin types MM and ZZ. Both proteins show similar secondary structure (45—50% α-helix, 15—20% β-structure), but the micro-environment in the vicinity of the three Trp residues is different in the MM and ZZ proteins. The possible significance of these findings in terms of α_1-antitrypsin deficiency was discussed.[638]

The binding of Ca^{2+}, but not Mg^{2+} or Mn^{2+}, to the protein modulator of cyclic nucleotide phosphodiesterase leads to an increase in helical content. Studies on the native protein and some chemically modified forms have been used to advance a model of Ca^{2+} activation of the modulator protein based on a three conformational state model.[639]

The small basic proteins clupeine YI, YII, and Z assume predominantly unordered conformations in aqueous solution but the addition of counter ions, such as perchlorate, or 2-chloroethanol, leads to the onset of right-handed α-helical conformations. In the range 0.01—0.4 M YII, in contrast to YI and Z, is insensitive to the addition of mono- and di-phosphate.[640]

A fragment of tetanus toxin may be prepared by treatment with papain. Although this fragment is immunoreactive, c.d. analysis shows that the structure of this protein is very different from that of the native toxin. The fragment shows considerable β-structure but little if any α-helix, compared to the native protein which has 29% α-helix and 23% β-structure.[641]

The far-u.v. c.d. spectra of bovine and human prothrombin, prothrombin fragment 1 and 2, prethrombin 1 and 2, and de-(1—13)-prethrombin 2 have been obtained and the α, β, and random structures estimated. Prothrombin and its activation components contain large amounts of aperiodic structures. The

[635] M. M. Ventura and J. B. Aragoa, *An. Acad. Bras. Cienc.*, 1978, **50**, 587.
[636] A. I. Shulmina, L. A. Dronova, V. E. Nazaikinskii, V. V. Shubin, and V. V. Mosolov, *Biokhimiya*, 1979, **44**, 514.
[637] T. A. Bewley and Y. Birk, *Int. J. Pept. Protein Res.*, 1978, **12**, 249.
[638] C. B. Glaser and L. Karic, *Int. J. Pept. Protein Res.*, 1978, **12**, 284.
[639] M. Walsh, F. C. Stevens, K. Oikawa, and C. M. Kay, *Can. J. Biochem.*, 1979, **57**, 267.
[640] C. Toniolo, G. M. Bonora, F. Marchiori, G. Borin, and B. Filippi, *Biochim. Biophys. Acta*, 1979, **576**, 429.
[641] J. P. Robinson, H. C. Chen, J. H. Hook, and D. Puett, *Mol. Cell. Biochem.*, 1978, **21**, 23.

hypothesis that prothrombin activation components exist as relatively non-co-operative domains within the prothrombin molecule was tested by comparison of the c.d. spectrum of prothrombin and the sum of the spectra of the components. Support for this idea was found in that no gross alterations in secondary structure as determined by c.d. were observed on activation.[642]

Temperature and salt denaturation studies on tropomyosin and tropomyosin–troponin complex using c.d. show that during complex formation the structures of the individual proteins are destabilized. The hydrophobic nucleus of the troponin molecule, however, interacts with the hydrophobic blocks in tropomyosin decreasing their accessibility to urea.[643]

Exposure of collagen and procollagen to the u.v. beam of a c.d. machine leads to random scission of the proteins. Minimizing the damage allowed an investigation of thermal transitions at low concentrations. Under these conditions both type I collagen and type I procollagen show the same T_m. These results demonstrate that the polypeptides found at both the *N*- and *C*-terminals of type I procollagen have no effect on the thermal stability of a triple-helical domain. The observation of u.v. scission in this work is worthy of note and suggests the identification of similar events in other c.d. studies should be made.[644]

Bovine myelin basic protein shows no significant α- or β-structure in aqueous solution. On binding to lipid bilayers of phosphatidylserine or phosphatidic acid the protein undergoes a major conformational change. Protein-cleavage experiments in association with c.d. measurements have shown that a major structure-forming region in the middle of the protein is disrupted by the scission of the Phe bond between 89 and 90.[645]

An interesting finding has been made that porcine and normal aorta elastin show identical c.d., but the peptides obtained from human pathological samples exhibit different spectra at different stages of atheromatous lesions, which have been attributed to conformational variations around the bridging zones.[646]

The molar ellipticity of the warfarin–albumin complex at 310 nm increases with pH from 6 to 9 in a manner parallel to that of albumin alone at 292 nm. The change in ellipticity with pH occurs in a smaller pH range following addition of Ca^{2+}. This is taken as evidence that the binding site of warfarin on the albumin is affected by the neutral-to-base transition in the protein.[647]

Leukoagglutinin from kidney bean shows close similarity to concanavalin A with a high amount of β-structure. Above pH = 11.2 the protein undergoes a conformational change attributed to the ionization of Tyr, but is stable to acid conditions down to pH = 2. Sodium dodecylsulphate disorganizes the protein particularly in the presence of acid when the protein rearranges to a conformation containing as much as 30% α-helix.[648]

Native human erythrocyte glycophorin A in aqueous solution is *ca.* 27% α-helix,

[642] J. W. Bloom and K. G. Mann, *Biochemistry*, 1979, **18**, 1957.
[643] V. M. Aslanyan, N. M. Gevorkyan, and M. S. Sarkisyan, *Biol. Zh. Arm.*, 1978, **31**, 683.
[644] T. Hayashi, S. Curran-Patel, and D. J. Prockop, *Biochemistry*, 1979, **18**, 4182.
[645] M. A. Keniry and R. Smith, *Biochim. Biophys. Acta*, 1979, **578**, 381.
[646] M. Davril, K. K. Han, and M. H. Loucheux-Lerfebvre, *Biopolymers*, 1979, **18**, 2195.
[647] J. Wilting, M. M. Weideman, A. Roomer, C. J. Anton, and J. H. Perrin, *Biochim. Biophys. Acta*, 1979, **579**, 469.
[648] B. Jirgensons, *Biochim. Biophys. Acta*, 1979, **577**, 307.

10% β-structure, and 63% disordered, and is only slightly affected by the presence of detergent. The four major tryptic peptides obtained from digestion of the protein show very different structures. T1A and T3A possess some β-structure but are mainly disordered. T6A is hydrophobic and completely helical, whilst T4A has some helix but is predominantly unordered. Interestingly it is found that the c.d. spectrum of the native protein is a simple sum of the spectra of the tryptic peptides, showing that the native conformation is determined solely by short-range interactions.[649]

Chromophoric Proteins. As in previous years a number of papers have appeared concerned with the c.d. characteristics of cytochromes. The cytochrome *c* peroxidase of *Pseudomonas aeruginosa* has been studied in the range 200—650 nm. The protein possesses 34% α-helix, and is not significantly changed by ligand binding. In the Soret region the oxidized, reduced, and CO complexes all give distinct c.d. spectra. In the oxidized form the two haems are degenerate and this degeneracy is lifted on reduction. The CO complex exhibits hyperchromicity and evidence is presented that the ligand reacts with only one of the haems. All forms show a maximum at 263 nm typical of the haem-transition region with ellipticity increasing in the order iron(III) < iron(II) < iron(II)—CO; these changes imply that histidine weakly interacts with haem iron in the iron(III) form and on reduction moves closer to the haem.[650, 651] The cytochrome *c* peroxidase and peroxide complex from *Saccharomyces cerevisiae* has also been studied and the nature of the haem binding site elucidated.[652]

The relatively new technique of stopped-flow circular dichroism has been applied to two studies of the reduction of cytochrome *c*. The reaction of cytochrome *c* with protohaem has been used as a model for the oxidase reaction. For the reduction of cytochrome *c* a marked triphasic conformational change in proximity to haem *c* was observed. The reverse reaction, however, was found to be a single monophasic process, suggesting that electron transfer out of the protein is not accompanied by any significant conformational change.[653] Rapid reduction of cytochrome *c* by dithionite showed two transient intermediates. The decay of the 402 nm band, signifying electron transfer to haem *c*, is synchronous with a transient characterized by a c.d. maximum at 413 nm. This process is followed by a slow decay to the native reduced form. These data have been interpreted in terms of changes in haem co-ordination.[654] C.d. studies in the Soret region of the cytochrome b–c_1 complex from yeast have revealed changes in the conformation of c_1, dependent on the redox state of *b*. This interaction was not altered by addition of antimycin A, which caused drastic changes in the c.d. of the complex overall. Excitation splitting has also been detected, which is induced by uncouplers and is consistent with haem–haem interaction between the two cytochrome *b* molecules.[655, 656]

[649] T. H. Schulte, H. Thomas, and V. T. Marchesi, *Biochemistry*, 1979, **18**, 275.
[650] M. Ronnberg, N. Ellfolk, and R. Soininen, *Biochim. Biophys. Acta*, 1979, **578**, 392.
[651] M. Ronnberg and N. Ellfolk, *Symp. Pap.—I.U.P.A.C. Int. Symp. Chem. Nat. Prod.*, 1978, **1**, 13.
[652] G. Sievers, *Symp. Pap.—I.U.P.A.C. Int. Symp. Chem. Nat. Prod.*, 1978, **1**, 17.
[653] I. Tabushi, K. Yamamura, and T. Nishiya, *J. Am. Chem. Soc.*, 1979, **101**, 2787.
[654] I. Tabushi, K. Yamamura, and T. Nishiya, *Tetrahedron Lett.*, 1978, **49**, 4921.
[655] J. Reed, T. A. Reed, and B. Hess, *Eur. J. Biochem.*, 1978, **91**, 255.
[656] J. Reed, T. A. Reed, and B. Hess, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1045.

No striking differences have been observed between the α-helical content observed for phenobarbitol and 3-methylcholanthrene induced P_{450} and P_{448}. However, near-u.v. c.d. shows marked differences between these proteins. Reduction of P_{450} and P_{448} leads to a remarkable decrease in the Soret c.d.; this suggests that this process produces large conformational changes around the haem, as does CO binding.[657]

C.d. has been used to study the R ⇌ T system in haemoglobin. Using abnormal haemoglobins with amino-acid substitutions in the $\alpha_1 \beta_2$ contact region it has been possible to evaluate the equilibrium parameters for the two-state model.[658] The far-u.v. c.d. of purified erythrocruorin from the mollusc *Planorbis corneus* indicates the presence of 75—80% α-helix. The near-u.v. c.d. of the oxy-, deoxy-, and CO-forms of the protein is more like that of vertebrate haemoglobins than that of annelid haemoglobin. Nevertheless the Soret c.d. has only half the rotational strength of that of human Hb A. On deoxygenation the Soret c.d. falls by a factor of two, whereas the band at 260 nm shows only a small decrease in ellipticity.[659]

The c.d. spectra of erythrocyte catalase and its CN^-, N_3^-, and F^- derivatives, in the region 210—700 nm, show that treatment with acid or alkali causes spectral changes consistent with dissociation of the enzyme into subunits. The protein conformation does not change appreciably on ligand binding.[660]

The formation of the peroxy-derivative of laccase is accompanied by a prominent c.d. change in the region 260—360 nm. These features confirm the nature of the intermediates as analogous to the oxygenated haemocyanins and oxytyrosinase. Fluoride modifies the near-u.v. c.d.[661] Two papers have appeared concerned with studies on lipoxygenase. The c.d. spectra of three forms of lipoxygenase from soybeans show characteristic differences in the region 300—600 nm. Native lipoxygenase-1 shows a negative band at *ca.* 330 nm. Yellow lipoxygenase, formed from reaction with 13-L-hydroperoxylinoleic acid, shows a positive Cotton effect at 425 nm and an increase in ellipticity at 330 nm. The blue enzyme, a complex with linoleic acid derivatives, exhibits a negative band at 580 nm and positive bands at 410 and 391 nm. Using the method of Chen the native enzyme is estimated to contain 34% α-helix, 27% β-structure, and 39% random-coil.[662] The lipoxygenase obtained from *Fusarium* shows large c.d. maxima at 400 nm and 490 nm and a negative at 428 nm in the oxidized form, whereas the reduced enzyme shows many complex peaks in this region. A reversible conversion occurs between the α-helix form (pH = 6.0) and the β-form (pH = 9.0), but at high pH (pH = 12) the far-u.v. c.d. is greatly diminished, suggesting an extensive change in backbone structure. Some of the changes in enzyme activity are explained in terms of these conformational alterations.[663]

[657] T. Shimizu, T. Nozawa, M. Hatano, H. Satake, Y. Imai, C. Hashimoto, and R. Sato, *Biochim. Biophys. Acta*, 1979, **579**, 122.
[658] S. Matsukawa, M. Nishibu, M. Nagai, K. Mawatari, and Y. Yoneyama, *J. Biol. Chem.*, 1979, **254**, 2358.
[659] E. J. Woodard and W. J. Gullick, *Biochim. Biophys. Acta*, 1979, **576**, 456.
[660] M. Palzic and H. B. Dunford, *Can. J. Biochem.*, 1979, **57**, 321.
[661] O. Farver, M. Goldberg, and I. Pecht, *FEBS Lett.*, 1978, **94**, 383.
[662] L. J. M. Spaapen, G. A. Veldink, T. J. Liefkens, J. F. G. Vliegenthart, and C. M. Kay, *Biochim. Biophys. Acta*, 1979, **574**, 301.
[663] Y. Matsuda, T. Beppu, and K. Arima, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 203.

The visible c.d. of bovine plasma amine oxidase indicates that the copper chelate structure in the enzyme is optically active. Heating can destroy the optical activity of the copper centre, apparently by altering the groups ligating the metal. The protein contains *ca.* 15% α-helical structure and some β-sheet. The visible c.d. shows a loss of copper activity and the far-u.v. c.d. a significant conformational change on addition of $(NH_4)HCO_3$ or H_2O_2. Other studies also showed that binding of CN^- to the enzyme leads to a conformational change, but the binding of F^- does not.[664]

Three papers have appeared covering the c.d. of rhodopsin. Digitonin micelles of rhodopsin show a negative band at 220 nm and two positive bands at 340 and 490 nm. The addition of Ca^{2+} decreases the ellipticity of all the bands but this effect is reversed by addition of edta.[665] Although cephalopod metarhodopsins have been considered as having no optical activity associated with their main absorption bands, octopus acid–metarhodopsin in digitonin has a positive band at 498 nm and a negative band at 436 nm, whereas alkaline metarhodopsin has a negative band at 381 nm. Evidence is given that the conformation of all-*trans* retinal in octopus metarhodopsin is influenced by the asymmetry of the nearby protein.[666] Squid rhodopsin has positive c.d. bands at wavelengths corresponding to the α- and β-absorption bands both at liquid-nitrogen and room temperatures. The rotational strength of the α-band is approximately twice that of the cattle protein. Batho-rhodopsin has a negative peak at 532 nm with slightly higher ellipticity than rhodopsin. The reversal of sign of the α-band may be related to a difference in isomerization of retinal between the two proteins. Lumirhodopsin has a small negative band at 490 nm and a large positive band at 290 nm not present in rhodopsin, probably due to a change in conformation of the opsin moiety.[667]

Extrinsic Chromophores. Two papers have appeared on the study of bilirubin–albumin interactions. Albumin has been found to possess one strong and 18—20 weak binding sites for bilirubin. At pH = 6.7 the free energy of binding was −6.5 to −8.6 kcal mol^{-1} and the binding constant was *ca.* 1.4 to 12 × 10^5 M^{-1} for the strong binding site. C.d. spectra show that binding to the high-affinity site disrupts intra- and inter-molecular hydrogen bonds in the bilirubin molecule. Subsequent bilirubin molecules are bound *via* hydrophobic bonds. The u.v. c.d. shows no significant protein conformational change at <10 bilirubins bound. At >10 bilirubins bound a decrease in α-helix occurs.[668] The effects of the binding of penicillin, benzoic acid, and various lower aliphatic alcohols on bilirubin–albumin complexes have been studied at neutral pH by c.d. spectroscopy. Significant changes in the visible range occur within a certain range of added ligand concentration. A scheme of equilibria among all complex components is proposed, which includes both dissociation of bilirubin and ternary complex formation. At least two types are apparent. Values for equilibrium parameters for the ligands have been obtained and related to their chemical structures.[669]

[664] H. Ishizaki and K. T. Yasunobu, *Adv. Exp. Med. Biol.*, 1976, **74**, 575.
[665] E. M. Slobodyanskaya, *Mekh. Sens. Retseptsii*, 1976, 112.
[666] M. Tsuda, *Biochim. Biophys Acta*, 1979, **578**, 372.
[667] Y. Schichida, F. Tokunoga, and T. Yoshizawa, *Biochim. Biophys. Acta*, 1978, **504**, 413.
[668] V. P. Kobzareva, E. P. Shapovalenko, and I. V. Kolasov, *Bioorg. Khim.*, 1978, **4**, 1635.
[669] E. Lavie and G. Blauer, *Arch. Biochem. Biophys.*, 1979, **193**, 191.

The c.d. and other spectral measurements have been reported for riboflavin, FAD, roseoflavin, the complexes of riboflavin and roseoflavin with riboflavin-binding protein (RBP), D-amino-acid oxidase (AO), and its complexes with ligands in the region 310—600 nm. It is found that the dimer of AO has higher affinities than the monomer. The c.d. spectra obtained showed higher sensitivity than m.c.d. to the environment around the isoalloxazine moiety.[670] The binding of NADP or NADPH to dihydrofolate reductase from *Lactobacillus casei* enhances the negative c.d. band at 340 nm, and binding of folate and dihydrofolate gives rise to substantial extrinsic c.d. bands. The binary complexes of the reductase and methotrexate or trimethoprin show strong c.d. bands, whereas ternary complexes containing NADPH show spectra which are not the sum of the spectra of the binary complexes. Addition of trimethoprin leads to the disappearance of the 340 nm band of NADPH, whereas in the methotrexate–reductase–NADPH complex a couplet c.d. is observed at long wavelength. The couplet may arise from a direct interaction between the dihydronicotinamide and pteridine rings in the complex.[671]

The introduction of the nitrotyrosine chromophore into proteins by treatment with tetranitromethane has provided a useful c.d. probe for investigations of Ca^{2+} binding to rabbit skeletal and bovine cardiac troponin. The introduction of the probe does not significantly alter the protein secondary structure and observation of c.d. changes at 275 nm allows Ca^{2+} binding to be followed. Binding parameters were obtained, and it was concluded that the sensitivity of the nitrotyrosyl moiety to subtle changes in its microenvironment make it a useful conformational probe in the study of Ca^{2+} binding to troponin and homologous molecules.[672]

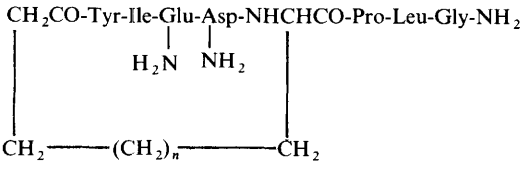
Addition of Ca^{2+} or Cd^{2+} to (Co^{2+})-concanavalin A induces slow time-dependent changes in the extrinsic Cotton effects. One equivalent of Ca^{2+} causes maximal change in the c.d. The time-dependent changes appear to be conformationally mediated.[673]

Rabbit carbonic anhydrase isoenzymes have been shown to react differently with acetazolamide. The anhydrase isoenzymes I and II induce pronounced asymmetry in added acetazolamide and allow titrations to be carried out by monitoring the c.d. at 272 nm. In contrast isoenzyme III does not induce ellipticity in the ligand, a fact attributed to a substantially lower affinity of the Zn in the enzyme for the sulphonamide group of the inhibitor.[674]

Hormones.—C.d. spectra have been measured of three analogues of deamino-oxytocin [I; structure (1)] of different ring sizes where the SS group is replaced by the $(CH_2)_n$ group. Their backbone rings comprise 19, 20, and 21 atoms for (1,6-aminopimelic acid)oxytocin ($n = 1$) (II), (1,6-aminosuberic acid)oxytocin ($n = 2$) (III), and (1,6-aminoazelaic acid)oxytocin ($n = 3$) (IV), respectively. The pH dependence of the c.d. spectra indicates that the conformation of (IV) is different from (II) and (III). The temperature dependence shows that the conformation of (II) is the most rigid. Their biological activities are related to the conformation and

[670] K. Shiga, K. Horiike, Y. Nishina, S. Otani, and H. Watari, *J. Biochem. (Tokyo)*, 1979, **85**, 931.
[671] K. Hood, P. M. Bayley, and G. C. K. Roberts, *Biochem. J.*, 1979, **177**, 425.
[672] W. McCubbin, M. T. Hincke, and C. M. Kay, *Cann. J. Biochem.*, 1979, **57**, 15.
[673] A. D. Cardin and W. D. Behnke, *Biochim. Biophys. Acta*, 1978, **537**, 436.
[674] M. K. Koester and E. A. Noltmann, *Biochemistry*, 1979, **18**, 343.

internal motions of the peptide hormone analogues.[675] In the oxytocin inhibitors (1-penicillamine)oxytocin(I) and (1-penicillamine-2-leucine)oxytocin(II) the longest



(I)

wavelength SS transition was found at 274—279 nm as a negative band; this indicates a dihedral angle of 110—115° with a right-handed chirality. The intensity of this band and especially that of the peptide amide transition at 220 nm indicates that (I) and (II) are more rigid than native oxytocin.[676]

C.d. has shown that the polyethyleneglycol ester (PEG) of [Val^4]-ACTH(1—10) is partially helical in CF_3CH_2OH and has a β-conformation in water, whereas the PEG ester of [Pro^1,Ala^2,Ala^3,Val^4]ACTH(1—10) is helical in both solvents. These experimental data are in reasonable agreement with theoretical calculations. A model for the most probable conformation of ACTH was proposed.[677]

Conformational differences between the vasoactive hormone bradykinin and its fragments have been identified and the importance of Arg-1 and -9 to the maintenance of specific folding has been established. These findings are discussed in relation to biological activity.[678]

The secondary structures of human somatotropin, human choriomammotropin, ovine and porcine prolactin, human, ovine, and porcine β-lipotropin, human and ovine lutropin, human thyrotropin, human corticotropin, α-melanotropin, and human β-melanotropin have been predicted by the method of Chou and Fasman. The predicted structures do not correspond well with the c.d. measurements.[679] The c.d. spectrum of thyrotropin-releasing hormone has been analysed by decomposing the spectrum into Gaussian bands.[680] A review of the structure of glycoprotein hormones with regard to disulphide bond assignments and c.d. spectra has appeared.[681]

The far-u.v. c.d. of insulin and relaxin are similar. Differences in the 222 nm region are attributed to dimerization of insulin, and a comparison of relaxin with desasparagyl–desalanyl insulin suggests a relaxin structure similar to insulin in solution and thus compatible with previous models based on insulin co-ordinates.[682]

[675] K. Kawano, Y. Kobayashi, Y. Kyogoku, S. Morikawa, and S. Sakakibara, *Biochim. Biophys. Acta*, 1979, **578**, 295.
[676] V. J. Hurby, K. K. Deb, J. Fox, T. Bjarnason, and A. T. Tu, *J. Biol. Chem.*, 1978, **253**, 6060.
[677] H. Mutter, M. Mutter, and E. Bayer, *Z. Naturforsch., Teil B.*, 1979, **34**, 874.
[678] K. Lintner, S. Fermandjian, and D. Regoli, *Biochimie*, 1979, **61**, 87.
[679] M. D. Jibson and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, **14**, 113.
[680] A. Attanasio, S. Fuchs, E. Pietrzik, and W. Voelter, *Hypothal. Horm.-Chem., Physiol., Clin. Appl. Proc. Eur. Colloq. Hypothal. Horm.*, 1976, 109.
[681] L. C. Giudice and J. G. Pierce, *Struct. Funct. Gonadotropins*, 1978, 81.
[682] C. Schwabe and S. J. Harmon, *Biochem. Biophys. Res. Commun.*, 1978, **84**, 374.

Immunoproteins.—A variation in the position of the A_{232} maximum for separated β_2-microglobulin has been found despite close similarities elsewhere in the c.d. spectrum. Whether this reflects (i) an as yet unidentified molecular polymorphism of human β_2-microglobulins, (ii) a predominant characteristic of any trace impurities, or (iii) the degree of dimerization in individual β_2-microglobulins remains to be determined.[683]

When native human haptoglobin type 2-1, acetylated, and iodinated haptoglobins are heated to 58 °C, conformational alterations occur as reflected by the gradual reduction in helical content. This was most pronounced in the iodinated preparation.[684]

Membrane Proteins.—A method has been published which allows for correction for differential absorption flattening, absorbance obscuring, and light scattering in c.d. measurements of membrane systems. The approach has been applied to purple membrane from *Halobacterium halobium*. The results obtained after correction agree well with those obtained by *X*-ray diffraction and electron microscopy.[685] The c.d. of oriented purple membrane films has been compared with results from solution measurements. C.d. spectra showed significant differences in the visible region, where the biphasic band in the solution spectrum was replaced by a single band at 555 nm, and in the far-u.v., where the 208 nm band was missing, whereas the 317 nm band remained constant. Based on excitonic interpretations the following conclusions were reached: (i) A relatively strong in-plane monomeric interaction between the retinal and apoprotein occurs, (ii) the helical axis of the membrane protein is normal to the membrane plane, (iii) the helical axis of the bleached protein is tilted more in-plane than is the case of the native protein. Additional conclusions were made concerning the magnetic dipole and coupling arrangements.[686]

Nuclear Proteins.—A number of papers have appeared concerning the interpretation and analysis of c.d. of chromatin and DNA–nuclear protein complexes.[687-690] Histones H4 from either *Ceratitis capitata* or higher animals show very close conformational similarity to a random coil at pH <5 and a more ordered structure at pH >8 at high ionic strength.[691]

The chromatin non-histone protein HMG-14 from calf thymus has a random structure. On interaction with DNA the protein binds ionically, but does not significantly alter the DNA structure.[692] The protein–DNA complex chromatin has been shown to undergo conformational changes on u.v. irradiation corresponding to changes in both the DNA and protein structures.[693]

[683] P. M. Johnson, P. M. Scopes, B. G. Solheim, and B. G. Johannson, *FEBS Lett.*, 1979, **100**, 141.
[684] W. Dobryszyeka, I. Katnik, and J. Lisowski, *Stud. Biophys.*, 1978, **73**, 177.
[685] D. W. Urry and M. M. Long, *Physiol. Membr. Disord.*, 1978, 107.
[686] D. D. Muccio and J. Y. Cassim, *Biophys. J.*, 1979, **26**, 427.
[687] G. D. Fasman, *NATO Adv. Study Inst., Ser. A*, 1978, **21**, 67.
[688] G. D. Fasman, *Methods Cell Biol.*, 1978, **18**, 327.
[689] G. D. Fasman, *Cell Nucl.*, 1978, **5**, 55.
[690] C. H. Huang and R. Baserga, *Cell Nucl.*, 1978, **5**, 99.
[691] J. Perera, *Insect Biochem.*, 1979, **9**, 39.
[692] K. Javaherian and S. Amini, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1385.
[693] M. Vorlickova, E. Palacek, and J. Sponar, *Radiat. Environ. Biophys.*, 1979, **16**, 117.

7 Magnetic Circular Dichroism

Contributed by T. Brittain

Reviews.—Reviews have appeared concerned with the m.c.d. approach to haem-protein analysis [694] and the theory and practice of m.c.d. in more general terms.[695]

Theory and Analysis.—A detailed theoretical discussion of deviations of m.c.d. for a system with a paramagnetic ground state from simple magnetic field–temperature dependence at high field and/or low temperature has been published. This theory of saturation has been applied to molecules in the point groups $D_{\infty}h$ to C_3. A single general formula applies in all cases. An analysis is also presented for the special case of accidental degeneracy of a $^2P_{3/2}$ ground state in a linear molecule. Various experimental considerations are discussed and a method for correcting for magnetic reluctance is given.[696]

To explain the temperature dependence of the m.c.d. in π–π^* bands of low-spin ferrihaem compounds a theoretical treatment based on the exchange type π–d interactions has been formulated. The transition between the ground and excited Kramers doublets gives rise to m.c.d. of two types. Instead of temperature-independent A-type effects usually seen in both *B*- and *Q*-bands of metal–porphyrins, there will be two A-type effects of the same sign and two temperature-dependent *C*-terms of opposite sign. The values of energy gaps between orbit–orbit split levels are predicted to be generally different for the *Q*- and *B*-band. Previously theoretical models are discussed and experimental evidence in favour of this new interpretation is outlined.[697] A theoretical analysis of two-photon m.c.d. and forbidden transitions has been published.[698, 699]

Model Compounds.—The studies in this area have been mainly concerned with porphyrins and haems as model systems of haemproteins. M.c.d. studies of neutral and anionic forms of a series of porphyrins, chlorins, and bacteriochlorins have appeared together with those on haem.[700, 701] Particular attention has turned to haem *a* as a model, to allow interpretation of previously obtained measurements on cytochrome *c* oxidase. Low-temperature m.c.d. of haem *a* derivatives showed that reduced haem *a* contains low-spin Fe^{2+} in pyridine and remains low-spin when oxidized in the presence of imidazole. Modification of the formyl group at position-8 on the porphyrin ring leads to a cytochrome *c* type spectrum. These findings have allowed a partial assignment of the electronic spectrum of haem *a*.[702] In similar studies, model low- and high-spin haem *a* derivatives were characterized by m.c.d. and their properties compared with those of cytochrome *c* oxidase. The formyl group at position-8 was also identified as

[694] M. Hatano and T. Nozawa, *Adv. Biophys.*, 1978, **11**, 95.
[695] M. Hatano, *Bunseki*, 1978, **6**, 369.
[696] P. N. Schatz, R. L. Mowery, and E. R. Krauss, *Mol. Phys.*, 1978, **35**, 1537.
[697] A. P. Mineyev and Y. A. Sharonov, *Theor. Chim. Acta*, 1978, **49**, 295.
[698] G. Wagniere, *Chem. Phys.*, 1979, **40**, 119.
[699] S. Lee, *Diss. Abstr. Int. B*, 1978, **39**, 1310.
[700] G. F. Kuipers, *Diss. Abstr. Int. B*, 1978, **39**, 2824.
[701] T. Nozawa, *Kagaku Zokan*, 1978, **76**, 113.
[702] T. Brittain, C. Greenwood, J. P. Springall, and A. J. Thomson, *Biochem. J.*, 1978, **173**, 411.

playing a significant role in the electronic properties of haem *a* in these studies.[703, 704]

Proteins.—The m.c.d. of the 4Fe–4S cluster in *Chromatium* high-potential iron protein (HIPIP), *Bacillus stearothermophilus* ferredoxin, and *Clostridium pasteurianum* ferredoxin varies significantly with cluster oxidation state, but is relatively insensitive to the nature of the protein. Comparison with the corresponding spectra of Fe–S proteins containing 2Fe clusters shows that m.c.d. is superior to absorption and natural c.d. spectroscopy for characterization of cluster type and oxidation state.[705]

M.c.d. studies have shown that in the cytochrome oxidase of *Pseudomonas aeruginosa* in the oxidized state the haem *c* is low spin either in the presence or absence of CN^-, as is haem *d*.[706]

M.c.d. difference spectra of cytochromes b_{562} and $b_{566/568}$ show that the bands at 566 and 558 nm belong to a single low-spin cytochrome $b_{556/558}$ with a highly asymmetric haem environment.[707]

Mixtures of high-spin and low-spin ferro-forms have been observed in non-equilibrium states of metmyoglobin, produced by reduction of oxidized forms of the protein by thermolysed electrons in aqueous glycerol glasses at 77 K. The content of high-spin species is higher in the F^- complex than in the native protein. Two intense peaks are found in the α-band region of metmyoglobin and its complexes with F^-, OH^-, and imidazole. Similarity of spectra of low-spin ferro-forms of the complexes are explained by the strong influence of the distal histidine. Low-temperature reduction of the N_3^- and CN^- complexes leads to formation of non-equilibrium low-spin ferro-forms, whose spectra demonstrate the maintenance of N_3^- and CN^- co-ordination.[708] Computer modelling has been carried out on the m.c.d. spectra of a myoglobin–nicotinic acid complex, and is consistent with the superimposition of two $\pi \rightarrow \pi^*$ transitions to doubly degenerate excited states, if it is postulated that the asymmetric environment of the haem destroys the natural degeneracy.[709] In a typical preparation of aquomethaemoglobin—by treatment of oxyhaemoglobin with ferricyanide—m.c.d. has shown that *ca.* 1% of the haemoglobin remains in the oxy-form. Using this method to detect a small contamination with the oxy-form it has been possible to redetermine the A_{570}/A_{630} ratio of methaemoglobin as 0.80, not as previously reported 0.83—0.87.[710] In a paper following the study of non-equilibrium states in myoglobin [708] similar experiments have been reported on peroxidase complexes. Mixtures of high-spin and low-spin ferro-forms have been found in peroxidase complexes with F^- and N_3^- with the

[703] G. T. Babcock, J. Van Steelandt, G. Palmer, L. E. Vickery, and I. Salmeen, *Dev. Biochem.*, 1978, **5**, 105.
[704] T. Nozawa, Y. Orii, A. Kaito, T. Yamamoto, and T. Masahiro, *Dev. Biochem.*, 1978, **5**, 117.
[705] P. J. Stephens, A. J. Thomson, T. A. Keiderling, J. Rawlings, K. K. Rao, and D. O. Hall, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 5273.
[706] C. Greenwood, T. Walsh, M. Johnson, D. Barber, J. Springall, and A. J. Thompson, *Front. Biol. Energ.*, 1978, **2**, 851.
[707] A. M. Arutyunya, Y. A. Kamenskii, E. Milgrom, S. Surkov, A. A. Konstantinov, and Y. A. Sharonov, *FEBS Lett.*, 1978, **95**, 40.
[708] S. N. Magonov, R. M. Davydov, L. A. Blyumenfeld, R. Vilu, A. M. Arutyunya, and Y. A. Sharonov, *Mol. Biol.*, 1978, **12**, 1182.
[709] A. M. Arutyunya, S. N. Magonov, and Y. A. Sharonov, *Mol. Biol.*, 1979, **13**, 438.
[710] R. E. Linder, R. Records, G. Barth, E. Bunnenberg, C. Djerassi, B. E. Hedlung, A. Rosenberg, E. S. Benson, L. Seamons, and A. Moscovitz, *Anal. Biochem.*, 1978, **90**, 474.

following order of high-spin content: N_3^- complex < peroxidase < F^- complex. Only a low-spin form is found following low-temperature reduction of the CN^- complex. In the case of N_3^- and CN^- complexes the existence of low-spin forms is seen as being due to the presence of these ligands in the iron co-ordination sphere.[711] A third paper in this series has investigated non-equilibrium states in carboxymethylated fluoride complex and formylated cytochrome *c*. Changes in spectral characteristics and the temperatures required to be attained for equilibration of states are dependent upon the type of derivative being studied. In general the spectra demonstrate that there are substantial differences in protein structure in the haem vicinity.[712]

Two papers have appeared concerned with the electronic structures of the copper sites in *Rhus vernicifera, Polyporus versicolor* laccase, and ceruloplasmin. Studies of the spectra of laccase and some of its ligand-bound derivatives rule out near-tetrahedral geometry for type 2 and 3 Cu but are consistent with structures based on tetragonal six- or five- or square-planar four-co-ordination.[713] The m.c.d. spectrum of ceruloplasmin features a single negative band at 14 600 cm^{-1}, which is quite similar to those of other blue Cu proteins. But, when taken in conjunction with natural c.d. measurements, a tetrahedral geometry for the type 2 and 3 Cu is ruled out. A tetragonal structure for type 2 Cu with four nitrogen donor ligands is suggested.[714]

8 Mössbauer Spectroscopy

Contributed by D. P. E. Dickson

The primary sources of literature information for this review are 'The Mössbauer Effect Reference and Data Journal' published by the Mössbauer Effect Data Center, University of North Carolina, Asheville, NC 28804, USA, and 'The Index of Publications in Mössbauer Spectroscopy of Biological Materials' by L. May, Catholic University of America, Washington DC 20017, USA.

During 1979 work has been reported on haem proteins, iron–sulphur proteins, and iron storage and transport proteins. There have also been a number of dynamical studies in which the Mössbauer technique has been used to obtain information on the mobility of the Mössbauer atom or of the group or molecule containing that atom rather than on its spectroscopic properties.

Work has also been reported on a novel form of vitamin B_{12}, in which ^{57}Co emission Mössbauer spectra yielded information on the freedom of movement of the corrin ring and the different conformation of the various derivatives,[715, 716] and on iron amino-acid dithiocarbamate complexes of sarcosine and proline, which indicated that the ligands co-ordinate to iron *via* the two sulphur atoms.[717]

[711] S. N. Mogonov, R. M. Davydov, L. A. Blumenfeld, A. M. Arutyunyan, and Y. A. Sharonov, *Mol. Biol.*, 1978, **12**, 1191.

[712] R. M. Davydov, S. N. Magonov, A. M. Arutyunyan, and Y. A. Sharonov, *Mol. Biol.*, 1978, **12**, 1341.

[713] D. M. Dooley, J. Rowlings, J. D. Dawson, P. J. Stephens, L. E. Andreasson, B. G. Malmstrom, and H. B. Gray, *J. Am. Chem. Soc.*, 1979, **101**, 5038.

[714] J. D. Dawson, D. M. Dooley, R. Clark, P. J. Stephens, and H. B. Gray, *J. Am. Chem. Soc.*, 1979, **101**, 5046.

[715] M. Katada, S. Tyagi, A. Nath, R. L. Petersen, and R. K. Gupta, *Biochim. Biophys. Acta*, 1979, **584**, 149.

[716] M. Katada, S. Tyagi, D. S. Rajoria, and A. Nath in 'Porphyrin Chemistry Advances', ed. F. R. Longo, Ann Arbor Service Publishers Inc., Ann Arbor, Michigan, 1979, Vol. 4B, p. 157.

[717] S. Wajda, *Pol. J. Chem.*, 1979, **53**, 973.

Haem Proteins.—This section covers experimental and theoretical Mössbauer studies on haem proteins as well as work on enzymes containing haem, and synthetic analogues for haem proteins. Münck has reviewed Mössbauer spectroscopic work on haem proteins [718] and Sams and Tsin [719] similarly on iron porphyrins. A review of Mössbauer studies on the iron–ligand binding in haem proteins and related compounds by Maeda [720] places special emphasis on the binding involved in the reversible oxygenation of oxygen carriers.

A study of low-spin iron(III) haem complexes has been carried out by Rhynard *et al.*[721] The Mössbauer spectra of the azide and cyanide complexes of myoglobin and cytochrome *c* peroxidase, and of the synthetic analogues haem *bis*-pyridine, haem cyanopyridine, and haem bis-cyanide, were recorded at 4.2 K in small applied magnetic fields. Well resolved paramagnetic structure was observed below 20 K and the results were analysed using a spin-Hamiltonian formalism and assuming a low-symmetry crystal field in order to obtain the crystal-field and spin-Hamiltonian parameters and the relative orientations of the magnetogyric (***g***), magnetic hyperfine (***A***), and electric-field gradient (***V***) tensors. Good fits were achieved and in particular the orientation of the ***g***-tensors obtained from these fits were found to be in good agreement with published results derived from single-crystal e.p.r. measurements. The results indicate that the normal to the haem plane is a principal axis for all three tensors. However, the authors point out the dangers of assuming that this is generally the case in systems where single-crystal measurements are not available. The Mössbauer measurements in the above study were carried out using polycrystalline samples, and information on the orientation of the various tensors with respect to the crystal axes was obtained by relating these measurements to single-crystal measurements obtained with a different technique. A more direct approach is to make the Mössbauer measurements using single crystals but it is usually difficult to obtain sufficiently large single crystals of proteins. Harami *et al.*[722, 723] report single-crystal Mössbauer studies of metmyoglobin, myoglobin fluoride, and myoglobin nitride. The ^{57}Fe-enriched crystals which were used in this investigation were grown to a size of *ca.* 1 mm × 1 mm × 2 mm over a period of one month. The experiments were carried out with a small external magnetic field along each of the crystal axes. Computer analysis of the spectra of the high-spin compounds (metmyoglobin and myoglobin fluoride) using a spin-Hamiltonian yielded ***A***-tensors that did not show the anisotropy that had been previously suggested. Analysis of the spectra of the low-spin iron(III) compound, myoglobin fluoride, showed that the principal axis of the electric-field gradient tensor lies close to the haem plane and at an angle of 32° to one of the four Fe—N directions.

A single-crystal Mössbauer study has also been carried out on the low-spin iron(II) compounds oxymyoglobin and carboxymyoglobin.[724] In oxymyoglobin the quadrupole interaction is negative and the principal axis of the electric-field

[718] E. Münck in 'The Porphyrins', ed. D. Dolphin, Academic Press, New York, 1979, Vol. 4B, p. 379.
[719] J. R. Sams and T. B. Tsin in 'The Porphyrins', ed. D. Dolphin, Academic Press, New York, 1979, Vol. 4B, p. 425.
[720] Y. Maeda, *J. Physique Colloq.*, 1979, **2**, 514.
[721] D. Rhynard, G. Lang, K. Spartalian, and T. Yonetani, *J. Chem. Phys.*, 1979, **71**, 3715.
[722] T. Harami, Y. Maeda, and Y. Morita, *J. Physique Colloq.*, 1979, **2**, 498.
[723] T. Harami, *J. Chem. Phys.*, 1979, **71**, 1309.
[724] Y. Maeda, T. Harami, Y. Morita, A. Trautwein, and U. Gonser, *J. Physique Colloq.*, 1979, **2**, 500.

gradient tensor lies nearly in the haem plane, whereas in carboxymyoglobin the quadrupole interaction is positive and the principal axis of the electric-field gradient tensor is nearly perpendicular to the haem plane. Measurements in large applied magnetic fields confirm that both compounds are in a diamagnetic state and that the quadrupole splittings are quite different.

Wilson and Philips [725] investigated the angular dependence of the Mössbauer spectrum of sickle-cell haemoglobin by quick freezing deoxygenated sickle-cell blood in liquid nitrogen to produce an ordered sample. The spectra were obtained with a small magnetic field of 0.4 T and with the γ-ray beam at 0°, 45°, and 90° to the field direction. The results can be interpreted to give information on the orientation of individual molecules within the sickle-cell strand structure.

A high magnetic field Mössbauer study of deoxymyoglobin, deoxyhaemoglobin, and the synthetic analogues iron(II)-2-methylimidazole *meso*-tetraphenylporphyrin and iron(II)-1,2-dimethylimidazole *meso*-tetraphenylporphyrin showed that at temperatures above 20 K all the compounds exhibited very similar spectra,[726] with negative quadrupole interactions and anisotropic internal magnetic fields decreasing as a function of $1/T$. This information was interpreted as indicating that the orbital of the odd electron is prolate in the ground-state quintet with little unquenched orbital angular momentum. The differences in the spectra obtained at 4.2 K suggest differences in the detailed structure within the quintet.

Elliott *et al.*[727] have used ^{119}Sn Mössbauer spectroscopy and other techniques to investigate the binding of triethyltin to cat haemoglobin. The Mössbauer measurements support the idea that the triethyltin is made penta-co-ordinate owing to binding to two amino-acid residues on the protein.

Garibov *et al.*[728] have observed the interaction of hydrazine and dimethylhydrazine with oxyhaemoglobin, both in polycrystalline form and in fresh red blood cells (erythrocytes) from rats, using Mössbauer spectroscopy. Addition of hydrazine to erythrocytes leads to two quadrupole-split doublet spectra, one corresponding to a low-spin iron(II) haemochrome-like component and the other to a high-spin iron(II) component. In air the latter component oxidizes to high-spin iron(III). The effect of hydrazine on the oxyhaemoglobin powder was similar. In the absence of air the high-spin iron(II) component was observed to diminish with time, with a simultaneous increase in the haemochrome-like component.

The effect of light on haem proteins is to produce photodissociation which can be monitored using Mössbauer spectroscopy. In an investigation of photodissociated carboxymyoglobin at low temperatures, Marcolin *et al.*[729] have identified an iron(II) high-spin complex with Mössbauer parameters which are different from deoxymyoglobin and which can exhibit several slightly different conformations with different recombination characteristics. The time dependence of the reactions was investigated using a constant-velocity Mössbauer spectrometer which monitored the intensity of a particular Mössbauer absorption line as a function of time.

[725] J. M. Wilson and T. J. Philips, *Bull. Am. Phys. Soc.*, 1979, **24**, 27.
[726] T. A. Kent, K. Spartalian, G. Lang, T. Yonetani, C. A. Reed, and J. P. Collman, *Biochim. Biophys. Acta*, 1979, **580**, 245.
[727] B. M. Elliott, W. N. Aldridge, and J. W. Bridges, *Biochem. J.*, 1979, **177**, 461.
[728] R. E. Garibov, E. V. Russkikh, and V. V. Khrapov, *Dokl. Akad. Nauk SSSR*, 1979, **245**, 238.
[729] H. E. Marcolin, R. Reschke, and A. Trautwein, *Eur. J. Biochem.*, 1979, **96**, 119.

This technique could be useful in other systems for investigations of reaction kinetics.

It can often be a problem to obtain good fits between the calculated and experimental Mössbauer spectra of proteins and this may be due to some form of heterogeneity of the site of the Mössbauer nucleus. A similar phenomenon observed in e.p.r. spectra has been attributed to a variation in the g-tensor known as 'g-strain'. Dwivedi *et al.*[730] have obtained good fits to the applied magnetic field Mössbauer spectra of cytochrome *c*-551 from *Pseudomonas aeruginosa* by assuming the g-strain used in fitting the e.p.r. spectra together with the normal spin-Hamiltonian formalism.

Certain haem proteins and enzymes are thought to contain iron in unusual valence and spin states. Two possible examples are the hydrogen peroxide derivative of myoglobin and compound II from horseradish peroxidase, for which $Fe^{4+}(S = 1)$ states have been suggested. Mössbauer spectra have been obtained from these compounds in large applied magnetic fields of up to 5.5 T and at temperatures between 4.2 and 120 K.[731] Fitting of these spectra using a spin-Hamiltonian formalism leads to a confirmation of these assignments together with values of the spin-Hamiltonian parameters.

As well as compound III (considered above), the enzymatic reaction of horseradish peroxidase with peroxide produces another intermediate, compound I, which is also thought to contain iron in an unusual oxidation state. Low-temperature applied magnetic field Mössbauer spectra of this compound have been fitted using a spin-Hamiltonian with the iron in an $Fe^{4+}(S = 1)$ state.[732] The fits confirm this valence assignment but also lead to the proposal that there is a spin coupling between the haem iron and a free radical which is in an $S = \frac{1}{2}$ state.

The enzyme catalase is present in nearly all cells containing a cytochrome system and catalyses the decomposition of H_2O_2 into H_2O and O_2. Each molecule contains four subunits with haem at the active centres but the detailed structure is not known. The enzyme appears to be strongly affected by pH and the buffer used and so the conditions under which measurements are made are rather important. Parak *et al.*[733] have made Mössbauer measurements on bacterial catalase at pH = 6.3 in an acetate buffer and have obtained spectra similar to those of myoglobin, indicating a closely similar level scheme.

The haemoglobin model compound protohaem-di-3-(1-imidazole propylamide) is a protoporphyrin in which the fifth and sixth ligands above and below the haem plane are imidazole nitrogen; the imidazole is in turn attached to a propylamide chain which is bound to the haem. The orientation of the imidazole with respect to the haem plane is thus constrained and it is therefore hoped that this may lead to a kinetic behaviour like that of a biological haem protein. Mössbauer measurements on this compound are indicative of a low-spin iron(III) state with a positive quadrupole interaction.[734]

[730] A. Dwivedi, W. A. Toscano, and P. G. Debrunner, *Biochim. Biophys. Acta*, 1979, **576**, 502.
[731] C. E. Schulz, R. Chiang, and P. G. Debrunner, *J. Physique Colloq.*, 1979, **2**, 534; *Bull. Am. Phys. Soc.*, 1979, **24**, 321.
[732] C. E. Schulz, P. W. Devaney, H. Winkler, P. G. Debrunner, N. Doan, R. Chiang, R. Rutter, and L. P. Hager, *FEBS Lett.*, 1979, **103**, 102.
[733] F. Parak, D. Bade, and A. L. Marie, *J. Physique Colloq.*, 1979, **2**, 528.
[734] E. V. Mielczarek, B. Balko, R. L. Berger, D. K. White, T. G. Traylor, and T. Mincey, *J. Physique Colloq.*, 1979, **2**, 495.

Synthetic model complexes which bind oxygen reversibly are of particular interest in that they may provide information about the mechanisms of oxygen transport in haem proteins. Mössbauer spectroscopy has been used in an investigation of the interaction of two polyphosphazene iron–porphyrin systems with oxygen and carbon monoxide.[735]

The interaction of bases with haem compounds is of interest because of the similarity of the resulting haemochromes with certain haem proteins. Mössbauer spectra have been obtained at room temperature for eight substituted pyridine haemochromes in order to relate the Mössbauer quadrupole splittings to the σ- and π-bonding characteristics.[736]

The Mössbauer hyperfine field is dependent on the electronic structure of the atom. The electronic distributions in metmyoglobin, myoglobin fluoride, and fluorohaempyridine have been calculated using the self-consistent charge-extended Hückel method and from these the hyperfine fields at the ^{57}Fe nuclei have been calculated for different configurations and compared with the values obtained experimentally by Mössbauer spectroscopy.[737] The results are interpreted to show that the iron atom can communicate very effectively the electronic changes produced by substitution at the sixth ligand (where oxygen is attached in haemoglobin) to other ligands. This result may be relevant to an understanding of the co-operativity of the binding of oxygen to haemoglobin.

Case *et al.*[738] have calculated the electronic distributions in a number of iron–porphyrin complexes which are models for the active site in oxy- and carboxy-haemoglobin. The calculations were made using an extended Pariser–Parr–Pople Hamiltonian and an X_α multiple-scattering method and were compared with previous results from extended Hückel and *ab initio* Hartree–Fock methods. A comparison of the Mössbauer quadrupole splittings derived from these calculations with those observed experimentally provides support for the inclusion of correlation effects involving doubly excited configurations. The iron–oxygen unit of oxyhaemoglobin is shown to be well represented as an equal mixture of Fe^{2+} ($S = 0$), O_2 ($S = 0$) and Fe^{2+} ($S = 1$), O_2 ($S = 1$) valence state pairs; the latter resembling ozone in certain respects. The iron–carbon monoxide unit corresponds closely to an idealized Fe^{2+} ($S = 0$), CO ($S = 0$) species.

A theoretical investigation of the ground state and low-lying excited states of oxyhaemoglobin, based on the interpretation of the temperature variation of experimental Mössbauer spectra, as well as magnetic susceptibility data, has been presented by Bacci *et al.*[739]

Gupta and Sharma[740] have made a theoretical interpretation of the Mössbauer data on haemoglobin cyanide.

Iron–Sulphur Proteins.—This section covers Mössbauer spectroscopic studies on iron–sulphur proteins, enzymes containing iron–sulphur centres, and synthetic analogues.

[735] H. R. Allcock, P. P. Greigger, J. E. Gardner, and J. L. Schmutz, *J. Am. Chem. Soc.*, 1979, **101**, 606.
[736] W. M. Connor and D. K. Straub, *Inorg. Chem.*, 1979, **18**, 866.
[737] S. K. Mun, J. C. Chang, and T. P. Das, *J. Am. Chem. Soc.*, 1979, **101**, 5562.
[738] D. A. Case, B. H. Huynh, and M. Karplus, *J. Am. Chem. Soc.*, 1979, **101**, 4433.
[739] M. Bacci, M. Cerdonio, and S. Vitale, *Biophys. Chem.*, 1979, **10**, 113.
[740] R. R. Gupta and R. R. Sharma, *Bull. Am. Phys. Soc.*, 1974, **24**, 319.

The two-iron ferredoxin from *Pseudomonas putida* differs significantly from the two-iron plant ferredoxins. Bertrand and Gayda [741] have presented a model for the electronic structure which explains the differences between the electric-field gradients in terms of variable mixing of the *d*-orbitals of the Fe^{2+} ion due to the differing amounts of rhombic distortion of the active site.

Winkler *et al.*[742] have obtained Mössbauer spectra from reduced rubredoxin between 10 and 150 K in applied magnetic fields and have fitted these using Blume's stochastic theory of lineshape in order to obtain information on the spin fluctuation rates of the high-spin iron(II) ion.

Compounds containing the anions $[Fe_4S_4(SR)_4]^{1-,2-,3-}$ provide analogues for the different oxidation states of the iron–sulphur proteins which have four-iron active centres. The effect of the terminal ligand R is important in determining the electronic properties and Papaefthymiou *et al.*[743] have investigated these effects in the case of the trianion, which is a model for reduced bacterial ferredoxin.

The enzyme nitrogenase, which has a crucial role in catalysing nitrogen fixation, contains a molybdenum–iron protein which has a cofactor with $S = \frac{3}{2}$ iron–molybdenum–sulphur active centres of a novel type. Huynh *et al.*[744] have obtained Mössbauer spectra of these cofactors in applied magnetic fields of up to 5 T and have deconvoluted the spectra to show six magnetic subsites, three with positive magnetic hyperfine coupling constants and three with negative. This indicates that the centre contains six iron atoms, spin coupled with three spin-up and three spin-down. Mössbauer spectroscopy was also used in a study of the electrochemical and solvent perturbation properties of the molybdenum–iron protein from nitrogenase.[745]

Another approach to understanding the nature of the iron–molybdenum–sulphur centres in nitrogenase has been to synthesize centres for which the stoicheiometry and structure can be found and then to compare their spectroscopic properties with those of the nitrogenase centre. Frankel *et al.*[746] report Mössbauer data on a possible analogue compound which contains cubic $[Fe_3MoS_4]$ clusters. Christou *et al.*[747] have investigated a closely related compound containing tungsten rather than molybdenum.

Iron-storage and -transport Proteins.—In an investigation of the bacterium *Escherichia coli* grown in an iron-rich medium Bauminger *et al.*[748] observed Mössbauer spectra which indicated the presence of an iron-storage material of a novel type. The spectra obtained are markedly different from those of the iron-storage protein ferritin, which is found in plants and higher animals, and there is spectral evidence that the material undergoes a magnetic-ordering transition at *ca.*

[741] P. Bertrand and J. P. Gayda, *Biochim. Biophys. Acta*, 1979, **579**, 107.
[742] H. Winkler, C. E. Schulz, and P. G. Debrunner, *Phys. Lett.*, 1979, **69A**, 360.
[743] G. C. Papaefthymiou, R. B. Frankel, E. J. Laskowski, and R. H. Holm, *Bull. Am. Phys. Soc.*, 1979, **24**, 345.
[744] B. H. Huynh, E. Münck, and W. H. Orme-Johnson, *Biochim. Biophys. Acta*, 1979, **576**, 192; *J. Physique. Colloq.*, 1979, **2**, 526.
[745] J. Rawlings, M. H. Emptage, and B. H. Huynh, *Fed. Proc.*, 1979, **28**, 735.
[746] R. B. Frankel, T. E. Wolff, and R. H. Holm, *Bull. Am. Phys. Soc.*, 1979, **24**, 345.
[747] G. Christou, C. D. Garner, T. J. King, C. E. Johnson, and J. D. Rush, *J. Chem. Soc., Chem. Commun.*, 1979, 503.
[748] E. R. Bauminger, S. G. Cohen, D. P. E. Dickson, A. Levy, S. Ofer, and J. Yariv, *J. Physique Colloq.*, 1979, **2**, 523.

2.6 K. Following these observations a protein with a relative molecular mass of 500 000 and an electron-dense core has been isolated from *E coli*. In a Mössbauer study of the bacterium *Proteus mirabilis*,[749] in which the distribution of iron components within the bacterium was investigated, very similar spectra were obtained to those seen in the above study of *E. coli*, indicating that the new iron-storage material is also present in this bacterium.

Bauminger *et al.*[750] have used Mössbauer spectroscopy to determine the quantities of ferritin-like iron in the red blood cells taken from patients with thalassaemia, sickle-cell anaemia, and other blood diseases in which the haemoglobin breaks down and deposits the resulting iron in the form of the iron-storage protein ferritin.

Enterobactin is the protein which forms complexes with iron and by means of which iron is transported into the bacterium *E. coli*. The complex involved is usually considered to contain Fe^{3+} but in a recent study it has been shown that the iron–enterobactin complex formed at low pH has Mössbauer parameters which clearly indicate that the iron is in a high-spin iron(II) form.[751]

Dynamical Studies.—Mössbauer spectroscopy is usually used to study the hyperfine interactions of iron atoms within biomolecules in order to obtain information on the electronic structure of the iron atom and its ligands. Another feature of the Mössbauer effect is the recoil-less fraction (the fraction of γ-ray emission or absorption events in which there is no energy loss to recoil) which determines the total spectral intensity. The recoil-less fraction and its temperature dependence are related to the dynamical characteristics of the system and thus investigations of the spectral intensity can be used for studying dynamical behaviour. A discussion of dynamical studies of biomolecules by Mössbauer spectroscopy has been presented by Parak.[752]

Dwivedi *et al.*[753] have measured the temperature dependence of the recoil-less fraction for oxymyoglobin, deoxymyoglobin, carboxymyoglobin, and rubredoxin in frozen solutions. The general pattern is similar for all four proteins and deviates from the predictions of the Debye model, indicating a low-temperature anharmonicity. Diffusional line broadening is noticeable for temperatures above 200 K in glycerol and above 230 K in aqueous samples. The data presented should provide a useful survey of the range of parameters to be expected for the recoil-less fraction in iron proteins. The molecular dynamics of these systems differ considerably from the dynamics of ordinary solids and present many intriguing problems to the Mössbauer spectroscopist.

Harrison *et al.*[754] have measured the ^{119}Sn Mössbauer recoil-less fraction in $\alpha,\beta,\gamma,\delta$-tetraphenylporphinatodihydroxytin(IV) in both bis-chloroform and bis-carbon tetrachloride solvate forms. The data were interpreted in terms of the effect of the porphyrin residue on the thermal excitation of the tin atom.

[749] D. P. E. Dickson and S. Rottem, *Eur. J. Biochem.*, 1979, **101**, 291.
[750] E. R. Bauminger, S. G. Cohen, S. Ofer, and E. A. Rachmilewitz, *Proc. Nat. Acad. Sci. USA*, 1979, **76**, 939; *J. Physique Colloq.*, 1979, **2**, 502.
[751] R. C. Hider, J. Silver, J. B. Neilands, I. E. G. Morrison, and L. V. C. Rees, *FEBS Lett.*, 1979, **102**, 325.
[752] F. Parak, *Bull. Am. Phys. Soc.*, 1979, **24**, 354.
[753] A. Dwivedi, T. Pederson, and P. G. Debrunner, *J. Phys. Colloq.*, 1979, **2**, 531.
[754] P. G. Harrison, K. Molloy, and E. W. Thornton, *Inorg. Chim. Acta*, 1979, **33**, 137.

Prusakov *et al.*[755] have monitored both the recoil-less fraction and the linewidth in a Mössbauer study of water–protein system dynamics using ^{57}Fe bound within or on the surface of hydrated albumin. The dependence of the recoil-less fraction on temperature is the same for both systems up to $-60\,^{\circ}C$ but above this the two systems differ in a way which was interpreted in terms of the different effects of the melting of water at the surface of the protein. In a similar study involving ^{57}Fe bound to the surface of α-chymotrypsin and human serum albumin Belonogova *et al.*[756] observed, *via* the Mössbauer recoil-less fraction, that the mobility of the iron increased with hydration for fractional saturations above 0.6 for albumin and above 0.7 for chymotrypsin. The data were interpreted to indicate that the first water molecules to interact with dry protein bind to specific sites, after which less specific binding and filling of the surface crevices occurs.

9 Dissociation and Association of Proteins

Contributed by E. J. Wood

Analytical Ultracentrifugation.—*Techniques.* The tabletop air-turbine ultracentrifuge (Beckman Airfuge ®) has a number of attractive features for the study of proteins and protein–protein interactions. Its use has been mentioned in a previous Report,[757] and a non-empirical method has now been developed to determine the molecular weights of unpurified proteins by sedimentation equilibrium.[758] Sedimentation of proteins in 100 μl buffer, adjusted to 0.5% total protein concentration with added albumin for density stabilization, was continued for 24 h in a fixed-angle rotor. After deceleration the tubes were placed in a vertical position and successive 10 μl-fractions withdrawn from the meniscus of the reorientated solution using a capillary pipette and syringe guided by a micromanipulator. The specific protein concentration of each fraction was determined by absorbance at 280 nm, by enzyme activity, or by radioactivity if labelled proteins were used. The radial distance of each fraction was then calculated by numerical integration, as the geometry of the tube and its orientation to the axis of rotation are known. Exact equations were developed by considering the centrifuge tube to consist of a cylinder and a hemispherical lower portion, both of internal radius a, orientated at an angle θ to the axis of rotation. The cross-section area of the solution during centrifugation is given by the sum of a variably truncated ellipse and a variably truncated circle whose radius varies. Plots of ln (protein concentration) *versus* r^2 were found to be linear, and molecular weights calculated from the slopes of such plots agreed well with published values. It is claimed that by the use of isotopically labelled proteins molecular weights over a range 6000—600 000 dalton may be determined to ± 5% at concentrations 1000-fold lower than with previous sedimentation equilibrium methods. Thus [^{125}I]insulin was studied at a concentration of 10^{-10} M and a speed of 96 000 rev min^{-1}. Under these conditions (previously inaccessible) insulin

[755] V. E. Prusakov, O. V. Belonogova, E. N. Frolov, R. A. Stukan, V. I. Gol'danskii, A. I. Berg, and L. May, *Mol. Biol. (Moscow)*, 1979, **13**, 443.

[756] O. V. Belonogova, E. H. Frolov, N. V. Illyustrov, and G. I. Likhtenshtein, *Mol. Biol. (Moscow)*, 1979, **13**, 567.

[757] E. J. Wood in 'Amino-acids, Peptides, and Proteins', ed. R. C. Sheppard (Specialist Periodical Reports), The Royal Society of Chemistry, London, 1979, Vol. 10, p. 272.

[758] R. J. Pollet, B. A. Haase, and M. L. Standaert, *J. Biol. Chem.*, 1979, **254**, 30.

behaved as a homogeneous species with a molecular weight of 5600 dalton. At higher concentrations (2×10^{-5}—2×10^{-4}M) self-association was detected which is consistent with the well recognized dimerization of the molecule in this concentration range.

The method of Bothwell *et al.*[759] was used as a basis for the determination of the molecular weights of proteins in heterogeneous mixtures and to analyse protein–protein interactions, using the same instrument.[760] The total fraction of each particular protein remaining in an upper fraction of the tube after centrifugation was determined by quantitative SDS–gel electrophoresis. However, the method yields 'native' molecular weights of the protein components, and the procedure allows a wide range of proteins to be analysed in a single experiment. This method is of course empirical, molecular weights being obtained by comparison with well characterized 'standard' proteins. Interactions were also studied and the values obtained for the association equilibrium constants for the systems lysozyme–ovalbumin and lysozyme–bovine serum albumin agreed well with published values. The Airfuge has also been used to study protein–small molecule interactions, for example, that between [^{3}H]-ouabain and Na^+, K^+-ATPase.[761] The advantages of this method are its simplicity, the small volumes required (100 μl), and the rapidity with which the separations of bound from free small molecules is achieved (*e.g.* 30 s).

On a larger scale, but still using the preparative ultracentrifuge, a simplifed procedure has been described for the determination of both the sedimentation coefficient and the partial specific volume of a protein from density-gradient centrifugation.[762] The method involves sedimentation of the protein through 5—20% sucrose gradients prepared in both H_2O and D_2O. Only a knowledge of the density of the sucrose solution is required; it is not necessary to know rotor dimensions, angular velocity, time of centrifugation, or solvent viscosity.

In conventional sedimentation equilibrium, it is reported that at high ionic strengths the apparent isopotential volume (ϕ_1') may increase considerably.[763] This can lead to considerable errors in molecular weight estimates if values for $\bar{v}$ obtained at low ionic strengths are used. For lobster muscle lactate dehydrogenase, which does not dissociate at relatively high ionic strengths (*e.g.* 1.2 M-ammonium sulphate), ϕ_2' was 0.767 ± 0.1 ml g^{-1} compared with 0.730 ± 0.01 ml g^{-1} at low ionic strength. Bull and Breese have given consideration to the problems of determining dry weight, and hence partial specific volumes, of proteins.[764]

Squire and Himmel examined hydrodynamic data from 21 proteins whose molecular dimensions were known from *X*-ray crystallography and presented two equations relating molecular weights and sedimentation coefficients.[765] For the 21 proteins the mean of the calculated hydration values was 0.53 ± 0.26 g H_2O g^{-1} protein. This is much higher, and has more variance than values previously obtained by n.m.r. measurements on frozen protein solutions (0.36 ± 0.04). A

[759] M. A. Bothwell, G. J. Howlett, and H. K. Schachman, *J. Biol. Chem.*, 1978, **253**, 2073.
[760] R. G. Clarke and G. J. Howlett, *Arch. Biochem. Biophys.*; 1979, **195**, 235.
[761] R. W. Albers and N. Krishnan, *Anal. Biochem.*, 1979, **96**, 395.
[762] J. E. Sadler, *J. Biol. Chem.*, 1979, **254**, 4443.
[763] P. Tuengler, G. L. Long, and H. Durchslag, *Anal. Biochem.*, 1979, **98**, 481.
[764] H. R. Bull and H. Breese, *Arch. Biochem. Biophys.*, 1979, **197**, 199.
[765] P. G. Squire and M. E. Himmel, *Arch. Biochem. Biophys.*, 1979, **196**, 165.

model of hemispherical clathrate cages was proposed which could account for these differences in data.

Theory. Although sedimentation equilibrium has become the standard method for estimating the molecular weights of proteins, for large asymmetric molecules or for unstable proteins the time taken to reach equilibrium can be so long that the method is of little use. A number of methods have been proposed and used for obtaining molecular weights from measurements taken during the approach to equilibrium, *e.g.* the Archibald method, thus shortening the period of the experiment. Holladay has examined the possibility of using data from the approach to equilibrium and Fujita's approximate solution of the Lamm equation to estimate s/D.[766] Values of C_r *versus* r during approach to equilibrium were analysed by a non-linear least-squares algorithm, and it was shown that molecular weights could be estimated in one-tenth of the time required to reach sedimentation equilibrium, and the procedure was tested using ribonuclease and ovalbumin. In a second paper [767] an approximate solution to the Lamm equation was derived in order to analyse sedimentation-velocity data which could yield estimates of s and s/D. Of more direct relevance to associating–dissociating systems is the approach used by De Voort *et al.* to study the interaction of α_{S1}- and κ-caseins.[768] In this system an oligomer (probably a trimer) of κ-casein interacts with the monomer or dimer of α_{S1}-casein, forming a complex of mol. wt. *ca.* 400 000 dalton. The method used was sedimentation equilibrium and the concentration distribution in the cell was determined with the u.v.-scanning optical system. The distribution of molecular weight was determined by multiple regression analysis in four series with exponentially spaced molecular weight scales shifted by a factor of $2^{\frac{1}{4}}$ between series. The simplex optimization routine used in conjunction with this provided a better fit to the data than the linear programming method of Scholte.

Examples. Sedimentation velocity and equilibrium methods were used to study the behaviour of apohaemoglobin in the presence of inositol hexaphosphate (IHP).[769] The presence of IHP at concentrations up to 1 mM favoured the formation of tetramers, but above this concentration dimer formation was promoted. This suggested binding with a stoicheiometry greater than 1 mol IHP per four subunits. In the absence of IHP the apohaemoglobin existed largely as a dimer with a sedimentation coefficient, $s^{\circ}_{20,w}$, of 2.6 S and a molecular weight of 32 000 dalton. Similar techniques were used to investigate the asymmetry of apolipoproteins A and I from human and rhesus monkey serum.[770] Both proteins undergo a concentration-dependent, reversible self-association in aqueous solution. Ultracentrifuge data taken together with viscometric and fluorescence polarization values indicated that the axial ratio of apolipoprotein A to I was 5.8 and could be represented as an elongated ellipsoid with a semi-major axis (a) of 75.6 Å and a semi-minor axis (b) of 12.6 Å. It seems likely that the monomers dimerize along their major axis and that the dimers produce elongated oligomers by an end-to-end association.

[766] L. A. Holladay, *Biophys. Chem.*, 1979, **10**, 183.
[767] L. A. Holladay, *Biophys. Chem.*, 1979, **10**, 187.
[768] F. R. De Voort, C.-Y. Ma, and S. Nakai, *Arch. Biochem. Biophys.*, 1979, **195**, 596.
[769] A. H. Chu and E. Bucci, *J. Biol. Chem.*, 1979, **254**, 371.
[770] D. L. Barbeau, A. Jonas, T. Teng, and A. M. Scanu, *Biochemistry*, 1979, **18**, 362.

Gel Chromatography.—Controlled-pore glass beads have a number of advantages as the stationary phase in exclusion chromatography, but so far have received little attention for use in the analysis of associating–dissociating systems. At relatively high protein concentrations, osmotic shrinkage of conventional gel chromatographic media complicates the analysis of association equilibria since neither the elution volumes of excluded polymers nor those of any partitioning species can be regarded as invariant. The use of controlled-pore glass beads overcomes the problem of osmotic shrinkage and if, in addition, the pore size is chosen so that only monomer distributes between mobile and stationary phases the chromatographic characteristics of all the polymeric forms are defined unequivocally and are identical. Nichol *et al.*[771] have explored the possibilities of such systems using bovine liver glutamate dehydrogenase and CPG-10 170B glass beads with a mean pore diameter of 15.9 nm. Glutamate dehydrogenase is a well characterized system in which the monomer undergoes indefinite association; the monomer has a Stokes diameter of 12 nm, and the dimer of 17.2 nm. The isodesmic equilibrium constant of 1.5 $\pm$ 0.3 $l\,g^{-1}$ found in this work was in reasonable agreement with previous reported values (1.4—2.4 $l\,g^{-1}$). The same workers investigated the possible association of haemoglobin molecules ($\alpha_2\beta_2$) at the high concentrations prevailing inside the red blood cell (*ca.* 320 $g\,l^{-1}$).[772] According to the results obtained with both bovine and human oxyhaemoglobins on GPG-10 120 glass beads, only half of the protein would be in the $\alpha_2\beta_2$ state under conditions of oxygen saturation and at 320 $g\,l^{-1}$. The exclusion chromatography data were consistent with the joint operation of thermodynamic non-ideality and reversible association of the $\alpha_2\beta_2$ species. Previous sedimentation equilibrium and osmotic-pressure measurements had indicated that over a wide concentration range no long-range intermolecular interactions operated in solutions of haemoglobin near the isoelectric point. From a consideration of the effect of 'DPG' (glycerate 2,3-bisphosphate) it was concluded that the control of oxygen binding by haemoglobin under physiological conditions involves both self-association and allosteric effects.

An extended theoretical and experimental study of interacting protein systems by scanning molecular sieve chromatography has appeared.[773] This method has been applied in a number of ways in the past, but this work concerned itself with large zone or saturation experiments which afford the most accurate determination of the solute partition cross-section and the partition coefficient. Molecular size and interaction parameters are readily derived from these quantities, but in addition large zone experiments can provide information on the time-dependent properties of solute profiles in molecular sieve chromatography. In the experimental system described, the gel filtration column is moved through a horizontally collimated beam of monochromatic light at a rate of 1 mm s^{-1} for 100 s during which time the output from a photomultiplier is fed to a computer which acquires and stores a specified number of transmittance readings. Instead of determining the rates of movement of the leading and trailing boundaries of zones by locating the centroid position of the scan, in the new difference-method the baseline records of successive

[771] L. W. Nichol, R. J. Siezen, and D. J. Winzor, *Biophys. Chem.*, 1979, **9**, 47.
[772] L. W. Nichol, R. J. Siezen, and D. J. Winzor, *Biophys. Chem.*, 1979, **10**, 17.
[773] E. E. Brumbaugh, E. E. Saffen, and P. W. Chun, *Biophys. Chem.*, 1979, **9**, 299; P. W. Chun and M. C. K. Young, *Biophys. Chem.*, 1979, **9**, 313; E. E. Saffen and P. W. Chun, *Biophys. Chem.*, 1979, **9**, 329.

scans are subtracted from each other, yielding difference profiles. The centroid position of each boundary is then located by finding the peak position of the difference profiles. The system was used to analyse the dimer–tetramer equilibria of oxyhaemoglobin and, in addition, the effect of kinetic parameters was investigated by computer-simulation techniques. It was found that the kinetically controlled effects of local equilibration along the column in either the mobile or the stationary phase influence the apparent equilibrium constant as a function of the distance co-ordinate.

An examination of the light-scattering behaviour of a number of gel matrices used in gel chromatography revealed that the possession of a combination, by the beads, of a very low refractive index increment (μ) and a very large scattering particle size leads to scattering of light almost exclusively in the forward direction.[774] This explains why the absorbance of protein solutions obeys Beer's law to a high degree of precision even though there is a loss of more than 90% of input light by scattering. Sephadexes therefore perform well while glass beads (high μ) do not.

High-pressure liquid-chromatography columns have been introduced for use in protein and peptide separations. A new resin, Synchropak GPC-100 (10 nm pore size), was used in a 25 × 0.46 cm column coupled to a fluorescamine detection system for the separation of μg-amounts of proteins and peptides with molecular weights in the range 5000—700 000.[775] The relationship between elution volume and molecular weight over this range was linear and the experiment took 30—45 min. Proteins were also subjected to high-speed gel filtration in the presence of 6 M-guanidine hydrochloride on columns of silica-based aqueous gels in conjunction with high-pressure liquid chromatography.[776] Here again run times were typically 50 min compared with 1–2 days by conventional gel chromatography using 'soft' gels such as Sephadex or Sepharose.

Examples. Gel-filtration chromatography has been used to investigate the subunit dissociation equilibria of tadpole haemoglobin as a function of pH,[777] the interaction between troponin subunits,[778] and the interaction between prealbumin and retinol-binding protein.[779] Another interesting system investigated was the enzyme prephenate dehydratase, from *Bacillus subtilis*.[780] The monomer (mol. wt. 38 000 dalton) was inactive, the octomer (mol. wt. 210 000 dalton) was fully active, while the dimer (mol. wt. 55 000 dalton) showed enzymic activity which was modifiable by allosteric effectors such as methionine and phenylalanine.

Light Scattering.—Conventional, or intensity, light-scattering was used to study the mutual influence of ligand binding and self-association of the enzyme phosphorylase *b*.[781] The inactive dimer may be converted into an active tetramer either covalently by phosphorylation of a seryl residue or non-covalently by the

[774] L. C. Davis, *Biophys. Chem.*, 1979, **10**, 55.
[775] K. A. Gruber, J. M. Whitaker, and M. Morris, *Anal. Biochem.*, 1979, **97**, 176.
[776] N. Ui, *Anal. Biochem.*, 1979, **97**, 65.
[777] D. H. Atha, A. Riggs, J. Bonaventura, and C. Bonaventura, *J. Biol. Chem.*, 1979, **254**, 3393.
[778] J. Horwitz, B. Bullard, and D. Mercola, *J. Biol. Chem.*, 1979, **254**, 350.
[779] G. Fex, P.-A. Albertsson, and B. Hansson, *Eur. J. Biochem.*, 1979, **99**, 353.
[780] R. G. Riepl and G. I. Glover, *J. Biol. Chem.*, 1979, **254**, 10321.
[781] R. F. Steiner, L. Greer, and R. Bhat, *Biochemistry*, 1979, **18**, 1380.

binding of AMP. The linked processes of AMP binding and tetramer formation were analysed quantitatively in terms of a set of stepwise equilibrium constants describing the equilibria between the various possible states of ligation and self-association.

A pressure perturbation apparatus has been described in which light-scattering is used to monitor self-association processes. Pressure perturbation in both the time domain and the frequency domain was monitored and the instrument was used to study the kinetics of self-association of beef liver glutamate dehydrogenase.[782] The kinetic behaviour was consistent with the random self-association model proposed previously.[783] The activation volume $\Delta V^{\ddagger}$ for association was positive. In the instrument, monochromatic light isolated from a xenon arc impinges on a cylindrical cell, and alternate positioning of the photomultiplier tube allowed monitoring of transmitted light or light emitted at 90°. The pressure was varied by driving a piezoelectric stack with the output of a programmable power supply. Pressure perturbation was synchronized with the phase-sensitive detector/integrator by means of a variable-phase variable-frequency function generator. In time-domain experiments the power supply was programmed with a square wave and the detector/integrator was replaced with a digital signal averager.

Stopped-flow light-scattering was used to study the assembly of the blood-clotting protein fibrin following its activation with thrombin.[784] The assembly proceeds in two steps. The first of these involves end-to-end polymerization of fibrin monomers to protofibrils, and the second, lateral association of these to fibres. The protofibrils consist of monomers laid end-to-end with an overlap equal to one-half of the monomer length (450 Å). The fibres eventually formed may consist of 100 or more protofibrils, and these are later linked covalently in an irregular network by an enzyme which introduces γ-glutamyl-ε-lysyl cross-links. Because the length of fibrin fibres is many times the wavelength of visible light, light-scattering measurements give not mass but the mass/length ratio of the fibres. Other important biological fibre-forming processes have been studied by turbidity measurements including collagen fibril formation [785] and intermediate filament assembly.[786]

Quasi-elastic Light-scattering. The use of quasi-elastic light-scattering with rapid-mixing techniques is being developed, but is limited by the time taken to measure the autocorrelation function. Such times can be as fast as 10—30 s with current instrumentation, and therefore systems can be investigated where this time is short compared with the reaction half-time. One system that has been studied is the assembly of bacteriophage T4.[787] Another problem with such a method is the convective disturbance arising from mixing, and Wei and Bloomfield have given details of an apparatus in which convection is minimized and very rapidly damped.[788] It was found, however, that the degree of turbulence depends very much on the rate at which the reaction mixture was pushed into the cell, and it was

[782] H. R. Halvorson, *Biochemistry*, 1979, **18**, 2480.
[783] D. Thusius, P. Dessen, and J. M. Jallon, *J. Mol. Biol.*, 1975, **92**, 413.
[784] R. R. Hantgan and J. Hermans, *J. Biol. Chem.*, 1979, **254**, 11272.
[785] R. A. Gelman, B. R. Williams, and K. A. Piez, *J. Biol. Chem.*, 1979, **254**, 180; R. A. Gelman, D. C. Poppke, and K. A. Piez, *J. Biol. Chem.*, 1979, **254**, 11741.
[786] R. V. Zackroff and R. D. Goldman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6226.
[787] J. A. Benbasat and V. A. Bloomfield, *J. Mol. Biol.*, 1975, **95**, 335.
[788] G. J. Wei and V. A. Bloomfield, *Anal. Biochem.*, 1979, **97**, 217.

best to push the syringe drive comparatively slowly (*ca.* 4 s). These same workers also reported a computer-simulation study of the effect of diffusion on the size distribution obtained by combining quasi-elastic light-scattering with isokinetic band sedimentation or electrophoresis.[789]

Lyophilized horse-liver alcohol dehydrogenase was shown by quasi-elastic light-scattering and ultracentrifugation to have an initial molecular weight, when freshly dissolved, of *ca.* 10^6 dalton and this increased with time.[790] Non-lyophilized enzyme had the expected molecular weight of 78 000 dalton. The rate of self-association of the lyophilized material increased with increase in temperature.

A report of the 1978 meeting on light-scattering held at the Royal Society, London, has now been published and is available as a separate volume.[791]

Transport Studies.—*Theory and Techniques.* A computer-simulation system has been developed for investigating the mass-transport properties of interacting, non-identical macromolecules where the association has the form $A + B \rightleftarrows C$, $C + B \rightleftarrows D$.[792] Calculations were simplified by using a rectangular approximation for the centrifuge cell, although this introduced a few percent error, and the program was also directly applicable to gel chromatography, simply by substituting flow for centrifugal field and dispersion for diffusion. Simulation of centrifuge results were compared with experimental results for two systems, nitrogenase components and an antigen–antibody interaction.

The method of active enzyme sedimentation or reacting enzyme sedimentation has proved extremely valuable, and has recently been used to identify the active form of complex regulatory enzymes (such as phosphofructokinase) capable of existing as multiple polymeric forms.[793] However, although this technique has a number of unique advantages over other types of analysis, its extreme sensitivity is at once its greatest advantage and its greatest limitation. Measurements above enzyme concentration of 0.1 $\mu g\,ml^{-1}$ are excluded for some enzymes with present methods and yet it would be of great interest to analyse in the concentration range 0.1 $\mu g\,ml^{-1}$ to 1 $mg\,ml^{-1}$, the region in which most enzymes exist *in vivo* and the range in which most enzymes undergo shifts in their association–dissociation equilibria. A way of studying this concentration range has now been proposed, called 'high-concentration active enzyme centrifugation'.[794] It is claimed to be able to extend the upper concentration limit by up to four orders of magnitude. The new theory is based largely on certain properties of Gaussian curves described previously.[795]

Mühlmann and Schönert have considered the determination of association constants of proteins by means of electrophoresis methods.[796] The concentration profile of a reversibly associating substance during electrophoresis is determined by diffusion, migration, and reaction. The influence of diffusion can be eliminated by

[789] G. J. Wei and V. A. Bloomfield, *Biophys. Chem.*, 1979, **9**, 97.
[790] J. B. A. Ross, E. L. Chang, and D. C. Teller, *Biophys. Chem.*, 1979, **10**, 217.
[791] 'Light-Scattering in Physics, Chemistry and Biology', The Royal Society, London, 1979 (see also *Phil. Trans. Roy. Soc.*, Series A, 1979, Vol. 293).
[792] L. C. Davis and M. S. Chen, *Arch. Biochem. Biophys.*, 1979, **194**, 37.
[793] L. K. Hesterberg and J. C. Lee, *Arch. Biochem. Biophys.*, 1979, **197**, 500.
[794] G. J. Wei and W. C. Deal, *Biochemistry*, 1979, **18**, 1129.
[795] G. J. Wei and W. C. Deal, *Anal. Biochem.*, 1976, **75**, 113; *ibid.*, 1979, **87**, 433.
[796] H. P. Mühlmann and H. Schönert, *Biophys. Chem.*, 1979, **9**, 149.

extrapolating suitably transformed concentration profiles taken at different times, to infinite time. The resulting profile is a Gilbert profile reflecting migration and reaction only, and the association constant can be deduced from this. The feasibility of the method was established with a well known system, β-lactoglobulin A. In contrast to eliminating the effect of diffusion, the use of continuous optical scanning of polyacrylamide gel zones was used to estimate the apparent diffusion coefficient of β-lactoglobulin B.[797] This apparatus allowed the determination of the standard deviation of the concentration profile (σ). The apparent diffusion coefficient was linearly related to current density and protein load but non-linearly related to ionic strength. Because of this latter, the apparent diffusion coefficients of proteins in gels appeared to be about 10-fold larger than under typical conditions of ionic strength used in sedimentation and diffusion experiments.

Examples. Sedimentation velocity analysis was used to investigate changes in the quaternary structure of phosphorylase *a* [798] (see also ref. 781). It was shown that dimers were formed from tetramers upon the separate addition of either caffeine or glucose (known inhibitors), and that there was co-operative promotion of dimer formation when they were added together. Weight-average sedimentation coefficients were calculated from the schlieren traces obtained during the sedimentation of bovine immunoglobulin G1 which was shown to dimerize in 0.32 M-NaCl, 0.01 M-Tris–HCL, pH = 8.0.[799] No self-aggregation under these conditions occurred when the Fc portion was removed by pepsin digestion, nor was it observed at pH = 6.0.

In order to investigate 'communication' between dissimilar subunits in aspartate transcarbamoylase, Hensley and Schachman constructed molecules containing native regulatory subunits and enzymically active, nitrated catalytic subunits having one sensitive nitrotyrosyl per polypeptide chain.[800] Upon the addition of CTP there was a shift towards the T-state as shown by the change in sedimentation coefficient and there was also a decrease in absorbance at 430 nm due to the altered environment of the nitrated residues.

Sedimentation velocity methods have found use in the study of virus protein assembly, especially tobacco mosaic virus.[801] The rates of incorporation of the 4S and 20S proteins could be measured directly by analytical ultracentrifugation. The overall assembly process started with a nucleation step requiring two or three 20S disc aggregates per RNA molecule and was followed by an elongation or growth phase. In this second phase the 4S protein was incorporated 50—70 times faster than 20S disc.

Electron Microscopy.—A number of important oligomeric macromolecules have been studied by scanning transmission electron microscopy (STEM). A modified method was developed for fabricating substrates consisting of very thin, very clean carbon films supported on very clean fenestrated plastic films *ca.* 1.5 nm thick.[802]

[797] B. Chen, A. Chrambach, and D. Rodbard, *Anal. Biochem.*, 1979, **96**, 120.
[798] S. G. Withers, B. D. Sykes, N. B. Madsen, and P. J. Kasvinsky, *Biochemistry*, 1979, **18**, 5432.
[799] T. K. S. Mukkur and G. D. Smith, *Biochem. J.*, 1979, **183**, 463.
[800] P. Hensley and H. K. Schachman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3732.
[801] S. J. Shire, J. J. Steckert, M. L. Adams, and T. M. Schuster, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2745; T. M. Schuster, R. B. Scheele, and L. H. Khairallah, *J. Mol. Biol.*, 1979, **127**, 461; S. J. Shire, J. J. Steckert, and T. M. Schuster, *J. Mol. Biol.*, 1979, **127**, 487.
[802] M. Ohtsuki, M. S. Isaacson, and A. U. Crewe, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1228.

Molecules examined using this method included glutamine synthetase and ferritin. Tobacco mosaic virus (TMV) was also studied with special reference to the effects of electron radiation damage on its helical structure.

Electron microscopy is a very powerful technique for investigating structural proteins. For example, the effect of ATP, inorganic phosphate (Pi), and Ca^{2+} and Mg^{2+} ions on myosin filament formation was studied.[803] In the absence of ATP or Pi, filaments some 30—50 nm wide and 5—15 μm long formed. Such filaments did not display tapered ends and were larger than physiological size. In the presence of ATP or Pi, but in the absence of divalent cations, regular filaments were not formed but instead long branched structures were observed. When both ATP or Pi and cations were present, filaments with tapered ends were formed, and those formed in the presence of Mg^{2+} had diameters close to the physiological value (15—17 nm). Ageing in the cold over several days resulted in loss of these abilities. Other structural materials that have been investigated are clathrin, the coat protein from coated vesicles found in the cytoplasm of many cells,[804] and which at pH = 6.5 formed basket-like structures, and the annular, oligomeric protein which forms the 'gap junction' or 'connexon', which links cells and is believed to mediate and regulate the passage of ions and small molecules between cell interiors.[805]

The complex structures of the haemocyanins from arthropods is gradually being unravelled with the help of electron microscopic studies.[806, 807] The largest of these haemocyanins, sedimentation coefficient 60S, consists of 48 subunit polypeptide chains in an assemblage of eight 'hexamers'. Not all arthropods possess the 60S aggregate, however, some having assemblages of four or two 'hexamers'. The assemblages may be dissociated in stepwise fashion by pH change and removing divalent cations to give, eventually, a 16S hexameric unit. This unit is made up of six 5S particles, probably arranged in a trigonal antiprism. The 5S monomer is heterogeneous, and 5—8 types of polypeptide chain have been separated. The native molecule may be re-assembled by mixing these separated units under the appropriate conditions;[808] this approach has yielded interesting data on the structural role of the various polypeptide chains in the formation of the larger aggregates.

The enzyme pyruvate carboxylase from yeast was a tetramer with a rhombic appearance in the electron microscope.[809] Two elongated and two rounded subunits appeared to be present, and an unusual model for the molecule has been proposed in which (i) the protomers are similar or identical, (ii) the subunits are prolate ellipsoids, (iii) they are arranged with the centres located at the corners of a rhombus, and (iv) diagonally opposite pairs of subunits lie in orthogonal planes. Computer-generated images based on this model were similar in appearance to the material observed in electron micrographs. The enzyme from mammalian and

[803] I. Pinset-Härström and J. Truffy, *J. Mol. Biol.*, 1979, **134**, 173, 189.
[804] W. Schook, S. Puszkin, W. Bloom, C. Ores, and S. Kochwa, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 116.
[805] P. N. T. Unwin and G. Zampighi, *Nature (London)*, 1980, **283**, 545.
[806] A. Klarman, J. Gottlieb, and E. Daniel, *Biochemistry*, 1979, **18**, 2239.
[807] P. D. Jeffrey, *Biochemistry*, 1979, **18**, 2508.
[808] M. M. C. Bijtholt, E. F. J. van Bruggen, and J. Bonaventura, *Eur. J. Biochem.*, 1979, **95**, 399.
[809] N. D. Cohen, M. F. Utter, N. G. Wrigley, and A. N. Barrett, *Biochemistry*, 1979, **18**, 2197.

avian liver had a similar structure,[810] but that from *Pseudomonas citronellolis* had a quite distinct three-dimensional structure.[811]

Electron microscopy was used to investigate the structure of rat β-glucuronidase which may be crystallized as thin sheets.[812] High-dispersion electron-diffraction patterns were obtained and used to produce a filtered image by Fourier methods with the aid of computer image-processing methods. The enzyme is composed of four identical subunits of mol. wt. 75 000 dalton arranged tetrahedrally. Electron microscopy was also used to study the self-assembly of protein S of *Myxococcus xanthus*.[813] At certain stages of the life-cycle of this organism protein S (mol. wt. 23 000 dalton) is induced and accumulates in very large amounts. Purified protein S will self-assemble around protein S-deficient spores in the presence of Ca^{2+} ions, and it is suggested that the protein has a function in spore–spore interaction in the fruiting body.

Kinetic Studies.—*Protein–Protein Interactions.* The kinetics of formation of an asymmetric CO-haemoglobin hybrid, $(\alpha\beta)^A(\alpha\beta)^C$, from $\alpha_2\beta_2{}^A$ and $\alpha_2\beta_2{}^C$ were studied by electrophoresis at sub-zero temperatures (−30 to −40 °C).[814] The support medium was an acrylamide–methacrylate copolymer in a dimethyl sulphoxide–water mixture. At low temperatures the hybrid species were stable enough to be isolated. It was shown that the rate of hybrid formation was strongly influenced by pH, decreasing by an order of magnitude between pH = 7.3 and 8.3. At pH > 10 at − 40 °C, the hybrid was stable for several hours.

The interactions between the domains of the immunoglobulin chains are considered to be important for the structure and function of the immunoglobulin molecule. Information on such interactions has been obtained by studying the kinetics of dimerization of a Bence–Jones protein (= light chain) and of its variable fragment.[815] The close similarities between the reactions of the two proteins suggested that dimerization occurred chiefly through interactions between the variable regions and that the constant region contributed very little. The results also suggested that conformational changes were involved and a good fit to the data was obtained by making the assumption that both monomers and dimers could exist in two states. The rate constant for the association and dissociation reactions were of the order of $10^7 M^{-1} s^{-1}$ and $10^2 s^{-1}$, and the isomerization rate constants for the conformational changes were of the order of $10 s^{-1}$.

The pressure perturbation studies on glutamate dehydrogenase have already been mentioned.[782] Lactate dehydrogenase has also been investigated by subjecting it to pressures of between 1 and 2000 bar.[816] There was an initial pressure-induced dissociation of the native (tetrameric) enzyme into subunits followed by subunit aggregation to form inactive high molecular weight particles. Subunit exchange chromatography has been mentioned in a previous report.[817] Its potentialities

[810] N. H. Goss, P. Y. Dyer, D. B. Keech, and J. C. Wallace, *J. Biol. Chem.*, 1979, **254**, 1734.
[811] N. D. Cohen, J. A. Duc, H. Beegen, and M. F. Utter, *J. Biol. Chem.*, 1979, **254**, 9262.
[812] M. R. Dickson, M. Stewart, D. E. Hawley, and C. A. Marsh, *Biochem. J.*, 1979, **181**, 1.
[813] M. Inouye, S. Inouye, and D. R. Zusman, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 209.
[814] M. Perrella, M. Samaja, and L. Rossi-Bernardi, *J. Biol. Chem.*, 1979, **254**, 8748.
[815] H. Maeda, E. Steffen, and J. Engel, *Biophys. Chem.*, 1978, **9**, 57.
[816] G. Schmid, H.-D. Lüdemann, and R. Jaenicke, *Eur. J. Biochem.*, 1979, **97**, 407.
[817] E. J. Wood in 'Amino-acids, Peptides, and Proteins', ed. R. C. Sheppard (Specialist Periodical Reports), The Royal Society of Chemistry, London, 1976, Vol. 8, p. 239.

have been re-investigated with special reference to the self-association of α-chymotrypsin.[818]

Protein–Small Molecule Interactions. Nucleotide binding to myosin is important in understanding the mechanism of muscular contraction, and the binding to myosin subfragment 1 (S1) was measured by fluorescence stopped-flow using the rate of enhancement or quenching of intrinsic tryptophan fluorescence when either ATP or ADP bound.[819] The binding of the fluorescent analogues of these nucleotides, $1,N^6$-ethanoadenosine 5′-triphosphate or -diphosphates, was also studied. On the basis of the data obtained and computer simulations a mechanism was proposed:

$$S1 + X \overset{1}{\rightleftharpoons} S1 \cdot X \overset{2}{\rightleftharpoons} S1^{\dagger} \cdot X \overset{3}{\rightleftharpoons} S1^{*} \cdot X$$

where X represents the nucleotide. The slow step (3) corresponds to the relatively slow first-order transition with ATP or ADP, whereas step (2) is responsible for the rapid phase observed with analogous nucleotides.

It has long been known that holothurians possess dimeric haemoglobins. These animals are quite closely related to vertebrates, and because of the possible role of the dimer in the co-operativity of human haemoglobin there is considerable interest in studying co-operativity in naturally occurring dimeric haemoglobins. Steinmeier and Parkhurst have made kinetic and equilibrium studies on O_2 and CO binding by *Thyonella gemmata* haemoglobin,[820] which has a Hill coefficient, *n*, of *ca.* 1.4 for both ligands but no Bohr effect in the range pH = 7—9. The kinetics of oxygen dissociation were biphasic, and the dissociation rate constants for the haems in the two polypeptide chains (non-identical) differed by a factor of *ca.* 5. The kinetics of O_2 association following Hb·CO photolysis were unusual in that the rate of O_2 binding was less than first power in O_2. A model was proposed in which the binding of O_2 to the initial photoproduct was some 30- to 40-fold slower than that to a second form derived from the first by a slow conformational change ($t_{\frac{1}{2}} \approx 1$ ms).

Valency-hybrid haemoglobins represent simpler systems which allow kinetic analysis of the mechanism of co-operativity of vertebrate, tetrameric haemoglobins. HbM Saskatoon ($\alpha_2\beta_2^{+63Tyr}$) binding of O_2 and CO was investigated by the stopped-flow method.[821] It was concluded that at least three functional states seemed to participate in ligand binding.

Protein–Small Molecule Equilibria.—Klotz and Hunston have considered the conceptions and misconceptions existing in published treatments of data for multiple binding of ligands to proteins.[822] When data are represented graphically as mol ligand bound/mol protein, *r*, as a function of free ligand concentration, *A* (usually as log *A*), it is tempting to use the binding data to try to estimate the total number of accessible sites, *n*, on the protein. This corresponds to finding *r* at infinite free concentration of *A*, yet often a plateau in *r* at high *A* is not discernible. There may indeed be no evidence from the available data that saturation has been

[818] E. Antonini, G. Carrea, P. Cremonesi, P. Pasta, M. R. Rossi-Fanelli, and E. Chiancone, *Anal. Biochem.*, 1979, **95**, 89.
[819] F. Garland and H. C. Cheung, *Biochemistry*, 1979, **18**, 5281.
[820] R. C. Steinmeier and L. J. Parkhurst, *Biochemistry*, 1979, **18**, 4645, 4651.
[821] N. Makino, Y. Sugita, and T. Nakamura, *J. Biol. Chem.*, 1979, **254**, 10862.
[822] I. M. Klotz and D. L. Hunston, *Arch. Biochem. Biophys.*, 1979, **193**, 314.

approached. When the same data are transformed, for example to r/A *versus* r, perceptions are often bent to wish and intercepts are chosen on the r-axis which correspond with preconceptions of the value of n. In addition, when the transformation generates a concave curve it is often inferred that the protein has two different classes of binding site. On this presumption binding data have often been correlated quantitatively by means of an expression containing two hyperbolic terms, one for each class of independent site. The two binding parameters are supposed to be the respective-site binding constants. When the oligomeric protein is constructed from n identical subunits, it is unlikely that the binding sites are distributed between two different independent classes. Such concave curves frequently result from interactions between initially identical sites and under these circumstances assignments of site-binding constants are almost universally incorrect. Klotz and Hunston illustrate these principles by analysing binding data for several different proteins having 2—6 sites.

The work of Cann and his co-workers on isoelectric focusing of interacting systems has been extended in a further two papers.[823] When macromolecules interact with each other and with ligands the isoelectric behaviour may show transient patterns; however, it is shown that the isoelectric point of a given complex cannot be determined from the transient patterns. These workers present a theory for an isoelectric-focusing procedure which permits the determination of intrinsic ligand-building constants.

The allosteric properties of two types of arthropod haemocyanins have been investigated extensively. The 17S 'monomer' of *Callianassa californiensis* haemocyanin, which contains six polypeptide chains, was found to be the allosteric unit which behaves as a self-contained co-operative entity.[824] This 'monomer' can exist in equilibrium with a 39S 'tetramer' depending upon the influence of Mg^{2+}, H^+, and oxygen. The binding data were interpreted in terms of a theory integrating the allosteric equilibria and association equilibrium. *Panulirus interruptus* haemocyanin in contrast exists only as the six-polypeptide-chain 'monomer', and the linkage between various ligands (O_2, H^+, Ca^{2+}, and Na^+) has been investigated.[825] The binding of Ca^{2+} or Na^+ was associated with the release of protons and there was a difference of *ca.* ten-fold in apparent affinity between Ca^{2+} and Na^+. The two cations may compete for the same site. Measurement of oxygen equilibrium as a function of Ca^{2+}, Na^+, and H^+ concentrations revealed specific effects of these cations on the function of the protein.

As usual many papers on the binding of oxygen and other ligands by haemoglobin have appeared, including two reviews.[826, 827] Mills and Ackers have investigated oxygen binding by human haemoglobin.[828] They found that $\alpha^2\beta^1$ dimers (superscripts denote intersubunit contacts) had a significantly lower oxygen affinity than triliganded tetramers ($\alpha_2\beta_2(O_2)_3$). This suggests that in these tetramers

[823] J. R. Cann and K. J. Gardiner, *Biophys. Chem.*, 1979, **10**, 203, 211.
[824] F. Arisaka and K. E. van Holde, *J. Mol. Biol.*, 1979, **134**, 41.
[825] H. A. Kuiper, L. Forlani, E. Chiancone, E. Antonini, M. Brunori, and J. Wyman, *Biochemistry*, 1979, **18**, 5849.
[826] M. F. Perutz, *Annu. Rev. Biochem.*, 1979, **48**, 327.
[827] S. J. Gill in 'Studies in Modern Thermodynamics', ed. M. N. Jones, Elsevier, Amsterdam, 1979, Vol. 1, p. 224.
[828] F. C. Mills and G. K. Ackers, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 273.

the interactions at the $\alpha^1\beta^2$ intersubunit contacts are propagated to the unliganded haem in such a manner as to increase its affinity for oxygen. This is in contrast with the observed *reduction* of oxygen affinity arising from the same contracts when unliganded dimers are assembled to form unliganded tetramers ($\alpha_2\beta_2$). In a second study [829] Ackers reported a new thermodynamic analysis of the linkage between the binding of two ligands, *e.g.* oxygen and organic phosphate, to the tetramer. The theory relates observable properties of binding isotherms for one ligand to the total concentrations of the second ligand and of the macromolecule. Imai has also reported an extensive study of the co-operativity in four-step oxygenation equilibria of haemoglobin.[830] In the presence of 0.1 M-Cl^- and 2 mM-glycerate 2,3-bisphosphate the T to R transition at any oxygenation step was endothermic; haemoglobin gains entropy upon the transition and the deoxy-T structure was stabilized by the entropy term. Mills *et al.*[831] also studied ligand binding as well as subunit association of normal human haemoglobin, and Atha *et al.*[823] investigated the linkage between oxygenation and subunit association in the human haemoglobin variant, Hb Kansas, which has a substitution at the $\alpha_1\beta_2$ interface resulting in an unusually low oxygen affinity, a low Hill coefficient, and a high degree of subunit dissociation of the liganded form. Other haemoglobins with amino-acid substitutions in the $\alpha_1\beta_2$ contact region have also been studied.[833] Other areas of haemoglobin function that have been investigated are the effect of temperature on CO binding to a Root effect haemoglobin (that from *Brevoortia tyrannus*),[834] CO binding by trout haemoglobin I,[835] the interaction of butene with human haemoglobin,[836] and the NO and CO equilibria of horse myoglobin.[837]

There is disagreement on the relative affinities of the monomeric and dimeric forms of α-chymotrypsin for competitive inhibitors. Using a combination of sedimentation equilibrium, gel chromatography, and enzyme kinetic experiments, Tellam *et al.* have tried to throw some light on this problem by evaluating the binding constants for the interactions of *N*-acetyl-L-tryptophan with the monomeric and dimeric forms.[838] It was shown that both forms bound *N*-acetyl-L-tryptophan, with equilibrium constants 1300 M^{-1} (monomer) and 250 M^{-1} (dimer). α-Chymotrypsin was also studied by means of a fluorescent probe, 1-anilinonaphthalene-8-sulphonate.[839] The results showed that fluorescent enhancements occurring upon the interaction of fluorescent probes with proteins may not always result from binding to hydrophobic sites on the protein and the authors stress the need for caution in the interpretation of fluorescence changes when such probes interact with macromolecules. It was shown for human serum albumin that, although L-tryptophan and bilirubin bind to the same region of the protein,

[829] G. K. Ackers, *Biochemistry*, 1979, **18**, 3372.
[830] K. Imai, *J. Mol. Biol.*, 1979, **133**, 233.
[831] F. C. Mills, G. K. Ackers, H. T. Gaud, and S. J. Gill, *J. Biol. Chem.*, 1979, **254**, 2875.
[832] D. H. Atha, M. L. Johnson, and A. F. Riggs, *J. Biol. Chem.*, 1979, **254**, 12390.
[833] S. Matsukawa, M. Nishibu, M. Nagai, K. Mawatari, and Y. Yoneyama, *J. Biol. Chem.*, 1979, **254**, 2358.
[834] W. A. Saffran and Q. H. Gibson, *J. Biol. Chem.*, 1979, **254**, 1666.
[835] B. G. Barisas and S. J. Gill, *Biophys. Chem.*, 1979, **9**, 235.
[836] C. Poyart, E. Bursaux, S. Guesnon, and B. Bohn, *Eur. J. Biochem.*, 1979, **99**, 85.
[837] R. W. Romberg and R. J. Kassner, *Biochemistry*, 1979, **18**, 5387.
[838] R. Tellam, J. de Jersey, and D. J. Winzor, *Biochemistry*, 1979, **18**, 5316.
[839] J. D. Johnson, M. A. El-Bayoumi, L. D. Weber, and A. Tulinsky, *Biochemistry*, 1979, **18**, 1292, 1297.

possibly in a cavity in the protein, such a cavity is large enough to contain both ligands.[840]

Ovadi *et al.* have investigated the effect of association and dissociation on the catalytic properties of glyceraldehyde 3-phosphate dehydrogenase which is normally tetrameric but reversibly dissociates into dimers and monomers on dilution.[841] Enzyme activity depended non-linearly on protein concentration over the range 3×10^{-8}—3×10^{-6}M and it seemed likely that a rapidly equilibrating monomer–dimer–tetramer system existed.

Subunit Structure of Proteins.—*SDS–Gel Electrophoresis.* Zone electrophoresis in a polyacrylamide support in the presence of sodium dodecylsulphate (SDS) is the most widely used method of determining the molecular weights of subunits of oligomeric proteins. The technique is rapid and inexpensive and researchers have not been slow to exploit all the possible variations in order to make it more versatile. Many workers are finding that it is advantageous to use a gradient of polyacrylamide. The combination of molecular sieving and electrophoresis gives greatly improved resolution and simple techniques are available for pouring gradients without the use of any special equipment.[842] Gel concentration can have an effect on the electrophoresis even if no gradient exists, as micelles of SDS in equilibrium with 'monomer' SDS are themselves subject to sieving.[843] The amount of protein-bound SDS present may be calculated by determination of dodecanol by g.l.c. after hydrolysis of the sulphate.[844]

It is claimed that SDS may be removed from protein fractions isolated following a gel electrophoresis separation by ion-pair extraction.[845] Isolation of macromolecules from a gel is rendered easier if the polyacrylamide gel is made up using *NN'*-diallyltartardiamide as the cross-linker; the gel is readily solubilized by periodic acid but of course this has the disadvantage that it also denatures the proteins.[846] Gels containing SDS must be run at room temperature otherwise precipitation occurs. This is especially troublesome at low pH values; for example even 1% SDS forms a precipitate at pH = 2.4 and at 2—4 °C. This problem is overcome if Li^+ or Tris $[(CH_2OH)_3CNH_3^+]$ dodecyl sulphates are used instead of the sodium form.[847] For highly charged membrane-protein subunits exhibiting anomalous behaviour in the presence of SDS, it may be advantageous to try a cationic detergent such as CTAB (cetyltrimethylammonium bromide).[848]

A number of two-dimensional electrophoresis techniques have been used successfully, typically using isoelectric focusing in the first direction and SDS-electrophoresis in the second. Such techniques have resolved the subunits of cytochrome *c* oxidase[849] and NADPH–ubiquinone oxidoreductase (26 polypeptides).[850] Alternatively a proteolytic enzyme may be added after the first

[840] C. Jacobsen and J. Jacobsen, *Biochem. J.*, 1979, **181**, 251.
[841] J. Ovádi, J. Batke, F. Bartha, and T. Keleti, *Arch. Biochem. Biophys.*, 1979, **193**, 28.
[842] R. Ruchel and J. Gross, *Anal. Biochem.*, 1979, **92**, 91.
[843] K. Kubo, T. Isemura, and T. Tagaki, *Anal. Biochem.* 1979, **92**, 243.
[844] M. Saraste and T. K. Korhonen, *Anal. Biochem.*, 1979, **92**, 444.
[845] L. E. Henderson, S. Oroszlan, and W. Konigsberg, *Anal. Biochem.*, 1979, **93**, 153.
[846] P. J. Späth and H. Koblet, *Anal. Biochem.*, 1979, **93**, 275.
[847] R. Lichtner and H. U. Wolf, *Biochem. J.*, 1979, **181**, 759.
[848] M. H. Eley, P. C. Burns, C. C. Kannapell, and P. S. Campbell, *Anal. Biochem.*, 1979, **92**, 411.
[849] J. A. Freedman, R. P. Tracy, and S. H. P. Chan, *J. Biol. Chem.*, 1979, **254**, 4305.
[850] C. Heron, S. Smith, and C. J. Ragan, *Biochem. J.*, 1979, **181**, 435.

electrophoresis, and the peptides produced analysed by electrophoresis in the second direction.[851] A computer-method for the quantitative analysis of two-dimensional electrophoretograms has been reported.[852]

The combination of cross-linking of oligomeric proteins using bifunctional reagents with SDS–gel electrophoresis continues to be a valuable technique for studying the topology of subunits. The method has been used to study the pyruvate dehydrogenase complexes of *Azotobacter vinelandii* and *E. coli*.[853] A cross-linked hexamer of algal phosphofructokinase has been prepared,[854] and its shape and dimensions determined. Such studies are useful because, so far, it has not been possible to isolate the different aggregated states of this enzyme in sufficient quantities to investigate their size and shape by hydrodynamic methods. The known aggregation processes have hitherto made it difficult to resolve the structure of the protomer within the assembly.

Examples. The yeast multienzyme complex, fatty-acid synthetase (mol. wt. 2.3×10^6 dalton), was dissociated by acylation with dimethyl maleic anhydride to obtain only two subunits, α and β.[855] The dissociation was reversible following hydrolysis of the protein dicarboxylic acid monoamides. It was possible to show that the isolated β-subunit (mol. wt. 180 000 dalton) contained the following different partial enzymic activities involved in long-chain fatty-acid synthesis: malonyl, palmitoyl, and acetyl transferase, and enoyl reductase and dehydratase.

Pig-heart fumarase, which exists as a tetramer, has been dissociated reversibly into inactive monomeric subunits by incubation in a buffered medium containing dithiothreitol, edta, glycerol (10%), and 6 M-urea.[856] Upon dialysis at 4 °C against the same medium, but omitting the urea, an inactive dimer formed, and after incubation of this at 25 °C for 3 h an active tetramer was formed. It was concluded that a small conformational change was associated with the conversion of dimer to tetramer, producing proper orientation of the active-site residues.

The molecular weights and molar ratio of the component enzymes of the *E. coli* pyruvate dehydrogenase multienzyme complex were determined by measuring the position and intensity of Coomassie Blue staining of the bands observed in SDS–gel electrophoresis.[857] The results were consistent with a polypeptide chain ratio of 24 : 24 : 12 (pyruvate decarboxylate : dihydrolipoyl transacetylase : dihydrolipoyl dehydrogenase) and a molecular weight of 4.8×10^6 dalton for the intact complex.

A study of human serum apolipoprotein B in guanidinium chloride and SDS solutions has confirmed the previously reported monomer molecular weight of 250 000 dalton and demonstrated that the dimeric state of the polypeptide chain, which occurs *in vivo*, is maintained in micellar detergent solution.[858]

[851] C. Bordier and A. Creltol-Järvinen, *J. Biol. Chem.*, 1979, **254**, 2565.
[852] J. Bossinger, M. J. Miller, K.-P. Vo, E. P. Geiduschek, and N.-H. Xuong, *J. Biol. Chem.*, 1979, **254**, 7986.
[853] R. A. de Abreu, J. de Vries, A. de Kok, and C. Veeger, *Eur. J. Biochem.*, 1978, **97**, 379.
[854] H. H. Paradies and W. Vettermann, *Arch. Biochem. Biophys.*, 1979, **194**, 88.
[855] F. Wieland, L. Renner, C. Verfürth, and F. Lynen, *Eur. J. Biochem.*, 1979, **94**, 189.
[856] S. Yamato and T. Murachi, *Eur. J. Biochem.*, 1979, **93**, 189.
[857] H. J. Angelides, S. K. Akiyama, and G. G. Hammes, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3279.
[858] J. C. H. Steele and J. A. Reynolds, *J. Biol. Chem.*, 1979, **254**, 1639.

Examples of Associating–Dissociating Systems.—*Sickle-cell Haemoglobins (HbS).* Very many studies of HbS have appeared in the last year and much of the emphasis has been on the way in which macrofibres are formed with the aim of understanding the pathological manifestations of sickle-cell disease, including the distortions of the red blood cells.[859] When solutions of chromatographically pure deoxy-HbS are stirred for a number of hours, the fibres align to produce well defined fascicles. Later long needles and twisted ribbons develop. Finally all forms are replaced by crystals which, according to electron microscopic and low-angle *X*-ray scattering data, have cell parameters indistinguishable from those of crystals grown in polyethyleneglycol at pH = 5—6.[860] Double filaments of molecules with an axial repeat of 64 Å were found to comprise the basic unit of both crystal and fibre structures.[861] It is proposed that the two filaments are held together through contacts between Val-6β of the molecules of one strand with hydrophobic side chains of the molecule in the neighbouring strand. These residues may be mapped by studying the inhibitory effects of other haemoglobins (*e.g.* HbA, HbF) on the polymerization of HbS. Thus the replacement of one of the β^S-chains of HbS by a γ (from HbF) appeared to inhibit polymerization through the formation of hybrids of the type $\alpha_2\beta^S\gamma$, and residues γ80 and γ87 were partly responsible for this inhibition.[862] Following further studies of binary mixtures of HbS and HbA_2 it was concluded that residues 22, 80, and 87 of the β-chain were implicated as intermolecular contact-sites stabilizing the deoxy-HbS polymer. The same residues seemed to be the same ones that stabilize the basic unit of the deoxy-crystal.[863] Sunshine *et al.*, however, concluded that the difference between polymerization properties of mixtures of deoxy-HbS with deoxy-HbA and -F could be attributed to the copolymerization of the $\alpha_2\beta^A\beta^S$ hybrid and the absence of any significant copolymerization of the $\alpha_2\gamma\beta^S$ hybrid.[864] These workers considered that the analysis used in a similar study by Cheetham *et al.*[865] had apparent empirical utility but was thermodynamically unsound.

Adachi and Asakura measured the solubilities of oxy- and deoxy-haemoglobins in concentrated phosphate buffers by a turbidimetric method[866] and studied aggregation by low-angle laser light-scattering.[867] Of the haemoglobins studied (S, C, C_{Harlem}, F, A), deoxy-HbS had the lowest solubility, and a 1 : 1 mixture of deoxy-HbA and deoxy-HbS aggregated in 2.42 M-phosphate buffer in which pure deoxy-HbA was totally soluble. However, they later reported that at 30 °C deoxy-HbA aggregated or formed gels in 1.5 M-phosphate buffer after a delay time; the aggregates or gels could be totally re-liquefied by cooling.[868] This suggested that the hydrophobic substitution (Val) at β6 in HbS was not essential for gelation under the

[859] F. K. Friedman and S. Beychok, *Annu. Rev. Biochem.*, 1979, **48**, 217.
[860] T. E. Wellems and R. Josephs, *J. Mol. Biol.*, 1979, **135**, 651.
[861] B. Magdoff-Fairchild and C. C. Chiu, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 223.
[862] R. L. Nagel, R. M. Bookchin, J. Johnson, D. Labie, H. Wajcman, W. A. Isaac-Sodeye, G. R. Honig, G. Schiliro, J. H. Crookston, and K. Matsutomo, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 670.
[863] R. L. Nagel, J. Johnson, R. M. Bookchin, M. C. Garel, J. Rosa, G. Schiliro, H. Wajcman, D. Labie, W. Moo-Penn, and D. Castro, *Nature (London)*, 1980, **283**, 832.
[864] H. R. Sunshine, J. Hofrichter, and W. A. Eaton, *J. Mol. Biol.*, 1979, **133**, 435.
[865] R. C. Cheetham, E. R. Huehns, and M. A. Rosemeyer, *J. Mol. Biol.*, 1979, **129**, 45.
[866] K. Adachi and T. Asakura, *J. Biol. Chem.*, 1979, **254**, 4079.
[867] K. Adachi, T. Asakura, and M. L. McConnell, *Biochim. Biophys. Acta*, 1979, **580**, 405.
[868] K. Adachi and T. Asakura, *J. Biol. Chem.*, 1979, **254**, 12273.

appropriate conditions. There are haemoglobin mutants which have substitutions in the α-chain in addition to the sickling mutation at β6. Substitutions of His for Asp at α47 resulted in an inhibition of polymerization, but by contrast the introduction of a Tyr at α75 and an Ala at α6 had the opposite effect.[869] These latter are the first reported examples of α-chain mutations which potentiate the gelation of HbS.

Studies on the nucleation process in HbS aggregation in concentrated phosphate buffer suggested that the size of the nucleus under these conditions was much smaller than previously reported and varied with the phosphate concentration.[870] The gelation of HbS in the presence of other proteins [871] and the association of the rod-like aggregates [872] have been tackled at a theoretical level. Using an embedding technique involving the use of tannic acid, which has been used to study microtubules, it was hoped to improve the resolution of HbS fibre structure in electron microscopy of thin sections.[873] By generating computer models it was possible to show that the distinctive fibre cross-sectional patterns in thin sections corresponded closely to the results expected for the 14-strand structure of the fibres.[874] Studies with CO-HbS showed that the polymerization reaction was nearly specific for deoxy-HbS.[875]

Aspirin and its derivatives are known to acetylate haemoglobin and are potential antisickling agents because they shift the conformational equilibrium towards the oxy-form. Such agents must be pharmacologically acceptable before clinical trials can be started. Recently, two double-headed aspirin derivatives, bis-(3,5-dibromosalicyl) succinate and bis-(3,5-dibromosalicyl) fumarate, have been found to be potent acylating agents of intracellular HbS *in vitro* and to reduce the gelation tendency.[876] These reagents cross-link the β-chains, probably at the β-cleft. Lyotropic salts can also affect the solubility of deoxy HbS and a number of these have been investigated.[877] Such salts exert specific effects determined by the nature of the ion(s) involved; cations and anions acted in an approximately additive fashion.

Microtubules. The assembly of the protein tubulin to form microtubules is, like that of HbS, an area of intense investigative activity, and the whole range of physical techniques is being employed to study this process. Johnson and Borisy have studied the equilibrium and kinetics of tubulin assembly at different temperatures,[878] and heat-capacity microcalorimetry measurements were made by Hinz *et al.*[879] The depolymerization kinetics of microtubules were studied by rapid dilution or drug addition,[880] and the effects of polyethyleneglycol in promoting

[869] R. E. Bennesch, S. Kwong, R. Edalji, and R. Bennesch, *J. Biol. Chem.*, 1979, **254**, 8169.
[870] K. Adachi and T. Asakura, *J. Biol. Chem.*, 1979, **254**, 7765.
[871] M. J. Behe and S. W. Englander, *J. Mol. Biol.*, 1979, **133**, 137.
[872] R. W. Briehl and J. Herzfeld, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2740.
[873] R. L. Garrell, R. H. Crepeau, and S. J. Edelstein, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1140.
[874] G. Dykes, R. H. Crepeau, and S. J. Edelstein, *Nature (London)*, 1978, **272**, 506.
[875] J. Hofrichter, *J. Mol. Biol.*, 1979, **128**, 335.
[876] J. A. Walder, R. H. Zaugg, R. Y. Walder, J. M. Steele, and I. M. Klotz, *Biochemistry*, 1979, **18**, 4265.
[877] W. N. Poilleu and J. F. Bertles, *J. Biol. Chem.*, 1979, **254**, 3462.
[878] K. A. Johnson and G. G. Borisy, *J. Mol. Biol.*, 1979, **133**, 199.
[879] H.-J. Hinz, M. J. Gorbunoff, B. Price, and S. N. Timasheff, *Biochemistry*, 1979, **18**, 3084.
[880] T. L. Karr and D. L. Purich, *J. Biol. Chem.*, 1979, **254**, 10885.

reconstitution were investigated and analysed by the linked-function theory of Wyman.[881]

In contrast to these comparatively simple *in vitro* systems, the situation *in vivo* is extremely complex, involving the so-called microtubule associated proteins, certain other proteins, and nucleotides. Thus recent data have implicated a dynein-like ATPase in microtubule-dependent cell movements. It was possible to show that dynein isolated from the flagellae of the alga *Chlamydomonas* bound *in vitro* to assembled brain microtubules and decorated them with periodic and angular projections.[882] Dynein appeared to induce ATP-dissociable cross-bridging between adjacent microtubules. The so-called microtubule-organizing centres have also been studied. Those from the flagellate, *Polytomella,* were demonstrated to function in microtubule initiation but not elongation.[883] Four polypeptides of mol. wt. 190 000—210 000 dalton having these initiation properties were extracted from the microtubule-organizing centres of *Polytomella.*

It has been shown that protein τ and the high molecular weight microtubule-associated proteins from chicken brain and from mouse fibroblasts were similar to the corresponding proteins from other sources, implying that both are widely distributed.[884] An improved method for the isolation of microtubule-associated proteins using Mg^{2+}-saturated phosphocellulose has been reported,[885] and a new protocol for the purification of tubulin itself has been developed.[886]

In addition to microtubules and microfilaments a third fibrous organelle from cytoplasm is now recognized. This is the '10 nm filament' or intermediate filament. Runge *et al.*[887] have reported that the major 68 000 dalton protein in cycled microtubule preparations, which may correspond to the tubulin assembly protein described by Lockwood,[888] is a constituent of neurofilaments which are 10 nm filaments. The tubulin assembly-stimulating activity of the protein was not observed unless it was released from the 10 nm filaments by heat treatment. Brain 10 nm filaments were isolated by a new procedure and it was believed that the 10 nm filaments that are formed in the early cycles of the tubulin-purification protocol were probably composed of neurofilament polypeptides.[889]

Inhibitors of microtubule formation have been studied very extensively and have been classified into two categories: (i) those inhibiting the polymerization-dependent GTPase activity of phosphocellulose-purified tubulin but inducing a significant polymerization-independent GTPase activity (examples include colchicine and griseofulvin) and (ii) those inhibiting GTPase activity associated with tubulin polymerization as well as that induced by inhibitors of the first class (examples include podophyllotoxin and the vinca-alkaloids).[890] Microtubule assembly is very efficiently inhibited by colchicine or podophyllotoxin at concentrations

[881] J. C. Lee and L. L. Y. Lee, *Biochemistry,* 1979, **18**, 5518.
[882] L. T. Haimo, B. R. Telzer, and J. L. Rosenbaum, *Proc. Natl. Acad., Sci. USA,* 1979, **76**, 5759.
[883] M. E. Stearns and D. L. Brown, *Proc. Natl. Acad. Sci. USA,* 1979, **76**, 5745.
[884] D. W. Cleveland, B. M. Spiegelman, and M. W. Kirschner, *J. Biol. Chem.,* 1979, **254**, 12670.
[885] R. C. Williams and H. W. Detrich, *Biochemistry,* 1979, **18**, 2499.
[886] T. L. Karr, H. D. White, and D. L. Purich, *J. Biol. Chem.,* 1979, **254**, 6107.
[887] M. S. Runge, H. W. Detrich, and R. C. Williams, *Biochemistry,* 1979, **18**, 1689.
[888] A. H. Lockwood, *Cell,* 1978, **13**, 613.
[889] R. Thorpe, A. Delacourte, M. Ayers, C. Bullock, and B. H. Anderton, *Biochem. J.,* 1978, **181**, 275.
[890] T. David-Pfeuty, C. Simon, and D. Pantaloni, *J. Biol. Chem.,* 1979, **254**, 11696.

substoicheiometric to the concentration of free tubulin, and such 'microtubule poisoning' has been explained as being due to binding of a tubulin-drug complex at the assembly end of the microtubule leaving the disassembly end free to depolymerize. However, the observations of Sternlicht and Ruigel were not consistent with this mechanism.[891] Under conditions where spontaneous nucleation was suppressed and assembly was restricted to an elongation polymerization, the microtubules formed were colchicine-tubulin dimer complex ('CD')–tubulin copolymers with the incorporated CD randomly distributed in the copolymer. The CD–tubulin copolymers possessed assembly-competent ends having association and dissociation rate constants which decreased as the CD : tubulin ratio in the copolymer increased, and the critical tubulin concentration required for microtubule assembly increased in the presence of CD. This suggested that the affinity of the copolymer decreased as the CD : tubulin ratio increased, and the critical tubulin concentration required for microtubule assembly increased in the presence of CD.

GTP-dependent polymerization of rat brain tubulin proteins was inhibited to the extent of 50% by 0.12 mol colchicine mol^{-1} tubulin and 0.14 mol podophyllotoxin mol^{-1} tubulin.[892] However, substitution of guanylyl-5′-methylene diphosphonate for GTP resulted in microtubule protein polymerization at such concentrations. This compound induced the formation of ribbon structures with three or four protofilaments. The analogue, 8-azidoguanosine 5′-triphosphate was used as a photoaffinity probe to examine the role of GTP in microtubule assembly.[893] Photolysis of this compound in the presence of microtubule protein resulted in the labelling of a GTP-specific site on the β-monomer. Experiments with ^{32}P-labelled analogues suggested that hydrolysis occurred concurrently with polymerization and that nucleoside diphosphate remained bound to the assembled microtubules. A new method was used to determine the dissociation constants for GDP binding to tubulin which was based on a quantitative analysis of ligand during exclusion chromatography.[894] The value obtained, 5.5×10^{-3} M, was in good agreement with that obtained independently by the Hummel and Dryer method. GTP bound at the same site (the E-site) some 2.8-fold more tightly.

10 Fluorescence

Contributed by R. Devonshire

The following review is intended to be representative of the literature during 1978 and 1979. It is restricted to studies of isolated and purified components and as in previous reports in this series the coverage of fluorescence studies in the areas of immunology and membranes is minimal.

Review Articles.—An extensive review (574 references) has been published of the intrinsic luminescence of proteins and the application of such studies to the investigation of protein structure.[895] A further review of protein luminescence, covering the period June 1977 to June 1978, emphasizes the importance of studies of

[891] H. Sternlicht and I. Ringel, *J. Biol. Chem.*, 1979, **254**, 10540.
[892] I. V. Sandoval and K. Weber, *J. Mol. Biol.*, 1979, **134**, 159.
[893] R. L. Geahleu and B. E. Haley, *J. Biol. Chem.*, 1979, **254**, 11982.
[894] B. Zeeberg and M. Caplow, *Biochemistry*, 1979, **18**, 3380.
[895] E. A. Burshtein, *Biofizika*, 1977, **6**, 1.

the isolated chromophores.[896] Recent work on the kinetics, thermodynamics, geometry, and solvent effects in exciplex systems has been discussed with particular emphasis on indole exciplexes and their relevance to fluorescence and phosphorescence studies of proteins.[897] A valuable and interesting review of bioluminescence discusses these phenomena in the framework of three classes based on different mechanisms of physiological control[898] and follows an earlier review by the authors of the previous year's published work in this area.[899] The measurement of calcium ion concentrations with bioluminescent photoproteins has been reviewed[900] and the recent literature (mainly 1977) concerned with the excited states of biomolecules has been surveyed.[901] The application of fluoresence energy transfer to measurements of inter-chromophore distances in proteins has been reviewed[902] and the measurement of large-scale rotational motions of proteins using fluorescence probes and spin labels has been discussed with emphasis on the recently developed e.s.r. technique of saturation transfer spectroscopy.[903] The mapping of the three-dimensional structure of proteins using the photochemical techniques of photoaffinity labelling and u.v. sensitization has been reviewed.[904] The origins and interpretation of circularly polarized luminescence (CPL) are discussed in a paper which includes a valuable section on suitable instrumentation and its calibration,[905] and the biochemical and biophysical applications of CPL have been reviewed in another article by the same author.[906] A study of tRNA is used to illustrate a discussion of the theory of fluorescence-detected circular dichroism.[907] The study of the phosphorescence of aromatic amino-acids and proteins has been described[908] and a brief review of the application of low-temperature luminescence to the study of protein interactions has appeared.[909] A general discussion of the interpretation of data on the solute quenching of protein fluorescence is contained in a paper which examines the iodide quenching of lysozyme fluorescence.[910] Studies of protein fluorescence are to be found in a general review of[911] and in a collection of review articles on[912] the applications of synchrotron radiation.[912] The chemistry of the excited states of aromatic amino-acids and peptides has been briefly reviewed.[913]

Instrumentation and Techniques.—A number of new instruments for measuring fluorescence lifetimes and fluorescence anisotropy decay have been described in the

[896] W. C. Galley and J. G. Milton, *Photochem. Photobiol.*, 1979, **29**, 179.
[897] R. Lumry and M. Hershberger, *Photochem. Photobiol.*, 1978, **27**, 819.
[898] J. P. Henry and A. M. Michelson, *Photochem. Photobiol.*, 1978, **28**, 292.
[899] J. P. Henry and A. M. Michelson, *Photochem. Photobiol.*, 1978, **27**, 855.
[900] J. R. Blinks, *Ann. N.Y. Acad. Sci.*, 1978, **307**, 71.
[901] T. Y. Lee and R. D. Fugate, *Photochem. Photobiol.*, 1978, **27**, 803.
[902] L. Stryer, *Annu. Rev. Biochem.*, 1978, **47**, 819.
[903] D. D. Thomas, *Biophys. J.*, 1978, **24**, 439.
[904] G. Jori and J. D. Spikes, *Photochem. Photobiol. Rev.*, 1978, **3**, 193.
[905] I. Z. Steinberg, *Methods Enzymol.*, 1978, **49**, 179.
[906] I. Z. Steinberg, *Annu. Rev. Biophys. Bioeng.*, 1978, **7**, 113.
[907] D. H. Turner, *Methods Enzymol.*, 1978, **48**, 199.
[908] M. Sokolovsky and E. Daniel, *Methods Enzymol.*, 1978, **49**, 236.
[909] C. Helene, T. Montenay-Garestier, and M. Monsigny, *J. Luminescence*, 1979, **18/19**, 577.
[910] S. S. Lehrer and P. C. Leavis, *Methods Enzymol.*, 1978, **48**, 222.
[911] J. H. Monro, *Chem. Br.*, 1979, **15**, 330.
[912] Proceedings of the Daresbury Study Weekend, 27—28 January, 1979, ed. R. B. Cundall and I. H. Munro, Daresbury SRC Laboratory Report DL/SCI/R13.
[913] E. Hayon, *Jerusalem Symp. Quantum Chem. Biochem.*, 1977, **10**, 113.

literature. The techniques employed in these instruments can usually be categorized by identifying their mode of operation as being in either the time or frequency domains. These categories, however, are directly related by a process of Fourier transformation and this equivalence is highlighted by a number of recent hybrid techniques which are also mentioned below.

The lifetime resolution in time-resolved studies is limited by transit time jitter in the detectors and inaccuracies in the electronics. Excitation light sources with excellent characteristics (in particular their high repetition rates and constant profiles) are now available, albeit at considerable expense. A recent subnanosecond-resolution single-photon counting instrument uses a synchronously pumped tunable dye laser for excitation.[914] The dye laser has an intrinsic pulse duration of 35 ps and this is clearly not the limiting factor in the instrument response function of 350 ps measured using time-correlated single-photon counting. A similar instrument was used in a study of *in vivo* chlorophyll fluorescence where lifetimes down to 110 ps were measured.[915] This investigation highlights another important characteristic of these sources, their low peak intensity relative to giant pulse lasers. Previous studies of chloroplasts using single-shot lasers with streak-camera detection gave much shorter lifetimes due to exciton annihilation effects. A study of flavin fluorescence has been published in which a mode-locked Ar^+ laser was used to excite directly the chromophore,[916] and tunable, highly stable subnanosecond u.v. pulses can be produced by a continuous-wave Ar^+ laser-pumped dye-laser system which can be operated in three different timing configurations: mode-locked, cavity-dumped, and a combination of these two.[917] The system can measure exponential decays as short as 50 ps. A careful study of the optimization of data acquisition rates in time-correlated single-photon fluorimetry examined the performance of four different operating configurations of a time-to-amplitude converter.[918] An inverted configuration with an interactive source was found to be the optimum. The long data-accumulation times necessary with some photon-counting systems is a serious restraint in the investigation of biological preparations. A recent paper describes a correction procedure which enables a high count rate to be used in the measurement of fluorescence anisotropy decays, thereby reducing the accumulation times considerably.[919] The measurement of fluorescence anisotropy decays with up to 100 ps resolution using a N_2 laser-pumped dye laser for excitation and a Boxcar integrator in the detection system has been described.[920]

An interesting fluorescence lifetime apparatus has been described which uses a mode-locked Ar^+ laser to excite a sample at the full laser repetition frequency of 114.8 MHz.[921] The high intensity of the source allows two-fold discrimination in the stop channel of the photon-counting detection system which gives excellent time

[914] V. J. Koester and R. M. Downben, *Rev. Sci. Instrum.*, 1978, **49**, 1186.
[915] G. S. Beddard, G. R. Fleming, G. Porter, G. F. W. Searle, and J. A. Synowiec, *Biochem. Biophys. Acta*, 1979, **545**, 165.
[916] A. J. W. G. Visser and A. Van Hoek, *J. Biochem. Biophys. Methods*, 1979, **1**, 195.
[917] K. G. Spears, L. E. Cramer, and L. D. Holland, *Rev. Sci. Instrum.*, 1978, **49**, 255.
[918] G. R. Haugen, B. W. Wallin, and F. E. Lytle, *Rev. Sci. Instrum.*, 1979, **50**, 64.
[919] T. Kouyama, *Jpn. J. Appl. Phys.*, 1978, **17**, 1409.
[920] M. Scholz, K. Teuchner, M. Naether, W. Becker, and S. Daehne, *Acta Phys. Pol. A*, 1978, **54**, 823.
[921] U. P. Wild, A. R. Holzwarth, and H. P. Good, *Rev. Sci. Instrum.*, 1977, **48**, 1621.

resolution. The high repetition rate establishes a periodic fluorescence signal which is deconvoluted with the apparatus function in Fourier space to give the Fourier transform of the fluorescence decay.

Variable-frequency phase fluorometry is a powerful technique for measuring fluorescence lifetimes and anisotropy decays. It requires much lower excitation intensities, can use radiation of much lower spectral width, and has simpler optics compared with pulsed-excitation time-resolved techniques. Details of such an instrument have been published and these show a tuning range from 10—500 MHz giving a time resolution of 1 ps.[922] The design and performance of a differential polarization phase fluorimeter [923] and of an interesting phase fluorimeter which uses optical methods for phase manipulation [924] have been described. In the latter technique a CW laser beam modulated at 170 MHz is split to give a reference and an excitation beam. The reference (with variable optical delay) and fluorescence beams are combined at a photocathode and a null point detected with an AM radio or a spectrum analyser. 4 ps resolution is claimed for the instrument. Subpicosecond fluorescence-lifetime measurements are discussed in a paper which points out the high harmonic content of high-repetition-rate short-duration optical pulses as found in synchrotron radiation and the output of mode-locked lasers.[925]

If the longitudinal modes of a laser are not phase-locked to produce a short duration pulse then the laser output acts as a randomly modulated source of excitation. If this excitation is cross-correlated in time with the sample emission a time decay is generated which is equivalent to that in a pulsed-excitation experiment. An instrument based on this principle has been constructed using a CW laser and an inexpensive detection system and was used to obtain a value of 0.66 ± 0.04 ns for the fluorescence lifetime of Rose Bengal in ethanol.[926]

An ingenious instrument has been constructed to measure small shifts in fluorescence emission spectra.[927] It is capable of measuring spectral shifts as small as 0.2 nm and clearly has many potential applications. Details of fluorescence polarization spectrometers which can give simultaneous measurements of orientation and mobility in uniaxial media,[928] use a T format and photon counting to give precise measurements at low signal-to-noise ratios,[929] and use a rotating polaroid sheet to give continuous measurements of the degree of polarization [930] have all been published.

There is a growing interest in chemi- and bio-luminescence and recently a number of timely review articles have appeared concerned with details of the methodology and instrumentation which have been developed for studies of these phenomena.[931-933]

[922] H.-P. Haar and M. Hauser, *Rev. Sci. Instrum.*, 1978, **49**, 632.
[923] W. W. Mantulin and G. Weber, *J. Chem. Phys.*, 1977, **66**, 4092.
[924] E. R. Menzel and Z. D. Popovic, *Rev. Sci. Instrum.*, 1978, **49**, 39.
[925] E. Gratton and R. Lopez Delgado, *Rev. Sci. Instrum.*, 1979, **50**, 789.
[926] C. C. Dorsey, M. J. Pelletier, and J. M. Harris, *Rev. Sci. Instrum.*, 1979, **50**, 333.
[927] L. D. Burtnick and I. Z. Steinberg, *Anal. Biochem.*, 1978, **90**, 488.
[928] (*a*) J. P. Jarry, P. Sergot, C. Pambrun, and L. Monnerie, *J. Phys. E*, 1978, **11**, 702; (*b*) J. P. Jarry and L. Monnerie, *J. Polym. Sci., Polym. Phys. Edn.*, 1978, **16**, 443.
[929] D. M. Jameson, G. Weber, R. D. Spencer, and G. Mitchell, *Rev. Sci. Instrum.*, 1978, **49**, 510.
[930] J. C. Smith, N. Graham, and B. Chance, *Rev. Sci. Instrum.*, 1978, **49**, 1491.
[931] J. E. Wampler, 'Bioluminescence Action', ed. P. J. Herring, Academic Press, London, 1978, pp. 1—48, 477—549.
[932] D. G. Bullock, R. A. Bunce, and T. J. N. Carter, *Anal. Lett.*, 1979, **12**, 841.

Data Treatment.—A particularly valuable paper has appeared which critically compares the available numerical methods for the deconvolution of photoluminescence data for their ease of use, accuracy of results, and resilience to noisy data.[934] The results were fairly clear. The iterative-convolution technique was the only one to cope well with the test situations, the most difficult of which is a double exponential decay excited by a lamp whose decay has a long tail. Two methods based on Fourier series performed poorly. A more recent paper has reached similar conclusions.[935] The method of deconvolution which uses modulating functions performed very well in the tests in the above papers.[936] The analysis of three-component fluorescence decays is discussed in the context of a model based on linear systems theory.[937]

The statistical accuracy of rotational correlation times determined by time-resolved fluorescence anisotropy decay has been investigated.[938] As might be expected the theory establishes the value of using a chromophore with a high intrinsic anisotropy and whose fluorescence lifetime, τ_F, is similar to the correlation time to be measured. For the case of $\tau = 20$ ns, correlation times ranging from 2 ns to 3 μs can be measured with an accuracy of 10%. The upper limit corresponds to a globular protein with molecular weight $>10^6$ dalton. In a brief review of time-resolved fluorescence depolarization the author points out that there is no deconvolution procedure, as yet, which gives the direct recovery of emission anisotropy decay functions.[939]

A very interesting paper has critically examined, experimentally, the basis for the measurement of microviscosities in biological systems, using fluorescence polarization.[940] Using three probes in all, they were able to show that, in different oils of the same macroscopic viscosity, widely different microviscosities were reported by the probes, presumably as a result of differences in the anisotropy of solute–solvent interactions. A non-linear least-squares minimization procedure has been described for use in the interpretation of fluorescence-quenching data in terms of binding affinity constants.[941]

Corrections to fluorescence-polarization measurements, necessitated by the use of finite apertures in fluorimeters, have been calculated,[942] and it has been shown that the use of total reflectance fluorescence instead of front-face fluorescence produces a substantial reduction in the inner-filter effect which arises from the re-absorption of emitted radiation.[943] Two graphical procedures for the correction of inner-filter effects in fluorescence titrations have been published.[944]

[933] J. M. Anderson, G. J. Faini, and J. E. Wampler, *Methods Enzymol.*, 1978, **57**, 529; N. Langerman, *ibid.*, p. 540; G. L. Picciolo, J. W. Denning, D. A. Nibley, and E. W. Chapelle, *ibid.*, p. 550; H. H. Seliger, *ibid.*, p. 560.

[934] A. E. McKinnon, A. G. Szabo, and D. R. Miller, *J. Phys. Chem.*, 1977, **81**, 1564.

[935] D. V. O'Connor, W. R. Ware, and J. C. Andre, *J. Phys. Chem.*, 1979, **83**, 1333.

[936] B. Valeur, *Chem. Phys.*, 1978, **30**, 85.

[937] J. Eisenfeld and C. C. Ford, *Biophys. J.*, 1979, **26**, 73.

[938] Ph. Wahl, *Chem. Phys.*, 1977, **22**, 245.

[939] R. E. Dale, *J. Luminescence*, 1979, **18/19**, 120.

[940] F. Hare and C. Lussan, *Biochem. Biophys. Acta*, 1977, **467**, 262.

[941] M. T. Flanagen, F. G. Tattam, and N. M. Green, *Immunochemistry*, 1978, **15**, 261.

[942] P. E. Zinsli, *J. Phys. E.*, 1978, **11**, 17.

[943] T. Hirschfeld, *Spectrochim. Acta*, 1978, **A34**, 693.

[944] R. A. Leese and E. L. Wehry, *Anal. Chem.*, 1978, **50**, 1193; M. L. Mertens and J. H. R. Kaegi, *Anal. Biochem.*, 1979, **96**, 448.

Theories of Fluorescence Phenomena.—*Fluorescence Quenching.* The addition of a quencher to a fluorescent system in solution can either increase or decrease the temperature dependence of the fluorescence quantum yield, ϕ_F. This arises because of the different temperature dependence of the two principle contributory factors: non-radiative processes within the fluorophore and the diffusion of the quencher and fluorophore in the liquid. This has been investigated for model systems of protein residues (indole and phenol in water and dioxan) and found to give a consistent picture of the effect and its origins.[945] A new model for the interpretation of fluorescence quenching experiments has been published. It avoids an assumption about ground-state complex formation previously invoked to explain observed differences between the results of time-resolved and steady-state quenching experiments when one is calculated from the other, by including a 'static' quenching term for quencher molecules lying between a collision distance and a 'kinetic' distance (the latter calculated from time-resolved studies of the excited fluorophore).[946] Theoretical expressions for the quenching of fluorescence in time-resolved and steady-state experiments have been obtained using the exact solution of the Smoluchowski equation with a Coulomb potential.[947]

Singlet Energy Transfer. There have been a number of significant papers dealing with the problem of determining the distribution of end-to-end distances in flexible molecules from measurements of the efficiency of energy transfer between donor, D, and acceptor, A, molecules attached to the chain ends. The assumption that distances between D and A are not correlated with the relative orientations of D and A was tested with Monte-Carlo type calculations of the chains Tyr-$(Ala)_n$-Tyr ($n = 4$ or 9) and found to be adequate even for the shorter chain when the conformations are dominated by entropic factors.[948] This results in a considerable simplification in the integral expression for the luminescence decay and permits a calculation of the orientation factor term from an analytical expression. Similar conclusions were reached from calculations which include long-range interactions,[949] the above being for the non-interacting case. The authors have applied the methods in the two papers to a study of the fluorescence of natural polypeptide chains.[950] An important paper to be read in conjunction with those above considers the case for transfer involving transitions of mixed polarization characterized by two or three transition moments.[951] It is shown that, for cases where the orientations of chromophores are fixed but unknown, the error in the D to A distances is smaller when the fluorescence polarizations of D and A are reduced and is unlikely to exceed $\pm 10\%$ for polarizations <0.3. Criticisms of earlier experimental work on the basis of restrictions in the orientational freedom of the luminophores were shown not to be valid. In contrast to examples of the dynamic averaging of relative orientations, it is possible to conceive of a situation where there exists a continuous stationary statistical orientational distribution of D, A

[945] G. Laustriat and D. Gerard, *J. Phys. Chem.*, 1978, **82**, 746.
[946] J. C. Andre, M. Niclause, and W. R. Ware, *Chem. Phys.*, 1978, **28**, 371.
[947] K. M. Hong and J. Noolandi, *J. Chem. Phys.*, 1978, **68**, 5172.
[948] A. Englert and M. Lederc, *Proc. Nat. Acad. Sci. USA*, 1978, **75**, 1050.
[949] M. Leclerc, S. Premilat, and A. Englert, *Biopolymers*, 1978, **17**, 2459.
[950] M. Leclerc, S. Premilat, and A. Englert in 'Peptides: Proceedings of the Fifth American Peptide Symposium', ed. M. Goodman and J. Meienhofer, Wiley, New York, 1977, p. 364.
[951] E. Haas, E. Katchalski-Katzir, and I. Z. Steinberg, *Biochemistry*, 1978, **17**, 5064.

pairs. The energy-transfer properties of systems exhibiting such static averaging have been compared with those for dynamic averaging.[952]

An interesting observation of stereoselective energy transfer has been made in a tryptophan complex.[953] Methanol solutions of D-tryptophan were observed to have an appreciably higher fluorescence efficiency than those of L-tryptophan when excited by left circularly polarized light (c.p.l.). Similarly, excitation of the tryptophan moiety in 5-dimethylaminonaphthalene-1-sulphonyl-D-tryptophan by left c.p.l. resulted in a considerably higher energy-transfer efficiency than in the corresponding L-tryptophan compound. A development of the Jablonski 'active sphere' model for concentration depolarization has been proposed.[954] Expressions for the mean probability of energy transfer from D to any A in the newly defined 'active shell' are derived for two- and three-dimensional systems and applied to experimental data in the literature. An earlier theory of the effect of electronic energy transfer on photoluminescence quantum yields has recently been extended to one-dimensional systems[955] and to multicomponent luminescent systems.[956]

Fluorescence Polarization. An interesting paper has appeared which derives expressions for time-dependent photoselected emission from systems where rotational motion of the fluorophore is negligible.[957] Expressions for the time dependence of photoselected emission and the normally measured emission anisotropy are derived for a number of cases including dual emission, energy transfer between unlike molecules, weakly interacting dimers, of identical molecules, and energy transfer among several identical molecules, the latter of which may be likened to a protein containing a number of tryptophan residues. In some cases the measurement and interpretation of the components of photoselected emission may be considerably simpler than for the anisotropy decay. A correction factor has been derived to account for the effect of polarization on luminescence quantum yields.[958]

A detailed model of the torsional dynamics and resultant fluorescence depolarization of linear molecules has been developed.[959] The model considers $N + 1$ identical rods connected at their ends by torsion springs and undergoing rotational Brownian motion about their fixed symmetry axes. Four zones in the fluorescence-anisotropy decay are identified with certain types of motion of the chain. The model is applied to results obtained for ethidium bound to DNA, as is another model which considers the macromolecule as a semiflexible chain.[960] The second model suggests that twisting motions of the chain are mainly responsible for the observed depolarization. A related treatment of acridine orange bound to DNA has also appeared.[961] Theories of fluorescence polarization in magnetically oriented

[952] R. E. Dale, *Acta Phys. Pol. A*, 1978, **54**, 743.
[953] C. H. Tran and J. H. Fendler, *J. Am. Chem. Soc.*, 1979, **101**, 1285.
[954] A. Kawski and K. Nowaczyk, *Acta Phys. Pol. A*, 1978, **54**, 777.
[955] C. Bojarski, *Acta Phys. Acad. Sci. Hung.*, 1978, **44**, 217.
[956] J. Kusba and C. Bojarski, *J. Luminescence*, 1979, **20**, 73.
[957] F. Tanaka and N. Mataga, *Photochem. Photobiol.*, 1979, **29**, 1091.
[958] J. A. Poole and A. Findeisen, *J. Chem. Phys.*, 1977, **67**, 5338.
[959] S. A. Allison and J. M. Schur, *Chem. Phys.*, 1979, **41**, 35.
[960] M. D. Barkley and B. H. Zimm, *J. Chem. Phys.*, 1979, **70**, 2991.
[961] G. P. Kishchenko and A. D. Gruzdev, *Biofizika*, 1978, **23**, 801.

photosynthetic systems,[962] uniaxial polymers,[928b] and uniaxial liquid crystals [963] have been published. A valuable paper develops a theory for the fluorescence-anisotropy decays of fluorophores embedded in a membrane.[964] The theory, which is applicable to any situation where orientations are restricted by the surrounding structure, characterizes the probe motion in terms of two parameters, a wobbling diffusion constant and a degree of orientational constraint. A general formalism to predict the fluorescence polarization of an aggregate of an arbitrary number of molecules in the limit of complete incoherent transfer is given in a paper on the light-harvesting chlorophyll *a*/*b* protein.[965] Expressions for the fluorescence polarization of a probe incorporated in a flexible fibre have been derived which contain a term for the bending elastic modulus of the fibre.[966]

Photophysics and Photochemistry of Aromatic Amino-acids.—*Emission Lifetimes.* The fluorescence decay of neutral aqueous solutions of tryptophan and some of its derivatives has been shown to be non-exponential. The reasons for this complexity have not as yet been clearly identified but this important observation will certainly make more difficult the task of characterizing the tryptophan residues in multiple tryptophan-containing proteins on the basis of their fluorescence lifetimes. Accurate time-resolved single-photon counting measurements were able to resolve the fluorescence decay on neutral aqueous tryptophan solutions into two components with mean lifetimes of 3.14 and 0.51 ns and also to show that the relative contribution of each was wavelength dependent with maxima at 350 and 335 nm, respectively.[967] The authors attribute the two emissions to differently solvated ^1L_a and ^1L_b states, respectively, and argue that the two states are not able to interconvert rapidly, as is normally assumed. An investigation of the same system using a giant-pulse laser system with streak camera detection system, limited to wavelengths >370 nm, also found a dual exponential decay with lifetimes of 2.11 and 5.4 ns.[968] The latter has since been shown to arise from a photoproduct so that, if the 2.11 ns component is associated with the 3.14 ns component in the previous paper, the results are consistent as the subnanosecond component would not be seen by the streak camera because it is further into the u.v. than 370 nm. The authors in the second paper suggest that the components may arise from different rotamers, trapped during the excited-state lifetime in different conformations. The time dependence of the emission spectrum of tryptophan in glycerol, water, and ethanol has been investigated.[969] In contrast to tryptophan, indole and a number of its derivatives display single exponential fluorescence decays. The effect of water interacting with the NH bond on this lifetime has been investigated in a study of indole, 1-methylindole, and 3-methylindole in water–dioxan mixtures.[970] A different interaction was found to operate in *N*-acetyltryptophanamide, probably

[962] R. S. Knox and M. A. Davidovich, *Biophys. J.*, 1978, **24**, 689.
[963] L. L. Chapoy and D. B. Dupre, *J. Chem. Phys.*, 1978, **69**, 519.
[964] K. Kinosita, jun., S. Kawato, and A. Ikegami, *Biophys. J.*, 1977, **20**, 289.
[965] R. L. Van Metter, *Biochim. Biophys. Acta*, 1977, **462**, 642.
[966] T. Yanagida and F. Oosawa, *J. Mol. Biol.*, 1978, **126**, 507.
[967] D. M. Rayner and A. G. Szabo, *Can. J. Chem.*, 1978, **56**, 743.
[968] G. R. Fleming, J. M. Morris, R. J. Robbins, G. J. Woolfe, P. J. Thistlethwaite, and G. W. Robinson, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4652.
[969] L. C. Pereira, I. C. Ferreira, M. P. F. Thomaz, and M. I. B. M. Jorge, *J. Photochem.*, 1978, **9**, 425.
[970] J. P. Privat, P. Wahl, and J. C. Auchet, *Biophys. Chem.*, 1979, **9**, 223.

an intramolecular quenching by the carbonyl group. An extensive investigation of the fluorescence lifetimes and quantum yields of a series of small peptides and peptide hormones, each containing a single tryptophan, has been published.[971] A number of valuable general conclusions are reached. In particular, it is suggested that, for a highly solvated protein tryptophan that is not specifically quenched, lifetimes similar to one in the longer peptides will be observed (0.5—1 ns and 3—4 ns). A longer lifetime is definitely diagnostic of either buried tryptophan or one which cannot freely sample the solvent environment.

Fluorescence Quenching. There have been several important investigations of fluorescence quenching of tryptophan and tyrosine by commonly used buffering reagents. A study of the fluorescence quenching of tryptophan and several derivatives by phosphate groups found that phosphate monoanions quench by the transfer of a proton from an acidic OH group to the indole chromophore.[972] Phosphate trianions probably quench the fluorescence by deprotonation of the NH indole group and phosphate dianions were found to have no effect on tryptophan or *N*-methyltryptophan but did quench other derivatives. The authors conclude that the fluorescence of tryptophanyl residues in protein–nucleic acid complexes will not be quenched unless an accessible residue is located near a phosphorylated polynucleotide chain end. An interesting paper has investigated the appearance of tyrosinate fluorescence, $\lambda_{max} \approx 340$ nm, in neutral solutions in the presence of high acetate buffer concentrations.[973] Previously tyrosinate fluorescence had only been observed at pH > pK (S_0) = 10.3. Carboxylate ions were shown to quench tyrosine fluorescence at 302 nm by proton transfer to give tyrosinate fluorescence. The results have considerable significance in studies of protein fluorescence where emission in the region of 340 nm has previously been attributed exclusively to tryptophan residues. The result is nicely illustrated by the observation of fluorescence at 345 nm in two cytotoxins known not to contain tryptophan.[974] Phosphate dianions have been shown to be efficient quenchers of tyrosine fluorescence.[975] Tyrosine fluorescence enhancement in troponin C has been attributed to a proton-transfer mechanism involving citrate (*vide infra*).[976]

Investigations of tryptophan or tryptophan residue fluorescence quenching by histidine,[977] *N*-bromosuccinimide,[978] and hydrogen peroxide,[979] and of indole fluorescence quenching by amides[980] and halogenoalkanes[981] have all been reported. The studies with *N*-bromosuccinimide were extended to include several proteins.

[971] T. C. Werner and L. S. Förster, *Photochem. Photobiol.*, 1979, **29**, 905.
[972] T. Alev-Behmoaras, J. J. Toulme, and C. Helene, *Biochimie*, 1979, **61**, 957.
[973] D. M. Rayner, D. T. Krajcarski, and A. G. Szabo, *Can. J. Chem.*, 1978, **56**, 1238.
[974] A. G. Szabo, K. R. Lynn, D. T. Krajcarski, and D. M. Rayner, *FEBS Lett.*, 1978, **94**, 249; A. G. Szabo, K. R. Lynn, D. T. Krajcarski, and D. M. Rayner, *J. Luminescence*, 1979, **18/19**, 582.
[975] O. Shimizu, J. Watanabe, and K. Imakubo, *Photochem. Photobiol.*, 1979, **29**, 915.
[976] P. C. Leavis and S. S. Lehrer, *Arch. Biochem. Biophys.*, 1978, **187**, 243.
[977] S. V. Konev, V. P. Khrapovitskii, and S. N. Cherenkevitch, *Zh. Prikl. Spektrosk.*, 1978, **29**, 37.
[978] B. F. Peterman and K. J. Laidler, *Can. J. Chem.*, 1979, **57**, 1471; B. J. Peterman and K. J. Laidler, *Biochim. Biophys. Acta*, 1979, **577**, 314.
[979] P. Cavatorto, R. Favilla, and A. Mazzini, *Biochim. Biophys. Acta*, 1979, **578**, 541.
[980] P. M. Froehlich and K. Nelson, *J. Phys. Chem.*, 1978, **82**, 2401.
[981] G. Lessard and G. Durocher, *Photochem. Photobiol.*, 1978, **29**, 399.

Spectroscopy. A particularly interesting paper discusses the possible role of a mixed valence-Rydberg state in the fluorescence of indoles.[982] A useful framework for understanding a number of features of indole photophysics is developed. Features recently observed in the vapour-phase absorption spectra of indole and 1-methylindole have been assigned to low-lying Rydberg states.[983] Two different approaches have been taken in studies which have been able to resolve the overlapping ^1L_a and ^1L_b transitions in indole and some derivatives; the first uses fluorescence excitation polarization [984] and the second environmental perturbation of absorption spectra.[985] The photoionization threshold of tryptophan in aqueous solution has been measured as 4.5 $\pm$ 0.1 eV.[986] This is 3.4 eV below the gas-phase ionization threshold. The result has some interesting consequences for the photochemistry of indole excited at different positions within the first absorption band. The fact that there are significant photochemical processes with wavelength-dependent quantum yields taking place in studies of protein luminescence is generally ignored. There have been interesting observations of the effect of solvent interactions on the photophysics and photochemistry of indole excited into its first, (^1L_a, ^1L_b), excited singlet states [987] and there have been flash-photolysis investigations of tyrosine and tyrosyl peptides [988] and of tryptophan and *N*-acetyl-L-tryptophanamide.[989]

This is an appropriate part of the report to mention that there have been a number of flash photolysis investigations of protein systems, including human serum albumin,[990] carboxy peptidase A,[991] lysozyme,[992] and bovine serum albumin.[993]

Intrinsic Protein Luminescence.—*General Interest.* Interesting studies have been made of the luminescence of proteins in either dry powder form or as moist powders with a known water–protein mole ratio in the temperature range from 0 to −196 °C. No special shifts in the fluorescence were observed for dry proteins over the whole temperature range but step-like changes were observed in one or two temperature ranges depending on whether or not the protein had buried or exposed tryptophan residues, respectively.[994] The lower temperature shift stabilized at a water content corresponding to a filled first hydration shell. In D_2O the corresponding shifts occurred at higher temperatures.[995] The results are interpreted in terms of the fast nanosecond mobility of two subsystems which contain

[982] H. Lami, *J. Chem. Phys.*, 1977, **67**, 3274.
[983] H. Lami, *Chem. Phys. Lett.*, 1977, **48**, 447.
[984] B. Valeur and G. Weber, *Photochem. Photobiol.*, 1977, **25**, 441.
[985] M. Martinaud and A. Kadiri, *Chem. Phys.*, 1978, **28**, 473.
[986] E. Amouyal, A. Bernas, and D. Grand, *Photochem. Photobiol.* 1978, **29**, 1070.
[987] I. Tatischeff, R. Klein, T. Zemb, and M. Duquesne, *Chem. Phys. Lett.*, 1978, **54**, 394; J. Zechner, G. Koehler, N. Getoff, I. Tatischeff, and R. Klein, *J. Photochem.*, 1978, **9**, 304.
[988] J. F. Baugher and L. I. Grossweiner, *Photochem. Photobiol.*, 1978, **28**, 175.
[989] W. A. Volkert, R. R. Kuntz, C. A. Ghiron, R. F. Evans, R. Santus, and M. Bazin, *Photochem. Photobiol.*, 1977, **26**, 3.
[990] B. Hicks, M. White, C. A. Ghiron, R. R. Kuntz, and W. A. Volkert, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 1172.
[991] J. Y. Lee and L. I. Grossweiner, *Photochem. Photobiol.*, 1978, **27**, 635.
[992] J. F. Baugher and L. I. Grossweiner, *Photochem. Photobiol.*, 1977, **25**, 305.
[993] M. White, R. R. Kuntz, C. A. Ghiron, and W. A. Volkert, *Photochem. Photobiol.*, 1978, **28**, 413.
[994] E. A. Permyakov and E. A. Burstein, *Stud. Biophys.*, 1977, **64**, 83.
[995] E. A. Permyakov and E. A. Burstein, *Stud. Biophys.*, 1977, **64**, 163.

the chromophore: protein with strongly bound water and hydrated protein side-chains with loosely bound water. An additional insight into the mechanism of protein fluorescence quenching is given in a paper from the same research group which considers the thermal quenching of protein fluorescence in fluid solutions.[996] The authors suggest that quenching of the fluorophore by vicinal groups occurs during large-scale fluctuations of the protein structure and that, just as in the case of macromolecule rotation, these are controlled by the solvent-diffusion coefficient (or T/η where T is the absolute temperature and η the solvent viscosity). This suggests that plots of $1/\phi_f$ (ϕ_f is the fluorescence quantum yield) *versus* T/η should be linear and this is found to be the case for a large number of proteins. The observation that the relationship does not hold for denatured proteins supports the hypothesis. The work may reveal a useful new measure of protein structural mobility. Related observations have been made of the temperature dependence of quenching by added quenchers.[997]

Tryptophan Fluorescence. A versatile technique has been described for immobilizing proteins by covalent attachment to derivatized porous glass beads.[998] In a study of the folding and refolding of chymotrypsinogen A using denaturants it was established that immobilization did not appear to affect the properties of this zymogen when compared to the native form. The technique was then applied to a study of the catalytic effect of sulphydryl oxidase on the rate of disulphide bond formation and polypeptide chain refolding in reductively denatured chymotrypsinogen A.[999] Pig heart lactate dehydrogenase (LDH) is a well characterized enzyme containing six tryptophans and four identical NADH binding sites which, when occupied, reduce the fluorescence to 14% of that in the free enzyme as a result of efficient tryptophan to NADH energy transfer. A good illustration of how far combined quenching and lifetime measurements can be of value in leading to assignments of fluorescence contributions in cases of multiple emitters is a recent study of LDH.[1000] Some tentative assignments were possible on the basis of lifetime changes induced by NADH, Cs^+, I^-, and acrylamide as quenchers. Three of the six tryptophans were not quenched by bound NADH and had a short lifetime (*ca.* 1 ns). In the free enzyme one of the remaining tryptophans has a lifetime of 8 ns and the remaining two lifetimes in the range 4—5 ns. Closely similar fluorescence behaviour was found in the related protein pig heart cytoplasmic malate dehydrogenase, thus supporting the previously recognized homology of the two proteins, something not apparent in steady-state fluorescence studies.[1001] There have been several studies of liver alcohol dehydrogenase (LADH) fluorescence. The sequence and structure of LADH are known. It is a dimeric enzyme comprising identical subunits. Each subunit has two tryptophan residues, Trp-15 which is exposed to the solvent and Trp-314 which is buried in a hydrophobic region at the subunit interface, clearly a situation where selective quenching effects may be

[996] T. L. Bushueva, E. P. Busel, and E. A. Burstein, *Biochem. Biophys. Acta*, 1978, **534**, 141.
[997] E. A. Burstein, T. L. Bushueva, and E. A. Permyakov, *Zh. Prikl. Spektrosk.*, 1978, **28**, 653.
[998] H. E. Swaisgood, V. G. Janolino, and H. R. Horton, *Arch. Biochem. Biophys.*, 1978, **191**, 259.
[999] V. G. Janolino, M. X. Sliwkowski, H. E. Swaisgood, and R. H. Horton, *Arch. Biochem. Biophys.*, 1978, **191**, 269.
[1000] T. Torikata, L. S. Förster, C. C. O'Neal, jun., and J. A. Rupley, *Biochemistry*, 1979, **18**, 385.
[1001] T. Torikata, L. S. Förster, R. E. Johnson, and J. A. Rupley, *J. Biol. Chem.*, 1979, **254**, 3516.

expected. Trp-15 is quenched by I^- and its emission is to lower energy than that of Trp-314.[1002] Selective alkaline quenching of Trp-314 was observed due to resonance energy transfer to tyrosinate, probably Tyr-286. Ternary complex formation was also found to quench Trp-314 selectively and this, interestingly, may be an ionization induced by a conformational change. Another study of the same system again highlights the difficulties involved in obtaining quantitative information from energy-transfer considerations.[1003] The authors suggest that conformational changes in LADH lead to the fluorescence quenching observed following the binding of NAD and NADH. The tryptophan residues can be differentiated on the basis of their emission lifetimes as shown in another study of LADH which also found that the quenching of the fluorescence by ionic and non-ionic quenchers was strongly temperature dependent.[1004] The fluorescence properties of native and chemically modified LADH have been compared.[1005]

An important pointer to future developments in the study of the dynamical behaviour of proteins using fluorescence is given in a paper which monitors the fluorescence anisotropy decay of tryptophan residues in a number of proteins.[1006] Subnanosecond time resolution is achieved using synchrotron radiation, and single-photon counting and rapid data accumulation (1.28 MHz) gave unusually short experiment times (10—40 min). The combination of high signal-to-noise ratio and subnanosecond time resolution made it possible to observe multiple rotational correlation times for single tryptophan residues. A wide range of tryptophan rotational freedom with respect to the macromolecule framework is observed. The residue in nuclease B rotates with the whole molecule whereas the residue in myelin basic protein has considerable internal rotational freedom. Readers are recommended to look at the paper.

The influence of ionic strength on the results of protein fluorescence quenching studies is commonly ignored. The consequences of not doing so are well illustrated in a study of glucose quenching of hexokinase isoenzymes A and B.[1007] Previously reported values for binding dissociation constants were found to be virtually meaningless. Fluorescence quenching titrations with Cs^+, I^-, and glucose have enabled the same authors to characterize the four tryptophan residues in the monomer subunit of the B isoenzyme.[1008] A detailed investigation of the fluorescence characteristics of the different forms of the pyridoxal phosphate enzyme aspartate aminotransferase has been published.[1009] There is a significant quenching of tryptophan fluorescence on binding of the coenzyme which, it is proposed, arises from both energy transfer to the coenzyme and conformational changes around buried tryptophan groups. A fluorescence study has shown that the two active sites of myosin ATPase are identical with respect to ATP binding and

[1002] W. R. Laws and J. D. Shore, *J. Biol. Chem.*, 1978, **253**, 8593.
[1003] M. A. Abdallah, J. F. Biellmann, P. Wiget, R. Joppich-Kuhn, and P. L. Luisi, *European J. Biochem.*, 1978, **89**, 397.
[1004] N. Barboy and J. Feitelson, *Biochemistry*, 1978, **17**, 4923.
[1005] D. M. Parker, M. J. Hardman, B. V. Plapp, J. J. Holbrook, and J. D. Shore, *Biochem., J.*, 1978, **173**, 269.
[1006] I. H. Munro, I. Pecht, and L. Stryer, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 56.
[1007] I. Feldman and D. C. Kramp, *Biochemistry*, 1978, **17**, 1541.
[1008] D. C. Kramp and I. Feldman, *Biochim. Biophys. Acta*, 1978, **537**, 406.
[1009] M. Arrio-Dupont, *Eur. J. Biochem.*, 1978, **91**, 369.

hydrolysis [1010] and that the fluorescence enhancement on ATP binding is representative of the rate of the initial phosphate burst and not of the conformational change that is induced.[1011] Nuclear Overhauser effects in ^{1}H n.m.r. spectroscopy at 360 MHz and fluorescence-quenching studies have produced good evidence for the location of a tryptophan residue(s) in the immediate vicinity of the purine binding site of creatine kinase.[1012] The fluorescence properties of the tryptophan residues in *Escherichia coli* DNA-binding protein reveal that they are located at or near the DNA binding site [1013] and those of the three tryptophan residues in the A-protein of tobacco mosaic virus indicate that they are buried within the structure.[1014] Enhanced fluorescence from cytochrome b_5 when the non-polar segment of the protein binds to phospholipid vesicles has been identified as coming from one of two adjacent residues, Trp-108 or Trp-109.[1015] The depth of penetration of the protein into the membrane was studied using energy transfer to trinitrophenyl or dansyl groups at the surface of the bilayer.[1016] A study of the circularly polarized luminescence of human plasminogen and its derivatives, ligand free and saturated with 6-aminohexanoic acid (known to impair the *in vivo* fibrinolytic action of plasmin), indicated that similar changes in the binding site were induced in all of them following binding.[1017] As a consequence the known gross structural change which occurs on binding to native unmodified plasminogen and which shows clearly in linearly polarized fluorescence studies most probably involves spatial changes of entire domains.

On the basis that any lowering in the value of fluorescence polarization from a group of 'identical' chromophores, such as a multiple tryptophan-containing protein, relative to that from an isolated, equally rigidly held chromophore is a consequence of energy transfer between the chromophores, it is possible to obtain structural information from depolarization studies. A recent study has compared the fluorescence absorption anisotropy spectrum of trypsin with that of the model compound *N*-acetyl-L-tryptophanamide (NATA).[1018] A higher polarization was observed for the denatured protein relative to the native form and this was interpreted as evidence of a conformation-dependent energy transfer between tryptophan residues. Lowering the temperature from 296 to 228 K had relatively little effect on the trypsin polarization, indicating that chromophore rotation was not responsible for the depolarization. Of the six possible pairings of tryptophan residues apparently only Trp-51 and Trp-237 are closer than the estimated Förster critical transfer distance, R_0 = 7—9 nm. There is a negligible contribution from tyrosine fluorescence which, contrary to earlier suggestions, is not a result of a very efficient Tyr → Trp energy-transfer process.

A study of the intrinsic u.v. fluorescence of bacteriorhodopsin has shown that a number of the tryptophan residues (variously reported as four and seven) are

[1010] S. P. Chock and E. Eisenberg, *J. Biol. Chem.*, 1979, **254**, 3229.
[1011] S. P. Chock, P. B. Chock, and E. Eisenberg, *J. Biol. Chem.*, 1979, **254**, 3236.
[1012] M. Vasak, K. Nagayama, K. Wüthrich, M. L. Mertens, and J. H. R. Kaegi, *Biochemistry*, 1979, **18**, 5050.
[1013] P. K. Bandyopadhyay and C.-W. Wu, *Biochemistry*, 1978, **17**, 4078.
[1014] A. Magne, D. Gerard, L. Hirth, and G. Laustriat, *Biochem. Biophys. Acta*, 1977, **495**, 189.
[1015] P. J. Fleming and P. Strittmatter, *J. Biol. Chem.*, 1978, **253**, 8198.
[1016] P. J. Fleming, D. E. Koppel, A. L. Y. Lau, and P. Strittmatter, *Biochemistry*, 1979, **18**, 5458.
[1017] S. Vuk-Pavlovic and A. Gafni, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 971.
[1018] C. A. Ghiron and J. W. Longworth, *Biochemistry*, 1979, **18**, 3828.

accessible to ionic quenchers and that these, and probably others, are significantly quenched in the transition to the photointermediate M_{410}.[1019]

Tryptophan Phosphorescence. A heavy-atom effect of mercury on the spectroscopy of indole and several derivatives has been demonstrated in frozen aqueous solutions of the indoles and 5-mercuri-2′-deoxyuridine monophosphate.[1020] The fluorescence is quenched and the phosphorescence greatly enhanced compared with that of the free indoles. This effect has been used in a study of the binding of sugars to wheat germ agglutinin (WGA).[1021] Thiomercuribenzoate derivatives of glycosides were prepared and their effect on the fluorescence at 298 and 77 K and phosphorescence at 77 K of WGA was studied. The presence of a tryptophan residue in one of three WGA binding subsites was indicated by the results. Evidence for the role of solvent–protein interactions in the energetics of the conformational flexibility of LADH has been found in a study of the phosphorescence from the buried tryptophan residue at the base of the nucleotide-binding domain in the temperature range 1—40 °C.[1022] The buried tryptophan residues in LADH, *Escherichia coli* alkali phosphatase, bovine pancreatic trypsine, rabbit muscle aldolase, and *Aspergillus oryzae* RNAase T_1 were found to be the only source of phosphorescence at room temperature in these systems; no tyrosine phosphorescence was observed.[1023] The phosphorescence lifetimes varied from 1—500 ms.

Tyrosine Fluorescence. Good evidence has been found that the decrease in β-trypsin fluorescence with increasing pH arises from a tryptophan to phenolate energy-transfer process.[1024] Mellitin is a small amphipathic peptide of 26 residues extracted from bee venom. There is only one fluorescence residue, Tyr-19, and its polarization was monitored in an investigation which was able to characterize a monomer ⇌ tetramer equilibrium and the binding of the tetramer to phospholipids.[1025] The Ca^{2+}-, Mg^{2+}-, and H^+-induced tyrosine fluorescence enhancement in troponin C has been identified as a dequenching by co-ordination or protonation of vicinal carboxylates, which normally quench tyrosine fluorescence by proton transfer.[1026] The interaction of bovine neurophysin and the hormones oxytocin and vasopressin has been investigated using the intrinsic tyrosine fluorescence of each of the molecules.[1027] A study of the binding of Ca^{2+} and Tb^{3+} to the hormone angiotensin produced evidence for an energy transfer from an intrinsic tyrosine residue to Tb^{3+}.[1028] As mentioned in an earlier section tyrosinate fluorescence with a maximum at 345 nm has been observed in proteins lacking tryptophan at pH = 7.[974] The two cytotoxins isolated from *Naja raja* venom each contain three tyrosine residues and it seems probable that there is an intramolecular excited-state proton transfer from the phenolic hydroxyl group of one or more of the tyrosine residues to a neighbouring group. Mitochondrial F_1–ATPase isolated from pig

[1019] R. A. Bogomolni, L. Stubbs, and J. K. Lanyi, *Biochemistry*, 1978, **17**, 1037.
[1020] C. Helene, *C.R. Hebd. Séances Acad. Sci., Ser. D*, 1979, **288**, 433.
[1021] M. Monsigny, F. Delmotte, and C. Helene, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 1324.
[1022] S. Kishner, E. Trepman, and W. C. Galley, *Can. J. Biochem.*, 1979, **57**, 1299.
[1023] Y. Kai and K. Imakubo, *Photochem. Photobiol.*, 1979, **29**, 261.
[1024] N. Ramachandran and C. A. Ghiron, *Biochim. Biophys. Acta*, 1978, **532**, 286.
[1025] J. F. Faucon, J. Dufourcq, and C. Lussan, *FEBS Lett.*, 1979, **102**, 187.
[1026] P. C. Leavis and S. S. Lehrer, *Arch. Biochem. Biophys.*, 1978, **187**, 243.
[1027] S. S. Sur, L. D. Rabbani, L. Libman, and E. Breslow, *Biochemistry*, 1979, **18**, 1026.
[1028] R. E. Lenkinski, J. D. Glickson, and R. Walter, *Bioinorg. Chem.*, 1978, **8**, 363.

hearts shows strong tyrosine fluorescence. A careful study of the fluorescence has revealed the presence of a previously undetected tryptophan emission.[1029] The tyrosine residues in cowpea chlorotic mottle virus protein were observed as a fast component in the fluorescence decay curve [1030] and a significant tyrosine fluorescence contribution to the total fluorescence of isolated myelin from bovine brain white matter [1031] has been observed.

Other Protein Chromophores. A study of several reduced flavin model compounds and flavoproteins has shed some light on the slightly confused picture of the fluorescence properties of these compounds.[1032] The flavoproteins exhibited multiple-component fluorescence decays and their fluorescence was less polarized than that exhibited by low-temperature solutions of the model compounds. There appear to be at least two transitions in the absorption spectrum of the reduced flavins above 300 nm. The cellular retinol- and cellular retinoic acid-binding proteins have been isolated from bovine retina and their interaction with their respective ligands investigated using ligand fluorescence properties.[1033] Studies of the fluorescence from the purple membrane protein (PM) of *Halobacterium halobium* have indicated that it is the purple membrane itself (PM 568) that is the fluorescence emitter and not the primary photoproduct K, and that the emitting state is not the one responsible for initiation of the photochemical cycle.[1034] In a good example of the use of c.p.l. to study protein systems it has been shown that the NADH binding sites of LDH and LADH have different structures and rigidities.[1035] Both the c.d. and c.p.l. of NADH showed opposite signs on binding of the nicotinamide to the two enzymes. An earlier c.p.l. study of the nearby adenine binding sites in the two enzymes using the fluorescence coenzyme analogue εNAD^+ showed these sites to be almost identical. The binding of metals by the antibiotic, ionophore lasalocid A (X-537A) has been investigated using the c.p.l. of the salicylic acid moiety which is induced by the folding process.[1036] The ionophore encircles the metal leaving a hydrophobic exterior. The phosphorescence decay rates found in a study of Cu-substituted cytochrome *c* were greater than those in the free Cu porphyrin,[1037] and it has been found that the fluorescence quantum yield of phytochrome bound to its apoprotein is significantly less than in phytochrome itself.[1038]

A number of interesting investigations of the photophysical properties of phycobiliproteins from phycobilisomes have been published. In a careful spectroscopic study the Förster's critical energy-transfer distances between the phycobilins of *Porphyridium cruentum* and *Nostoc sp.* were calculated and on the basis of Förster's theory it was predicted that excitation energy is transferred in the sequence phycoerythrin → phycocyanin → allophycocyanin → chlorophyll *a*

[1029] T. Keira, C. Godinot, and D. C. Gautheron, *Experientia,* 1978, **34**, 1548.
[1030] J. Kruese, B. J. M. Verduin, and A. J. W. G. Visser, *Eur. J. Biochem.,* 1979, **95**, 21.
[1031] A. J. Crang and M. G. Rumsby, *Biochem. J.,* 1979, **177**, 739.
[1032] A. J. W. G. Visser, S. Ghisla, V. Massey, F. Mueller, and C. Veeger, *Eur. J. Biochem.,* 1979, **101**, 13.
[1033] J. C. Saari, S. Futterman, and L. Bredberg, *J. Biol. Chem.,* 1978, **253**, 6432.
[1034] R. Govindjee, B. Becher, and T. G. Ebrey, *Biophys. J.,* 1978, **22**, 67.
[1035] A. Gafni, *Biochemistry,* 1978, **17**, 1301.
[1036] B. Ehrenberg, I. Z. Steinberg, R. Panigel, and G. Navon, *Biophys. Chem.,* 1977, **7**, 217.
[1037] P. Glatz, B. Chance, and J. M. Vanderkooi, *Biochemistry,* 1979, **18**, 3466.
[1038] P.-S. Song, Q. Chae, and J. D. Gardner, *Biochim. Biophys. Acta,* 1979, **576**, 479.

and not directly to chlorophyll *a* from each of the phycobiliproteins.[1039] A steady-state study[1040] and a classic picosecond time-resolved study[1041] have demonstrated part or all of this process. An investigation of the absorption and emission properties of phycoerythrin and their perturbation by concentration effects, pH, and various ions has appeared[1042] and fluorescence studies contributed to a study of the molecular topography of the phycocyanin photoreceptor from *Chroomonas* algae.[1043]

The 525 nm emission from the bile pigment bilirubin has been interpreted as fluorescence, not phosphorescence as previously reported, following a measurement of its lifetime at low temperature.[1044] Visible-light irradiation of the 1 : 1 complexes formed between bilirubin and bovine and human serum albumin has been found to lead to significant changes in histidyl and tryptophyl residues.[1045]

An interesting collection of papers on bioluminescence and chemiluminescence has been published.[1046] The green fluorescent protein (GFP) from *Renilla reniformis* has been obtained as a pure preparation and its absorption and emission properties measured.[1047] The nature of the chromophore is not known. An interesting example of protein–protein energy transfer is indicated when the purified GFP is mixed with luciferin and luciferase. The emission spectrum shifts from the green to the blue and the quantum yield increases by more than a factor of three. Amino-acid analysis of the GFP from *Aequorea forskalea* has shown an absence of tryptophan residues.[1048] The blue fluorescent protein (BFP) from *Photobacterium fischeri* has been isolated.[1049] Its molecular weight (70 000 dalton) was found to be significantly greater than that of the BFP from *P. phosphoreum* (22 000 dalton).

Covalent Probes.—Table 1 is taken from a paper which reports an investigation of the fluorescence depolarization of pyrene butyryl (FB) bound to fumarase.[1050] The paper emphasizes the care necessary when interpreting the results of dye-labelled protein studies. In contrast to an earlier investigation of fumarase which used covalently bound 5-dimethylaminonaphthalene-1-sulphonyl (DNS) as a probe no evidence for a temperature-induced change in conformation was found. The pyrene chromophore is also found in a new fluorescent phosphonate label for serine hydrolases, pyrene methylphosphonofluoridate,[1051] and pyrene maleimide has

[1039] J. Grabowski and E. Gantt, *Photochem. Photobiol.*, 1978, **28**, 39.
[1040] J. Grabowski and E. Gantt, *Photochem. Photobiol.*, 1978, **28**, 47.
[1041] G. Porter, C. J. Tredwell, G. F. W. Searle, and J. Barber, *Biochim. Biophys. Acta*, 1978, **501**, 232.
[1042] D. Frackowiak, J. Dudkiewicz, J. Grabowski, K. Fiksinski, and H. Manikowski, *Photosynthetica*, 1979, **13**, 21.
[1043] J. Jung, P.-S. Song, R. J. Paxton, M. S. Edelstein, R. Swanson, and E. E. Hazen, *Biochemistry*, 1980, **19**, 24.
[1044] J. Dalton, L. R. Milgrom, and R. Bonnett, *Chem. Phys. Lett.*, 1979, **61**, 242.
[1045] F. F. Rubatelli and G. Jori, *Photochem. Photobiol.*, 1979, **29**, 991.
[1046] 'Bioluminescence and Chemiluminescence', *Methods in Enzymology*, Vol. 57, ed. M. A. Deluca, Academic Press, New York, 1978.
[1047] W. W. Ward and M. J. Cormier, *J. Biol. Chem.*, 1979, **254**, 781.
[1048] F. G. Prendergast and K. G. Mann, *Biochemistry*, 1978, **17**, 3448.
[1049] J. Lee, E. D. Small, Y. M. Liu, and S. Sinha, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1241.
[1050] R. P. McIntosh and P. Johnson, *Biopolymers*, 1978, **17**, 2373.
[1051] A. H. Berman and P. Taylor, *Biochemistry*, 1978, **17**, 1704.

Table 1 *Summary of protein and dye properties preventing a simple interpretation of rotational relaxation times derived from measurements of fluorescence depolarization*[a]

(from R. P. McIntosh and P. Johnson, ref. 1050)

Protein properties	*Dye properties*
Asymmetric molecule	Dye rotates about bond to protein with temperature increase
Dissociation of subunits	ρ_0 varies with temperature or viscosity
Flexible molecule	Lifetime not measured in relevant conditions
Solvent viscosity near protein surface different from bulk	Lifetime affected by temperature or solution additives
Protein structure modified by dye	Multiple dye environments (*i.e.* τ and ρ_0 values not unique)
Protein structure modified by temperature or solution additives	Dye–protein bond unstable
Asymmetric protein with non-random orientation of dye about geometric axes of protein molecule	Fluorescent impurities interfering

[a]Rotational relaxation time, ρ, calculated from the simple Perrin equation $(1/\rho - 1/3) = (1/\rho_0 - 1/3)(1 + 3\tau/\rho)$, where ρ = polarization of fluorescence (see ref. 901), ρ_0 = limiting polarization at low temperature or high viscosity, and τ = fluorescent lifetime

been used to label both luciferase [1052] and the pyruvate dehydrogenase multienzyme complex from *E. coli* [1053] in energy-transfer experiments.

A valuable new reagent for the attachment of an excited-state proton-transfer fluorescence probe, 2-naphthol, to primary amines has been developed.[1054] It is the lactone of 2-hydroxyl-1-naphthaleneacetic acid and it was evaluated by studying its reaction with bovine serum albumin. The studies indicated that, following a rapid adsorption, the lactone reacted with BSA at a unique single site on the protein. The BSA conjugate has a complex fluorescence decay and this is discussed by the authors in terms partly of a proton-accepting group located at or near the dye binding site.

Evidence that conformation change may be induced by the binding of the ligand to the maltose receptor of *E. coli* has been obtained from a distant reporter-group method based on the measurement of fluorescent lifetimes.[1055] The fluorescence-decay characteristics of the two intrinsic tryptophans changed on maltose binding as did the spectrum of the dye-labelled protein formed on reaction of 5-(iodoacetamido)fluorescein with a methionine residue of the maltose binding protein. The fluorescein chromophore has also been used to induce anti-fluorescyl antibodies of the IgG type and study their properties by iodide-quenching experiments,[1056] to label the initiation factor IF3 in a study of its binding to 305

[1052] S.-S. Tu, C.-W. Wu, and J. W. Hastings, *Biochemistry*, 1978, **17**, 987.
[1053] K. J. Angelides and G. G. Hammes, *Biochemistry*, 1979, **18**, 1223.
[1054] W. R. Laws, G. H. Posner, and L. Brand, *Arch. Biochem. Biophys.*, 1979, **193**, 88.
[1055] R. S. Zukin, *Biochemistry*, 1979, **18**, 2139.
[1056] R. M. Watt and E. W. Voss, jun., *J. Biol. Chem.*, 1979, **254**, 1684.

ribosomal subunits,[1057] and to label plasma Factor XIIIa in a study of protein–protein interactions in blood clotting.[1058]

Several studies have been reported of the time-resolved fluorescence depolarization of 1,5-IAEDANS [*N*-iodoacetyl-*N'*-(5-sulpho-1-naphthyl)ethylenediamine]-labelled actin, the major protein found in the thin filaments of muscle. 1,5-IAEDANS reacts with sulphydryl groups and probably attaches to the cysteine-373 residue. It has a long emission lifetime and has an emission spectrum well separated from that of the protein itself. Bound to actin the dye showed two rotational correlation times.[1059] One was only several ns and was attributed to a local protein motion and the other was several hundred ns. The latter became much greater when actin interacted with heavy meromyosin, as a result of complex formation. The complexes of actin with the Ca^{2+} regulatory proteins tropomyosin and troponin were found to rotate more rapidly in the presence of Ca^{2+}. A separate study of tropomyosin labelled with the same dye showed it to be a flexible molecule in both its monomer and polymer forms.[1060] Some inconsistencies were noted in the study of actin and a fuller investigation with careful control of actin concentration and ionic strength to regulate the relative amounts of G-actin (a monomer) and F-actin (a helical aggregate) gave a more consistent picture, albeit more complicated.[1061] The small value of the free F-actin correlation time was interpreted as evidence for a high protomer peptide chain flexibility around its *C*-terminus. Another study of 1,5-IAEDANS and dansyl chloride-labelled F- and G-actin points out the unusually large range of correlation times reported for F-actin using a variety of physical methods.[1062] The study found a rotational correlation time for G-actin consistent with a compact globular protein of its known molecular weight. However, a value of only 500 ns was found for the correlation time of F-actin, much too low for a molecule of its known size, but in agreement with other studies using labels. The author suggests that what is really being measured is label flexibility and that for any investigation of large protein aggregates with intrinsic rotational correlation times $\gg$ 500 ns this possible artefact must be considered. It is possible that the effect could usefully be monitored as a probe of actin interactions and evidence for this is given in the study mentioned above in which the interaction with heavy meromyosin was measured. A fluorescence-lifetime and intensity-quenching study of 1,5-IAEDANS-labelled F- and G-actin has been made using acrylamide as a quencher.[1063] Two components to the fluorescence lifetime of G-actin were observed. The major component was associated with the main Cys-373 binding site and the other to a relatively less polar site(s) of unknown location. Polymerization and interaction with tropomyosin both reduced access to the main binding site. The intrinsic segmental flexibility of the 5—1 moiety of myosin has been investigated using the same dye label [1064] and a study which labelled the same subfragment with (iodoacetamido)fluorescein and labelled actin with 1,5-IAEDANS showed that

[1057] J. Weiel, J. W. B. Hershey, and S. A. Levison, *FEBS Lett.*, 1978, **87**, 103.
[1058] J. M. Froyssinet, B. A. Lewis, J. J. Holbrook, and J. D. Shore, *Biochem. J.*, 1978, **169**, 403.
[1059] K. Tawada, P. Wahl, and J.-C. Auchet, *Eur. J. Biochem.*, 1978, **88**, 411.
[1060] P. Wahl, K. Tawada, and J.-C. Auchet, *Eur. J. Biochem.*, 1978, **88**, 421.
[1061] T. Ikkai, P. Wahl, and J.-C. Auchet, *Eur. J. Biochem.*, 1979, **93**, 397.
[1062] T. Tao, *FEBS Lett.*, 1978, **93**, 146.
[1063] T. Tao and J. Cho, *Biochemistry*, 1979, **18**, 2759.
[1064] R. Mendelson and P. H.-C. Cheung, *Biochemistry*, 1978, **17**, 2139.

there was a 30% energy transfer from the latter dye to the former in their rigor complex.[1065] 1,5-IAEDANS has also been used in studies of ribosomal proteins from *E. coli*,[1066] haemoglobin A, and its subunits [1067] and the recombination of light and heavy chains of immunoglobulins.[1068]

A surprising result has been obtained in a study of the reaction of the purple membrane of *Halobacterium halobium* with dansyl(5-dimethylaminonaphthalene-1-sulphonyl) chloride.[1069] An amino-acid analysis indicated that the major dansylated site of the retinyl protein, which apparently retained its chromophoric and functional properties, was Lys-40. As a consequence the authors propose that the protein folds so as to expose Lys-40 at the membrane surface and that the side chain is not involved in the proton-pump mechanism. These findings are contrary to current models of bacteriorhodopsin. The shape of the apolipoprotein A-I (apo A-I) of human serum high-density lipoprotein in solution has been investigated using sedimentation, viscometric, and fluorescence polarization measurements.[1070] The probe used was dansyl chloride and the results indicate an elongated ellipsoid with an axial ratio of 6:1. A similar study of the shape of elongation factor 2 used a dansyl-labelling technique which avoided exposing the protein to organic solvents.[1071] Dansyl chloride was also used to label immunoglobulins in a study of their rotational relaxation times [1072] and the fluorescence of dansylaziridine-labelled troponin C was used to monitor Ca^{2+} binding.[1073] Another study of Ca^{2+} binding to troponin C subunit was made using different probes, *N*-[*p*-(2-benzimidazolyl)phenyl]maleimide (BIPM) and *N*-ethylmaleimide (NEM).[1074] An energy-transfer study has established that the active sites of all four component enzymes of the pyruvate dehydrogenase complex isolated from *Azotobacter vinelandii* are at least 4 nm apart.[1075] A variety of maleimides and other sulphhydryl reagents were used to label the complex.

Non-covalent Probes.—Interest in proton-transfer fluorescent probes will probably grow rapidly over the next few years. An illustration of the potential of such studies is given in a recent paper which examines the properties of the oxidized NAD^+–pyruvate adduct when in solution and when bound to the coenzyme binding sites in NAD^+ linked dehydrogenases.[1076] The acid and base forms of the adduct were found to have easily distinguishable fluorescence properties. The interaction in the binding site of LDH was strong enough to result in deprotonation even in the ground state but the results for three other proteins were different enough to suggest that excited-state deprotonation could distinguish between their binding sites.

[1065] T. Takashi, *Biochemistry*, 1979, **18**, 5164.
[1066] (*a*) G. Y. Chu and C. R. Cantor, *Nucleic Acids Res.*, 1979, **6**, 2363; (*b*) C. Kang, B. Wells, and C. R. Cantor, *J. Biol. Chem.*, 1979, **254**, 6667.
[1067] E. Bucci, C. Fronticelli, K. Flanigan, J. Perlman, and R. F. Steiner, *Biopolymers*, 1979, **18**, 1261.
[1068] R. M. Watt and E. W. Voss, *J. Biol. Chem.*, 1979, **254**, 7105.
[1069] G. Harris, R. Renthal, J. Tuley, and N. Robinson, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 926.
[1070] D. L. Barbeau, A. Jonas, T.-L. Teng, and A. M. Scanu, *Biochemistry*, 1979, **18**, 362.
[1071] L. Montanaro and S. Sperti, *Methods Enzymol.*, 1979, **60**, 712.
[1072] E. I. Dudich, R. S. Nezlin, and F. Franek, *FEBS Lett.*, 1978, **89**, 89.
[1073] J. D. Johnson, J. H. Collins, and J. D. Potter, *J. Biol. Chem.*, 1978, **253**, 6451.
[1074] T. Iio and H. Kondo, *J. Biochem. (Tokyo)*, 1979, **86**, 1883.
[1075] W. H. Scouten, A. C. De Graaf-Hess, A. De Kok, H. J. Grande, A. J. W. G. Visser, and C. Veeger, *Eur. J. Biochem.*, 1978, **84**, 17; see also ref. 155.
[1076] A. Gafni, *Biochemistry*, 1980, **19**, 237.

The 'non-specific' probe 8,1-ANS (8-*N*-arylamino-1-naphthalene sulphonate) continues to attract as much attention to itself as do the systems it is used to study. Studies of 8,1-ANS have cast serious doubts on its use to specify binding-site polarity. A detailed fluorescence and *X*-ray crystallography study has been published of the binding of 8,1-ANS to α-chymotrypsin.[1077] The fluorescence enhancement observed on binding indicates that emission takes place from a predominantly non-polar singlet state, $S_1(np)$, and this would normally be regarded as evidence for a non-polar hydrophobic binding site. The *X*-ray results, however, show that the 8,1-ANS is found on the surface of a generally polar region near an array of charged amino-acid residues. It is strongly interacting with a disulphide group and a number of ordered solvent molecules. In another valuable study the fluorescence properties of a number of 8,1-ANS and 6,2-ANS derivatives bound to apomyoglobin have been compared.[1078] On the basis of well established solvent polarity scales for each of the two series of compounds it is shown that they indicate very different binding site 'polarities'. The emission properties of the probe are a result of specific interactions between the site and the probe. The most effective use of the probe would be to monitor changes in the site brought about by a perturbation. Laser flash-photolysis studies of 8,1-ANS in water and in ethanol have indicated that the very low fluorescence quantum yield in polar solvents is a result of photoionization.[1079]

The fluorescent probe 2,6-TNS (2-*p*-toluidinonaphthalene 6-sulphonate) has been used in studies of the conformation of fructose 1,6-diphosphatase,[1080] the kinetics of the porcine pancreatic α-amylase-catalysed hydrolysis of amylose,[1081] and the methotrexate binding site of dihydrofolate reductase.[1082] The related probe 1,8-TNS has been used to study the non-polar sites on the protein salmine A1 [1083] and the interaction between *E. coli* pyruvate oxidase and a detergent activator.[1084]

The validity of a new technique based on measurements of the fluorescence decay of lanthanide ions for the determination of the number of metal-co-ordinated water molecules has been established using a number of structurally well characterized compounds including two proteins, thermolysin and parvalbumin.[1085] A pulsed dye laser was used to excite the *f*-electron levels of Eu^{III} and Tb^{III} and the changes in the decay constants when H_2O was replaced by D_2O were found to correlate well with the number of co-ordinated water molecules. The authors have recently described another method in which the luminescence of Tb^{III} is directly excited rather than being excited by energy transfer from residues such as tryptophan as is

[1077] (*a*) J. D. Johnson, M. A. El-Bayoumi, L. D. Weber, and A. Tulinsky, *Biochemistry*, 1979, **18**, 1292; (*b*) L. D. Weber, A. Tulinsky, J. D. Johnson, and M. A. El-Bayoumi, *Biochemistry*, 1979, **18**, 1297.
[1078] H. Doduik, H. Kanety, and E. M. Kosower, *J. Phys. Chem.*, 1979, **83**, 515.
[1079] (*a*) G. R. Fleming, G. Porter, R. J. Robbins, and J. A. Synowiec, *Chem. Phys. Lett.*, 1977, **52**, 288; (*b*) G. W. Robinson, R. J. Robbins, G. R. Fleming, J. M. Morris, A. E. W. Knight, and R. J. S. Morrison, *J. Am. Chem. Soc.*, 1978, **100**, 7145.
[1080] T. Takabe, H. Ishikawa, M. Miyakawa, K. Takenaka, and S. Nikai, *J. Biochem.* (*Tokyo*), 1979, **85**, 203.
[1081] H. Kondo, H. Nakatani, K. Hiromi, and R. Matsuno, *J. Biochem.* (*Tokyo*), 1978, **84**, 403.
[1082] C. C. Fan, *Dev. Biochem.*, 1978, **4**, 413.
[1083] S. F. Russo and R. G. Engel, *Physiol. Chem. Phys.*, 1978, **10**, 163.
[1084] T. A. O'Brien and R. B. Gennis, *Biochemistry*, 1979, **18**, 804.
[1085] W. D. Horrocks, jun. and D. R. Sudnick, *J. Am. Chem. Soc.*, 1979, **101**, 334.

normally the case.[1086] The 488 nm line from a CW Ar^+ laser was used to populate the $^5D_4$ state of Tb^{III} and it was possible to differentiate the Ca^{II} binding sites of parvalbumin and thermolysin on the basis of differences in the fine structure of the emission, something not revealed in previous c.p.l. studies. Thermolysin is a neutral metalloendopeptidase with a Zn^{II} ion site at the base of the cleft between the two lobes of its structure and four Ca^{II} ions to provide stability. In an experiment in which Zn^{II} was replaced by Co^{II} and half of the Ca^{II} by Tb^{III} it was possible to see changes in the energy-transfer efficiency to Tb^{III} following 280 nm excitation over the temperature range 25—80 °C.[1087] The thermal denaturation had not been detected in previous studies of this system using a variety of techniques. The fluorescence properties of the metalloprotein formed by apo-alkaline phosphatase and Tb^{III} have been investigated.[1088]

A potentially very useful observation has been made concerning the fluorescence of nicotinamide $1,N^6$-ethenoadenine dinucleotide bound to several dehydrogenases.[1089] In contrast to a glycerol solution of the free dinucleotide the bound molecules showed significant changes in their linear polarization of fluorescence across the emission band and these changes were different for each of the enzymes. The authors attribute the differences to environmentally sensitive intensity borrowing by the weak etheneadenine transition. The fluorescence quenching of ethenoadenine derivatives by iodide and methionine has been compared for when the nucleotides are bound to several dehydrogenases and when in free solution [1090] and the fluorescence decay of $1,N^6$-ethenoadenine diphosphoribose has been compared with that of the intact coenzyme analogue, nicotinamide $1,N^6$-ethenoadenine dinucleotide, when each is bound to rabbit muscle glyceraldehyde phosphate dehydrogenase.[1091] The latter study shows that the negative co-operativity observed in NAD binding may originate from the nicotinamide base. The binding of DNase I to G-actin has been investigated using as a probe the CPL of $1,N^3$-ethenoadenosine 5′-triphosphate (ε-ATP) bound to the nucleotide binding site of G-actin.[1092] RNase I was previously known to stabilize the monomer form of actin relative to the polymer form but this study showed that it also appears to stabilize actin against denaturing forces. The conformation of F-actin and its interaction with heavy meromyosin and S-1 have been investigated using polarized fluorescence by incorporating $1,N^6$-ethenoadenosine 5′-diphosphate (ε-ADP) into F-actin in a myosin free ghost single fibre.[1093] The distance between the nucleotide binding site and Cys-373 of G-actin has been estimated as *ca.* 3 nm following an energy-transfer study using ε-ATP and *N*-(4-dimethylamino-3,5-dinitrophenyl)-maleimide.[1094] The mechanism of nucleotide binding to myosin S-1 has been studied using ε-ATP and ε-ADP.[1095] The stopped-flow technique was used and the

[1086] D. R. Sudnick and W. D. Horrocks, *Biochim. Biophys. Acta*, 1979, **578**, 135.
[1087] S. M. Khan, E. R. Birnbaum, and D. W. Darnall, *Biochemistry*, 1978, **17**, 4669.
[1088] A. D. Sherry, S. Au-Young, and L. G. Cottan, *Arch. Biochem. Biophys.*, 1978, **189**, 277.
[1089] A. Gafni, J. Schlessinger, and I. Z. Steinberg, *J. Am. Chem. Soc.*, 1979, **101**, 463.
[1090] A. Gafni, *Biochemistry*, 1979, **18**, 1540.
[1091] A. Gafni, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 285.
[1092] L. D. Burtnick and P. L. Schaar, *FEBS Lett.*, 1979, **97**, 166.
[1093] T. Yanagida and F. Oosawa, *J. Mol. Biol.*, 1978, **126**, 507.
[1094] M. Miki and K. Mihashi, *Biochem. Biophys. Acta*, 1978, **533**, 163.
[1095] F. Garland and H. C. Cheung, *Biochemistry*, 1979, **18**, 5287.

results indicated that at least three steps are required to describe the binding. Fluorescent polynucleotides of $1,N^6$-ethenoadenine (ε-A) and $3,N^4$-ethenocytosine (ε-C) have been prepared and their interaction with TMV protein studied.[1096] Two fluorescent AMP analogues, ε-AMP and the deoxy-form ε-dAMP, have been used to investigate the relation between the nucleotide binding kinetics and the activation of glycogen phosphorylase *b* in a temperature-jump relaxation study.[1097] ε-dAMP does not activate the enzyme but binds to the same site as ε-AMP. The fluorescent nucleotide analogue linear benzo-ATP has been found to activate *E. coli* aspartate transcarbamylase to the same extent as ATP.[1098]

The preparation and properties of a new class of fluorescent nucleotide substrates for DNA-dependent RNA polymerases have been described.[1099] The 1-aminonaphthalene-5-sulphonate chromophore was attached to purine and pyrimidine derivatives *via* a γ-phosphoamidate bond. The kinetics of the binding of fluorescent formycin nucleotides to chloroplast coupling factor (CF_1) have been investigated.[1100]

Dansyl derivatives have been used in studies of the L-tryptophan binding site on serum albumin (dansyl-sarcosine),[1101] the inhibition of horse serum cholinesterase (dansyl-alkanetrimethylammonium salts),[1102] the competitive inhibition of yeast hexokinase (dansyl-glucose),[1103] the binding of arginine derivatives by trypsin and trypsinogen (dansyl-L-arginine),[1104] and the inhibition of thrombin [dansyl-arginine *N*-(3-ethylpentane-1,5-diyl)amide].[1105]

An interesting paper has reported the preparation of highly fluorescent retinyl derivatives and their use in a study of the retinal binding site in bacteriorhodopsin.[1106] Evidence for energy transfer from aromatic amino-acids in the protein to the retinyl moiety was obtained.

[1096] R. K. Ledneva, A. P. Razjwin, A. A. Kost, and A. A. Bogdanov, *Nucleic Acids Res.*, 1978, **5**, 4225.
[1097] B. Vandenbunder, M. Dreyfus, and H. Buc, *Biochemistry*, 1978, **17**, 4153.
[1098] P. Van der Lijn, J. R. Barrio, and N. J. Leonard, *J. Biol. Chem.*, 1978, **253**, 8694.
[1099] L. R. Yarbrough, J. G. Schlageck, and M. Baughman, *J. Biol. Chem.* 1979, **254**, 12069.
[1100] V. Shoshan, N. Shavit, and D. M. Chipman, *Biochim. Biophys. Acta*, 1978, **504**, 108.
[1101] H. Bruderlein and J. Bernstein, *J. Biol. Chem.*, 1979, **254**, 11570.
[1102] R. Narayanan and P. Balaram, *FEBS Lett.*, 1978, **93**, 38.
[1103] P. A. Lartey and M. Derechin, *Prep. Biochem.*, 1979, **9**, 85.
[1104] S. Goto and G. P. Hess, *J. Biochem. (Tokyo)*, 1979, **85**, 961.
[1105] M. E. Nesheim, F. G. Prendergast, and K. G. Mann, *Biochemistry*, 1979, **18**, 996.
[1106] T. Schreckenbach, B. Walckhoff, and D. Oesterhelt, *Photochem. Photobiol.*, 1978, **28**, 205.

3
Peptide Synthesis

BY I. J. GALPIN
Appendices compiled by
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1 Introduction

The general layout of this chapter is similar to that of previous years. The last year has seen relatively few major syntheses, although the number of reported syntheses of small peptides/neuropeptides has continued to increase. Once again the emphasis seems to have been on the actual preparation of peptides either by the solution or solid-phase approach rather than the development of new techniques. The role of the peptide chemist has been discussed on two occasions.[1,2] In both cases the present state of the art is discussed and the future, with particular reference to genetic engineering, is examined.

The proceedings of the Sixth American Peptide Symposium,[3] the proceedings of the Fifteenth Japanese Peptide Symposium,[4] and the proceedings of the Fifteenth European Peptide Symposium [5] have all been published. Little attempt has been made to review the contents of these volumes individually as much of the material contained therein has already been published in other places. A symposium discussing the versatility of proteins has also been held.[6]

Several interesting new texts have been published this year, including 'The Peptides; Analysis, Synthesis, Biology'.[7] This text serves to replace the now outdated Schröder and Lubke. More general aspects of peptide chemistry are covered in 'Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins' [8] and 'Methods in Enzymology Vol. 47'.[9] A concise review outlining the introduction of the major protecting groups and their removal has appeared.[10] Of particular

[1] P. Newmark, *Nature (London)*, 1979, **280**, 637.
[2] H. D. Niall and G. W. Tregear, *New Engl. J. Med.*, 1979, **301**, 940.
[3] 'Peptides, structure and biological function', Proceedings of the 6th American Peptide Symposium, ed. E. Gross and J. Meienhofer, Pierce Chemical Company, Rockford, 1979.
[4] 'Peptide Chemistry, 1977, Proceedings of the 15th Symposium on Peptide Chemistry, Osaka', ed. T. Shiba, Protein Research Foundation, Osaka, 1978.
[5] 'Peptides 1978, Proceedings of the 15th European Peptide Symposium, Gdansk', ed. I. Z. Siemion and G. Kupryszewski, Wroctaw University Press, Wroctaw, 1979.
[6] 'Versatility of Proteins, Proceedings of International Symposium on Proteins, Taipei', ed. C. H. Li, Academic Press, New York, 1979.
[7] 'The Peptides, Analysis, Synthesis, Biology', Volume 1, ed. E. Gross and J. Meienhofer, Academic Press, New York, 1979.
[8] 'Chemistry and Biochemistry of Amino-acids, Peptides, and Proteins', Volume 5, ed. B. Weinstein, Marcel Dekker AG, Basle, 1979.
[9] 'Enzyme Structure, Part E, Volume 47, Methods in Enzymology', ed. C. H. W. Hirs and S. N. Timasheff, Academic Press, New York, 1979.
[10] P. M. Hardy, *Chem. Ind. (London)*, 1979, **18**, 617.

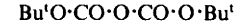
interest here is the inclusion of a number of relatively new techniques which are used in peptide chemistry; the application of the various protecting groups is well illustrated.

^{1}H N.m.r. data which is generally useful has appeared in two papers. The first deals with the parameters for individual amino-acids situated in a tetrapeptide chain and gives a useful guide to the resonances which may be expected.[11] The second gives proton amide-titration shifts for use in conformational studies.[12] A survey[13] of the hydration of 78 peptides has been made and the presence of water associated with peptides in the crystal lattice has been examined. This is of considerable interest as many peptide chemists find it difficult or impossible to remove water of hydration from analytical samples.

The years' coverage follows that established previously and lists of synthetic peptides and useful derivatives are presented in the appendices.

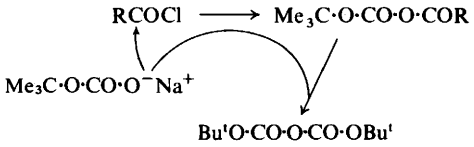
2 Methods

Protective Groups.—*Established Methods of Amino-group Protection.* The preparation of di-t-butyl pyrocarbonate (1) from sodium t-butylcarbonate and a



(1)

variety of carboxylic acid chlorides has been described.[14] A range of carboxylic acid chlorides as shown in Scheme 1 were investigated. A new method has been described[15] for the introduction of the most well known of all protecting groups.

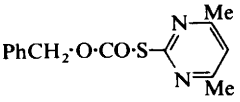


(1)

where R = 2-nitrophenyl; 2,4- and 3,5-dinitrophenyl; 2-chloro-5-nitrophenyl, 2,4,6-trinitrophenyl, or trichloromethyl

Scheme 1

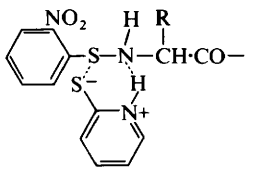
The method uses the dimethylpyrimidinyl thiocarbonate (2) to introduce the benzyloxycarbonyl function and claims to be an improvement over previous methods giving supression of by-product formation. The removal of the



(2)

[11] A. Bundi and K. Wüthrich, *Biopolymers*, 1979, **18**, 285.
[12] A. Bundi and K. Wüthrich, *Biopolymers*, 1979, **18**, 299.
[13] C.-H. Yang, J. N. Brown, and K. D. Kopple, *Int. J. Pept. Protein Res.*, 1979, **14**, 12.
[14] V. F. Pozdnev, E. A. Smirnova, N. N. Podgornova, N. K. Zentsova, and U. O. Kalei, *Zh. Org. Khim.*, 1979, **15**, 106; (*Chem. Abs.*, 1979, **90**, 168 196a).
[15] M. D. Fenn, *Proc. Aust. Biochem. Soc.*, 1979, **12**, 16.

benzyloxycarbonyl group and the tosyl group by electrochemical methods has also been studied.[16] Side reactions during the use of the Nps group have been known for some time. Migration of the Nps group from a carboxyl component to an amino component during coupling has been studied [17] and the rate has been found to be dependent on the type of ester protection employed. For example, pentachlorophenyl active esters tend to show migration to a greater extent than the corresponding methyl esters. Also when activation has been achieved by reaction with isobutylchloroformate, migration is scarcely detectable in dimethyl formamide or tetrahydrofuran, whereas in chloroform between 32 and 52% migration is observed. However, using di-cyclohexyl-carbodi-imide or hydroxysuccinimide active esters for the activation of Nps-D-valine, no migration was observed in chloroform. Such a side reaction is highly deletereous as it generates a new amino component which can react with the activated carboxyl component which is present. Other side reactions associated with the use of the Nps protecting group have also been studied.[18] The acidolysis of the Nps group is not an ideal means of removal as it can give rise to partial fission of t-butyl groups and modification of tryptophanyl residues. Removal by thiolysis has been known for some time, particularly easy cleavage has recently been found using 2-mercaptopyridine.[19] This reagent is thought to act by forming a six-membered cyclic transition state as shown (3). Such a transition state leads to the formation of an unprotonated amino component; the cleavage is very rapid and quantitative at room temperature. A variety of solvents have been studied and under no circumstances has damaged tryptophan been observed.



(3)

Further studies on the phosphinyl and phosphinothioyl amino-acids have been made.[20, 21] As has been reported in the 1979 Specialist Periodical Reports, the diphenylthiophosphinyl derivatives can be prepared by direct reaction of the amino-acid with the corresponding thiophosphinyl chloride. It appears [20] that reaction with the corresponding amino-acid ester followed by hydrolysis is a better route of preparation as higher yields are ultimately obtained. The best method of cleavage for this particular group is treatment with 0.6 M-HCl in formic acid for 20 min. The diphenylthiophosphinyl derivatives may be coupled by any of the generally accepted methods as no steric hindrance is observed. The derivatives have been utilized in solid-phase peptide synthesis, using the caesium salt method for the

[16] N. F. Loginova, V. G. Mairanovsky, and G. S. Katrukha, *Bioorg. Khim.*, 1979, **5**, 1579.
[17] V. N. Lashkov and G. P. Vlasov, *J. Gen. Chem. USSR* (*Engl. Transl.*), 1979, 1710.
[18] R. E. Vegner, G. I. Chipens, and I. K. Perkone, *Bioorg. Khim.*, 1979, **5**, 645.
[19] M. Stern, A. Warshawsky, and M. Fridkin, *Int. J. Pept. Protein Res.*, 1979, 315.
[20] S. Ikeda, F. Tonegawa, E. Shikano, K. Shinozaki, and M. Ueki, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1431.
[21] M. Ueki, T. Inazu, S. Ikeda, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2424.

initial attachment to the resin. Dimethylthiophosphinyl derivatives (4) have also been described. They may be prepared either by direct reaction of the thiophosphinyl chloride with the corresponding amino-acid under Schotten–Baumann

(4)

conditions or *via* the amino-acid ester followed by removal of the ester function by hydrolysis.[21] The latter method gives the highest yield as the dimethylthiophosphinyl chloride is hydrolysed rather rapidly under alkaline conditions; the use of low temperature and excess reagent alleviates this problem to a certain extent. The protecting group may be removed using HCl in dichloromethane but is more efficiently removed by treatment with triphenylphosphine dihydrochloride in dichloromethane. Under these conditions the group is removed at 60 times the rate observed for a BOC group.

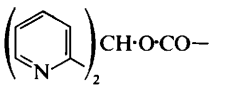
The base-labile fluorenylmethyloxycarbonyl group (Fmoc) has been found to be labile to some extent to amino components during peptide coupling.[22] Slow but significant cleavage of the Fmoc function from Fmoc-alanine or Fmoc-glycine was observed on treatment with several amino-acid esters. The rate of cleavage being fastest for *N*-terminal proline, which might be expected in view of the higher basicity of this imino- rather than amino-acid. Di-isopropylethylamine had little effect on this protecting group, whereas triethylamine caused almost complete cleavage within a day and significant cleavage within one hour. In actual coupling environments the basicity decreases with time, being quite rapid at the beginning of the reaction. 2,4-Dinitrophenol and pentachlorophenol were found to inhibit[22] this cleavage by amino components as the first step in this cleavage is proton abstraction, although they did not completely stop loss of the protecting group. It has also recently been found that the Fmoc protecting group may be cleaved by hydrogenolysis.[23, 24] This is in direct contrast to findings by the workers who originally introduced this protecting group.[25–27] In the presence of 10% palladium on charcoal as catalyst hydrogenolysis of Fmoc-alanine using methanol solvent[24] was complete in 4 h at 0 °C, similarly the $t_{0.5}$ for Fmoc-aniline using 20% acetic acid in methanol was found to be 6 h.[23] Also, rather unexpectedly, partial decomposition of Fmoc-glycine was noticed on prolonged standing in dimethyl formamide, dimethy acetamide, or *N*-methylpyrrolidone. In 1—2 h (representing one cycle of a solid-phase synthesis), this corresponds to 0.03—0.06% decomposition in DMF and 0.006—0.012% in dimethyl acetamide. *N*-Methylpyrrolidone was found to be highly unsuitable for use with the Fmoc protecting group.

[22] M. Bodanszky, S. S. Deshmane, and J. Martinez, *J. Org. Chem.*, 1979, 1622.
[23] E. Atherton, C. Bury, R. C. Sheppard, and B. J. Williams, *Tetrahedron Lett.*, 1979, 3041.
[24] J. Martinez, J. C. Tolle, and M. Bodanszky, *J. Org. Chem.*, 1979, **44**, 3596.
[25] L. A. Carpino and G. Y. Han, *J. Am. Chem. Soc.*, 1970, **92**, 5748.
[26] L. A. Carpino and G. Y. Han, *J. Org. Chem.*, 1972, **37**, 3404.
[27] L. A. Carpino and G. Y. Han, *J. Org. Chem.*, 1973, **38**, 4128.

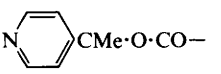
Formylation of amino-acid esters or peptides using dicyclohexyl-carbodi-imide in formic acid [28] or a water-soluble carbodi-imide in formic acid [29] have both been reported. The latter method, which achieves formylation through formic anhydride is claimed to be particularly efficient as impurities may be removed by washing with dilute acid and base. Trifluoroacetylation of amino-acids and peptides using ethyltrifluoroacetate as the acylating species in the presence of an equivalent of base (triethylamine or tetramethylguanidine) employing methanol as the solvent has been reported,[30] with serine and tyrosine no *O*-trifluoroacetylation was observed.

More details have emerged of the use of the supernucleophilic cobalt(I) phthalocyanin for the removal of the trichloroethoxycarbonyl and other β-halogenated protecting groups.[31]

New Methods of Amino-group Protection. Relatively few papers describing *N*-terminal protecting groups have been published. A range of new urethane protecting groups containing a 'basic handle' [32] have been prepared. The derivatives (5—8) were prepared in an attempt to increase acid stability. A protected



(5)



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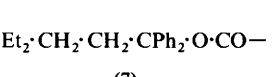
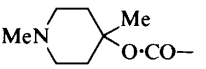


(7)



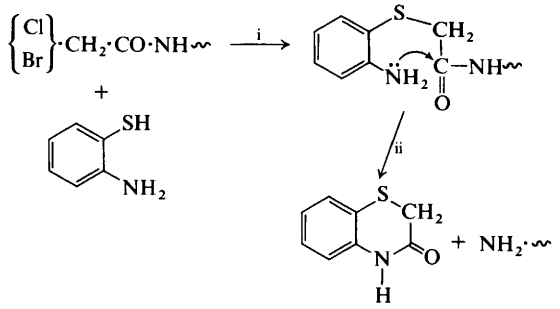
(8)

hydrazide with (7) as amino protection required 1 h for complete deblocking with trifluoroacetic acid, and (8) was found to be more stable to trifluoroacetic acid than the t-butyloxycarbonyl group. The group (8) was found to be much more stable than the corresponding 1-methylcyclohexyl function which is cleaved by trifluoroacetic acid in 1 min at 25 °C. The group (8) was also found to be stable to hydrogenolysis and to be cleaved by treatment with HBr in acetic acid. The use of these 'basic handle' type urethane protecting groups was demonstrated in synthesis of several peptides.

The use of halogeno-acetyl groups for amino protection has been proposed.[33] This rather reactive protecting group has been shown to react with 2-amino-thiophenol in weak alkali (see Scheme 2). The resulting derivatives giving rise to the free peptide after acidolysis. This reversible protection of α-amino functions may be achieved by reaction with *N*-halogenoacetoxysuccinimide; this reagent gives rapid and specific acylation of amino groups. The quantitative chloro-acylation of lima bean trypsin inhibitor is described along with the removal of the protecting group from this protein.

The conversion of bromoethoxycarbonyl amino-acids to the corresponding phosphonium salts has been studied in more detail.[34] The use of 2-bromoethyl

[28] A. R. Day and R. J. Freer, *Int. J. Pept. Protein Res.*, 1979, 334.
[29] F. M. F. Chen and N. L. Benoiton, *Synthesis*, 1979, 709.
[30] T. J. Curphey, *J. Org. Chem.*, 1979, 2805.
[31] H. Eckert and I. Ugi, *Justus Liebigs Ann. Chem.*, 1979, 278.
[32] S. Coyle, O. Keller and G. T. Young, *J. Chem. Soc., Perkin Trans.* 1, 1979, 1459.
[33] J. D. Glass, M. Pelzig, and C. S. Pande, *Int. J. Pept. Protein Res.*, 1979, **13**, 28.
[34] H.-H. Bechtolsheimer, M. Buchholz, and H. Kunz, *Justus Liebigs Ann. Chem.*, 1979, 1908.



Reagents: i, HCO_3^- ; ii, H^+

Scheme 2

esters for carboxyl protection is also examined, as reaction with triphenylphosphine followed by base treatment gives a mild base cleavage of the ester function. The preparation of *N*-2-phosphonoethyl (9) amino-acids has also been reported,[35] as

$$(EtO)_2\overset{\overset{O}{\|}}{P}-CH_2\cdot CH_2-$$

(9)

has conversion of a 2-methylthioethoxycarbonyl group to a 2-thiocyanoethyoxycarbonyl group by treatment with cyanogen bromide.[36]

In an attempt to prepare solubilizing acid-labile protecting groups the alcohol (10) was prepared.[37] This could be converted to the corresponding phenyl mixed carbonate which was then used to introduce the group as an amino-protecting function in the presence of triton B; a 74% yield is obtained in the case of alanine.

$$Me(O\cdot CH_2\cdot CH_2)_3\cdot O\cdot CH_2\cdot CH_2\cdot CMe_2\cdot O-$$

(10)

Protection of Terminal Carboxy Group.—The solubilizing protecting group used for amino protection based on the alcohol (10) has also been used for the preparation of acid labile esters which may be used for carboxyl protection.[37] The esters are comparable in lability to the corresponding t-butyl esters and may be formed by a reaction of the corresponding *Z* amino-acid with the alcohol (10) in the presence of dimethylaminopyridine and dicyclohexylcarbodi-imide. The 2-bromo- and 2-chloro-ethyl esters mentioned in passing earlier have been described in more detail.[38] The bromo or chloro group may be replaced by iodine by treatment with sodium iodide in DMF; the iodo compound may then be cleaved by treatment with zinc. Alternatively reaction with trimethylamine gives corresponding choline esters which are stable to acid but are cleaved without racemization at a pH = 10—10.5 using dioxan as solvent; 10^{-4} M sodium hydroxide was used for the cleavage.

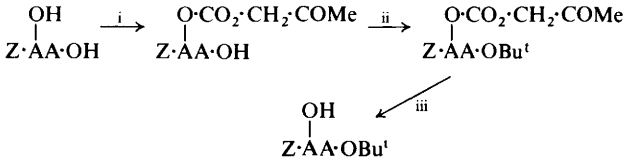
[35] K. Issleib, H. Wache, and A. Balszuweit, *Z. Chem.*, 1979, **19**, 212; (*Chem. Abs.*, 1979, **91**, 141 197k).
[36] R. Barthels and H. Kunz. *Z. Naturforsch., Teil B*, 1979, **34**, 1121.
[37] H. Anzinger, M. Mutter, and E. Bayer, *Angew. Chem.*, 1979, **91**, 747.
[38] H. Kunz and M. Buchholz, *Chem. Ber.*, 1979, **112**, 2145.

Full details of the use of phenyl esters in peptide synthesis have now emerged.[39] The phenyl esters are prepared by a reaction of the *N*-protected amino-acid with phenol in the presence of dicyclohexylcarbodi-imide generally using ethyl acetate as solvent, with or without the addition of pyridine. This ester function is stable to HBr in acetic acid, hydrogenolysis, and trifluoroacetic acid and its use has been exemplified in the synthesis of parts of gastrin and lysozyme. The protecting group may be removed by treatment with alkali at pH = 10.5 using hydrogen peroxide as catalyst. Cleavage is generally complete in 5—20 min using a variety of solvents, and no racemization is observed. Dimethyl sulphide should be added as a scavenger to protect *S*-substituted cysteine residues, methionine, and tryptophan. Transpeptidation is not a problem with aspartyl residues as rearrangement to the β-peptide has been shown to be <1% under the conditions employed in deprotection.

Two new methods for the preparation of amino-acid esters from the corresponding *Z* amino-acid and alkyl halides have been described. The first[40] employs phase transfer catalysis, using trioctylmethylammonium chloride, aqueous sodium bicarbonate, and dichloromethane. The reaction which is carried out with vigorous stirring occurs in the organic phase where the carboxylate anion is alkylated. The product is isolated from the dichloromethane layer and no racemization is observed. A second method,[41] employing sodium bicarbonate in dimethyl formamide has been demonstrated. The method gives no racemization and may be carried out at room temperature.

A method of preparing the toluene-*p*-sulphonates of amino-acid ethyl esters has been put forward.[42] The method utilizes the reaction between an amino-acid and ethyl toluene-*p*-sulphonate under ethanol reflux, giving the toluene-*p*-sulphonate salt of the ester directly. The method appears to be particularly useful for acid-sensitive residues such as tryptophan, the ester salt of which was obtained in 81% yield using this method.

A reaction sequence (see Scheme 3) employing acetoacetyl as intermediate protection for hydroxylic functions has enabled the t-butyl esters of threonine,



Reagents; i, diketene; ii, $Me_2C = CH_2/H^+$; iii, 2 equiv. $NH_2 \cdot NH_2$

Scheme 3

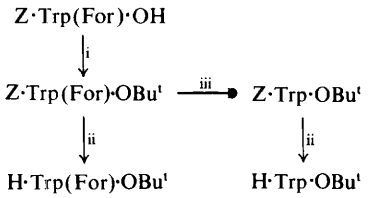
serine, and hydroxyproline to be prepared.[43] The *Z* amino-acid is treated with diketene in dichloromethane to give the side-chain protected acetoacetyl derivative, treatment with isobutene in the presence of acid in the usual way gave the corresponding blocked t-butyl ester. The acetoacetyl function could then be

[39] I. J. Galpin, P. M. Hardy, G. W. Kenner, J. R. McDermott, R. Ramage, J. H. Seely, and R. G. Tyson, *Tetrahedron*, 1979, **35**, 2577.
[40] V. Bocchi, G. Casnati, A. Dossena, and R. Marchelli, *Synthesis*, 1979, 957.
[41] V. Bocchi, G. Casnati, A. Dossena, and R. Marchelli, *Synthesis*, 1979, 961.
[42] K. Ueda, *Bull. Chem. Soc. Jpn.*, 1979, 1879.
[43] H. Kinoshita, H. Ishikawa, and H. Kotake, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3111.

removed by treatment with a two-fold excess of hydrazine giving the *Z* amino-acid t-butyl ester.

Side-chain Protection and Reaction of Amino-acids. A paper reporting the use of minimal side-chain protection in the synthesis of enkephalins and LH–RH analogues by the solid-phase method has appeared.[44] The paper advocates that tyrosine, serine, and arginine should remain unprotected as no modification of these groups occurred even in the presence of a three-fold excess of the acylating species. Histidine, however, was protected as the dinitrophenyl derivative. Although successful in this case, it seems doubtful whether a solid-phase synthesis employing HF for final deprotection would be successful when more ambitious sequences were tackled, particularly if acylation-termination steps were required.

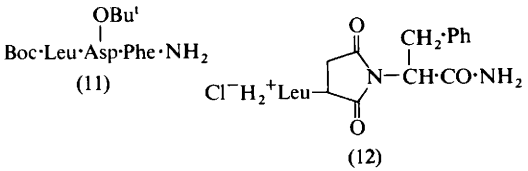
Further studies on the removal of t-butyl-based protection by trifluoroacetic acid has confirmed the fact that *C*-t-butylation of tyrosine takes place to an extent of 0.5—1% in 30 min, and that the butylation proceeds through t-butyl trifluoroacetate, which is formed during the acidolysis. In all cases 3′-t-butyl tyrosine was the major product.[45] The use of N^{in}-formyl protection for tryptophan [46] has been found to prevent t-butylation during acidolysis. Also this has been used in the preparation of tryptophan t-butyl ester in good yield. The reaction sequence shown in Scheme 4 was utilized for the preparation of the tryptophan derivatives indicated. Care must be used, however, when hydrogenolysis is carried out, as the 2,3-dihydroindole may be produced as a side product.



Reagents: i, $Me_2C{=}CH_2{-}H^+$; ii, H_2/Pd; iii, OH^-

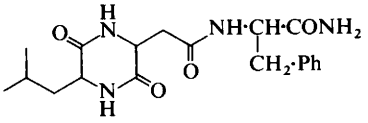
Scheme 4

The rearrangement of aspartyl peptides and the concommitant formation of aminosuccinyl peptides has once again been examined. It was found [47] that the tripeptides (11) were rearranged by treatment with 4M-HCl in ethyl acetate giving the aminosuccinyl peptide (12). This compound converts to the *β*-linked tripeptide during chromatography on silica gel using ethyl acetate–pyridine–acetic



[44] D. H. Coy and N. Branyas, *Int. J. Pept. Protein Res.*, 1979, **14**, 339.
[45] B. F. Lundt, N. L. Johansen, and J. Markussen, *Int. J. Pept. Protein Res.*, 1979, **14**, 344.
[46] M. Löw and L. Kisfaludy, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 13.
[47] I. Schoen and L. Kisfaludy, *Int. J. Pept. Protein Res.*, 1979, **14**, 485.

acid–water (60 : 20 : 6 : 11). In contrast, when trifluoroacetic acid was used no imide was formed. Conversion of the aminosuccinyl tripeptide to the corresponding piperazine-2,5-dione (13) was observed in neutral solution. The rate of conversion



(13)

of related tripeptides to the corresponding piperazine-2,5-diones was found to be dependent on both steric and electronic factors at the carboxyl rather than the amino terminus of the tripeptide. Cyclopentyl esters[48] and cyclohexyl esters[49] have both been proposed as a means of suppressing succinimide formation, with particular application in solid-phase peptide synthesis. Both ester functions are stable to trifluoroacetic acid but are cleaved by hydrogen fluoride. It is important, however, that the cleavage be carried out at a low temperature (between −20 and 0 °C) as at higher temperatures considerable rearrangement may be observed (60% rearrangement at 20 °C). Under the conditions generally employed in solid-phase synthesis the cyclopentyl ester gave between 0.5 and 1% rearrangement per cycle, whereas the cyclohexyl ester gave <0.002%; with both esters triethylamine was more active in promoting rearrangement than di-isopropylethylamine.

The use of mesitylene-2-sulphonyl as a protecting group for arginine has been studied.[50] In syntheses of substance P and neurotensin, deprotection of the protected arginine function was achieved by treatment with methane sulphonic acid in the presence of anisole; the sulphoxide function used to protect methionine was reduced with mercaptoethanol after removal of the guanidine protecting group. It is important that the scavenging system, anisole–thioanisole–*o*-cresol (1 : 1 : 1 vol.) be used as this suppresses mesitylene sulphonation of tyrosine residues.

Sulphonyl derivatives have also been used for the protection of histidine. The N^{im}-*p*-methoxyphenylsulphonyl derivatives are the most useful in synthesis.[51] The group is rapidly cleaved by treatment with trifluoroacetic acid in the presence of dimethyl sulphide; quantitative cleavage is achieved in 40—60 min, in contrast to the toluene-*p*-sulphonyl group which is removed in 90 min under the same conditions. Unfortunately this protecting group is also removed by treatment with *N*-hydroxybenzotriazole–sodium hydroxide (1 M) in 1 h or partially removed by hydrazine in 24 h. In this case the position of substitution in the imidazole ring is not specified. However, as was reported in last years' report, specifically protected π- or τ-imidazole-substituted derivatives may be prepared. Further information on these compounds has now appeared.[52] The *N*-π-phenacyl-protected histidine is used in

[48] J. Blake, *Int. J. Pept. Protein Res.*, 1979, 418.

[49] J. P. Tam, T.-W. Wong, M. W. Riemen, F.-S.Tjoeng, and R. B. Merrifield, *Tetrahedron Lett.*, 1979, 4033.

[50] H. Yajima, K. Akaji, K. Mitani, N. Fujii, S. Funakoshi, H. Adachi, M. Oishi, and Y. Akazawa, *Int. J. Pept. Protein Res.*, 1979, **14**, 169.

[51] K. Kitagawa, K. Kitade, Y. Kiso, T. Akita, S. Funakoshi, N. Fujii and H. Yajima, *J. Chem. Soc., Chem. Commun.*, 1979, 955.

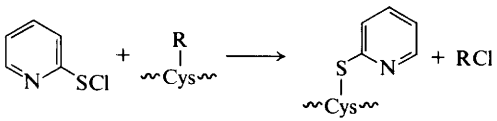
[52] A. R. Fletcher, J. H. Jones, W. I. Ramage, and A. V. Stachulski, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2261.

the synthesis of thyrotropin releasing hormone; final deprotection is achieved either by treatment with zinc in acetic acid or by photolysis. The effect of bulky N^{π}-substituents on imidazole ring substitution in *N*-alkylhistamine have also been studied,[53] some degree of successful direction being achieved using N^{π}-phthalyl protection. The stability of the 1-benzyloxycarbonylamino-2,2,2-trifluoroethyl derivative of histidine under the conditions of transesterification used in solid-phase synthesis has been studied.[54]

The rate of acylation of tyrosine in benzyloxycarbonyltyrosine ethyl ester using t-butyloxycarbonylglycine nitrophenyl ester has been studied again.[55] This present study showed that the rate of acylation was solvent dependent, increasing in the order dioxan > THF > ethyl acetate > DMF > chloroform > acetonitrile. Thus the dipolar aprotic solvents tend to reduce *O*-acylation. Acylation of tyrosyl, seryl, and threonyl hydroxyl functions is most pronounced in the active-ester couplings of histidine-containing peptides.[56] However, it has been found that the addition of 2,4-dinitrophenol or pentachlorophenol can minimize this unwanted acylation. It has been found[57] that *O*-sulphated tyrosine may be introduced directly as the tyrosine sulphate barium salt as the zwitterionic amino-acid may be prepared by hydrogenolysis of benzyloxycarbonyltyrosine-*O*-sulphate barium salt. The method was used to prepare (28-threonine, 31-leucine) CCKPZ (25—33).

The protection of cysteine has continued to receive attention. Studies of the removal of the trityl group have been carried out by two groups of workers. In one case, removal was by iodine in the presence of aqueous acetic acid[58] and in the second, iodine in methanol or DMF was used.[59] The first paper examined the mechanism of the cleavage in some detail. Once again the acetomidomethyl function has been found to be difficult to remove,[60] in a synthesis of porcine pancreatic secretory trypsin inhibitor-II only 70% of the thiol functions were liberated as indicated by Ellman reaction.

The removal of *S*-cysteine protecting groups using 2-pyridine sulphenyl chloride and subsequent formation of disulphide bonds has been reported.[61] The pyridine-sulphenyl chloride reacts with cysteine according to Scheme 5 giving the compound (14). The pyridinesulphenyl chloride is decomposed by moisture but is stable in



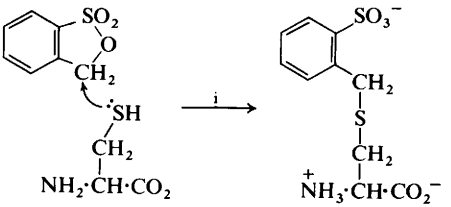
where R = Trt, Dpm, Acm, Bu^t, and SBu^t

Scheme 5

[53] J. C. Emmett, F. H. Holloway, and J. L. Turner, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1341.
[54] E. M. S. Salem, *Pol. J. Chem.*, 1979, 1341.
[55] S. K. Girin and Y. P. Shvachkin, *J. Gen. Chem. USSR* (*Engl. Transl.*), 1979, **48**, 1720.
[56] J. Martinez, J. C. Tolle, and M. Bodanszky, *Int. J. Pept. Protein Res.*, 1979, **13**, 22.
[57] L. Moroder, L. Wilschowitz, E. Jaeger, S. Knof, P. Thamm, and E. Wuensch, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, 787.
[58] W. Konig and K. Kernebeck, *Justus Liebigs Ann. Chem.*, 1979, 227.
[59] B. Kamber and W. Rittel, *Justus Liebigs Ann. Chem.*, 1979, 1928.
[60] R. Tomatis, M. Guarneri, A. Guggi, S. Salvadori, and R. Rocchi, *Int. J. Pept. Protein Res.*, 1979, **14**, 347.
[61] J. V. Castell and A. Tun-Kyi, *Helv. Chim. Acta*, 1979, **62**, 2507.

glacial acetic acid. Solutions of the compound are yellow and the colour is discharged by the addition of the *S*-substituted cysteine derivatives indicated in Scheme 5. Compounds of the type (14) are stable to acid showing no significant decomposition, but they are unstable to alkali as they disproportionate to give two symmetrical disulphides. In the presence of excess thiol the mercaptopyridyl moiety is removed leaving the free cysteine thiol function. In the presence of cysteine derivatives containing a free thiol function, compound (14) gave the anticipated unsymmetrical disulphide. In model studies of peptides containing both acetamidomethyl and benzyl cysteine residues, only the former was cleaved by pyridylsulphenyl chloride; the reagent did not interfere with the t-butyloxycarbonyl, benzyloxycarbonyl, or benzyl functions. However, some side-reactions were observed in the presence of tryptophanyl residues. In contrast to earlier work on the use of *o*-nitrosulphenylchloride it was found that the cleavage of *S*-substituted cysteine derivatives was rapid and quantitative, whereas cleavage with Nps chloride [62] had taken *ca.* 10 min; also the cysteine Nps mixed disulphide was much less reactive in unsymmetrical disulphide-bond formation.

The 2-sulphobenzyl protecting group has been proposed as a reversible protecting group with solubilizing properties for cysteine.[63] The derivative is prepared by alkylation of cysteine under mildly alkaline conditions with benzylsultone as shown in Scheme 6. The derivative is very soluble in water and

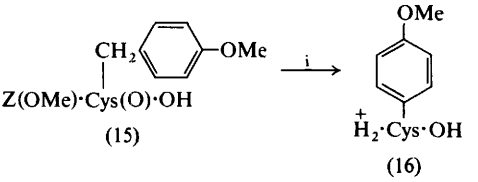


Reagents: i, Mild akaline

Scheme 6

resistant to acid hydrolysis, it is not cleaved by hydrogen fluoride in 2 h at 20 °C. However, it is removed by treatment with sodium in liquid ammonia. The group was used in syntheses of fragments of the insulin A- and B-chains. The behaviour of *S*-substituted cysteine sulphoxides under acidolytic deprotection conditions has been examined.[64] Deprotection of the sulphoxide (15) under reductive conditions (sodium in liquid ammonia) gave free cysteine but treatment of this cysteine derivative with hydrogen fluoride or methane sulphonic acid in the presence of anisole gave (16) (see Scheme 7). If the *p*-methoxybenzyl group is replaced by a simple benzyl function then no cleavage of the side-chain function is observed using sodium in liquid ammonia or acidic media. This work certainly suggests that *S*-substituted cysteine sulphoxides must be eliminated before the final deprotection step whether they have been used for side-chain protection in the synthesis or have arisen by an oxidative side reaction; removal is best achieved by treatment with thiophenol.

[62] L. Moroder, F. Marchiori, G. Borin, and E. Scoffone, *Biopolymers*, 1973, **12**, 493.
[63] U. T. Rüegg, D. Jarvis, and J. Rudinger, *Biochem. J.*, 1979, **179**, 127.
[64] H. Yajima, S. Funakoshi, N. Fujii, K. Akaji, and H. Irie, *Chem. Pharm. Bull.*, 1979, **27**, 1060.

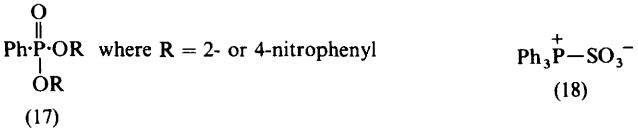


(15) (16)

Reagents: i, HF or $MeSO_3H$–amide

Scheme 7

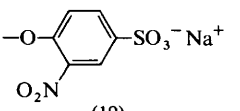
Formation of the Peptide Bond.—Phenylphosphonic esters of the type (17) have been used as coupling reagents.[65] These esters form mixed anhydrides which are converted directly into the dipeptides in high chemical yield although they are not completely free of racemization. The use of diethylphosphocyanidate in peptide coupling has also been reviewed.[66] Alkyltriphenylphosphonium halides, where the



(17) (18)

alkyl group is methyl or n-butyl, have been applied to peptide synthesis.[67] It is claimed that these reagents do not give racemization when coupling is carried out at 30 °C in the presence of pyridine. The triphenylphosphine–sulphur trioxide complex (18)[68] and several other sulphur trioxide adducts have been prepared. The reagent acts as a condensing agent in HMPA, DMF, DMSO, and pyridine but will not effect condensation in dichloromethane. This is due to the transferrence of sulphur trioxide to the solvent prior to activation of the carboxyl component. It is thus related to the DMF–SO_3 or pyridine–SO_3 complexes which have been used previously. Although the sulphur trioxide mixed anhydride was rapidly formed, a small degree of participation by the symmetrical anhydride was also observed in the absence of an amino component. Racemization was clearly base dependent; 8.9% is observed with tetramethylguanidine as base, but 32.4% is observed with lithium methoxide.

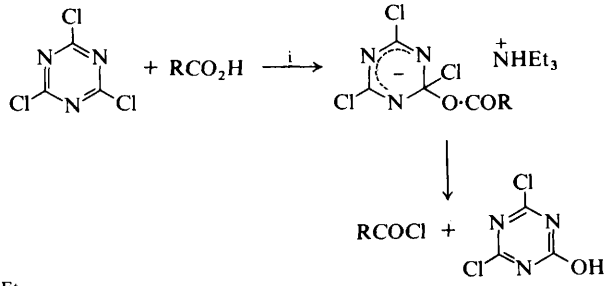
The use of *m*-nitrophenyl esters[69] and the water soluble 2-nitro-4-sulphophenyl esters[70] (19) have been reported. Both of these esters are prepared by reaction of the *N*-protected amino-acid with a corresponding phenol in the presence of dicyclohexylcarbodi-imide. The latter esters are generally used at pH = 8—9.



(19)

[65] T. Mukaiyama, N. Morito, and Y. Watanabe, *Chem. Lett.*, 1979, 1305.
[66] T. Shiori, *Yuki Gosei Kagaku Kyokai Shi*, 1979, **37**, 856.
[67] N. Yamazaki and M. Yamaguchi, *Synthesis*, 1979, **5**, 355.
[68] I. J. Galpin, G. W. Kenner, and A. Marston, *Bioorg. Chem.*, 1979, **8**, 323.
[69] H. Ito, *Synthesis*, 1979, 465.
[70] A. A. Gershkovich and S. B. Serebryany, *Bioorg. Khim.*, 1979, **5**, 1125.

Two reagents have been reported for preparing acyl halides for use as peptide-coupling reagents. The first, cyanuric-chloride [71] reacts with *N*-protected amino-acids to produce the complex shown in Scheme 8, which in turn decomposes to give the acid chloride. Similarly, 2-fluoro-1,3,5-trinitrobenzene was used to generate the

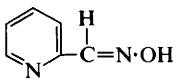


Reagents: i, NEt_3

Scheme 8

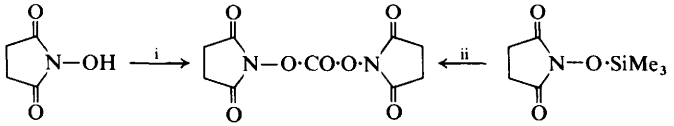
acyl fluoride from the corresponding amino-acid derivative.[72] Predictably, both methods gave racemization; however, 98% optically pure products were obtained when the latter reagent was used in the presence of two equivalents of hydroxy-succinimide. Clearly, however, both methods are of limited use as coupling reagents.

Cyclohexanone oxime [73] and picoline aldehyde oxime [74] (20) have both been used to prepare active esters. The latter compound is particularly interesting as the



(20)

rate of acylation is comparable to that of *o*-nitrophenyl esters and shows marked acid catalysis. A new method of introducing the most favoured of the *N*-hydroxy compounds, *N*-hydroxysuccinimide,[75] has been developed. The new reagent *N,N*-disuccinimidylcarbonate (21) may be prepared according to Scheme 9. The stable crystalline solid may be treated with *N*-protected amino-acids in the presence of pyridine at room temperature to give the *N*-hydroxysuccinimide ester; an additional mole of hydroxysuccinimide and carbon dioxide are liberated during this



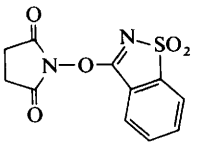
(21)

Reagents: i, $ClCO{\cdot}O{\cdot}CCl_3$; ii, $COCl_2$, 0 °C

Scheme 9

[71] K. Venkataraman and D. R. Wagle, *Tetrahedron Lett.*, 1979, 3037.
[72] H. Kinoshita, K. Inomata, O. Miyano, and H. Kotake, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 2619.
[73] P. Kumar and A. K. Mukerjee, *Synthesis*, 1979, 726.
[74] P. Y. Romanovskii, A. Y. Krikis, and G. I. Chipens, *J. Gen. Chem. USSR (Engl. Transl.)*, 1979, 1697.
[75] H. Ogura, T. Kobayashi, K. Shimizu, K. Kawabe, and K. Takeda, *Tetrahedron Lett.*, 1979, 4745.

reaction. In a one-pot coupling reaction no racemization was observed and excess reagent was decomposed by the addition of water with carbon dioxide and *N*-hydroxysuccinimide as the only by-products. The reagent (22) has also been proposed as a new means of introducing this active ester;[76] the ester is prepared in high yield using this compound. Alternatively, a one-pot procedure may be adopted, once again giving no racemization. The activated derivative of hydroxybenzotriazole (23) has been applied in peptide coupling. It appears to be an



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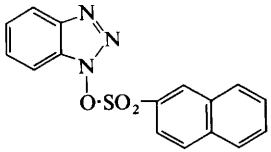


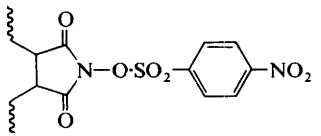
(23)

efficient reagent giving no side reactions and no racemization as determined by the Anderson test.[77]

A modified fragment coupling procedure has been developed;[78] in this particular instance a (6 + 3) fragment coupling was carried out using a ten-fold excess of dicyclohexylcarbodi-imide and a 20-fold excess of hydroxybenzotriazole in the presence of pentafluorophenol.

The REMA method has recently been applied to the synthesis of antanamide.[79] In this synthesis a 1.5—2-fold excess of Ddz amino-acids was used; activation was carried out for 8 min at −15 °C with s-butoxycarbonylchloride in the presence of *N*-methylmorpholine. An efficient synthesis was achieved, giving yields of 77—89% for each of the coupling steps.

A polymeric coupling reagent based on the co-polymer formed between styrene and *N*-hydroxymaleimide activated by a *p*-nitrobenzenesulphonate group has been reported.[80] The activation provided by the *p*-nitrobenzenesulphonate (24) gave a 90% coupling in 30 min. In the presence of one equivalent of pyridine 0.1%



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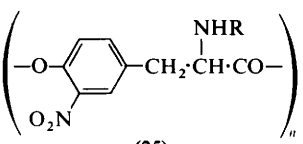


(25)

racemization was observed. This was lower than the 1% which was observed when *N*-methylmorpholine was used as base. Polymeric active esters based on 3-nitrotyrosine have also been reported.[81, 82] Active polymers of the type (25) were used to prepare an enkephalin analogue containing 3-nitrotyrosine in addition to other sequential polypeptides.

[76] K. Inomata, H. Kinoshita, H. Fukuda, O. Miyano, Y. Yamashiro, and H. Kotake, *Chem. Lett.*, 1979, 1265.
[77] B. Devadas, R. K. Pandey, and K. B. Mathur, *Indian J. Chem., Sect. B*, 1978, **16**, 1026.
[78] J. Hlaváček, T. Barth, K. Bláha, and K. Jošt, *Collect. Czech. Chem. Commun.*, 1979, **44**, 275.
[79] C. Birr, M. Nassal, and R. Pipkorn, *Int. J. Pept. Protein Res.*, 1979, 287.
[80] Y. A. Davidovitch and U. Ragnarsson, *Acta Chem. Scand., Ser. B*, 1979, 311.
[81] F. H. C. Stewart, *Aust. J. Chem.*, 1979, **32**, 661.
[82] F. H. C. Stewart, *Aust. J. Chem.*, 1979, **32**, 405.

A range of oxazolones have been prepared using the water-soluble carbodi-imide ethyl-γ-dimethyl aminopropyldi-imide hydrochloride in dichloromethane.[83] The method is advantageous as it does not suffer from the disadvantages encountered when acetic anhydride is used to form oxazolones. After washing with water and sodium carbonate, optically active oxazolones were obtained after a 15 min activation. The oxazolones are stable to sodium bicarbonate but their optical rotation in dichloromethane solution decreases at a rate of 1% per minute at 5 °C. The oxazolones racemized very slowly in chloroform, but even at −5 °C racemization was still observed. The paper also describes the preparation of peptide oxazolones although most examples of these are racemic.

Relative rates of coupling in liquid-phase peptide synthesis have been measured.[84] Table 1 shows the relative rate of acylation by mainly Boc amino-acids

Table 1 *Rates of acylation of H·*Ala*·polyethylene glycol by Boc·amino-acids relative to that for glycine*

Gly, Pro, Ala	80—100
Cys(Pr^i), Gln, Asp(OBzl), Tyr(Bzl), Phe, Met	55—80
Leu, Z·Arg(Z$_2$), Ser(Bzl), Lys(Z), His(Boc), Asn, Glu(OBzl)	30—55
Trp, Thr(Bzl), Val, Ile	<30

based on a variety of coupling methods; the species undergoing acylation is alanylpolyethylene glycol 6000. Clearly steric factors are important. Relative rates are not highly solvent dependent, the rate always being greatest when dimethylformamide is used as solvent. Although it is not revealed by these experiments, the conformation of the growing chain must also be an important feature in determining the rate of acylation.

Interest in enzyme-mediated reactions has continued to flourish. A highly unusual synthesis involving chain extension at the *C*-terminus using the support (26)[85] has been carried out. Extension of the chain was achieved using a water-soluble carbodi-imide at a pH = 6—8.5 with an appropriate amino-acid ethyl ester. The ester protection was then cleaved from the *C*-terminus by the use of an

[83] F. M. F. Chen, K. Kuroda, and N. L. Benoiton, *Synthesis*, 1979, 230.
[84] M. Mutter, *Int. J. Pept. Protein Res.*, 1979, **13**, 274.
[85] G. P. Royer and G. M. Anantharmaiah, *J. Am. Chem. Soc.*, 1979, **101**, 3394.

immobilized carboxypeptidase at pH = 8.5, leaving the chain free for extension at the carboxyl terminus. Final cleavage from the support was brought about by treatment with cyanogen bromide. As the esterase and peptidase pH optima are

$$PEG(CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}Met{\cdot}OH)_2$$

(26)

very different for this enzyme, ester cleavage was achieved without affecting the amide linkages, this gave optically pure H-Leu-Phe-Leu-OEt. On another occasion, however, slight racemization (0.7%) was observed. The method was demonstrated to be satisfactory when side-chain protected amino-acids were used. Thermolysin has been used to catalyse condensation of *N*-substituted aspartic and glutamic acids with phenylalanylalkyl esters.[86] Coupling was achieved between pH = 6—8 at 30 °C over 3—5 h. Only the L-isomer of the ester salt was found to react and only the α-carboxyl of side-chain free aspartic acid was coupled using the enzyme. Papain has also been used to catalyse peptide bond formation,[87] the enzyme is highly sensitive to pH (5.6 is optimal), and the addition of alcohols appears to give rise in general to increased yields which lie between 51 and 96% over a three day reaction. Low salt concentrations are favoured and only citrate buffer was found to be successful in synthesis; the presence of 10% trifluoroethanol totally inhibited the enzyme. Relatively few successful syntheses were achieved and on a few occasions unpredictable products were produced.

Racemization.—A series of lysyl di- and tri-peptide derivatives have been made as model systems in racemization studies.[88, 89] The dipeptides of general structure (27)

$$Ph{\cdot}CO{\cdot}X{\cdot}Lys(Z){\cdot}OMe$$

(27)

where X = Ala, Leu, Val, Ile, Phe, or Pro

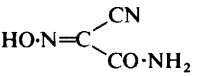
were formed by coupling the appropriately protected amino-acids with the lysine derivative shown, using trithylamine as base and EEDQ as the coupling reagent. Saponification with NaOH (1 M) in tetrahydrofuran for 30 min, followed by catalytic hydrogenation, gave the free dipeptide. The coupling conditions for reagents in question were studied by examination of the dipeptide diastereoisomers, either by amino-acid analysis [88] or by n.m.r. spectroscopy.[89] In the n.m.r. spectrum no separation was obtained for leucyl dipeptides with a variety of amino-protecting groups. The benzoyl group at the amino-terminus is clearly required in order to maximize the separation of the n.m.r. signals arising from each of the various diastereoisomers. In this work it was shown that benzoyl amino-acid could be coupled without racemization and that the dicyclohexylcarbodi-imide–hydroxybenzotriazole method was only racemization-free when dichloromethane is used as solvent. It is certainly not free of racemization when dimethyl formamide is used as solvent. Therefore when dimethyl formamide is used, it is preferable to use hydroxysuccinimide as the additive rather than the hydroxybenzotriazole.

[86] Y. Isowa, M. Ohmori, T. Ichikawa, K. Mori, Y. Nonaka, K. Kihara, K. Oyama, H. Satoh, and S. Nishimura, *Tetrahedron Lett.*, 1979, 2611.
[87] G. Anderson and P. L. Luisi, *Helv. Chim. Acta*, 1979, **62**, 488.
[88] N. L. Benoiton, K. Kuroda, S. T. Cheung, and F. M. F. Chen, *Can. J. Biochem.*, 1979, **57**, 776.
[89] N. L. Benoiton, K. Kuroda, and F. M. F. Chen, *Int. J. Pept. Protein Res.*, 1979, **13**, 403.

The ease of racemization of oxazolones has been under discussion,[83, 90] particularly since oxazolones can in fact be obtained from urethane-protected amino-acids. Although these oxazolones can be isolated they do not show the great tendency to racemize which is normally associated with 2-alkyl- or 2-aryl-oxazolones.

The racemization of amino-acid residues in a peptide chain at the penultimate *C*-terminal amino-acid has been studied,[91] as has the use of tritiated amino-acids for determining the level of racemization by a variety of coupling methods. The rate of *O*-acylurea and *N*-acylurea formation was monitored in these studies.[92, 93]

The compound (28) was examined as an additive in DCCl coupling.[94] It was found that on coupling benzoyl-leucine to glycine-ethyl ester in DMF or



(28)

dichloromethane a marked suppression of racemization was observed; the formation of by-products was also suppressed. Activation of protected amino-acids with t-butyl, n-hexyl, and cyclohexyl isocyanide appears to give rise to racemization.[95] However, in the presence of hydroxysuccinimide no racemization is observed as the amide bond-forming step appears to take place *via* the hydroxysuccinimide ester. In a Ugi four-component condensation[96] it was found that relatively high racemization occurred in DMF. However, by increasing the methanol concentration racemization was decreased. Similarly, lowering the temperature decreased the extent of racemization; in addition it increased yields considerably. In DMF solution containing methanol (0.1 M) at 0 °C only 0.6% racemization was observed for the Ugi reaction.

In a careful study of the racemization of cysteine peptides,[97] it has been shown that racemization does not occur through reversible β-elimination and that the α-proton never becomes fully detatched. For other amino-acids a correlation was found between the electron-withdrawing ability of the side chain and racemization. It was shown, however, that the cysteine sulphur atom has a special ability to stabilize a negative charge at the α-carbon atom, and that this interaction appears to occur through space across the intervening methylene group rather than by resonance. It is important therefore that base concentrations be carefully monitored when *S*-substituted cysteine derivatives are used in coupling, since racemization is particularly facile.

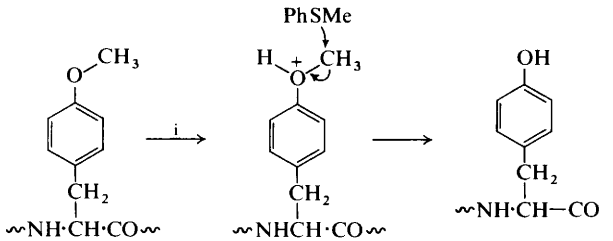
It has recently been found that amino-acid oxidases are clearly able to distinguish between D- and L-histidine.[98] This finding is in direct contrast to that of Gerwin *et*

[90] J. L. Fox, *Chem. Eng. News*, 1979, **57**, 20.
[91] M. Dzieduszycka, M. Smulkowski, and E. Taszner, *Pol. J. Chem.*, 1979, **53**, 1095.
[92] A. M. Kolodziejczyk and A. Arendt, *Pol. J. Chem.*, 1979, **53**, 611; (*Chem. Abs.*, 1979, **91**, 175715t).
[93] A. Arendt and A. M. Kolodziejczyk, *Pol. J. Chem.*, 1979, **53**, 791; (*Chem. Abs.*, 1979, **91**, 193623r).
[94] J. Izdebski, *Pol. J. Chem.*, 1979, **53**, 1049; (*Chem. Abs.*, **91**, 175728z).
[95] L. Wackerle, *Synthesis*, 1979, 197.
[96] M. Waki, Y. Minematsu, J. Meienhofer, and N. Izumiya, *Chem. Lett.*, 1979, 823.
[97] M. Barber, J. H. Jones, and M. J. Witty, *J. Chem. Soc., Perkin Trans. 1*, 1979, **10**, 2425.
[98] J. H. Jones and W. I. Ramage, *Int. J. Pept. Protein Res.*, 1979, 65.

al.[99] who had misquoted an earlier paper[100] on the enzymic determination of racemization of histidine. In order that the method may be used two important conditions must be observed. Firstly, the incubation with the enzyme must be short, and secondly, an accurate maintenance of pH must be observed. In addition, the enzyme is considerably inhibited by the presence of zinc ions, and in fact the presence of trace metals seems to be crucial if a reliable figure for racemization is to be obtained.

General Deprotection.—As has been mentioned earlier,[64] the use of *S*-benzylcysteine and *S*-*p*-methoxybenzylcysteine sulphoxides can give rise to problems on deprotection. However, these problems can be overcome[101] if the peptides are reduced before HF or methanesulphonic acid deprotection. It is recommended that the peptide sulphoxide should be treated with thiophenol (20 equivalents) at 85 °C for 6 h in order to achieve total reduction. Incomplete reduction is observed, however, if less rigorous conditions are employed and if other thiols are used as reducing agents.

The *O*-sulphonation of tyrosine during methanesulphonic acid deprotection of *p*-methoxybenzenesulphonylarginine-4-glutamine neurotensin has been studied.[102] The scavenging system anisole–thianisole–*o*-cresol (1 : 1 : 1) was found to suppress *O*-sulphonation although the liberated cations seemed to attack the tyrosine even in the presence of scavengers. The use of thioanisole–trifluoromethanesulphonic acid and thioanisole–methanesulphonic acid as deblocking agents has been studied.[103, 104] Deprotection of *O*-methyltyrosine is achieved by a push–pull mechanism (see Scheme 10). From this it can be seen that the sulphur atom of



Reagents: i, $CF_3 \cdot SO_3H$ or Me_3SO_3H

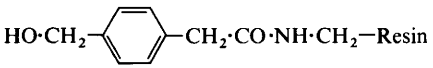
Scheme 10

thioanisole, which is a soft base, attacks the methyl group of protonated *O*-methyltyrosine, which is a soft acid, with the liberation of the free phenolic OH group. Under these conditions no migration from oxygen to the aromatic ring is observed.

[99] H. J. Goren, E. Katchalski-Katzir, and M. Fridkin, *Biopolymers*, 1977, **16**, 2265.
[100] H. V. Malmstadt and T. P. Hadjiioannou, *Anal. Chem.*, 1963, **35**, 14.
[101] S. Funakoshi, N. Fujii, K. Akaji, H. Irie, and H. Yajima, *Chem. Pharm. Bull.*, 1979, **27**, 2151.
[102] H. Yajima, K. Akaji, H. Saito, H. Adachi, M. Oishi, and Y. Akazawa, *Chem. Pharm. Bull.*, 1979, **27**, 2238.
[103] Y. Kiso, K. Ito, S. Nakamura, K. Kitagawa, T. Akita, and H. Moritoki, *Chem. Pharm. Bull.*, 1979, 1472.
[104] Y. Kiso, S. Nakamura, K. Ito, K. Ukawa, K. Kitagawa, T. Akita, and H. Moritoki, *J. Chem. Soc., Chem. Commun.*, 1979, 971.

It has been shown [105] that benzyl-based protecting groups may be removed from peptides by catalytic transfer hydrogenation using formic acid as the donor. This acid is an excellent solvent in contrast to other hydrogen donors such as cyclohexene or cyclohexadiene. Using palladium-black in 88% formic acid deprotection occurs within 30 s at room temperature. Nitro-arginine may also be cleaved using this system and methionine presents no problems. Using 4.4% formic acid in methanol, removal of benzylic-based protecing groups may be achieved in the presence of other acid-labile groups by passage of the compound dissolved in the solvent through a short column of palladium-black; the evolution of carbon dioxide does not interfere, and this type of deprotection may easily be automated. Trimethylsilyliodide has also been used to cleave benzyl-based protecting groups.[106] It is particularly useful for *O*-benzyltyrosine as no rearrangement can be observed under these conditions. Cleavage proceeds by S_N2 attack of iodide on the alkyl group with no carbonium ions formed. Thus t-butyloxycarbonyl and benzyloxycarbonyl may both be cleanly removed using this reagent. At the end of the reaction it is a straightforward matter to quench with methanol. Cleavage is efficiently achieved in the presence of *S*-benzylcysteine, nitroarginine, or benzylhistidine which are all unaffected by this reagent.

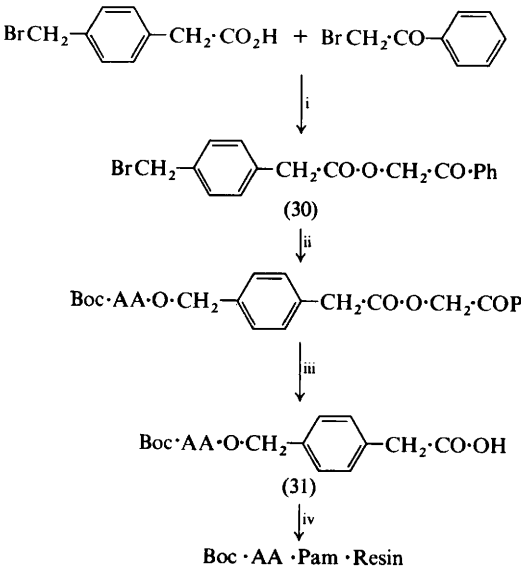
Repetitive Methods of Peptide Synthesis.—*Solid-phase Peptide Synthesis.* Two reviews of the solid-phase approach have appeared,[107, 108] the latter by Merrifield gives an up to date account of techniques which are used for the synthesis of polypeptides. The 4-(hydroxymethyl)phenylacetomidomethyl resin (Pam resin) (29) has been used for the synthesis of 4-Gln-neurotensin.[109] The increased acid



(29) Pam Resin

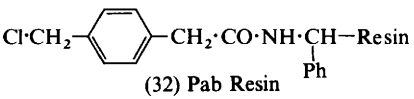
stability in comparison to the conventional Merrifield resin gave a higher yield and fewer purification steps were required. The *C*-terminal residue (leucine) was attached by reaction of Boc-leucine with Pam resin in the presence of carbonyldiimidazole; the resin is then acylated prior to continuing with the synthesis. Aminoacylated Pam resin may also be prepared by the route shown in Scheme 11.[110] Potassium fluoride in acetonitrile was used to catalyse the reaction forming the phenacyl derivative (30). The Boc-amino-acid is then added under similar conditions with the displacement of bromide; the carboxylic acid (31) generated by cleavage of the phenacyl function with zinc in acetic acid was then coupled to the amino-methylpolystyrene with dicyclohexylcarbodi-imide in the usual way. In this way a more efficient route to the amino-acyl Pam resin was achieved. An alternative resin, 4-chloromethylphenylacetomidobenzhydrylamine (Pab) polystyrene has been put forward.[111] The Pab resin (32) is derived from a benzhydrylamine polymer

[105] B. ElAmin, G. M. Anantharamaiah, G. P. Royer, and G. E. Means, *J. Org. Chem.*, 1979, **44**, 3442.
[106] R. S. Lott, V. S. Chauhan, and C. H. Stammer, *J. Chem. Soc., Chem. Commun.*, 1979, 495.
[107] S. Tamagaki, *Kagaku Kogaku*, 1979, **34**, 342.
[108] R. B. Merrifield, *Pure Appl. Chem.*, 1978, **50**, 643.
[109] D. Chang, G. Rackur, and K. Folkers, *Bioorg. Chem.*, 1979, 41.
[110] J. P. Tam, S. B. H. Kent, T. W. Wong, and R. B. Merrifield, *Synthesis*, 1979, 955.
[111] E. Giralt, D. Andreu, and L. Rafecas, *Tetrahedron Lett.*, 1979, 3587.



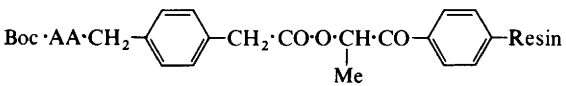
Reagents: i, KF–MeCN; ii, Boc·AA·OH–KF; iii, Zn–HOAc; iv, DCCI–aminomethyl resin

Scheme 11

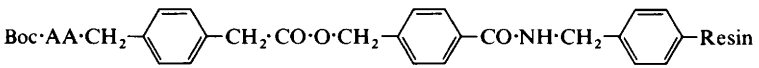


(32) Pab Resin

and is comparable to the Pam resin. However, it is claimed that the method of preparation in this case is simpler and that the rate of HF cleavage at the end of the synthesis is comparable to that found for the benzhydrylamine polymer; the difference is that partial acid cleavage during the synthesis is avoided. The Pop (33) and Pon (34) resins have been put forward as successors to the Pam resin.[112] Using



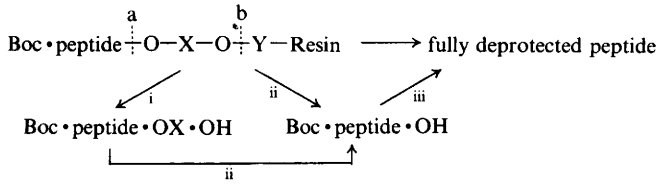
(33) Boc·AA·Pop·Resin



(34) Boc·AA·Pon·Resin

the Pop and Pon resins, a variety of alternative cleavages are available (see Scheme 12). Two selectively cleavable bonds occur between the amino-acid and the resin. Thus orthogonal cleavage may be achieved. Bond *a* is acid labile and is a normal hydrogenolysable benzyl ester. Bond *b* on the other hand may be cleaved by

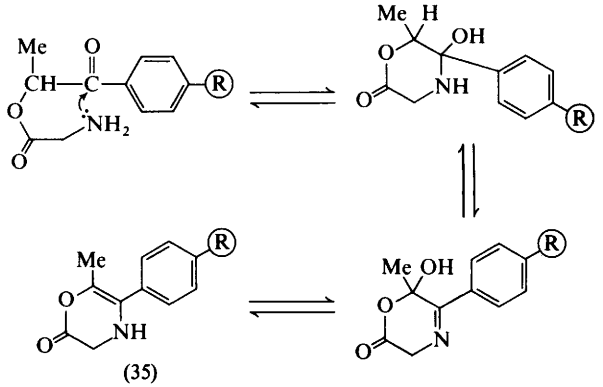
[112] J. P. Tam, F. S. Tjoeng, and R. B. Merrifield, *Tetrahedron Lett.*, 1979, 4935.



Reagents: i, *hν* or RSH; ii, CN^- or OH^-; iii, H^+

Scheme 12

unsolvated nucleophiles such as cyanide. The use of an oxymethylphenylacetic acid spacer in contrast to the straightforward phenacyl resin prevents dihydrooxazanone (35) formation when the phenacyl carbonyl is attacked by the amino-acid amino function [113] (see Scheme 13). This side reaction could be minimized by addition of the two *C*-terminal residues as a protected dipeptide.

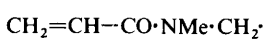


Scheme 13

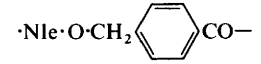
Solid-phase synthesis on a polyamide support has now become a viable alternative to the conventional polystyrene-based polymers.[114] These resins, which may be used for both polypeptide and polynucleotide synthesis give near quantitative yields of products; the properties of the matrix is directly comparable to that of the growing chain. The resins swell to a greater extent than the polystyrene resins, for example, the dimethyl acetamide-based resins show a ten-fold increase in volume on solvation. Such a polar support has been prepared [115] by emulsion co-polymerization of ethylene bis-acrylamide, dimethyl acrylamide, and the monomer (36). The resin is converted to a usable form by reaction with excess ethylenediamine. This resin was used in the synthesis of substance P and a nonadeoxyribonucleotide. The growing peptide chain was linked to the resin through a spacer unit (37). The synthesis also employed the base-labile Fmoc group for amino

[113] F. S. Tjoeng, J. P. Tam, and R. B. Merrifield, *Int. J. Pept. Protein Res.*, 1979, 262.
[114] E. Atherton, M. J. Gait, R. C. Sheppard, and B. J. Williams, *Bioorg. Chem.*, 1979, **8**, 351.
[115] R. Arshady, E. Atherton, M. J. Gait, K. Lee, and R. C. Sheppard, *J. Chem. Soc., Chem. Commun.*, 1979, 423.

protection. A basic polymer, containing a piperizine unit based on the polyacrylylmorpholine, (Enzacryl K2) has been developed for the cleavage of the Fmoc group.[116] The physical properties of the polymer were carefully selected to give

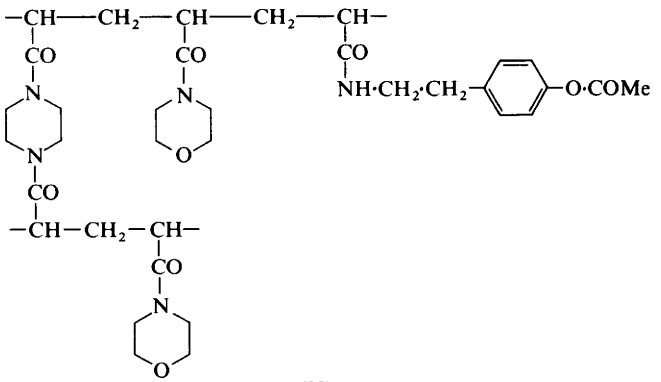


(36)



(37)

optimal cleavage of the Fmoc function. A phenolic polyacrylylmorpholine based resin has also been developed for use in solid-phase synthesis.[117] This resin, based on the unit (38) is highly solvated throughout; thus a gel is produced, which is used for peptide synthesis. The resin which swells readily in dimethylformamide,

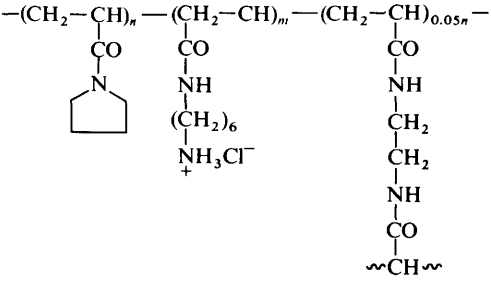


(38)

dimethylacetamide, and dichloromethane was used to prepare the Merrifield test peptide; a total yield of 65% of the fully deprotected peptide is obtained. The initial linkage to the resin was achieved by reaction of Boc-valine with the phenolic polymer in the presence of dicyclohexylcarbodi-imide and dimethylaminopyridine.

An entirely beaded polydimethylacrylamide support has been synthesized[118] by careful reverse-phase suspension polymerization. A beaded poly-*N*-acrylylpyrolidine resin has also been prepared by the reverse-phase suspension copolymerization method. The resulting polymer (Pap) (39) has been used in bidirectional synthesis of cysteine, histidine, glutamine, or glutamic acid-containing peptides. Boc-cysteine could be linked to the resin by coupling with a ten-fold excess of 1,6-dicyanatohexane to give an *S*-carbamoyl linkage.[119] Histidine was linked through 1,5-difluoro-2,4-dinitrobenzene. A more conventional linkage was achieved by using 4-chloromethylphenylacetic acid. Cleavage of peptide chains linked through this ester bridge was best achieved by three equivalents of sodium hydroxide in methanol. An oxytocin[120] analogue was synthesized in order to prove

[116] R. Arshady, E. Atherton, and R. C. Sheppard, *Tetrahedron Lett.*, 1979, 1521.
[117] R. Epton, P. Goddard, G. Marr, J. V. McLaren, and G. J. Morgan, *Polymer*, 1979, **20**, 1444.
[118] G. L. Stahl, C. W. Smith, and R. Walter, *J. Org. Chem.*, 1979, **44**, 3424.
[119] C. W. Smith, G. L. Stahl, and R. Walter, *Int. J. Pept. Protein Res.*, 1979, **13**, 109.
[120] G. L. Stahl, R. Walter, and C. W. Smith, *J. Am. Chem. Soc.*, 1979, **101**, 5383.



(39)

the potential of this resin. An isoluble non-cross-linked support for solid-phase synthesis has been developed.[121] The hydroxymethylphenylacetamidomethyl (Kel F-g-styrene) resin which is stable to acidolysis and cleaved by HF in the presence of anisole was used to prepare a small test peptide.

The mechanism of trifluoroacetylation during solid-phase synthesis has been examined.[122] Trifluoroacetylation was found to be independent of the coupling step and arose by transfer of the acyl moiety from trifluoroacetoxymethyl functions which are formed from hydroxymethyl groups during TFA treatment or during acidolysis of benzyl ester resin links. Transfer of trifluoroacetyl from the hydroxyl to amino functions occurs on neutralization with tertiary base. In a simulated acid–base cycle 1—2% trifluoroacetylation took place. It is interesting that such a trifluoroacetylation mechanism operates both with the hydroxymethyl resin (where there may be free OH groups) as well as on the Merrifield chloromethyl resin, when it is brought about by acidolysis. The use of the Pam resin mentioned above (34) prevented such trifluoroacetylation.

Deprotection and resin cleavage with trifluoromethanesulphonic acid has been reported.[123] The trifluoromethanesulphonic acid–anisole system was able to cleave all protecting groups with the exception of nitro-arginine which was only cleaved to an extent of 25%; remaining groups were all cleaved in excess of 92%. In the synthesis of a large peptide such incomplete deprotection (92%) may lead to considerable heterogeneity in the product.

The Merrifield test peptide has been used to study the use of the base-labile Fmoc protecting group in solid-phase peptide synthesis.[124] A *p*-benzyloxybenzyl ester resin was used and the Fmoc amino-acids were coupled as their symmetric anhydrides. Final cleavage of the tetrapeptides from the resin using 55% trifluoroacetic acid in dichloromethane gave a homogeneous product in 87% yield. Intermediate cleavage of the Fmoc function was brought about by treatment with 50% piperidine in dichloromethane. It was shown that under the conditions used for the removal of the Fmoc function, no cleavage from the resin was observed in 5 days. The use of a water–dioxane (1 : 2)wash facilitated decomposition of the intermediate

[121] S. B. H. Kent and R. B. Merrifield, *Isr. J. Chem.*, 1978, **17**, 243.
[122] S. B. H. Kent, A. R. Mitchell, M. Engelhard, and R. B. Merrifield, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2180.
[123] M. K. Chaudhuri and V. A. Najjar, *Anal. Biochem.*, 1979, **95**, 305.
[124] J. Meienhofer, M. Waki, E. P. Heimer, T. J. Lambros, R. C. Makofske, and C.-D. Chang, *Int. J. Pept. Protein Res.*, 1979, **13**, 35.

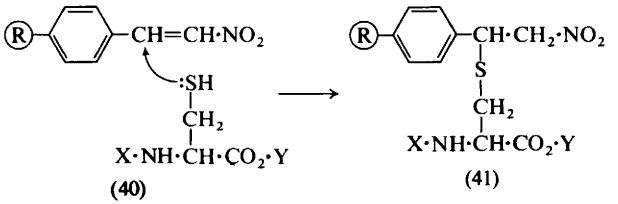
carbamate. Di-isopropylethylamine (1%) was added during the last 15 min of coupling in order to eliminate the possibility of protonation; such conditions do not give rise to deblocking of the Fmoc function at this concentration.

Fragment condensation on a solid support, after prior synthesis and purification of the fully blocked fragments, has been used in the syntheses of glycine valinomycin [125] and mast cell degranulating peptide.[126] The latter synthesis, which used the Ddz protecting group extensively, afforded biologically active MCD, representing a reasonable synthesis of this otherwise quite difficult peptide.

Techniques for Monitoring Solid-phase Synthesis. The Pam resin mentioned above (29) has been found to be particularly useful and to show high efficiency in the solid-phase Edman degradation of synthetic peptides.[127] The advantage of this resin in this application is its acid resistance, which is particularly important during Edman degradation. The use of the picrate method [128] for analysis during solid-phase synthesis has been used during the synthesis of histone fragments.[129] Either picric acid or imidazolium picrate can be used but it is very important that non-specifically absorbed picrate be removed. It appears that absolute levels of amino-groups are difficult to determine, but that highly consistent results may be obtained on repeated picrate titration. A quantitative method for the analysis of dithiasuccinyl amino-acids has also been published.[130]

The use of computer control in solid-phase peptide synthesis has been demonstrated.[131] Feedback control of deblocking or coupling cycles, based on perchloric acid titration or u.v. absorption allows the synthesis to be under the complete control of a computer. Automatic repetition of any particular cycle is carried out when the yield drops below a certain pre-determined value.

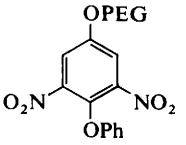
Other Repetitive Methods. Bidirectional peptide synthesis based on cysteine linked through a thiocarbamoyl linkage has been mentioned above.[119] A similarly applicable cysteine derivative may be obtained by addition of cysteine or a derivative to β-nitrostyrene;[132] addition to the polymeric styrene (40) (see Scheme 14) gives the resin-bound cysteine derivative (41) which may be extended at either the amino or carboxyl terminus depending on the nature of the protecting groups X



Scheme 14

[125] B. F. Gisin and A. R. Dhundale, *Int. J. Pept. Protein Res.*, 1979, **14**, 356.
[126] C. Birr and M. Wengert-Müller, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 147.
[127] G. R. Matsueda and M. N. Margolies, *FEBS Lett.*, 1979, **106**, 89.
[128] D. H. Live, W. C. Agosta, and D. Cowburn, *J. Org. Chem.*, 1977, **42**, 3556.
[129] D. E. Krieger, G. Vadali, B. W. Erickson, V. G. Allfrey, and R. B. Merrifield, *Bioorg. Chem.*, 1979, **8**, 409.
[130] G. Barany and R. B. Merrifield, *Anal. Biochem.*, 1979, **95**, 160.
[131] P. Villemoes, T. Christensen, and K. Brunfeldt, *Acta Chem. Scand., Ser. B*, 1978, **32**, 703.
[132] G. Heusel and G. Jung, *Justus Liebigs Ann. Chem.*, 1979, 1173.

and Y. Cleavage from the resin may be achieved in a few minutes by treatment with 2 M-sodium hydroxide in absolute ethanol, alternatively 0.5 M-sodium hydroxide will bring about the cleavage in under an hour. The polyethyleneglycol derivative (42) may be used for the reversible attachment of cysteine.[133] The phenoxide group

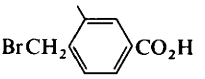


(42)

is rapidly displaced by the thiol function of cysteine but more slowly by other groups, thus a suitable cysteine derivative may be attached to the polyethyleneglycol-based support. The linkage is sensitive to thiols at pH = 7 (particularly mercaptoethanol) and has been used in the synthesis of bovine insulin B-chain. As might be expected the support is soluble in water and many organic solvents.

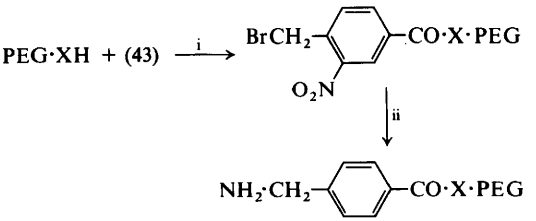
The original polyethyleneglycol support method has been used to prepare alanine and valine oligomers[134] and the undecapeptide alamethicin.[135] The growing chain achieved a helical conformation during synthesis which was carried out by active-ester addition of protected amino-acids. Although a model study used PEG 6000, it was found that PEG 10 000 gave better solubility properties when the larger peptide was synthesized.

Two groups of workers have used the spacer (43) in connection with polyethyleneglycol-based supports. The carboxylic acid (43) was coupled according



(43)

to the route shown in Scheme 15 to give the aminomethyl support (44).[136] The general liquid-phase approach was then used to build up the polypeptide chain on the amino-function of the support (44). Cleavage was achieved by photolysis at



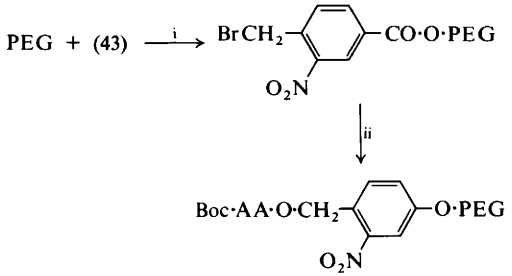
(44)

Reagents: i, DCCl; ii, potassium phthalimide, $NH_2 \cdot NH_2$ where X = O or NH

Scheme 15

[133] J. D. Glass, L. Silver, J. Sondheimer, C. S. Pande, and J. Coderre, *Biopolymers*, 1979, **18**, 383.
[134] G. M. Bonora and C. Toniolo, *Makromol. Chem.*, 1979, **180**, 2095.
[135] W. Mayr. R. Oekonomopulos, and G. Jung, *Biopolymers*, 1979, **18**, 425.
[136] V. N. R. Pillai, M. Mutter, and E. Bayer, *Tetrahedron Lett.*, 1979, 3409.

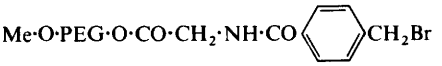
350 nm; up to a 90% cleavage is observed. Alternatively carboxylic acid (43) may be used in the way indicated in Scheme 16;[137] (45) is used to build up a peptide chain in the usual way. In this case cleavage may be effected by hydrogenolysis with a



Reagents: i, DCCl, 4-dimethylaminopyridine; ii, Boc·AA·OH, di-isopropylethylamine

Scheme 16

palladium catalyst in the presence of barium sulphate. An efficient cleavage may be achieved but other benzylic groups are also cleaved and the hydrogenolysis is precluded by the presence of sulphur. The modified support (46) has also been synthesized.[138] After the attachment of Boc-alanine by the caesium salt method the



(46)

chain was extended in the usual way using Boc-amino-acids and dicyclohexylcarbodi-imide; deprotection is by the use of 50% trifluoroacetic acid in dichloromethane. Deprotection with 1 M-sodium hydroxide in dioxane liberated the protected polypeptide; the remaining protecting groups are then cleaved by treatment with hydrogen fluoride in the presence of anisole. Purification by ion exchange on carboxymethyl cellulose gave the free decapeptide (47).

$$H \cdot Glu \cdot Arg \cdot Gly \cdot Phe_2 \cdot Tyr \cdot Thr \cdot Pro \cdot Lys \cdot Ala \cdot OH$$

(47)

The synthesis of a tripeptide flanked by two polyethyleneglycol chains has been reported.[139] The compound (48) is used in conformational studies.

$$EtO \cdot PEG \cdot CO \cdot CH_2 \cdot CH_2 \cdot CO \cdot Gly \cdot Trp \cdot Gly \cdot PEG \cdot OEt$$

(48)

Fragment condensation on a soluble chloromethylated resin using the azide method has also been reported.[140] Fragments were readily coupled and no steric hindrance was observed; this approach led to $<0.5\%$ racemization on fragment condensation.

[137] F.-S. Tjoeng and R. S. Hodges, *Tetrahedron Lett.*, 1979, 1273.
[138] B. Hemmasi, W. Woiwode, and E. Bayer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1775.
[139] M. Joppich and P. L. Luisi, *Makromol. Chem.*, 1979, **180**, 1381.
[140] M. Narita, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1229.

Synthesis of Polymeric Peptides.—The synthesis of Z-(β-isobutyl-aspartyl)$_n$ · OEt where n = 1—10 has been prepared by step-wise synthesis in order to carry out a conformational investigation.[141] The functionalisation of poly-γ-methylglutamate has been achieved [142] by reaction with ethylenechlorohydrin, ethylenecyanohydrin, or 2,2,2-trifluoroethanol. These reactive functional groups were aminolized in order to introduce a variety of other functional groups. A number of sequential and random co-polymers of lysine with aromatic amino-acids have been synthesized.[143] The syntheses used Boc for N^{α}-protection and Z for N^{ε}-protection of lysine. The di- and tri-peptide fragments were assembled using the dicyclohexylcarbodi-imide–*N*-hydroxysuccinimide method with *N*-ethylmorpholine as base. The di- and tri-peptide units were then linked together using hydroxysuccinimide active esters. The Z-functions were removed from the side chain at the end of the synthesis by treatment with HBr in acetic acid. The block co-polypeptide $(Leu_{67}, Lys_{33})_{100}Orn_{20}$ has been prepared.[144] The ornithyl portion of the molecule was prepared by polymerization of N^{ε}–TFA–ornithine *N*-carboxyanhydride initiated with *N*-propylamine. This fragment was coupled to the leucine–lysine co-polymer by the DCCl–HOSu method. After removal of protecting groups the conformation of this co-polypeptide was studied. Diastereoisomeric homo-oligopepties of L-isoleucine and D-alloisoleucine were prepared by stepwise elongation using the isobutylchloroformate mixed-anhydride method.[145] The peptides of general formula (49) were again used in conformational studies.

$$\text{Boc} \cdot (\text{AA})_n \cdot \text{OMe} \quad n = 2—6$$
(49)

The minimal critical chain-length for the formation of α-helical and β-pleated sheet conformation in a solid state has been studied.[146] Peptides of the type (50) and (51) were prepared by a combination of stepwise elongation using Nps-*N*-carboxyanhydrides and fragment coupling using the DCCl–HONSu method. The

$$\text{Nps} \cdot (\text{Leu} \cdot \text{Ala})_n \cdot \text{OEt} \quad n = 1—6 \text{ and } 8$$
(50)

$$\text{Nps} \cdot \text{Ala} \cdot (\text{Leu} \cdot \text{Ala})_m \cdot \text{OEt} \quad m = 1—5$$
(51)

i.r. and *X*-ray methods used showed that α-helical structure appeared at the decapeptide level, whereas β-pleated sheet conformation appeared at the heptapeptide level.

The mechanism of *N*-carboxyanhydride polymerization with the amino-acids glycine, alanine, and sarcosine has been studied [147] with triethylamine or pyridine used for initiation.

Purification Methods.—The use of ion-exchange chromatography, h.p.l.c., and reverse-phase h.p.l.c. continue to be important as a means of analysis and

[141] H. Yuki, Y. Okamoto, and Y. Doi, *J. Polym. Sci., Polym. Chem. Ed.*, 1979, **17**, 1911.
[142] A. Kudo, T. Endo, Y. Tosabayashi, and M. Okawara, *J. Polym. Sci., Polym. Chem. Ed.*, 1979, **17**, 789.
[143] G. Seipke, H.-A. Arfmann, and K. G. Wagner, *Biopolymers*, 1979, **18**, 855.
[144] P. Costantino, S. Verdini, P. DeSantis, R. Rizzo, and M. Savino, *Biopolymers*, 1979, **18**, 9.
[145] U. Widmer, G. P. Lorenzi, and P. Pino, *Biopolymers*, 1979, **18**, 239.
[146] R. Katakai, *J. Chem. Soc., Perkin Trans. 1*, 1979, 905.
[147] H. R. Kricheldorf, *J. Polym. Sci., Polym. Chem. Ed.*, 1979, **17**, 97.

purification for peptides. The separation of diastereoisomeric peptides by partition chromatography and the separation of D- and L-amino-acid derivatives using an optical phase have also been widely studied. References relating to the various forms of purification which have been employed are collected together in Table 2.

Table 2 *Purification methods*

Application	*Ref.*
h.p.l.c.	
Separation of proteins using h.p.l.c. on Spheron ion exchangers	148
Reverse-phase h.p.l.c. as a purity check in solid-phase peptide synthesis	149
H.p.l.c. separation of amino-acid enantiomers	150, 151
H.p.l.c. separation of intermediates in the synthesis of oxytocin	152
H.p.l.c. separation of oxytocin diastereoisomers	153
H.p.l.c. separation of opiate peptides	154
Separation of diastereoisomers by reverse-phase h.p.l.c.	155
g.l.c.	
Separation of amino-acid enantiomers	156, 157
Separation of amino-acid enantiomers on glass capillary columns	158
Ion exchange	
Separation of amino-acid enantiomers using a chiral eluant	159
Partition chromatography	
Purification of peptides by partition chromatography based on a hydrophobicity scale	160

Synthetic Operations with Peptides of Biological Origin.—The number of peptides which have been subjected to semisynthetic studies have continued to increase. A book by Offord entitled 'Semisynthetic Proteins' [161] summarizes the state of the art to date; it gives considerable experimental detail which is useful to newcomers to this area.

Horse heart cytochrome *c* (see also these Reports, Vol. 10, p. 340) has been further studied by three groups of workers; in each case the semisynthesis depends on initial cyanogen bromide cleavage at methionine-65 and/or methionine-80. In

[148] O. Mikes, *Int. J. Pept. Protein Res.*, 1979, **14**, 393.
[149] M. T. W. Hearn, C. A. Bishop, W. S. Hancock, D. R. K. Harding, and G. D. Reynolds, *J. Liq. Chromatogr.*, 1979, **2**, 1.
[150] G. Guebitz, W. Jellenz, G. Leufler, and W. Santi, *J. High Resolut. Chromatogr.*, 1979, **2**, 145.
[151] S. Hora and A. Dobashi, *J. High. Resolut. Chromatogr.*, 1979, **2**, 531.
[152] F. Nachtmann, *J. Chromatogr.*, 1979, **176**, 391.
[153] B. Larsen, B. L. Fox, M. F. Burke, and V. J. Hruby, *Int. J. Pept. Protein Res.*, 1979, **13**, 12.
[154] R. V. Lewis, S. Stein, and S. Udenfriend, *Int. J. Pept. Protein Res.*, 1979, **13**, 493.
[155] V. Viswanatha, B. Larsen, and V. J. Hruby, *Tetrahedron*, 1979, **35**, 1575.
[156] I. Abe, T. Kohno, and S. Musha, *Chromatographia*, 1978, **11**, 393.
[157] C. H. Lochmeuller and J. V. Hinshaw, jun., *J. Chromatogr.*, 1979, **171**, 407.
[158] G. J. Nickolson, H. Frank, and E. Bayer, *J. High Resolut. Chromatogr.*, 1979, **2**, 411.
[159] P. E. Hare and E. Gil-Av, *Science*, 1979, **204**, 1226.
[160] D. Yamashiro, *Int. J. Pept. Protein Res.*, 1979, **13**, 5.
[161] R. E. Offord, 'Semisynthetic Protiens', John Wiley and Sons Ltd., Chichester, 1979.

the first study[162-164] the (66—79) fragment was synthesized by solid-phase methods, and was used to introduce substitutions at positions 67 and 74. The (66—80), (81—104), or (66—104) fragments were treated with Boc-N_3, benzyloxycarbonyl chloride, *p*-nitrobenzyloxycarbonylazide, or *p*-methoxybenzyloxycarbonylazide to give the side-chain protected fragments. These protected fragments in combination with the synthetic (solid-phase synthesized) fragment were ultimately used to produce semi-synthetic cytochrome *c*. In the second study,[165] the methylsulphonylethoxycarbonyl (Msc) group was used to protect side chains. Cyanogen bromide cleavage of the protein gave the usual three fragments; the (81—104) fragment was isolated and then acylated with Boc-Met-ONSu to give the side-chain protected Boc-(80—104) fragment. This fragment was then condensed with fully synthetic protected (66—79) azide; the Msc groups were then removed by treatment with base to give the free (66—104) fragment. This was then acylated with Hse-65 cytochrome *c* (1—65) lactone. This coupling which is conformationally directed appears to provide a rapid route to modified cytochrome *c* although inevitably with this approach the methionine-65 is always replaced by homoserine. In the third piece of work,[166] lysine is protected by acetimidylation with methylacetimidate prior to cyanogen bromide cleavage; the resulting fragments were separated and further side-chain protection added where this was required. The (66—78) and (66—79) fragments of the protein, and the Asp-66 analogue of the (66—79) fragment were prepared. Also the *o*-fluorophenylalanine-82 analogue of the (81—104) fragment and the N^{ε}-trifluoroacetyl-Lys-79, N^{ε}-dinitrophenyl-Lys-79, and *S*-acetamidomethyl-Cys-79 analogues of the (79—104) fragment were prepared. *N*-terminal modification of fragments was achieved by removing the original *N*-terminal residue by Edman degradation and then replacing it with appropriately activated amino-acid derivatives. In all cases the homoserine residue, which was originally methionine-80 in the native protein, was removed by action of carboxypeptidase and the *N*-terminus of the (81—104) fragment was extended to include the methionine residue. Coupling of these fragments lead to the production of the (66—104) analogue sequences. One such sequence is coupled to the original homoserine lactone-65 (1—65) peptide with the production of a fully chemically and biologically characterized semisynthetic homoserine-65 cytochrome *c*.

Semisynthetic work on insulin and proinsulin has continued, des-B (22—30) porcine insulin has been prepared by the action of trypsin on porcine insulin.[167] Although DCCI can be used to couple the synthetic octapeptide which replaces the original (22—30) sequence, trypsin can also be used synthetically in this case. The des-octapeptide insulin was treated with Boc-azide to give N^{A1},N^{B1}-Boc_2-des-octapeptide insulin. This could then be coupled with conventionally synthesized octapeptide containing threonine at B-30 using trypsin in 0.025 M tris buffer in the presence of DMF at pH = 6.5 at 37 °C. The 20 h coupling gave a 58% conversion

[162] D. J. Ledden, P. T. Nix, and P. K. Warme, *Biochim. Biophys. Acta*, 1979, **578**, 401.
[163] A. K. Koul, G. F. Wasserman, and P. K. Warme, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 1253.
[164] P. T. Nix and P. K. Warme, *Biochim. Biophys. Acta*, 1979, **578**, 413.
[165] P. J. Boon, G. I. Tesser, and R. J. F. Nivard, *Proc. Natl. Acad. Sci.*, 1979, **76**, 61.
[166] C. J. A. Wallace and R. E. Offord, *Biochem. J.*, 1979, **179**, 169.
[167] K. Inouye, K. Watanabe, K. Morihara, Y. Tochino, T. Kanaya, J. Emura, and S. Sakakibara, *J. Am. Chem. Soc.*, 1979, **101**, 751.

to human insulin after deprotection with TFA and gel filtration chromatography. In a somewhat simpler approach [168, 169] residue B-30 was removed by treatment with carboxypeptidase and threonine *O*-t-butyl ester was coupled using trypsin. When a large excess of the threonine-t-butyl ester was used in organic co-solvents, a good yield of the human insulin was obtained. A range of methylsulphonylethoxycarbonyl insulins have been prepared.[170] Treatment of insulin with Msc-hydroxysuccinimide ester at pH = 5.8 gave a 60% yield of N^{A1},N^{B1} bis-Msc-insulin, and in DMSO the same reagent gave N^{A1},N^{B29} bis-Msc-insulin. Using N^{A1} citraconyl insulin N^{B1},N^{B29}, bis-Msc-insulin was ultimately obtained.

Bovine insulin analogues with methionine, arginine, or lysine at the N^{B1}-position have been synthesized using N^{A1},N^{B29} bis-Boc-insulin.[171] A five—ten-fold molar excess of the appropriately protected Boc-amino-acid active ester gave the N^{B1}-substituted insulin. In a study of des-(1—21)-preproinsulin [172] the mixed carbonic carboxylic mixed anhydride method was used to couple the BOC·Ala·Gln·Ala·tripeptide to proinsulin in 47% yield. Ideal conditions for growing semisynthetic bovine insulin crystals have also been described.[173]

Myoglobin has been converted into des-valine^1,$N^{\varepsilon 19}$-acetimidomethyl myoglobin by the selective removal of the valine residue.[174] This was achieved by treating $N^{\varepsilon 19}$-acetimidomyoglobin with 3-sulphophenylisothiocyanate. the haem unit is re-introduced after treatment with TFA to remove the valine residue. The semisynthesis of human grown hormone analogues[175] and the modification of the hormone by trinitrophenylation with picryl sulphonic acid at pH = 8.5 have been studied.[176] In the reactivity study it was found that the order of reactivity was the *N*-α-amino-function followed by the Lys-179, -143, -69, -111, -170, and -166 in decreasing order. In the bovine hormone residue-156 was unmodified in contrast to the equine hormone. The selective reduction of the disulphide bonds of ovine prolactin with dithiotreitol has been investigated.[177] Cleavage of all three disulphide bonds completely inactivates the molecule as evidenced by reduction and carbamidomethylation of the (4—11) and (191—199) disulphides. However, the reduction of the (4—11) bridge increases potency. After reduction of the (4—11) bridge the ovine prolactin gives a hormone which is more similar to the more active teleost hormone.

Reduction and re-oxidiation studies on (1—123) hen egg white lysozyme[178] and on bovine pancreatic trypsin inhibitor[179] have been carried out. The latter study,

[168] T. Oka and K. Morihara, Ref. 4 p. 79.
[169] K. Morihara, T. Oka, and H. Tsuzuki, *Nature (London)*, 1979, **280**, 412.
[170] A. Schüttler and D. Brandenburg, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1721.
[171] C. W. T. Yeung, M. L. Moule, and C. C. Yip, *J. Biol Chem.*, 1979, **254**, 9453.
[172] V. K. Naithani, E. E. Buellesbach, and H. Zahn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1363.
[173] S. M. Cutfield, G. C. Dodson, E. Schwertner, and H. Zahn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 783.
[174] R. D. DiMarchi, G. W. Neireiter, W. H. Garner, F. R. N. Gard, *Biochemistry*, 1979, **18**, 3101.
[175] C. H. Li and J. Blake, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6124.
[176] M. J. Biscoglio de Jimenez Bonino, O. Cascone, and J. A. Santome, *Int. J. Pept. Protein Res.*, 1979, **14**, 107.
[177] B. A. Doneen, T. A. Bewley, and C. H. Li, *Biochemistry*, 1979, **18**, 4851.
[178] A. Vizet, Y. Looze, J.-P. Perrauchin, J. Depreter, M. Deconinck, A.-G. Schnek, and J. Leonis, *Arch. Int. Physiol. Biochem.*, 1978, **86**, 972.
[179] G. Wagner, A. J. Kalb, and K. Wuethrich, *Eur. J. Biochem.*, 1979, **95**, 249.

which was carried out by ^{1}H n.m.r., investigated the influence of charged groups on the folding of the polypeptide.

The 4-pyridylmethyl group has been suggested as a masking function for thiols during semisynthesis.[180] The group which may be introduced by the reaction of cysteine residues with 4-pyridylmethyl chloride gives highly water soluble derivatives which are very stable to acid, but which may be removed by electrolytic cleavage. This protecting group has been used to mask the thiol function of the insulin A and B chains after reduction.

3 Syntheses Achieved

This section deals with peptides which have been prepared by either solution methods or solid-phase synthesis. As a very large number of syntheses have been reported, many appear only in the Appendices which follow this section. However, most major groups of peptides, particularly hormones, are covered here.

ACTH and MSH.—A total synthesis of *Squalus acanthias* ACTH[181] has been reported. The synthesis uses fragment coupling of the 1—10, 11—14, 15—19, and 20—39 fragments. The dicyclohexylcarbodi-imide–hydroxybenzotriazole method and the azide method have both been used. However, the relatively unusual 5-chloro-8-quinolyl and 4-oxymorpholinyl active esters were used in the assembly of some of the fragments. Benzylic protection was used with the exception of that for arginine, which was protected as the methoxybenzenesulphonyl derivative. At the end of the synthesis deblocking was achieved by treatment with hydrogen fluoride in the presence of anisole and 2% ethane dithiol; the product was then incubated with methanol–acetic acid at 60 °C for 14 h in order to remove dicyclohexylamido moiety which had become attached to the histidine imidazole function during coupling with dicyclohexylcarbodi-imide and hydroxybenzotriazole. Treatment with dithiothreitol (50 °C for 24 h) was also required in order to reduce the Met-sulphoxide. A number of ACTH analogues containing cysteine bridges have been prepared.[182] A solid-phase synthesis of the linear peptide with N^{in}-formyl for tryptophan protection was carried out with ultimate deprotection being achieved by HF treatment. After potassium ferricyanide oxidation, cyclic analogues were produced. The compound, which had a covalent structural lock, had reduced biological activity. Analogues in which the Trp-9 had been butylated have also been prepared[183, 184] in an attempt to enhance resistance to degradative enzymes and therefore enhance the steroidogenic activity. His[2,5-^{3}H]-6-ACTH (1—24) has been synthesized by reductive de-iodination of the corresponding di-iodo-histidine (1—24) peptide.[185] In this conventional synthesis both the dicyclohexylcarbodi-imide–hydroxybenzotriazole and the azide method were used. In the union of Glu(OBu^t)-5 to His(I_2)-6 in a (5 + 5) fragment coupling both methods gave very poor yields. In order to assemble this part of the hormone a (4 + 6) fragment

[180] U. T. Rüegg, D. Jarvis, and J. Rudinger, *Biochem. J.*, 1979, **179**, 119.
[181] K. O. Kamoto, K. Yasumura, S. Shimamura, M. Nakamura, A. Tanaka, and H. Yajima, *Chem. Pharm. Bull.*, 1979, **27**, 499.
[182] J. Blake, A. J. Rao, and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, 346.
[183] N. Löw, L. Kisfaludy, and M. Sárközi, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1.
[184] M. Sajgó, M. Löw, and L. Kisfaludy, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 9.
[185] M. C. Allen, D. E. Brundish, and R. Wade, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2057.

coupling was finally chosen as this gave a slightly better yield; but even then only a 28% yield using the azide method was achieved. The poor couplings in this sequence were not due to steric hinderance but were thought to be due to a possible side reaction which may occur when unprotected serine is used in the presence of histidine.

The synthesis of tritiated Tyr-2-α-MSH through the di-iodo-Tyr-α-MSH has been reported; the synthesis is achieved by standard step-wise methods and fragment condensation.[186] In a synthesis of Leu-9-α-MSH,[187] which employed solution synthesis and the azide method for coupling fragments, base-labile side-chain and *C*-terminal protecting groups were used. The toluenesulphonylethoxy group was used for carboxyl protection and side-chain amino-functions were protected as methanesulphonyloxycarbonyl derivatives. These groups which were stable during the hydrogenolysis of benzyloxycarbonyl groups used during the extension of the main chain were removed at the end of the synthesis by β-elimination under alkaline conditions in 10 min.

Enkephalins and Endorphins.—Several syntheses of enkephalins have been reported.[188–192] A full report has appeared of the synthesis of compound (52) and 25 other related analogues, usually containing methionine sulphoxide.[189] The

$$H \cdot Tyr \cdot \underset{D}{Ala} \cdot Gly \cdot (Me)Phe \cdot Met(O) \cdot ol$$

(52)

syntheses employed fundamentally simple solution techniques, the Boc-group was used for amino-protection and the mixed anhydride method for assembling fragments, which were combined by the dicyclohexylcarbodi-imide—hydroxybenzotriazole method. Isosteric analogues[190] have been synthesized in which the amide linkages are replaced by ethylene or aminomethylene linkages. The synthesis of these compounds was carried out using a phenolic polymer; the final product is removed by transesterification with dimethylaminoethanol in DMF. The resulting ester is hydrolysed at pH = 9.7. This phenolic polymer gives no loss from the resin during acidolysis in contrast to the basic Merrifield polymer and gave good yields of both linear and cyclic enkephalin analogues.

The compounds (53) and (54)[191] have been prepared for photoaffinity labelling studies. The compound (54) is attached to a cross-linking group after the removal of

$$Boc \cdot Tyr \cdot \underset{D}{Ala} \cdot Gly \cdot Phe(4\text{–}NO_2) \cdot Leu \cdot NH_2$$

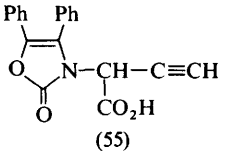
(53)

$$Boc \cdot Tyr \cdot \underset{D}{Ala} \cdot Gly \cdot Phe \cdot Leu \cdot Lys(Z) \cdot NH_2$$

(54)

[186] A. Eberle and W. Huebscher, *Helv. Chim. Acta*, 1979, **62**, 2460.
[187] A. Eberle and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 2452.
[188] B. Filippi, P. Giusti, L. Cima, G. Borin, F. Ricchelli, and F. Marchiori, *Int. J. Pept. Protein Res.*, 1979, **14**, 34.
[189] J. Pless, W. Bauer, F. Cardinaux, A. Closse, D. Hauser, R. Huguenin, D. Roemer, H.-H. Buescher, and R. C. Hill, *Helv. Chim. Acta*, 1979, **62**, 398.
[190] D. Hudson, G. W. Kenner, R. Sharpe, and M. Szelke, *Int. J. Pept. Protein Res.*, 1979, **14**, 177.
[191] J. V. Castell, A. N. del Eberle, V. M. Kriwaczek, A. Tun-Kyi, P. W. Schiller, K. Q. Do, P. Thanei, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 525.

the benzyloxycarbonyl side-chain protecting group on lysine. The highly fatty amino-acids carboranylalanine, adamantanylalanine, and t-butyl glycine have been incorporated into enkephalin.[192] In the synthesis of the carboranylalanyl analogues the 4,5-diphenyl-4-oxazoline-2-one derivative of propargyl glycine (55) was used as an intermediate as Boc-carboranylalanine nitrophenyl ester is unstable. But using the propargyl derivative (55), the peptide could be assembled and then



(55)

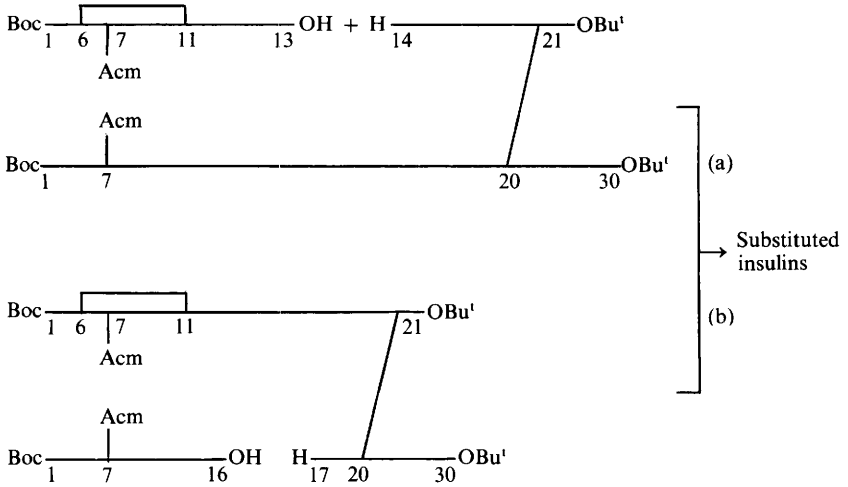
treated with decaborane to give the carboranylalanyl peptide. The oxazolinone function could not be removed by reduction or by treatment with hydrogen fluoride; however, straightforward hydrolysis with hydrochloric acid gave the free amine hydrochloride.

A review of the chemistry of β-endorphin [193] and the synthesis of endorphin analogues [194–196] have appeared. The conventional synthesis of α- and γ-porcine endorphins have been described.[194] The syntheses used a fragment-condensation approach with benzylic-based side-chain protection; methionine is protected as its sulphoxide. Final deprotection was achieved with methanesulphonic acid; the methionine sulphoxide function is converted into the thioether with mecaptoethanol. The synthesis of three analogues of β-endorphin in which disulphide bridges have been introduced in order to provide a more rigid conformation have been reported.[195] Interestingly, a (17—26) or (11—26) disulphide bridge gave fully biologically active peptides (guinea-pig ileum assay), whereas the analogue containing a (7—26) bridge showed reduced activity. However, none of these analogues showed significant immunoreactivity in radioimmunoassay. This was thought to be due to restriction of the ability of the solid-phase synthesized material to fold into its correct conformation. In another study it was found that deletion of a single amino-acid, Leu-14 or Asp-20,[196] totally abolishes immunoreactivity, whereas opiate potency is retained.

Insulin.—The Second International Symposium on Insulin was held at Aachen, where the chemistry, structure, and function of the hormone were discussed.[197]

Insulin analogues with single amino-acid replacements at the 2, 5, 6, 7, 8, and 11 positions in the A-chain and 5, 7, 13, and 16 positions in the B-chain have been synthesized using the selective disulphide-formation approach (see Scheme 17).[198] Two distinct approaches were used: in the first A10 modified (A1—13) fragments

[192] J.-L. Fauchere, O. Leukart, A. Eberle, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 1385.
[193] C. H. Li, *Adv. Biochem. Psychopharmacol.*, 1979, **20**, 145.
[194] M. Kubota, T. Hirayama, O. Nagase, and H. Yajima, *Chem. Pharm. Bull.*, 1979, **27**, 1050.
[195] J. Blake, W.-C. Chang, and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, 275.
[196] C. H. Li, W.-C. Chang, D. Yamashiro, and L.-F. Tseng, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 693.
[197] 'Insulin Chemistry, Structure and Function of Insulin and Related Hormones', 2nd International Symposium Aachen, ed. D. Brandenburg, Walter de Gruyter, London, 1980.
[198] F. Märki, M. de Gasparo, K. Eisler, B. Kamber, B. Riniker, W. Rittel, and P. Sieber, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1619.



Scheme 17

were coupled to the (A14—21–B1—30) disulphide; the second route, which allowed variation in the amino-terminal region of the B-chain, used condensation of (B1—16) with the (A1—21–B17—30) disulphide. A marked variation in potency was observed and little or no correlation between radioimmunoassay and biological activity was established; eight of the insulin analogues were obtained in crystalline form. A range of analogues containing D-cysteine residues at A6, A7, A11, A6 and 11, and B7 were synthesized by a related route using selective disulphide-bond formation.[199] Most of the analogues had low biological activity (up to a maximum of 1% in a glucose-oxidation assay in contrast to 7—70% in the antibody-binding assay). Synthesis of insulin, by use of fragment condensation in combination with solid-phase synthesis to give fragments which are used in selective formation of the disulphides, has also been reported.[200] The (1—3), (4—14), and (15—21) fragments were assembled on a 0.5% cross-linked polystyrene using Ddz-amino-acids. Gln-5, Gln-15, and Asn-18 were introduced as the corresponding benzyl esters, being converted later to the amides by aminolysis. Aspartic acid-21 is linked through the side chain to the resin by a 2-oxoethylester linkage. A 15-fold excess of the protected (1—3) fragment was coupled to the (4—14)) portion which was bound to the resin using the dicyclohexylcarbodi-imide–hydroxybenzotriazole method to give an 86% yield of the (1—14) fragment after cleavage from the carrier. The cleavage was achieved using 0.5 M-triethylamine in methanol–dioxane and 1 M-sodium hydroxide. The (15—21) fragment was then aminolysed from the resin and condensed with the (1—14) portion using the same method as previously; 4 days are required to give complete coupling in DMF. *S*-Butyl groups at A-6 and A-11 were then removed by tri-n-butylphosphine allowing selective disulphide-bond formation between cysteine at residues 6 and 11 by air oxidation. TFA was then used to remove t-butyl-

[199] K. Eisler, B. Kamber, B. Riniker, W. Rittel, P. Sieber, M. de Gasparo, and F. Märki, *Bioorg. Chem.*, 1979, **8**, 443.

[200] C. Birr and R. Pipkorn, *Angew. Chem., Int. Ed. Engl. 1979*, **18**, 536.

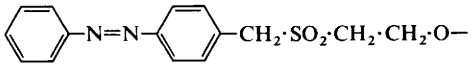
based protection and *S*-*p*-methoxybenzyl groups were removed by treatment with hydrogen fluoride in pyridine–anisole. The A-chain containing the free thiol was then dissolved in 30% acetic acid and reduced natural B-chain was added. After oxidation to form the A-20–B-19 disulphide the A-7–B-7 disulphide bridge was formed by iodine oxidation with concomitant removal of Acm-protection. The resultant insulin was crystallized as its zinc complex and had full biological activity.

A number of Lysine A-13A chain analogues have been reported.[201-204] The syntheses, which use standard solution methods, employed *S*-butyl for cysteine protection; the *S*-butyl function is directly converted to *S*-sulphonate by oxidative sulphitolysis at pH = 7.6. Thus, the A-chain analogues were obtained in the form of their tetra-*S*-sulphonates. In a detailed study of the *S*-sulphonates of the A- and B-chains [205] it was found that repeated sulphitolysis with sulphite and tetrathionite was required in order to obtain pure *S*-sulphonated fragments. After repeated sulphitolysis only one ion exchange column was required in order to obtain the pure B-chain *S*-sulphonate. In order to avoid the introduction of other thiols it was found that the *S*-sulphonates are best reduced with tri-n-butylphosphine giving the isolated chain in 95—100% yield with respect to the free thiol. Conditions for oxidation were also examined.

The *S*-(methoxycarbonylthio)-B-chain of porcine insulin has been prepared by treatment of the B-chain bis-*S*-sulphonate with methoxycarbonylsulphenyl chloride.[206] Reaction of this activated B-chain with thioglycolic acid or cysteine in acidic solutions gave unsymmetrical disulphides in 80% yield.

Somatostatin.—Two syntheses of somatostatin have been noted.[207, 208] The latter synthesis was carried out on a large scale to give the hormone in 5% overall yield. The synthesis was carried out in a stepwise fashion by active-ester coupling except that the terminal Ala-Gly dipeptide was added using the dicyclohexylcarbodiimide–hydroxybenzotriazole method. Acidic deprotection was carried out in the presence of 6% anisole and 10% ethane dithiol; the latter thiol is superior to mercaptoethanol in its scavenging properties. The synthesis which used the 2-methanesulphonylethoxycarbonyl protection for lysine also uses the base-labile group (56) for *C*-terminal protection as this is removed under the same conditions as the above MSE-group and is easily visible during t.l.c. and c.c.d. operations. The Acm-group which was used for sulphur protection was prepared from hydroxymethylacetamide, which in turn had been prepared from polyoxymethylene (paraformaldehyde) in place of the corresponding aqueous solution to give a highly improved preparation of the cysteine derivative. The Acm-group in this synthesis was removed by treatment with silver(I) in acetic acid.

[201] H.-G. Gielen, G. Wolf, H. Berndt, and H. Zahn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1535.
[202] G. Wolf, H. Berndt, and D. Brandenburg, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1549.
[203] W. Wolf, H. Berndt, and D. Brandenburg, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1559.
[204] G. Wolf, H. Berndt, and D. Brandenburg, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1569.
[205] R. C. Paynovich and F. H. Carpenter, *Int. J. Pept. Protein Res.*, 1979, **13**, 113.
[206] K. Nokihara and H. Berndt, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, 773.
[207] Y. P. Shvachkin, A. P. Smirnova, A. A. Shishkina, N. M. Ermak, V. P. Fedotov, I. S. Komolov, L. G. Morozoba, G. N. Pluzhnikova, and N. V. Sadovnikova, *Bioorg. Khim.*, 1979, **5**, 169.
[208] J. Diaz, R. Guegan, M. Baumont, J. Benoit, J. Clement, C. Fauchard, D. Galtier, J. Millan, C. Muneaux, Y. Muneaux, M. Vedel, and R. Schwyzer, *Bioorg. Chem.*, 1979, **8**, 429.



(56)

Thymic Peptides.—Several papers have described the syntheses of the serum thymic factor (57) and analogues [209-212], the peptides being prepared under a wide variety of solid phase and solution conditions. The (32—36),[213] (32—41),[214] and (33—41)[214] fragments of thymopoietin (thymic hormone) and the ubiquitous immunopoietic polypeptide [215] have also been prepared.

Glp · Ala · Lys · Ser · Gln · Gly_2 · Ser · Asn

(57)

Thymosin-α_1 has also received considerable attention.[216-218] A total synthesis of the 28 residue peptide being achieved [218] by fragment condensation of the (1—6), (7—12), (13—19), (20—24), and (25—28) fragments using the dicyclohexylcarbodiimide method with the addition of hydroxybenzotriazole. Z- and Ddz-protection was used for α-amino-functions, with side chains protected by t-butyl-based protecting groups. Stepwise assembly of fragments was achieved by the mixed anhydride method using isobutyl chloroformate and *N*-methylmorpholine. In this synthesis hydrogenolysis in 2,2,2-trifluoroethanol was used for particularly difficult peptide fragments.

Miscellaneous Peptides.—A convenient synthesis of human calcitonin which employs the repetitive excess mixed-anhydride method has been reported.[219] The (1—7), (8—10), (11—19), and (20—32) fragments were prepared by the REMA method and the fragments coupled by either the azide or the dicyclohexylcarbodiimide–hydroxybenzotriazole method. Protecting groups employed were removed by treatment with cold concentrated hydrochloric acid, as the paper describes the use of liquid hydrogen fluoride as 'giving disastrous results'. Final material was purified by h.p.l.c.

The peptide galline which contains repeating blocks of arginine has been synthesized by preparation of the related ornithine peptide.[220] The ornithyl peptides were amidated by treatment with 1-guanyl-3,5-dimethylpyrazole nitrate or *O*-methylisourea. In all cases essentially complete conversion to the guanidine function was observed. In order to prevent modification of the amino-terminus, the protecting group in this position should be retained while the amidation is carried out. Similar successful guanidation has been achieved using *O*-methylurea in the

[209] D. Blanot, J. Martinez, G. Auger, and E. Bricas, *Int. J. Pept. Protein Res.*, 1979, **14**, 41.
[210] T. Abiko, M. Kumikawa, S. Dazai, H. Sekino, and H. Higuchi, *Chem. Pharm. Bull.*, 1979, **27**, 2207.
[211] Y.-P. Wan and K. Folkers, *Bioorg. Chem.*, 1979, 35.
[212] R. G. Strachan, W. J. Paleveda, jun., S. J. Bergstrand, R. F. Nutt, F. W. Holly, and D. F. Veber, *J. Med. Chem.*, 1979, **22**, 586.
[213] G. Goldstein, M. P. Scheid, E. A. Boyse, D. H. Schlesinger, and J. van Wauwe, *Science*, 1979, **204**, 1309.
[214] T. Abiko, M. Kumikawa, and H. Sekino, *Chem. Pharm. Bull., 1979*, **27**, 2233.
[215] G. Goldstein and D. H. Schlesinger, *Belg. Patent* 864, 122; (*Chem. Abs.*, 1979, **90**, 138 206).
[216] T. Abiko, I. Onodera, and H. Sekino, *Chem. Pharm. Bull.*, 1979, **27**, 3171.
[217] S.-S. Wang, I. D. Kulesha, and D. P. Winter, *J. Am. Chem. Soc.*, 1979, **101**, 253.
[218] C. Birr and U. Stollenwerk, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 394.
[219] J. Hirt, P. Kranenburg and H. C. Beyerman, *Recl. Trav. Chim. Pays-Bas*, 1979, **98**, 143.
[220] F. Marchiori, G. Borin, B. Filippi, V. Moretto, G. M. Bonora, and C. Toniolo, *Int. J. Pept. Protein Res.*, 1979, **14**, 143.

preparation of apamin derivatives in which lysine-13 and -14 have been replaced by homoarginine.[221] The highly basic mast-cell degranulating peptide has also been synthesized.[222] Three fragments were synthesized with basic handle-type protecting groups for side-chain protection. The fragments were then condensed using dicyclohexylcarbodi-imide in the presence of hydroxybenztriazole. This method is preferred as in the case of the (1—6) fragment the hydrazide could not be converted into the corresponding azide. The pure fully protected peptide was obtained by gel filtration on Sephadex LH20.

A review of the chemistry of the gastrins has appeared [223] and the corrected sequence of porcine big gastrin has been published.[224] Using immunochemical methods with synthetic gastrin peptides [225] it was shown that the sequence error occured in the (7—9) region and that the originally proposed sequence of His-7, Pro-8, Pro-9 be revised to Pro-7, Pro-8, His-9 as fragments containing the latter sequence gave virtually full immunoreactivity whereas fragments containing the former sequence gave approximately 1000th of the correct immunoreactivity.

A synthesis of ribonuclease-12-[β-3-pyridylalanine]*S*-peptide has been reported.[226] The synthesis utilized solution methods with standard methods of fragment condensation; the resulting free peptide binds to the *S*-protein but gives an inactive complex. The synthesis of the (24—104) sequence of ribonuclease T_1 has also appeared.[227] Out of the total of 80 peptide bonds involved in this fragment 36% were formed by the azide method and 64% by the active ester method. The workers point out that triethylamine should be replaced by di-isopropylethylamine as base and that 1% ethanedithiol appears to give the best scavenging properties. As many of the fragments are highly insoluble, most of the couplings were carried out in dimethyl formamide or dimethyl sulphoxide, conformational factors are the major features which determine the yield of a coupling reaction. From the analytical data presented, it is clear that failure sequences are absent although as is stated some racemization cannot be excluded on the basis of the results obtained by digestion with APM and prolidase. The use of end-group analysis and diagnostic ratios point to the product being homogeneous and no evidence of α- to β-transpeptidation at the aspartyl-glycine linkage (29—30) was observed.

A series of papers [228–232] describe the approaches to, and the synthesis of, the

[221] B. E. B. Sandberg, *Int. J. Pept. Protein Res.*, 1979, **13**, 327.
[222] D. M. Bratby, S. Coyle, R. P. Gregson, G. W. Hardy, and G. T. Young, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1901.
[223] R. A. Gregory, *Bioorg. Chem.*, 1979, **8**, 497.
[224] G. J. Dockray, R. A. Gregory, L. Hood, and M. Hunkapiller, *Bioorg. Chem.*, 1979, **8**, 465.
[225] A. M. Choudhury, K. Y. Chu, G. W. Kenner, S. Moore, K. L. Ramachandran, and R. Ramage, *Bioorg. Chem.*, 1979, **8**, 471.
[226] I. Voskuyl-Holtkamp and C. Schattenkerk, *Int. J. Pept. Protein Res.*, 1979, **13**, 185.
[227] H. Romovacek, S. R. Dowd, J. Kawasaki, N. Nishi, and K. Hofmann, *J. Am. Chem. Soc.*, 1979, **101**, 6081.
[228] G. W. Kenner, R. Ramage, and R. C. Sheppard, *Tetrahedron*, 1979, **35**, 2767.
[229] I. J. Galpin, F. E. Hancock, B. K. Handa, A. G. Jackson, G. W. Kenner, R. Ramage, and B. Singh, *Tetrahedron*, 1979, **35**, 2771.
[230] I. J. Galpin, F. E. Hancock, B. K. Handa, A. G. Jackson, G. W. Kenner, R. Ramage, B. Singh, and R. G. Tyson, *Tetrahedron*, 1979, **35**, 2779.
[231] I. J. Galpin, G. W. Kenner, S. R. Ohlsen, R. Ramage, R. C. Sheppard, and R. G. Tyson, *Tetrahedron*, 1979, **35**, 2785.
[232] I. J. Galpin, F. E. Hancock, B. K. Handa, A. G. Jackson, G. W. Kenner, R. Ramage, and B. Singh, *Tetrahedron*, 1979, **35**, 2791.

(1—37) fragment of an analogue of hen egg white lysozyme. Full details are given of the build up of the (1—16), (17—26), (27—37), and (1—37) fully protected fragments. The overall approach to the synthesis, which has been described in preceding volumes of these Reports, is based on maximal protection with the use of acid-labile protecting groups for side-chain protection. *C*-Terminal protection is afforded by phenyl esters. The fully protected (1—37) fragment was shown to be homogeneous by a variety of techniques.

The synthesis of the (1—16) and (17—29) fragments[233,234] of the human leukemic lysozyme which has an additional glycine residue to 'normal' lysozyme between residues 47 and 48 have been prepared. The solution syntheses use active-ester and azide-coupling methods with t-butyl-based side-chain protection for glutamic acid and lysine, tosyl protection for arginine, and methoxybenzyl protection for cysteine. The fragments are prepared in preparation for a semisynthetic study of the human leukemic lysozyme.

4 Appendix 1: A List of Syntheses Reported during 1979

Natural Peptides, Proteins, Analogues, and Partial Sequences.—The syntheses are listed under the name of the peptide or protein to which they relate, as in previous years.

Peptide	*Ref.*
Actinomycin	
Actinomycin D and analogues	17
Adrenocorticotropic hormone (ACTH)	
ACTH-(1—24)-peptide	185
ACTH-(1—10)-, ACTH-(11—23)-peptide	235
[Trp(1-Bu^t)^9] and [Trp(2,3,7-Bu$^t_3$)^9]-ACTH	183, 184
ACTH Ava^2, Cys^5, Cys^{10}-(2—19)-peptide, Ava^2, Cys^3, Cys^{10}-(2—19)-peptide, and Gly^1, Cys^2, Cys^{10}-(1—19)-peptide	182
Dogfish ACTH-(1—39)-peptide	181
α-Amino-oxy analogues of ACTH	236
Adrenodoxin	
Bovine adrenodoxin-(50—56)-peptide and -(90—96)-peptides	237
Bovine adrenodoxin-(50—63)-peptide	238
Alamethicin	
Alamethicin α-helix	135
Alamethicin analogue	239
Amastatin	240
Angiotensin	
α-amino-oxy analogues of angiotensin	236
4-substituted analogues of [Asn^1, Ile^5]-angiotensin II	241
β-Homoamino acid analogues of angiotensin	242

[233] S. Yoshimura, K. Izumi, and Y. Shimonishi, *Bull. Chem. Soc. Jpn.*, 1979, 1672.
[234] S. Yoshimura and Y. Shimonishi, *Bull. Chem. Soc. Jpn.*, 1979, 1677.
[235] H. Mutter, M. Mutter, and E. Bayer, *Z. Naturforsch., Teil B*, 1979, **34**, 874.
[236] L. Kisfaludy, M. Low, I. Schon, and O. Nyeki, *Acta Chim. Acad. Sci. Hung.*, 1979, **100**, 101.
[237] Y. Okada, C. Kawasaki, M. Okinaka, and K. Kawasaki, *Chem. Pharm. Bull.*, 1979, 1360.
[238] Y. Okada, M. Okinaka, Y. Tsuda, and K. Kawasaki, *Chem. Pharm. Bull.*, 1979, **27**, 3015.
[239] R. Oekonomopulos and G. Jung, *Justus Liebigs Ann. Chem.*, 1979, 1151.
[240] H. Tobe, H. Morishima, H. Naganawa, T. Takita, T. Aoyagi, and H. Umezawa, *Agric. Biol Chem.*, 1979, **43**, 591.
[241] K. Hsieh, I. C. Kiraly-Olah, E. C. Jorgensen, and T. C. Lee, *J. Med. Chem.*, 1979, **22**, 1044.
[242] K. Stachowiak, M. C. Khosla, K. Plucinska, P. A. Khairallah, and F. M. Bumpus, *J. Med. Chem.*, 1979, 1128.

Peptide	*Ref.*
[1,7-di-Sar,8-X]angiotensin II analogues	243
1-Asn(N-Me_3) angiotensin II	244
[Asn[1], Aza-α^1-homo-Val[5]-Angiotensin II	245
[Asn[1], Ile[5], X[6]-Angiotensin II	246
Analogues of angiotensin II substituted in position 7	247
[Asn[1], Ile[5], X[8]-Angiotensin II	248
[Sar[1]-Angiotensin II	249
[Val[5]-Angiotensin II	112
Antamanide	
Antamanide analogues	79
Antimalarials	
Antimalarial threonine containing peptides	250
Apamin	
[Lys[13]]- and [Lys[14]]-apamin and guanidinated derivatives	221
Apolipoprotein	
Apolipoprotein-A-II-(17—31)-peptide, -(12—31)-peptide, and -(7—31)-peptide	251
Aspartame	
Amino-oxy analogues of aspartame	252
Bombesin	
Heptapeptide related to bombesin	253
Bombinin	
Analogue of toad bombinin	254
Bovine serum albumin	
Peptides related to antigenic site of bovine serum albumin	255
Bradykinin	
[Gly[6]]- and [Arg[9]]-bradykinin	256
[Ile[5]]- and [Ile[8]]-bradykinin, [di-Ile[5,8]]-bradykinin	257
[β-Ala[1,9], GABA[6]]-bradykinin	258
Bradykinin analogues	259
19 Analogues	260
Various analogues	261
Calcitonin	
Human calcitonin	219
Capreomycin	
Capreomycin analogues	262

[243] G. J. Moore, E. M. Oudeman, D. Ko, and D. M. Nystrom, *J. Med. Chem.*, 1979, 1147.
[244] P. Cordopatis, J. Brison, J. Dirkx, and D. Theodorpoulos, *Experientia*, 1979, **35**, 849.
[245] J. J. Ancans, N. A. Makarova, I. P. Misinya, and G. I. Chipens, *Bioorg. Khim., 1979*, **5**, 1295.
[246] K. Hsieh, E. C. Jorgensen, and T. C. Lee, *J. Med. Chem.*, 1979, **22**, 1199.
[247] G. J. Moore and E. M. Ko, *Can. J. Physiol. Pharmacol.*, 1979, **57**, 763.
[248] K. Hsieh, E. C. Jorgensen, and T. C. Lee, *J. Med. Chem.*, 1979, **22**, 1038.
[249] O. Leukart, E. Escher, D. Regoli, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 546.
[250] H. Gershon and I. S. Krull, *J. Med. Chem.*, 1979, 877.
[251] T. C. Chen, J. T. Sparrow, A. M. Gotto, jun., and J. D. Morrisett, *Biochemistry*, 1979, **18**, 1617.
[252] M. T. Briggs and J. S. Morley, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2138.
[253] K. Kitagawa, K. Ujita, Y. Kiso, T. Akita, Y. Nakata, N. Nakamoto, T. Segawa, and H. Yajima, *Chem. Pharm. Bull.*, 1979, **27**, 48.
[254] K. Friedel, G. Jonissek, A. Bauer, and M. Rimpler, *Tetrahedron Lett.*, 1979, 2331.
[255] M. Z. Atassi, S. Sakata, and A. L. Kazim, *Biochem. J., 1979*, **179**, 327.
[256] M. P. Filatova, N. A. Krit, N. V. Beschastnaya, and S. Reissmann, *Bioorg. Khim.*, 1979, **5**, 1445.
[257] A. T. Chiu, A. R. Day, J. A. Radding, and R. J. Freer, *J. Med. Chem.*, 1979, **22**, 334.
[258] V. Skaric, M. Topic-Bulic, and D. Skaric, *Croat. Chem. Acta*, 1978, **51**, 347.
[259] V. S. Chauhan, G. T. Young, and N. G. Bowery, *Bioorg. Chem.*, 1979, **8**, 333.
[260] S. St-Pierre, P. Gaudreau, J. N. Drouin, D. Regoli, and S. Lemaire, *Can. J. Biochem.*, 1979, **57**, 1084.
[261] L. W. Redman, D. Regoli, and E. R. Tustanoff, *Can. J. Biochem.*, 1979, **57**, 529.
[262] S. Nomoto and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1979, 1709.

Peptide	*Ref.*
Chemotactic peptide	
Synthesis and n.m.r. data	263
Syntheses chemotactic peptide	28, 264
Cholecystokinin-pancreozymin (CCK-PZ)	
[Thr^{28}, Leu^{31}]-(25—33)-peptide	57
C-terminal octapeptide of CCK-PZ	265
Chymotrypsin	
Substrates for chymotrypsin	266, 267
Cytochrome *c*	
Semisynthesis of fragments of horse heart cytochrome *c*	162—166
Synthesis of fragments of horse heart cytochrome *c*	268—270
Edeine antibiotics	
Analogue of edeine-D-(1—3)-peptide	271
Eledoisin	
Synthesis of ring-closed form of eledoisin	272
Encephalitogenic peptide	
^{13}C-Enriched human encephalitogenic peptide	273
Endorphin	
Chemistry of β-endorphin	193
α- and γ-endorphin	194
Human β-endorphin analogues	195
Deletion of amino-acid deletes immunoreactivity of β-endorphin	196
β-endorphin analogues	274
Enkephalin	
[Leu^5]-enkephalin	21, 72, 76, 112, 131, 275, 276
[Met^5]-enkephalin	44, 68, 103, 189, 190, 277
Analogues of [D-Ala^2]-enkephalin	21, 275
[Car^4, Leu^5]-enkephalin	192
Enkephalin with Phe and Tyr replaced by Car	249
Enkephalin amides	191, 275
Isosteric analogues of enkephalin	190
Analogues of enkephalin	81, 104, 188, 278
Enkephalin gene	279

[263] E. L. Becker, H. E. Bleich, A. R. Day, R. J. Freer, J. A. Glasel, and J. Visintainer, *Biochemistry*, 1979, **18**, 4656.

[264] P. C. Wilkinson, *Immunology*, 1979, **36**, 579.

[265] D. Gillessen, A. Trzeciak, R. K. M. Müller, and R. O. Studer, *Int. J. Pept. Protein Res.*, 1979, **13**, 130.

[266] E. G. DelMar, C. Largman, J. W. Brodrick, and M. C. Geokas, *Anal. Biochem.*, 1979, **99**, 316.

[267] M. Obara, Y. Karasaki, and M. Ohno, *J. Biochem. (Tokyo)*, 1979, **86**, 461.

[268] N. E. Vol'skaya, G. A. Vasilieva, A. F. Mironov, and R. P. Evstigneeva, *Bioorg. Khim.*, 1979. 181.

[269] N. E. Vol'skaya, G. A. Vasilieva, V. N. Amelina, N. V. Filenkova, and R. P. Evstigneeva, *Bioorg. Khim.*, 1979, **5**, 1302.

[270] N. E. Vol'skaya, G. A. Vasilieva, M. B. Efros, and R. P. Evstigneeva, *Bioorg. Khim.*, 1979, **5**, 1310.

[271] J. Grzybowska, H. Wojciechowska, R. Andruszkiewicz, and E. Borowski, *Pol. J. Chem.*, 1979, 1267.

[272] A. Failli, H. Immer, and M. Götz, *Can. J. Chem.*, 1979, **57**, 3257.

[273] C. M. Deber and M. E. M. Young, *J. Biol. Chem.*, 1979, **254**, 6341.

[274] C. H. Li, D. Yamashiro, L.-F. Tseng, W.-C. Chang, and P. Ferrara, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3276.

[275] R. Tomatis, S. Salvadori, E. Menegatti, and M. Guarneri, *Farmaco Ed. Sci.*, 1979, **34**, 496.

[276] W. A. Klis, E. Nawrocka, I. Z. Siemion, A. Plech, Z. Stachura, and Z. H. Herman, *Bioorg. Chem.*, 1979, **8**, 255.

[277] G. Losse and K.-D. Wehrstedt, *J. Prakt. Chem.*, 1978, **320**, 963.

[278] E. Hazum, K.-J. Chang, Y. Shechter, S. Wikinson, and P. Cuatreeases, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 841.

[279] E. D. Sverdlov, G. M. Polganov, G. S. Monastyrskaya, E. M. Khodkova, A. V. Chestukhin, and M. F. Shemyakin, *Bioorg. Khim.*, 1979, **5**, 1112.

Peptide	*Ref.*
Ferridoxin	
Two sequences of apoferridoxin-(7—20)-peptides and -(21—34)-peptide	280
Fibrinogen clotting inhibitor	281
Galline	
Galline-(52—65)-peptide	220
Gastrin	
Gastrins	223
Porcine big gastrin	39, 224, 225
Minigastrin	282
α-amino-oxy analogues of pentagastrin	236
Tetragastrin	68, 252
Gastrin fragments	283
Growth hormone	
Bovine GH	176
Bovine and ovine GH-(125—133)-peptide	284
Human GH	285
Growth hormone releasing hormone (GHRH)	
GHRH analogue	286
Haemoglobin	
β-Chain human haemoglobin-(89—100)-peptide	287
Histones	
Diacylated histone H4-(1—37)-peptide	129
Fragments of histones H1 and H5	288
Immunoglobulin	
Human immunoglobulin E-(496—504)-peptide, -(497—504)-peptide, and -(497—506)-peptide	289
Immunopoietic polypeptide	215
Insulin	
Insulin	197
Analogues and fragments of A- and B-chains	63, 198, 199, 205, 290
Analogues and fragments of A-chains	58, 201, 202, 203, 204
Derivative of A-chain of bovine insulin	291
Analogues and fragments of B-chains	133, 138, 167, 171, 206, 292, 293, 294, 295

[280] M. J. Gunter, B. Ridge, H. N. Rydon, and R. Sharpe, *Bioorg. Chem.*, 1979, **8**, 371.
[281] S. A. Poyarkova, V. K. Kibirev, D. M. Fedoryak, and S. B. Serebryany, *Bioorg. Khim.*, 1979, **5**, 508.
[282] R. A. Gregory, M. J. Tracy, J. I. Harris, M. J. Runswick, S. Moore, G. W. Kenner, and R. Ramage, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 73.
[283] A. N. Prusakov, M. A. Samartsev, and V. F. Martynov, *Bioorg. Khim.*, 1979, **5**, 497.
[284] F. Chillemi, R. Colombo, A. Pecile, and V. R. Olgiati, *J. Chem. Soc., Perkin Trans. 1*, 1979, 31.
[285] J. D. Wade and J. Bornstein, *Proc. Aust. Biochem. Soc.*, 1979, 17.
[286] P. Giori, M. Guarneri, E. Rosaspina, D. Mazzotta, G. Vertuani, and C. A. Benassi, *Gazz. Chim. Ital.*, 1978, **108**, 51.
[287] G. A. Zheltukhina, E. I. Filippovich, M. V. Sidorova, V. I. Lagutkina and R. P. Evstigneeva, *J. Gen. Chem. USSR (Engl. Transl.)*, 1978, **48**, 1495.
[288] S. L. Kielland, P. Mathiaparanam, L. A. Slotin, and R. E. Williams, *Can. J. Chem.*, 1979, **57**, 267.
[289] D. R. Stanworth, M. Kings, P. D. Roy, J. M. Moran, and D. M. Moran, *Biochem. J.*, 1979, **180**, 665.
[290] N. Ferderigos, A. Cosmatos, A. Ferderigos, and P. G. Katsoyannis, *Int. J. Pept. Protein Res.*, 1979, **13**, 43.
[291] B. R. D. Easter and S. E. Drewes, *Z. Physiol. Chem.*, 1979, **360**, 1335.
[292] A. Cosmatos, N. Ferderigos, and P. G. Katsoyannis, *Int. J. Pept. Protein Res.*, 1979, **14**, 457.
[293] G. Losse and H. Stange, *J. Prakt. Chem.*, 1979, **321**, 1036.
[294] G. Losse and H. Stange, *J. Prakt. Chem.*, 1979, **321**, 308.
[295] Y. P. Shvachkin, V. F. Krivtsov, G. A. Shmeleva, V. P. Fedotov, V. V. Abramova, and A. I. Ivanova, *J. Gen. Chem. USSR (Engl. Transl.)*, 1978, **48**, 1500.

Peptide	*Ref.*
Semisynthesis of insulins	169, 173, 180, 200
Insulin derivatives	170, 296, 297
Human proinsulin	298, 299, 300
Preproinsulin	172
Iron–sulphur protein	301
Luteinizing hormone releasing hormone (LHRH)	
LHRH analogues	44, 302
Analogues of LHRH substituted at positions 1, 6, and 10	303
Analogues of LHRH substituted at positions 3 or 8	304, 305
α-aza-analogues of LHRH	306, 307
Lysozyme	
Lysozyme	228
Lysozyme-(1—16)-peptide	229
Lysozyme-(17—26)-peptide	230
Lysozyme-(27—37)-peptide	231
Lysozyme-(1—37)-peptide	232
Reoxidation hen egg-white enzyme-(1—123)-peptide	178
Human lysozyme-(1—16)-peptide	233
Human lysozyme-(17—29)-peptide	234
Mast cell degranulating peptide (MCD)	
Synthesis MCD	126, 222
Melanocyte stimulating hormone (MSH)	
[(^{3}H)Tyr^2]-α-MSH	186
[Leu^9]-α-MSH	187
[Nle^7]-β-MSH	308
Melanocyte stimulating hormone release inhibiting hormone (MIF)	
Tripeptide analogues of MIF	309
Myelin protein	
Bovine myelin protein-(69—84)-peptide	310
Encephalitogenic fragment, myelin protein-(70—74)-peptide	311
Myelin protein-(90—104)-peptide	312
Myeloma immunoglobulin (M603)	
Myeloma immunoglobulin-(27—42)-peptide	113
Myoglobin	
[des-Val^1, $N^{\varepsilon 19}$-acetimido]myoglobin	174

[296] J. G. R. Hurrell and R. Conway, *Proc. Aust. Biochem. Soc.*, 1979, 17.
[297] D. Keglevic, S. Vuksan, B. Mulac, B. Ladesic, S. Iskric, and S. Kveder, *Croat. Chem. Acta*, 1978, **51**, 167.
[298] H. Berndt, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 765.
[299] N. A. Sasaki, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 761.
[300] H. Berndt, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 747.
[301] N. Ohta, C. Kawasaki, M. Maeda, S. Tani, and K. Kawasaki, *Chem. Pharm. Bull.*, 1979, **27**, 2968.
[302] J. Humpries, W.-P. Wan, T. Wasiak, K. Folkers, and C. Y. Bowers, *J. Med. Chem.*, 1979, **22**, 774.
[303] G. W. Moersch, M. C. Rebstock, E. L. Wittle, F. J. Tinney, E. D. Nicolaides, M. P. Hutt, T. F. Mich, J. M. Vandenbelt, R. E. Edgren, J. R. Reel, W. C. Dermody, and R. R. Humphrey, *J. Med. Chem.*, 1979, 935.
[304] Y. Yabe, C. Miura, H. Horikoshi, H. Miyagawa, and Y. Baba, *Chem. Pharm. Bull.*, 1979, **27**, 1907.
[305] G. R. Pettit and T. H. Smith, *J. Pharm. Sci.*, 1979, **68**, 1013.
[306] A. S. Dutta, B. J. A. Furr, and M. B. Giles, *J. Chem. Soc., Perkin Trans. 1*, 1979, 389.
[307] A. S. Dutta, B. J. A. Furr, and M. B. Giles, *J. Chem. Soc., Perkin Trans. 1*, 1979, 379.
[308] C. B. Heward, Y. C. S. Yang, T. K. Sawyer, M. D. Bergman, B. B. Fuller, V. J. Hruby, and M. E. Hadley, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 266.
[309] S. Bjoerkman, S. Castensson, and H. Sievertsson, *J. Med. Chem.*, 1979, 931.
[310] G. A. Hashim, E. F. Carvalho, and R. D. Sharpe, *J. Immunol.*, 1978, **121**, 665.
[311] S. J. Pasaribut, *Aust. J. Chem.*, 1979, **32**, 1575.
[312] J. D. Young, D. Tsuchiya, D. E. Sandlin, and M. J. Holroyde, *Biochemistry*, 1979, **18**, 4444.

Peptide	*Ref.*
Neurotensin	
Neurotensin	50
[Gln[4]]-neurotensin	102, 109
Nucleic acid binding peptide	313
Oxytocin	314
[Met[8]]-oxytocin	315
[D-Tyr[2]]-oxytocin	316, 323
[Glu(OMe)[4]]- and [Mpr[1], Glu(OMe)[4]]-oxytocin	317
[Asn(NMe_2)[5]]-oxytocin	318
7-Azetidine-2-carboxylic acid oxytocin	319
Diastereoisomers of oxytocin	153, 155, 323
Deamino-oxytocin	119, 120
[Glu[4]]-deamino-1-carba-oxytocin	320, 321
[Tyr[2], Glu[4]]-deamino-1-carba-oxytocin	321
[Glu[4]]-deamino-1-carba-oxytocin esters	321, 322
Oxytocin analogues, role of [Thr[4]]	324
Cyclo-[COTyr[2], Lys[6]]-oxytocin	325
Oxytocin intermediates	152
Pancreatic hormone III	
Avian pancreatic hormone III, *C*-terminal pentapeptide	326
Peptidoglycans	327—329
Bacterial peptidoglycans	330—332
Proctolin	
Analogues of proctolin	333
Prolactin	
Ovine prolactin	177
Red pigment concentrating hormone	
Analogue of red pigment concentrating hormone	334

[313] B. Gutte, M. Daeumigen, and E. Wittschieber, *Nature, (London)*, 1979, **281**, 650.
[314] G. Flouret, S. Terada, T. Kato, R. Gualtieri, and A. Lipkowski, *Int. J. Pept. Protein Res.*, 1979, **13**, 137.
[315] M. Bodanszky, N. Chandramouli, J. Martinez, and R. Walter, *J. Med. Chem.*, 1979, **22**, 270.
[316] V. J. Hruby, D. A. Upson, D. M. Yamamoto, C. W. Smith, and R. Walter, *J. Am. Chem. Soc.*, 1979, **101**, 2717.
[317] I.Photaki, C. Tzougraki, and C. Kotsira-Engonopoulos, *Int. J. Pept. Protein Res.*, 1979, 426.
[318] R. Walter, G. L. Stahl, T. Caplaneris, P. Cordopatis, and D. Theodoropoulos, *J. Med. Chem.*, 1979, **22**, 890.
[319] M. Barber, J. H. Jones, A. V. Stachulski, G. W. Bisset, H. S. Chowdrey, and A. L. Hudson, *Int. J. Pept. Protein Res.*, 1979, **14**, 247.
[320] M. Lebl, A. Dimeli, V. Bojanovska, J. Slaninova, T. Barth, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, 2556.
[321] M. Lebl, V. Bojanovska, T. Barth, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2573.
[322] M. Lebl, T. Barth, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2563.
[323] V. Viswanatha and V. J. Hruby, *J. Org. Chem.*, 1979, 2892.
[324] J. Lowbridge, M. Manning, J. Seto, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, 1979, **22**, 565.
[325] L. K. Polevaya, G. Cipens, R. Vegners, and Z. Auna, *Latv. PSR Zinat. Vestis, Kim. Ser.*, 1978, 753.
[326] P. Kanellis, D. F. Dyckes, and R. L. Hazelwood, *Int. J. Pept. Protein Res.*, 1979, **13**, 201.
[327] P. Lafrancier, M. Petitou, M. Level, M. Derrien, J. Choay, and E. Lederer, *Int. J. Pept. Protein Res.*, 1979, **14**, 437.
[328] S. Kusumoto, K. Ilkenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1177.
[329] R. Kaifu and T. Osawa, *Carbohydr. Res.*, 1979, **69**, 79.
[330] P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, 1979, 4013.
[331] S. Kusumoto, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1979, 1665.
[332] B. C. Das and E. Nebelin, *FEBS Lett.*, 1979, **107**, 254.
[333] A. N. Starratt and B. E. Brown, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 1125.
[334] M. Christensen, J. Carlsen, and L. Josefsson, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1051.

Peptide	*Ref.*
Ribonuclease	
12-β-3-pyridylalanine-RNase-(1—14)-*S*-peptide	226
β-4-pyridyl-L-Ala-RNase-(1—14)-*S*-peptide	335
RNase T_1-(24—104)-peptide	227
Bovine RNase-(32—34)-peptide, -(33—35)-peptide, and -(33—38)-peptide	336
RNase amino terminal-(1—15)-peptide	337
Serum thymic factor (STF)	
Serum thymic factor	211
Analogues of STF	209, 210, 211
Proposed thymic factor	212
Somatostatin	207, 208
Somatotropin	
Analogues of human somatotropin	175
Substance P	
Substance P synthesis	50, 115
Seven analogues of substance P	338
Analogues of substance P containing (Car)	249
D-Amino-acid analogues of substance P	339
Tachykinin	
Heptapeptides related to tachykinin	253
Thymopoietin II	
Analogue of STF related to thymopoietin	211
Thymopoietin activity of -(32—36)-peptide	213
Thymopoietin II-(32—41)-peptide, and -(33—41)-peptide	214
Thymosin	
Synthesis of thymosin α_1	217, 218
Bovine thymosin α_1-(19—21)-peptide	216
Thyrotrophin releasing hormone (TRH)	
Synthesis of TRH	52, 72
23 Analogues of TRH	340
Trichoderma metabolites	
Peptides related to metabolites of Trichoderma species	341
Trypsin	
Substrate for trypsin-like enzyme	342
Tuftsin	
Synthesis of tuftsin	123
[Leu^3]-tuftsin	19
[Leu^4]-tuftsin	343
Analogues of tuftsin	344—346

[335] C. Hoes, P. Hoogerhout, W. Bloemhoff, and K. E. T. Kerling, *Recl. Trav. Chim. Pays-Bas*, 1979, **98**, 137.
[336] H. G. Garg and R. W. Jeanloz, *Carbohydr. Res.*, 1979, **70**, 47.
[337] C.-H. Niu, S. Matsuura, H. Shindo, and J. S. Cohen, *J. Biol. Chem.*, 1979, **254**, 3788.
[338] G. Rackur, I. Yamaguchi, J. J. Leban, U. Bjoerkroth, S. Rosell, and K. Folkers, *Acta Chem. Scand., Ser. B*, 1979, **33**, 375.
[339] I. Yamaguchi, G. Rackur, J. J. Leban, U. Bjoerkroth, S. Rosell, and K. Folkers, *Acta Chem. Scand., Ser. B*, 1979, **33**, 63.
[340] L. G. Bauce and H. J. Goren, *Int. J. Pept. Protein Res.*, 1979, 216.
[341] I. M. Shaw and A. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1866.
[342] G. D. J. Green and E. Shaw, *Anal. Biochem.*, 1979, **93**, 223.
[343] D. Konopinska, I. Z. Siemion, and E. Baros-Stuliglowa, *Pol. J. Chem.*, 1979, 2255.
[344] D. Konopinska, E. Nawrocka, I. Z. Siemion, S. Szymaniec, and S. Slopek, *Arch. Immunol. Ther. Exp.*, 1978, **27**, 151.
[345] M. Blumenstein, P. P. Layne, and V. A. Najjar, *Biochemistry*, 1979, **18**, 5247.
[346] D. Konopinska, I. Z. Siemion, S. Szymaniec, and S. Slopek, *Pol. J. Chem.*, 1979, 343.

Peptide	*Ref.*
Valinomycin	
[Lys[1]]-valinomycin	125
Vasointestinal peptide (VIP)	
C-terminus VIP 13 residue peptide	347
Chicken VIP 28 residue peptide	348
Vasopressin	
Analogues of arginine vasopressin	349
[D-Tyr[2]] arginine vasopressin	316, 323
[Mpr[1], Tyr(Me)[2], D-Arg[8]]-vasopressin	350
8-[α-amino-β-glycylaminopropionic acid]-vasopressin	351
Desamino-[D-Arg[8]]-vasopressin	352
Desamino-[L-Nor-Arg[8]]- and desamino-[D-Nor-Arg[8]]-vasopressin	353
Desamino-[L-Dpr[8]]- and desamino-[D-Dpr[8]]-vasopressin	354
Desamino-[L-β-guanidino-Ala[8]]- and desamino-[D-β-guanidino-Ala[8]]-vasopressin	355
7-Azetidine-2-carboxylic acid lysine vasopressin	319
Sequential Oligo- and Poly-peptides	
Poly-(β-Ala)	356
Poly-(γ-Me-Glu)	142
Poly-Z-(α-isobutyl-aspartate)	141
Diastereoisomeric homo-oligopeptides of L-Ile and D-*allo*-Ile	145
Boc-(Nva)$_7$OMe	357
Nps-Glu(OBzl)$_6$-NHEt	358
Poly-azoaromatic amino-acids	359
Boc-X$_n$-Gly-OPEG; X = Ala, n = 1—8; X = Val, n = 1—6	134
Poly-(Leu-Ala)	146
Oligopeptides Gly-Trp-(Gly)$_n$-Trp-Gly-OH with n = 0—2	360
Block co-polymers ABA with A = poly-(γ-Bzl-L-Glu) and B = poly(butadiene)	361
Poly-(L-Met-co-L-homo-Cys)	362
Poly-(Lys(Z)-Val-Lys(Z))	363
Poly-(Lys-Val-Lys)	364
Poly-(Val-Val-Lys)	365
Oligopeptides of Leu, Met, Phe, and Tyr	87

[347] M. Bodanszky, A. Bodanszky, S. S. Deshame, and J. Martinez, *Bioorg. Chem.*, 1979, **8**, 399.
[348] H. Yajima, M. Takeyama, K. Koyama, T. Tobe, K. Inoue, T. Kawano, and H. Adachi, *Bull. Chem. Soc. Jpn.*, 1979, **27**, 3199.
[349] C. R. Botos, C. W. Smith, Y.-L. Chan, and R. Walter, *J. Med. Chem.*, 1979, 926.
[350] V. Krchňák and M. Zaoral, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1642.
[351] M. Zaoral, V. Krchňák, J. Škopkova, and A. Machová, *Collect. Czech. Chem. Commun.*, 1979, **44**, 3133.
[352] V. Krchňák and M. Zaoral, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1173.
[353] M. Zaoral, F. Brtnik, M. Flegel, T. Barth, and A. Machova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1179.
[354] V. Krachňák, M. Zaoral, and A. Machova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2161.
[355] M. Zaoral, V. Krchnák, F. Brtnik, A. Machová, and J. Škopkova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2447.
[356] K. Hanabusa, K. Kondo, and K. Takemoto, *Makromol. Chem.*, 1979, **180**, 307.
[357] P. Spadon and A. Del Pra, *Makromol. Chem.*, 1979, **180**, 1385.
[358] K. Okahashi and S. Ikeda, *Biopolymers*, 1979, **18**, 2105.
[359] A. Ueno, J. Anzai, and T. Osa, *J. Polym. Sci., Polym. Lett.*, 1979, 149.
[360] P. Skrabal, V. Rizzo, A. Baici, F. Bangerter, and P. L. Luisi, *Biopolymers*, 1979, **18**, 995.
[361] A. Nakajima, T. Hayashi, K. Kugo, and K. Shinoda, *Macromolecules*, 1979, **12**, 840.
[362] B. Nakajima, N. Nishi, and J. Noguchi, *Kobunshi Robanshu*, 1979, **36**, 629.
[363] R. Ciaschi and M. D'Alagui, *Macromol. Chem.*, 1979, **180**, 2893.
[364] R. Ciashi, M. D'Alagui, and G. Mignucci, *Macromol. Chem.*, 1979, **180**, 2883.
[365] M. D'Alagui, *Macromol. Chem.*, 1979, **180**, 2903.

Peptide	*Ref.*
Oligopeptides, $(Pro)_2$-X–X-Pro-X	366
Sequential and random co-polymers of Lys and aromatic amino-acids	143
Block co-polypeptide $(Leu^{67}, Lys^{33})_{100}$ Orn_{20}	144
Polypeptides as collagen models	367
Polymers as visual pigment models	368
Immunopoietic polypeptide	215
Enzyme Inhibitors	
2-Phosphonopyrrolidine inhibitors of angiotensin converting enzyme	369
Angiotensin converting enzyme inhibitors	370
Porcine pancreatic elastase inhibitors	371
Proteinase inhibitors	372
Trypsin inhibitor	33
Porcine pancreatic secretory trypsin inhibitor II-(1—52)-peptide	60
Bovine pancreatic trypsin inhibitor	179
Miscellaneous Peptides	
H-Gly-Gly-X-Ala-OH	11
Ppt-Pro-Leu-Gly-OMe	20
Boc-Leu-Asp(OBu^t)-Phe-NH_2	47
Ala-Asp-X-Phe where X = Gly or Leu	48
Aspartyl peptides	49
Boc-Ser(Bzl)-His(Ztf)-Leu-Val-OMe	54
Z-Phe-Gly-OEt	65
Z-Leu-Val-Gly-Ala-OBzl	69
Gly-Glu-Phe-Trp-Ala-Glu-OBzl	70
Boc-Leu-Val-OBu^t	80
Gly-Met-Ala_2-Cys-Lys	85
Z-Asp-Phe-OMe	86
X-Lys, Lys-Y, Gly-X-Lys, and Gly-Lys-Y where X and Y = Ala, Leu Val, Ile, Phe, and Pro	88
Bz-X-Lys(Z)-OMe where X = Ala, Leu, Val, Phe, and Ile	89
Bz-Leu-Gly-OEt	94
Gly-Ala-Leu	96
Leu-Ala-Gly-Val	121
Lys-Ala-Gly-Val	122
Peptide-flanked by two poly (ethylene oxide) chains, PEO-Gly-Trp-Gly-PEO	139
Boc-Tyr-Gly_2-Phe-X-OMe_3 where X = Leu, Gly, or Val	140
Merrifield test peptide (Leu-Ala-Gly-Val)	117, 124
Cyclic peptides containing Phe and Pro or Ala	373
Tryptophan dipeptides	374
Valine peptides	375
Racemic *N*-hydroxypeptides	376

[366] R. Deslauriers, J. H. Becker, A. S. Steinfeld, and F. Naider, *Biopolymers*, 1979, **18**, 523.
[367] W. Roth, K. Heppenheimer, and E. R. Heidemann, *Makromol. Chem.*, 1979, **180**, 905.
[368] M. Nanasawa and H. Kamogawa, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1727.
[369] E. W. Petrillo, jun. and E. R. Spitzmiller, *Tetrahedron Lett.*, 1979, 4929.
[370] G. H. Fischer and J. W. Ryan, *FEBS Lett.*, 1979, **107**, 273.
[371] C. H. Hassall, W. H. Johnson, and N. A. Roberts, *Bioorg. Chem.*, 1979, **8**, 229.
[372] A. Light, C. S. Paude, and J. S. Liepnieks, *Int. J. Pept. Protein Res.*, 1979, **13**, 88.
[373] S. F. Brady, S. L. Varga, R. M. Freidinger, D. A. Schwenk, M. Mendlowski, F. W. Holly, and D. F. Veber, *J. Org. Chem.*, 1979, **44**, 3101.
[374] G. R. Pettit, T. S. Krupa, and R. M. Reynolds, *Int. J. Pept. Protein Res.*, 1979, 193.
[375] K. Okada, Y. Kurosawa, and S. Nagai, *Chem. Pharm. Bull.*, 1979, **27**, 2163.
[376] M. Akiyama, M. Hasegawa, H. Takeuchi, and K. Shimizu, *Tetrahedron Lett.*, 1979, 2599.

Peptide	*Ref.*
α-Aib containing peptides	377
Depsipeptides	378
Peptides containing 1,2,3,4-tetrahydroquinoline-2-carboxylic acid	379
Phosphonic dipeptide analogues	380
Z-Aib-Pro-NHMe and related peptides	381
s-Triazine derivatives of dipeptide esters	382
3-Iodo-L-tyrosyl peptides	383
Alkali cation binding of bicyclodecapeptide	384
Methyl esters of valine *C*-terminal peptides	385
Diastereoisomeric tripeptides	386

5 Appendix II: Amino-acid Derivatives Useful in Synthesis

As before, this list includes both new derivatives and known ones for which new physical data or preparations have been reported. We have adhered to the recent practice of dividing the derivatives into two groups: those of the coded amino-acids and those of other amino-acids.

Abbreviations used

Bop = 2-benzyloxyphenyl; Mpt = dimethylthiophospinyl; MPS = 4-methoxyphenylsulphonyl; mNp = 2-nitrophenyl; Pfp = pentafluorophenyl; Pht = phthalyl; Ppt = diphenylthiophosphinyl; PSE = 2-[4-(phenylazo)-phenylsulphonyl]-ethoxy; Tfp = trifluoropropionyl; and Tmb = trimethoxybenzyl.

Coded Amino-acids

Compound	*m.p.*/°C	$[\alpha]_D$*[1]	*Conc.*	*Solvent*	*Ref.*
Alanine					
Fmoc-Ala-OH	144—145	−17.8	2.5	DMF	124
Mpt-Ala,Dcha	147—148	−10.0	1	EtOH	21
Ppt-Ala,Dcha	177—178	− 3.7	1	EtOH	20
Ppt-Ala-OH	120	−13.7	1	EtOH	20
Tfa-Ala-OH	70—71	—	—	—	30
$TosO^-H_2^+$-Ala-OPh	94—96	−48.2	2	EtOH	39
Z-Ala-OPh	94—96	−48.2	2	EtOH	39
Arginine					
$Cl^-H_2^+$Arg(Tos)-OBu^t	108—109	− 4.2	0.5	EtOH	284
Ppt-Arg(NO_2),Dcha	159—161	+ 5.0	1	MeOH	20
Ppt-Arg(NO_2)-OH	129—132	+ 2.5	1	MeOH	20

[377] R. Nagaraj, N. Shamala, and P. Balaram, *J. Am. Chem. Soc.*, 1979, **101**, 16.
[378] C. Gilon, Y. Klausner, and A. Hassner, *Tetrahedron Lett.*, 1979, 3811.
[379] S. Cerrini, W. Fedeli, F. Mazza, G. Lucente, M. Paradisi, and A. Romeo, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1013.
[380] A. Arendt, M. Hoffman, A. Kolodziejczyk, A. Sobczak, T. Sokolowska, and C. Wasielewski, *Pol. J. Chem.*, 1979, **53**, 447.
[381] B. V. V. Prasad, N. Shamala, R. Nagaraj, R. Chandrasekaran, and P. Balaram, *Biopolymers*, 1979, **18**, 1635.
[382] N. Oi, H. Takeda, H. Shimada, and O. Hiroaki, *Bunseki Kagaku*, 1979, **28**, 69.
[383] J. Tarnawski, B. Rzeszotarska, B. Nadolska, and K. Pawelczak, *Justus Liebig's Ann. Chem.*, 1979, 761.
[384] H. J. Moeschler, D. F. Sargent, A. Tun-Kyi, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 2442.
[385] B. Calas, J.-P. Gilbert, J. Mery, J. Parello, and C. Tisse, *C. R. Acad. Sci. C.*, 1979, **288**, 449.
[386] M. Matsuzaki and I. Tomida, *Agric. Biol. Chem.*, 1979, **43**, 925.

*[1] $[\alpha]_D$ recorded at 589 nm unless indicated by superscript: [a]546 nm, and measured at 20—25 °C; *[2] $[\alpha]_D$ measured at 18 °C; *[3] $[\alpha]_D$ measured at 26 °C; *[4] $[\alpha]_D$ measured at 27 °C; *[5] $[\alpha]_D$ measured at 30 °C; *[6] $[\alpha]_D$ measured at 19 °C; *[7] $[\alpha]_D$ variations in $[\alpha]_D$ with temperature given; *[8] $[\alpha]_D$ measured at 29 °C; *[9] $[\alpha]_D$ measured at 28 °C.

Compound	*m.p./°C*	$[\alpha]_D$*[1]	*Conc.*	*Solvent*	*Ref.*
Ppt-Arg(Tos)-OH	199—201	+ 4.9	1	DMF	20
Z-Arg(Adoc)$_2$-OPh	75—77	− 6.5	1	DMF	39
Asparagine					
Ppt-Asn-OH	163—164	− 5.0	1	MeOH	20
Aspartic acid					
Boc-Asp(*O*-cyclopentyl)-OH	87—89	+ 27	1	MeOH + 1.2 equiv DIPEA	48
Boc-Asp(*O*-cyclohex)	93—95	—	—	—	49
Mpt-Asp(OBu^t),Dcha	154	−20.0	1	EtOH	21
Ppt-Asp(OBzl),Dcha	156—157	−13.7	1	EtOH	20
Cysteine					
Boc-Cys(Acm)-OPSE	75—77	−24.0	1	MeOH	208
Boc-Cys(Bzl)-OBzl	84—86	−40.4*[4]	2	DMF	315
Boc-Cys(MBzl)(O)-OH	176—177	−71.6	1.4	DMF	101
Boc-Cys(O_3Na)-OH	234	−17.4	1	H_2O	388
Boc-Cys(O_3Na)-ONp	166	−18.8	1	DMF	388
Boc-Cys(O_3Na)-OSu	179	−25.6	1	DMF	388
Bpoc-Cys(Acm)-OH	93—98	—	—	—	231
Bpoc-Cys(Acm)-OH,Dcha	39—40	−36.0	2	DMF	231
$Cl^-H_2^+$-Cys(Acm)-OH	159—163	−30.7	1	H_2O	208
Cys(Bzl),Dcha	162—164 (d)	+10.0	1	EtOH	21
H-Cys(Acm)-OH,H_2O	205—206	−40.3	1	H_2O	208
H-Cys(MBzl)(O)-OH	176—177	+ 6.7	0.7	50%HOAc	101
Pht-Cys(O_3K)-OH	279	− 1.7	1	H_2O	388
Pht-Cys(O_3K)-ONp	230	0	1	DMF	388
Pht-Cys(Bzl),Dcha	170—171	+22.5	1	MeOH	20
Ppt-Cys(Ppt),Cha	141—144	+ 7.5	1	EtOH	20
Z-Cys(MBzl)(O)-OH	133—135	−54.1	1	DMF	101
Z-Cys(O_3Na)-OH	209	−14.3	1	H_2O	388
Z-Cys(O_3Na)-ONp	187	− 8.1	1	DMF	388
Z(OMe)-Cys(MBzl)(O)-OH	148—151	−72.8	1.1	DMF	101
Glutamic acid					
Boc-Glu(O-cyclohex)-OH Dcha	135—136	—	—	—	49
Ppt-Glu(OBzl),TBA	94—98	+10.0	1	EtOH	20
Z-Glu(OBu^t)-OPh	61	−30.6	1	DMF	282
Glutamine					
Ppt-Gln,Dcha	172—174	+ 8.7	1	EtOH	20
Ppt-Gln-OH	77—79	+22.5	1	EtOH	20
Glycine					
$Br^-H_2^+$-Gly-OPh	221—226	—	—	—	39
$Br^-H_2^+$-Gly-mNp	217—221	—	—	—	69
Mpt-Gly,Dcha	131—133	—	—	—	21
Ppt-Gly-OH	118—119	—	—	—	20
Z-Gly-mNp	72	—	—	—	69
Z-Gly-OPh	67—68	—	—	—	39
Histidine					
Boc-His(MPS)-OH	128—131	—	—	—	51
H-His(MPS)-OH	155—158	—	—	—	51

[387] S. Kuwata, T. Yamada, T. Shinogi, N. Yamagomi, F. Kitabashi, T. Miyazawa, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3326.

[388] A. Hubbuch, W. Danho and H. Zahn, *Justus Liebig's Ann. Chem.*, 1979, 776.

Compound	*m.p./°C*	$[\alpha]_D$*[1]	*Conc.*	*Solvent*	*Ref.*
N^{α}-Boc-N^{π}-Pac-His-OH	181—183	+ 9.4	1	MeOH	52
N^{α}-Boc-N^{π}-Pac-His-OMe	139—141	− 9.3	1	MeOH	52
N^{α}-Z-N^{π}-Pac-His-OH	205—208 (d)	+10.9	0.5	Pyridine	52
N^{α}-Z-N^{π}-Pac-His-OMe,HBr	123—127	−12.5	1	MeOH	52
Z-His(MPS)-OH	64—65	—	—	—	51
Z(OMe)-His(MPS)-OH	79—81	—	—	—	51
Isoleucine					
Ile-NCA	69—70	—	—	—	145
Mpt-Ile,Cha	146—158	−13.8	1	EtOH	21
Ppt-Ile,Cha	181—183	− 7.5	1	EtOH	20
Leucine					
Fmoc-Leu-OH	153—155	− 6.93	1	EtOAc	124
HCO-Leu-OBu^t	oil	−49.9	2	EtOH	29
HCO-Leu-OMe	oil	−42.6	2	EtOH	29
Mpt-Leu,Dcha	146—156	−30.0	1	EtOH	21
Ppt-Leu,Dcha	137—138	−15.0	1	EtOH	20
Ppt-Leu-OH	60—70	−17.5	1	EtOH	20
$TosO^-H_2^+$-Leu-OEt	153—154	+ 6.0*[2]	4	DMF	42
$TosO^-H_2^+$-Leu-OPh	172.5—175	+ 5.6	2	DMF	39
Z-Leu-OPh	oil	—	—	—	39
Lysine					
Adoc-Lys(Adoc)-OH	65	− 0.9	1	DMF	229
H-Lys(Adoc)-OH	227—229	− 1.3	1	MeOH	229
Mpt-Lys(Z),Dcha	123—124	+ 5.0	1	EtOH	21
Lys(Tfa)	260	+14.8	3	Dichloroacetic acid	144
Lys(Tfa),NCA	100	—	—	—	144
Z-Lys(Adoc)-OCp	—	—	—	—	229
Z-Lys(Adoc)-OH	112	+ 0.9*[5]	1	DMF	229
Methionine					
Boc-Met-OPh	73—75	−48.4	1	MeOH	39
Mpt-Met,Dcha	147—149	−10.0	1	EtOH	21
Ppt-Met,Dcha	145—146	− 1.2	1	EtOH	20
Phenylalanine					
$Br^-H_2^+$-Phe-OPh	232—233	+13.7*[4]	1	H_2O	39
Mpt-Phe,Dcha	165—170	− 7.5	1	EtOH	21
Ppt-Phe,Dcha	190—191	+ 8.7	1	EtOH	20
Z-Phe-OPh	105—108	−18.9	1	EtOH	39
Proline					
$Cl^-H_2^+$Pro-OBop	144—146	−27.6	1	MeOH	17
Mpt-Pro,Dcha	131—133	−75.0	1	EtOH	21
Nps-Pro-OBop	88—89	−54.4	1	MeOH	17
Ppt-Pro,Dcha	194—195	−40.0	1	EtOH	20
Ppt-Pro-OH	128—130	−15.0	1	EtOH	20
$(Tfp\text{-}Pro)_2O$	78—80	−50.5	0.5	Benzene	386
Tfp-Pro-OH	114—115	−54.0	0.5	MeOH	386
Serine					
Boc-Ser(Me)-OH	115—117	+17.5	2	MeOH	391

[389] N. T. Boggs III, B. Goldsmith, R. E. Gawley, K. A. Koehler, and R. G. Hiskey, *J. Org. Chem.*, 1979, **44**, 2262.

[390] I. Photaki, I. Samouilidis, S. Caranikas, and L. Zervas, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2599.

[391] F. M. F. Chen and N. L. Benoiton, *J. Org. Chem.*, 1979, 2299.

Compound	*m.p.*/°C	$[\alpha]_D$*[1]	*Conc.*	*Solvent*	*Ref.*
H-CO-Ser-OBzl	77—78	−18.2	2	EtOH	29
Ppt-Ser(Bzl), Dcha	138—139	+ 5.0	1	EtOH	20
Ppt-Ser, Dcha	157—158	− 5.0	1	EtOH	20
$TosO^-H_2^+$-Ser(Bu^t)-OPh	158.5—159	+28.3	2	$CHCl_3$	39
Z-Ser-OBu^t	93—95	+16.5*[3]	1.03	EtOH	43
Threonine					
Boc-Thr-(Me)-OH	125—127	+ 7.0	2	MeOH	391
		+ 2.6	2	DMF	
HCO-Thr-OBzl	84—85	−18.7	2	EtOH	29
Ppt-Thr, Dcha	147—149	−10.0	1	EtOH	20
Z-Thr(Bzl)-OPfp	90—92	−30.6	0.5	EtOH	284
Z-Thr-OBu^t	66—67	−20.6*[3]	1.07	EtOH	43
Tryptophan					
Boc-Trp(HCO)-OSu	154—155	−26.1	1	MeOH	46
Boc-Trp-OPh	154—155	−19.8	1	DMF	39
$Cl^-H_2^+$-Trp-OPh	210(d)	+34.5	1	DMF	39
H-Trp(HCO)-OBu^t oxalate	174—175	+17.6	1	MeOH	46
H-Trp-OBu^t	155—156	+19.7	1	MeOH	46
Mpt-Trp, Dcha	180—185	−12.5	1	MeOH	21
Ppt-Trp, Dcha	190—191	+ 7.5	1	EtOH	20
Ppt-Trp-OH	76—82	− 8.7	1	EtOH	20
$TosO^-H_2^+$-TrpOEt	139—140	+15.4*[5]	4	EtOH	42
Z-Trp(HCO)-OH	108—110	—	—	—	46
Z-Trp(HCO)-OH, Dcha	154—155	+25.0	1	MeOH	46
Z-Trp(HCO)-OSu	106—107	−52.0	1	DMF	46
Tyrosine					
Boc-Tyr(Me)-OH	153—155	+34.4	0.5	MeOH	350
H-Tyr(Me)OMe, HCl	194—196	+68	1.04	Pyridine	303
H-Tyr($SO_3Ba_{\frac{1}{2}}$)-OH	232	−24.8	1	95% DMF	57
Mpt-Tyr, Dcha	160—161	−10.0	1	EtOH	21
Mpt-Tyr(Bzl), Dcha	169—172	−10.0	1	EtOH	21
Ppt-Tyr(Bzl), Dcha	182—184	+15.0	1	MeOH	20
Ppt-Tyr, Dcha	192—193	− 1.2	1	MeOH	20
Ppt-Tyr(Ppt)-OH	117—124	−17.5	1	EtOH	20
Z-Tyr($SO_3Ba_{\frac{1}{2}}$)-$OBa_{\frac{1}{2}}$	—	+18.9	1	DMF	57
Valine					
Fmoc-Val-OH	144—145	+4.79	1	EtOAc	124
HCO-Val-OBu^t	63—64	−33.7	2	EtOH	29
HCO-Val-OBzl	44—45	−40.2	2	EtOH	29
Mpt-Val, Dcha	148—149	−12.5	1	EtOH	21
Ppt-Val, Dcha	149—151	−10.0	1	EtOH	20
Ppt-Val-OH	112—114	−17.5	1	EtOH	20
$TosO^-H_2^+$-Val-OEt	147—149	+12.4	4	DMF	42
Other Amino-acids					
Alanine related					
Mpt-D-Ala, Dcha	172—173	+ 7.5	1	EtOH	21
Z-β-Ala-OTcp	78—79	—	—	—	259
(+)-β-(2-furyl)-β-Ala, quinine salt	205—207	+12.9	1	H_2O	387
Z-(+)-β-(2-furyl)-β-Ala, quinine salt	109—111	−86.0	1	MeOH	387

Compound	*m.p.*/°C	$[\alpha]_D$*[1]	*Conc.*	*Solvent*	*Ref.*
Z-β-(2-furyl)-β-Ala, quinine salt	123—125	+55.9	1	MeOH	387
Bmv-(3-pyridyl)Ala-OH, Dcha	150—153	−182	0.35	MeOH	226
(+)-β-(2-thienyl)-β-Ala, quinine salt	206—208	+15.3	1	H_2O	387
Z-(+)-β-(2-thienyl)-β-Ala, quinine salt	128—129	+45.4	1	MeOH	387
Carboranylalanine (Car)					
Boc-Car-OH	158	−22.6	0.86	MeOH	192
Boc-Car-OMe	121—122	−30.2	0.7	MeOH	192
N-Methylcysteine [(Me)Cys]					
S-4-pyridyl(Me)Cys	219—221	−11.5	2.4	5M-HCl	180
Diaminopropionic acid (Dpr)					
Boc-D-Dpr(Tos)-OH	143—145	+3.13	1	MeOH	354
Boc-Dpr(Tos)-OH	142—143	+3.0	1	MeOH	354
Boc-D-Dpr(Tos)-OH	142—144	+9.6	1	MeOH	354
Z-Dpr(Tos)-OH	143—145	−11.7	1	MeOH	354
γ-Carboxyglutamic acid (Gla)					
H-Gla(OBu^t)$_2$-OH	163—164	−5.7*[3]	1	MeOH	389
Z-D-Gla(OBu^t)$_2$-$NHNH_2$	63.5—65	− 6.2	1	$CHCl_3$	389
Z-Gla-(OBu^t)$_2$-OH	85—86	−12.1	1.1	MeOH	389
Z-Gla-(OBu^t)$_2$quinine salt	149—150	−21.6	1	MeOH	389
4-Hydroxyproline (Hyp)					
Z-Hyp-OBu^t	62—63	−68.6*[4]	1.09	EtOH	43
allo-Isoleucine					
$Cl^-H_2{}^+$-*allo* Ile-OMe	124—125	−22.3	1	H_2O	145
Boc-D-*allo* Ile-OH	64—66	+2.86	1	AcOH	145
		−23.1	1	EtOAc	
Boc-D-*allo* Ile-OH, Dcha	140—141	—	—	—	145
D-*allo*-Ile-NCA	39—40	—	—	—	145
Lanthionine (Lan)					
N^{α}→Z-(D → L)-Lan dimethyl ester oxalate	156—157	+30.8*[5]	1	MeOH	390
N^{α}-Z-(D → L)-Lan-α-Np-α′-methyldiester, HCl	181—183	+36.2*[6]	2	DMF	390
N^{α}-Z-L-Lan dimethyl ester, HCl	183	−26.8	2.3	MeOH	390
N^{α}-Z-L-Lan-α′-methyl ester, HCl	148	−19.4*[9]	2	MeOH	390
N^{α}-Z-L-Lan-α-phenacyl α′-methyl diester,HCl	96—99	−19.7*[2]	2	MeOH	390
N^{α}-Z-L-Lan-α-pNp-α′-methyl diester, HCl	157—158	−25.7*[6]	2	DMF	390
N^{α}-Z,$N^{\alpha'}$-Trt(D → L) Lan-α^1-methyl ester	60—75	+77.3	2.5	MeOH	390
N^{α}→Z,$N^{\alpha'}$-Trt-(D → L)-Lan-α-pNp-α′-methyl diester	58—70	+76.5*[5]	2.3	EtOAc	390
N^{α}-Z,$N^{\alpha'}$-Trt-Lan-α′-methyl ester	80	+38.9*[4]	2.5	MeOH	390
N^{α}-Z,$N^{\alpha'}$-Trt-Lan bis diethyl ammonium salt	154—156	+ 8.9*[8]	2	MeOH	390

Compound	*m.p./°C*	$[\alpha]_D$*[1]	*Conc.*	*Solvent*	*Ref.*
Z-(D → L)-Lan	200—201	+23.1*[6]	3	1 M-HCl	390
Z-(L → D)-Lan	187—188	−23.3*[6]	3	1 M-HCl	390
Z-L-Lan	192—193	−24.3*[7]	2	1 M-HCl	390
N-Methyl-leucine [(Me)Leu]					
HCO-(Me)-Leu-OMe	oil	−49.4	2	EtOH	29
N-Methylphenylalanine [(Me)Phe]					
Boc-(Me)Phe(4-NO_2)-OH	—	−73.9	1.1	95% HOAc	189
H-(Me)Phe(4-NO_2)-OH	155(d)	+31	1	6 M-HCl	189
Ornithine (Orn)					
Boc-Orn(Adoc)-OCp	95—98	−21.3*[5]	1	DMF	229
Bpoc-Orn(Adoc)-OH	110—112	+ 8.6*[5]	0.5	DMF	229
H-Orn(Adoc)-OH	214—216	− 7.8	1	MeOH	229
Z-Orn(Adoc)-OH, Dcha	136—138	+ 7.6*[3]	1	DMF	230
Propargylglycine (Pra)					
Boc-Pra-OH	154—155	+41.1	1.3	MeOH	192
$Cl^-H_2^+$-Pra-OBu^t	195	−17.2	1	MeOH	192
Sarcosine (Sar)					
N-2-Bromethoxycarbonyl-Sar-OBu^t	oil b.pt. 159 °C/16 Torr				34
N-2-Bromethoxycarbonyl-Sar-OH	oil				34
Tryptophan related					
pentadeuterio-L-Trp	—	−30	0.5	H_2O	360
(α-L, β-*S*) (αβ-$^2H_2$)-Trp	—	−26.1	0.5	H_2O	360
Tyrosine related					
Boc-D-Tyr, Dcha salt	213.5—214.5	−44.4[a]	2.1	DMF	316
Boc-D-Tyr-OH	93—95	—	—	—	316
D-Tyr(Me)OMe, HCl	189—190	−68	1.04	Pyridine	303
3-I-Tyr(Bzl)	210—212	− 3.0[a]	2	70% HOAc	383
Boc-3-I-Tyr(Bzl)	126.5—127	+17.0[a]	1	EtOAc	383
$Cl^-H_2^+$-3-I-Tyr(Bzl)OMe	168—170.5	+ 9.0[a]	1	MeOH	383
Boc-Tyr(3-NO_2)-OH	144	+26.6	0.5	MeOH	81
Boc-Tyr(3-NO_2)-OTmb	89 and 114	−15.8	0.5	DMF	81
$Cl^-H_2^+$-Tyr(3-NO_2)-OTmb	173—174	−30.4	0.5	DMF	81
Z-Tyr(3-NO_2)-$NHNH_2$	188—191	—	—	—	276
Z-Tyr(3-NO_2)-OMe	100—101	− 1.0[a]	1	MeOH	276
D-Valine					
Nps-D-Val-ONSu	138—139	+109.0	0.9	MeOH	17

4
Peptides with Structural Features not Typical of Protein

BY B. W. BYCROFT

1 Introduction

This chapter describes work published during 1978—1979 in the general field indicated by the title. Attention is concentrated on those aspects relating to the modification of reactivity and other properties produced by the presence of an atypical structural unit within a peptide. The synthetic analogues of hormones are excluded from this coverage since they are more appropriately dealt with in the following chapter, and the emphasis here centres largely on naturally occurring peptide antibiotics and ionophores.

The increasing size and complexity of many of these compounds is apparent, and the structures of alamethicin, berninamycin, vancomycin, among others, represent some of the most complex natural products so far characterized. The problems associated with structural elucidation, even with the battery of spectroscopic methods now available, are by no means straightforward, as the recent necessity to revise a small but significant part of the structure of the important antitumour agent bleomycin illustrates.

The β-lactam field is growing at such a prodigious rate that only an outline of developments has been possible. The synthetic achievements in this area can only be described as outstanding and are largely due to impressive advances in synthetic organic chemistry. However, there is evidence here of the blending of the special skills derived from peptide chemistry. The significance of this interaction is more apparent in the synthesis of other types of peptide antibiotics.

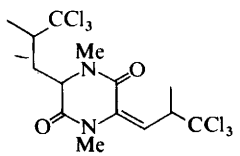
Conformational studies on both natural and synthetic peptides continue apace and slowly the significance of many of the atypical units to the overall topology of the molecules containing them is emerging.

2 Cyclic Peptides (Homodectic Peptides)

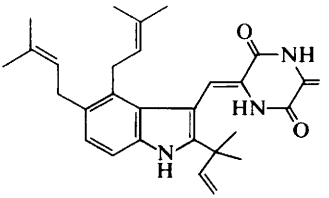
2,5-Dioxopiperazines (Cyclic Dipeptides).—Two further reviews covering naturally occurring cyclic dipeptides have appeared.[1,2] In the main, the new compounds isolated belong to the well-established groups. The chlorine-containing metabolite (1) from an Australian sponge [3] is an exception and is another example of an halogenated amino-acid derivative from a marine source.

The structure of cryptoechinulin G (2) followed from detailed analysis of spectral data [4] and represents a variation on the now familiar group of dioxopiperazines

[1] M. J. O. Anteunis, *Bull. Soc. Chem. Belg.*, 1978, **87**, 627.
[2] B. W. Bycroft, Comprehensive Organic Chemistry, ed. D. Barton and W. D. Ollis, Oxford, 1979, Vol. 6, p. 141.
[3] R. Kazlauskas, P. T. Murphy, and R. J. Wells, *Tetrahedron Lett*, 1978, 4945.
[4] G. Gatti, R. Cardillo and G. Fuganti, *Tetrahedron Lett*, 1978, 2605.

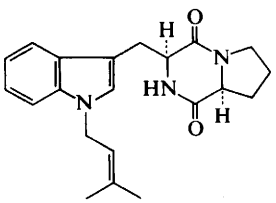


(1)

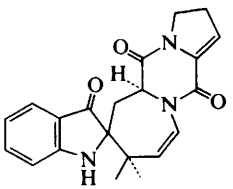


(2)

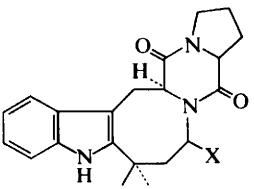
derived from tryptophan and another amino-acid, modified by isoprenylation and other reactions. The uncertainty with respect to the stereochemistry of the double bonds in isoechinulin B [5] and roquefortine [6] has been resolved by interpretation of n.m.r. data. Incorporation studies employing radiolabelled and stable isotope-containing precursors of the neurotoxin roquefortine [7] and echinulin [8] have, as expected, confirmed their derivation from tryptophan and C_5 units. Some aspects of the stereochemistry of the isoprenylation have also been established. An attempted biomimetic synthesis of brevianamide E from the proposed precursor (3) foundered, when the C_5 unit failed to rearrange to the 2-position of the indole ring under the influence of a variety of Lewis acids.[9] Whereas the fungal toxin austamide (4) is the latest of the complex dioxopiperazines to succumb to the synthetic assault of the Havard group. The cornerstone of this synthesis involved the construction of (5), its stereospecific oxidation to a 3-hydroxyindolenine, and subsequent pinacol-type rearrangement.[10]



(3)



(4)

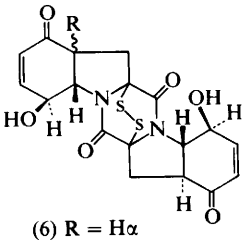


(5)

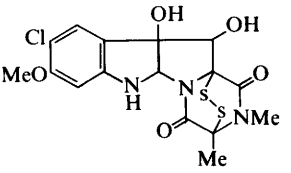
Within the epithiodioxopiperazine group, conformation of the structure of epicorazine A (6) has resulted from crystallographic data which has also established the complete stereochemistry.[11,12] The co-metabolite epicorazine B (7) differs from

[5] H. Nagasawa, A. Isogai, A. Suzuki, and S. Tamura, *Agric. Biol. Chem. (Japan)*, 1979, **43**, 1759.
[6] P. M. Scott, J. Polonsky, and M. A. Merrien, *J. Agric. Food Chem.*, 1979, **27**, 201.
[7] K. D. Barrow, P. W. Colley, and D. E. Tribe, *J. Chem. Soc., Chem. Commun.*, 1979, 225.
[8] J. K. Allen, K. D. Barrow, and A. J. Jones, *J. Chem. Soc., Chem. Commun.*, 1979, 280.
[9] P. G. Sammes and A. C. Weedon, *J. Chem. Soc., Perkin Trans. 1*, 1979, 3048 and 3053.
[10] A. J. Hutchinson and Y. Kishi, *J. Am. Chem. Soc.*, 1979, **101**, 6786.
[11] G. Deffieux, M. A. Baute, R. Baute, and M. J. Filleau, *J. Antibiotics*, 1978, **31**, 1102.
[12] G. Deffieux, M. Gadret, J. M. Leger, and A. Carpy, *Acta Crystallogr.*, 1979, **B35**, 2358.

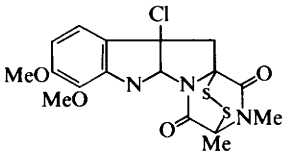
epicorazine A only in the stereochemistry at one of the ring junctions.[13] Two further additions (8) and (9) to the sporidesmin family have been isolated from *Pithomyces chartarum*. The structure of the latter is only a tentative proposal.[14]



(6) R = Hα
(7) R = Hβ

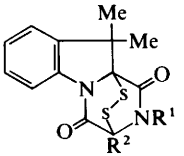


(8)

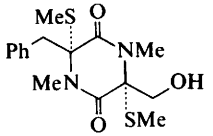


(9)

The potent antiviral and antibiotic activity of many members of this group continues to stimulate synthetic effort. A number of the basic analogues of gliotoxin (10) all of which are inhibitors of reverse transcription have been reported.[15] An elegant method of resolution of the racemic products was employed and it is noteworthy that, in the case investigated, both enantiomers retained full biological activity.[16] A novel approach for the introduction of sulphur into cyclic dipeptides has been developed for a synthesis of gliovictin (11). One of the α-positions was activated by formylation which facilitated the introduction of a thiomethyl group from methyl sulphenyl chloride under mildly basic conditions.[17]



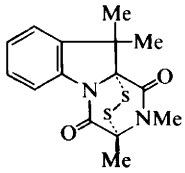
(10)



(11)

The controversy relating to the stereochemistry of the desulphurization reaction of epithiodioxopiperazines with triphenylphosphine has been resolved, at least in the case of (12). The resulting monosulphide has been shown conclusively to possess inverted chirality at the bridge-head carbon atoms. A tentative mechanism for this

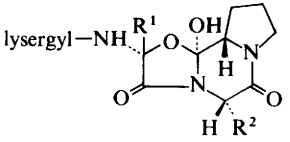
[13] G. Deffieux, M. J. Filleau, and R. Baute, *J. Antibiotics*, 1978, **31**, 1106.
[14] R. Rahman, S. Safe, and A. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1476.
[15] H. C. J. Ottenheijm, J. D. M. Herscheid, M. W. Tijhuis, M. Oosterbaan, and E. de Clercq, *J. Med. Chem.*, 1978, **21**, 796.
[16] H. C. J. Ottenheijm, J. D. M. Herscheid, M. W. Tijhuis, R. J. F. Nivard, E. de Clercq, and P. A. J. Prick, *J. Med. Chem.*, 1978, **21**, 799.
[17] R. M. Williams and W. H. Rastetter, *Tetrahedron Lett.*, 1979, 1187.



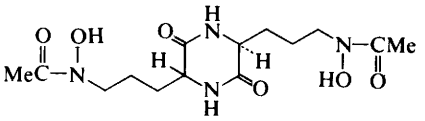
(12)

unusual reaction has been proposed.[18] Extended Hückel calculations on the conformational preferences of the sulphur and carbon–sulphur bridging groups accord with the available *X*-ray data.[19] On the biosynthetic front, fairly strong evidence that only the L,L-isomer of *cyclo*-(Phe-Ser) is the direct precursor of gliotoxin in *Trichoderma viride* has been presented.[20]

It is surprising, in view of the significance of the ergot peptides over the past three decades, that new metabolites are still emerging from commercial strains of *Claviceps purpurea*. 8-Hydroxyergotamine has been obtained from the mother liquors remaining after the isolation of ergotamine,[21] also the peptide alkaloids (13), (14), and (15) which had been obtained previously by total synthesis.[22] The biosynthesis of these important compounds still remains something of an enigma. However, a recent observation that protoplasts from *Claviceps* are capable of synthesizing ergot peptides offers a new avenue of approach in this area.[23]



(13) R^1 = Et, R^2 = CH_2·$CHMe_2$
(14) R^1 = Me, R^2 = $CHMe_2$
(15) R^1 = Et, R^2 = $CHMe_2$



(16)

A considerable deal of interest has been generated in recent years by the isolation of low molecular weight chelating agents which serve as iron-transporting agents in micro-organisms. The cyclic dipeptide rhodotorulic acid (16) from the yeast *Rhodotorula pilimanae* forms a dimeric iron complex (see Figure 1) with three molecules of (16). The potential of (16) as an iron-transport agent for facilitating iron mobilization in man is currently under evaluation.[24] The novel dioxopiperazine (17) which contains a cyclic substituted hydroxamic acid group has been described as part of a programme related to unnatural cyclic amino-acids.[25] The synthesis of the rearrangement product (18) of the complex antibiotic bicyclomycin

[18] J. D. M. Herscheid, M. W. Tijhuis, J. H. Noordik, and H. C. J. Ottenheijm, *J. Am. Chem. Soc.*, 1979, **107**, 1159.
[19] A. R. Gregory and M. Przybylska, *J. Am. Chem. Soc.*, 1978, **100**, 943.
[20] G. W. Kirby, G. L. Patrick, and D. J. Robins, *J. Chem. Soc., Perkin Trans. 1*, 1978, 1336.
[21] A. Krajicek, B. Trtik, J. Spacil, P. Sedmera, J. Vokoun, and Z. Rehacek, *Coll. Czech. Chem. Commun.*, 1979, **44**, 2255.
[22] R. Brunner, P. L. Stutz, H. Tscherter, and P. A. Stadler, *Can. J. Chem.*, 1979, **57**, 1638.
[23] J. E. Robbens, L. J. Cheng, J. A. Anderson, and H. G. Floss, *J. Nat. Prod.*, 1979, **42**, 537.
[24] C. J. Carrano and K. N. Raymond, *J. Chem. Soc., Chem. Commun.*, 1978, 501.
[25] V. J. Lee and R. B. Woodward, *J. Org. Chem.*, 1979, **44**, 2487.

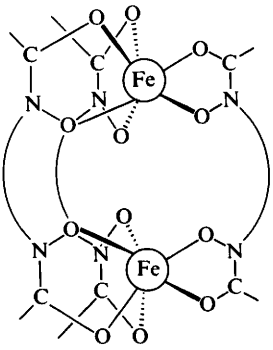
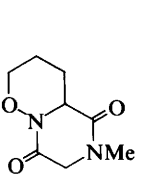
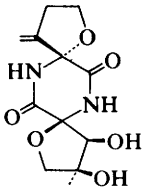


Figure 1 *Proposed structure of the iron*(III) *rhodotorulate dimer*



(17)



(18)

is a notable achievement [26] and will no doubt be extended in due time to a total synthesis. The asymmetric synthesis of amino-acids by the catalytic hydrogenation of cyclic dipeptides containing a L-amino-acid and an α,β-dehydroamino-acid residue is now established as an efficient chiral synthetic method,[27, 28] and the consequent interest in these systems has led to a detailed examination of their geometry.[29] The product of hydrogenation is the thermodynamically unstable L,L-isomer which under basic conditions epimerizes to the more stable L,D-isomer. The ease of this base-catalysed epimerization has been investigated and *N*-alkylation shown to enhance the rate of epimerization.[30]

The dioxopiperazine ring in L,D-isomers or glycine-containing derivatives adopts a predominantly planar conformation, whereas in all the L,L-isomers the ring is buckled (boat conformation) and proline, in particular, accentuates the buckling. Aromatic residues in the amino-acid side-chains lead to the so-called flagpole boat conformation. Further evidence from crystallographic data on *cyclo*-(L-Pro-D-t-Leu),[31] *cyclo*-(L-Ser-L-His),[32] and *cyclo*-(L-His-L-Asp)[33] n.m.r. data on *cyclo*-(L-Asp-L-Pro)[34] and a series of L-phenylalanine-containing cyclic peptides,[35] and

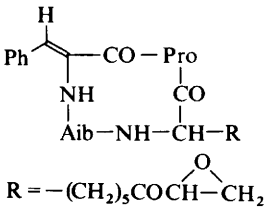
[26] H. Maag, J. F. Blount, D. L. Coffen, T. V. Steppe, and F. Wong, *J. Am. Chem. Soc.*, 1978, **100**, 6787.
[27] S. Lee, T. Kanmera, H. Aoyagi, and N. Izumiya, *Int. J. Pept. Protein Res.*, 1979, **13**, 207.
[28] T. Kanmera, S. Lee, H. Aoyagi, and N. Izumiya, *Tetrahedron Lett.*, 1979, 4483.
[29] C. Shin, M. Hayakawa, T. Suzuki, A. Ohtsuka, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 550.
[30] P. Gund and D. F. Veber, *J. Am. Chem. Soc.*, 1979, **101**, 1885.
[31] I. Skenar and J. Jecny, *Acta Crystallogr.*, 1979, **B35**, 513.
[32] M. Cotrait and M. Ptak, *Acta Crystallogr.*, 1978, **B34**, 528.
[33] R. Ramani, K. Venkatesan, and R. E. Marsh, *J. Am. Chem. Soc.*, 1978, **100**, 949.
[34] F. Piriou, F. Toma, J. Savrda, and S. Fermandjian, *Tetrahedron*, 1979, **35**, 441.
[35] B. Liberek and M. Bednarek, *Pol. J. Chem.*, 1978, **52**, 1099.

circular dichroism on a series of cyclic dipeptides containing L-proline[36] have provided support for these general observations. An extensive analysis of the mass spectral fragmentation patterns of *N*-trifluoroacetyl derivatives of dioxopiperazines claims to offer an alternative method of identification of individual compounds.[37]

Naturally Occurring Cyclic Peptides.—This section, as in previous years, deals with cyclic peptides other than 2,5-dioxopiperazines or compounds related to the major groups described under the headings below. The order in which they are considered relates to the number of amino-acid residues in the ring.

Chlamydocin (19) is the only known cyclic tetrapeptide in which all the four peptide bonds possess the *trans* geometry. The [Ala[4]]-analogue was obtained in low yield by cyclization of the trichlorophenyl ester of (20) and on catalytic hydrogenation the dehydrophenylalanine unit is stereospecifically reduced to a L-Phe residue.[38]

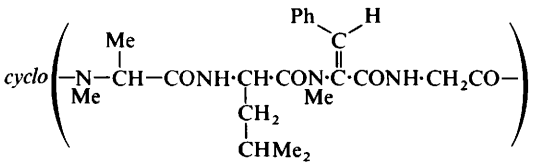
The details of the three alternative methods employed for the synthesis of the plant toxin tentoxin (21) and several analogues have been described. Once again the linear precursors were cyclized using the trichlorophenyl ester method.[39]



(19)

Aib-Phe[(z)Δ]-D-Pro-Ala·OH

(20)



(21)

A re-examination of the solution conformation of (21) as well as an investigation on the [Pro[1]]-analogue has led to the conclusion that previously proposed conformation is incorrect. An alternative is proposed (Figure 2) which is novel for any 12-membered tetrapeptide ring system.[40] D-[Me-Ala[1]]-Tentoxin, on the other hand, exists in two predominant conformers which can be separated by thin layer chromatography and isolated at 4 °C. A detailed spectroscopic study on these conformers has been reported and the activation energy for the equilibrium calculated.[41] Bouvaridine, a plant peptide with high antitumour activity, the

[36] P. Pancoska, I. Fric, and K. Blaha, *Coll. Czech. Chem. Commun.*, 1979, **44**, 1296.
[37] G. P. Slater and L. R. Hogge, *Can. J. Chem.*, 1979, **57**, 2037 and 2052.
[38] D. H. Rich and R. D. Jasensky, *J. Am. Chem. Soc.*, 1979, **101**, 5412.
[39] D. H. Rich, P. K. Bhatnagar, P. Mathiaparanam, J. A. Grant, and J. P. Tam, *J. Org. Chem.* 1978, **43**, 296.
[40] D. H. Rich and P. K. Bhatnagar, *J. Am. Chem. Soc.*, 1978, **100**, 2212.
[41] D. H. Rich and P. K. Bhatnagar, *J. Am. Chem. Soc.*, 1978, **100**, 2218.

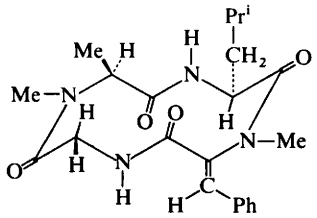
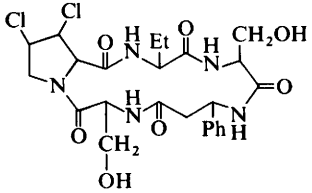
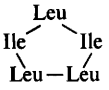


Figure 2 *Revised solution conformation for tentoxin*
(Reproduced by permission from *J. Am. Chem. Soc.*, 1978, **100**, 2212)

structure of which was reported two years ago, also exists in two different conformations which can be separated.[42] The implications of these observations with respect to the analysis of cyclic peptide antibiotics was noted. The cyclic pentapeptide malformin A with a disulphide bridge has fewer degrees of freedom about the ring bonds and a spectral study suggests that in solution one stable conformer predominates.[43] Mass spectrometric analysis of a partial hydrolysate of the potent fungal toxin cyclochlorotine (22) which contains the unusual β-phenylalanine and dichloroproline residues yielded sequence information in accord with the already proposed structure.[44] The cyclic pentapeptide viscumamide from mistletoe contains three leucine and two isoleucine residues. Four of the possible isomers have been synthesized and (23) shown to be identical with the natural product.[45]



(22)

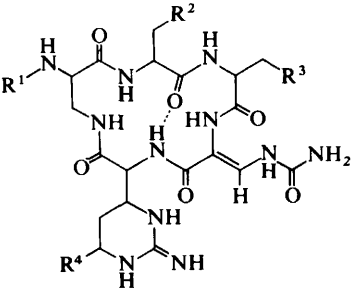


(23)

Viomycin, Capreomycin, Tuberactinomycin, and Related Antibiotics.—The details of the total syntheses of capreomycin IA (24) and IB (25) have now been reported[46] in full. The same approach was adopted for the syntheses of capreomycin IIA (26) and IIB (27) as well as the analogues (28)—(31).[47] As part of the same programme, tuberactinomycin N (32) was obtained by coupling tuberactinamine N (33) with *N,N*-dibenzyloxylcarbonyl-*O*-t-butyl-β-lysine 1-succinimidyl ester and subsequent deprotection.[48] A convenient procedure for the preparation of radiolabelled tuberactinomycin O has been described.[49]

The conformational aspects relating to viomycin (34) continue to attract attention. Hydrogen–deuterium exchange in the ^{1}H, ^{13}C, and ^{15}N n.m.r. spectra of

[42] J. J. Hoffmann, J. Sterling, and J. R. Cole, *J. Chromatogr.*, 1979, **17**, 287.
[43] A. E. Tonelli, *Biopolymers*, 1978, **17**, 1175.
[44] R. J. Anderegg, K. Biemann, A. Manmade, and A. C. Ghosh, *Biomed. Mass Spectrom.*, 1979, **6**, 129.
[45] A. Sakurai and Y. Okumura, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 540.
[46] S. Nomoto, T. Teshima, T. Wakamiya, and T. Shiba, *Tetrahedron*, 1978, **34**, 921.
[47] S. Nomoto and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1709.
[48] T. Shiba, T. Ando, and T. Teshima, *J. Antibiotics*, 1979, 1078.
[49] S. Nomoto, T. Teshima, and T. Shiba, *J. Antibiotics*, 1978, **31**, 792.



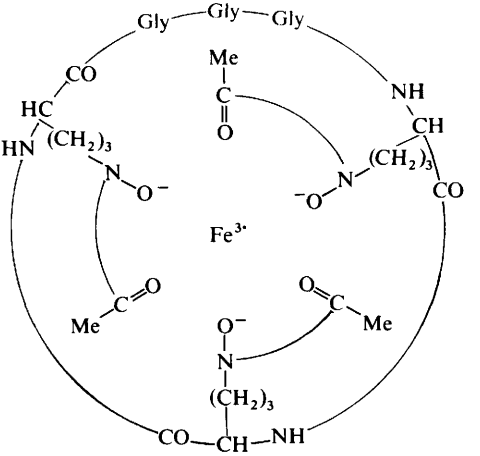
(24) R^1 = H, R^2 = OH, R^3 = NH·β-Lys, R^4 = H
(25) R^1 = H, R^2 = H, R^3 = NH-β-Lys, R^4 = H
(26) R^1 = H, R^2 = OH, R^3 = NH_2, R^4 = H
(27) R^1 = H, R^2 = H, R^3 = NH_2, R^4 = H
(28) R^1 = β-Lys, R^2 = H, R^3 = NH_2, R^4 = H
(29) R^1 = H, R^2 = NH_2, R^3 = H, R^4 = H
(30) R^1 = H, R^2 = H, R^3 = $CH_2CH_2NH_2$, R^4 = H
(31) R^1 = β-Lys, R^2 = H, R^3 = NH·β-Lys, R^4 = H
(32) R^1 = γ-hydroxy-β-Lys, R^2 = OH, R^3 = OH, R^4 = H
(33) R^1 = H, R^2 = OH, R^3 = OH, R^4 = H
(34) R^1 = β-Lys, R^2 = OH, R^3 = OH, R^4 = OH

the antibiotic have been used to assign the carbonyl resonances,[50] and the relationship between conformation and activity explored in a series of simple derivatives.[51]

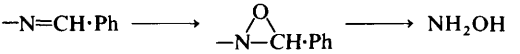
Iron-containing Cyclic Peptides.—All aspects relating to iron–complexing agents are covered in a valuable general review article.[52] A new approach to the synthesis of sideramines makes use of conventional peptide chemistry. The ornithine residues in the cyclic hexapeptide corresponding to ferrichrome (35) can be converted to a hydroxylamino groups *via* an oxaziridine intermediate (Scheme 1). This method has been applied to the synthesis of *enantio*-ferrichrome (36) which exhibited similar biological activity to that of the natural sideramine.[53] Ferrichrome and related peptides are known to undergo pronounced conformational changes on binding metal ions. High-resolution n.m.r. spectroscopy, in particular ^1H [54, 55] and ^{15}N [56] can be employed as sensitive indicators of these changes.

Polymyxins and Related Peptides Containing Fatty Acid Residues.—The polymyxins are a relatively large group of bacterial cyclic lipopeptide antibiotics containing several α,γ-diaminobutyric acid units and a fatty acid residue. The literature on this group has been reviewed up to the beginning of 1977 in a comprehensive article embracing the chemistry, biochemistry, and mode of action.[57] The group of

[50] G. E. Hawkes, E. W. Randall, W. E. Hull, D. Gattegno, and F. Conti, *Biochemistry*, 1978, **17**, 3986.
[51] T. Kitagawa, T. Miura, and H. Kurose, *Chem. Pharm. Bull.*, 1979, **27**, 2551.
[52] K. N. Raymond and C. J. Carrano, *Acc. Chem. Res.*, 1979, **12**, 183.
[53] H. U. Naegeli and W. Keller-Schierlein, *Helv. Chim. Acta*, 1978, **61**, 2088.
[54] A. Demarco, M. Llinas, and K. Wuethrich, *Biopolymers*, 1978, **17**, 2727.
[55] M. Llinas, M. P. Klein, and K. Wuethrich, *Biophys. J.* 1978, **24**, 849.
[56] A. Demarco, M. Llinas, and K. Wuethrich, *Biopolymers*, 1978, **17**, 637.
[57] D. R. Storm, K. S. Rosenthal, and P. E. Saranson, *Annu. Rev. Biochem.*, 1977, **46**, 723.



(35) All L-form
(36) All D-form

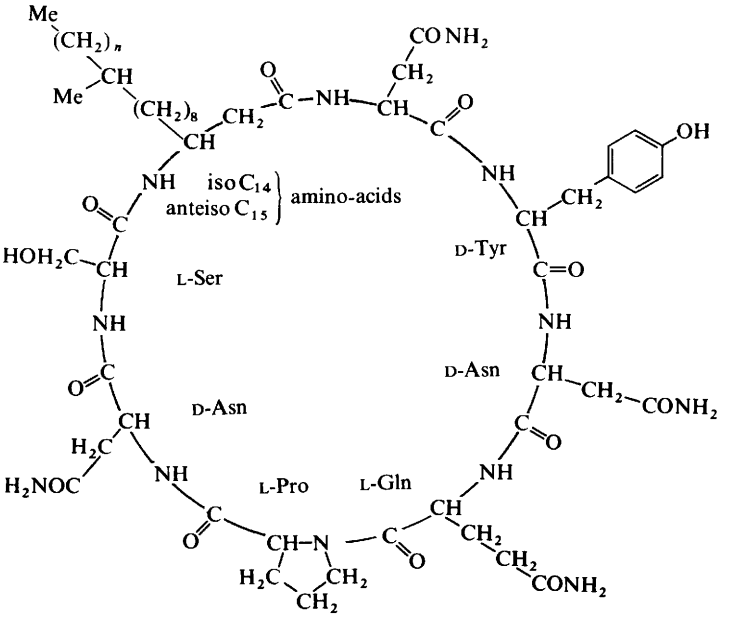


Scheme 1

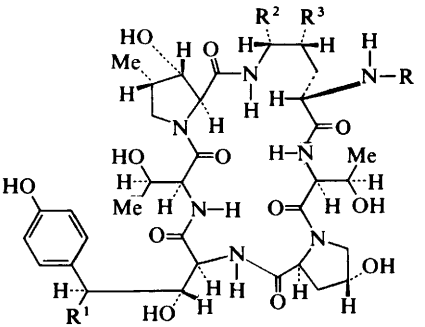
antifungal lipopeptides termed iturines from various strains of *Bacillus subtilis* [58] contain a fatty-acid residue which is bound within the ring structure as a β-amino-acid. The biological activity is associated principally with iturine A (37) and the structure and amino-acid sequence of this component,[59] along with that of the minor component iturine C (38) [60] have followed from chemical and mass spectrometric analysis. The assignment of the absolute configuration to the β-centre of the long chain β-amino-acid (iturinic acid) obtained from the hydrolysate of iturin A is based on an application of the so-called *dnp*-aromatic rule.[61] The antibacterial activity of iturin A and a number of its methylated and acetylated derivatives,[62] as well as preliminary spectral studies concerning the solution conformation [63] have been reported.

Echinocandin C (39) and D (40) are new members of the echinocandin family and have been isolated from the fungus *Aspergillus rugulosus*. The structural assignments rest on spectral and chemical comparisons with the evidence obtained on echinocandin B, the structure of which rests on an *X*-ray analysis. In this group the fatty-acid residue is bound once again within the ring structure as a complex α,γ-amino-acid.[64]

[58] F. Besson, F. Peypoux, G. Michel, and L. Delcambe, *J. Antibiotics*, 1978, **31**, 284.
[59] F. Peypoux, M. Guinand, G. Michel, L. Delcambe, B. C. Das, and E. Lederer, *Biochemistry*, 1978, **17**, 3992.
[60] F. Peypoux, F. Besson, G. Michel, L. Delcambe, and B. C. Das, *Tetrahedron*, 1978, **34**, 1147.
[61] U. Nagai, F. Besson, and F. Peypoux, *Tetrahedron Lett.*, 1979, 2359.
[62] F. Peypoux, F. Besson, G. Michel, and L. Delcambe, *J. Antibiotics*, 1979, **32**, 136.
[63] C. G. Jaureguiberry, B. P. Rogues, L. Delcambe, F. Peypoux, and G. Michel, *FEBS Lett.*, 1978, **93**, 151.
[64] R. Traber, C. Keller-Juslen, H. R. Loosli, M. Kuhn, and A. von Wartburg, *Helv. Chim. Acta*, 1979, **62**, 1252.



(37) R = NH_2
(38) R = OH

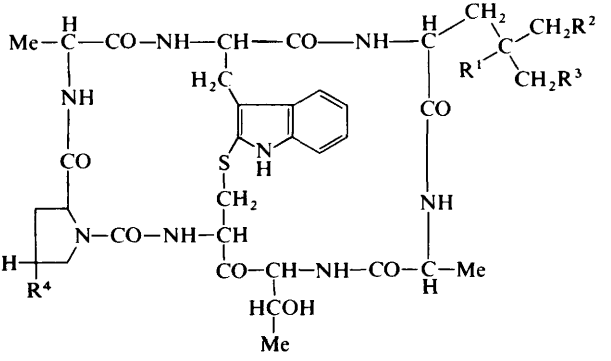


R = linoleoyl
(39) R^1 = H, R^2 = R^3 = OH
(40) R^1 = R^2 = R^3 = H

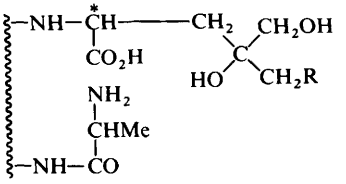
Cyclic Peptides from *Amanita phalloides*.—The isolation of only one new minor peptide in the past two years suggests that the majority of cyclic peptides from this prolific source have now been characterized. Consequently an authoritative review covering the three families of toxic compounds, the amatoxins, the phallotoxins, and phallolysins, is very timely.[65] The new component, prophalloin (41), is in fact

[65] T. Wieland and H. Faulstich, *CRC, Crit. Rev. Biochem.*, 1978, **5**, 185.

non-toxic, but it is suggested that it is the precursor of the toxin phalloin, and (41) has been synthesized using a simple modification of the method already developed for the synthesis of phalloin.[66] The very specific structural requirements for toxicity are further illustrated by an interesting reaction of phalloidin (42) and phallysin (43). Selective ring opening affords the corresponding seco-compounds (44) which can be epimerized at the α-centre of the lactone entity. Recyclization yields a mixture of (42) and (43) together with the diastereoisomers at the centres indicated,

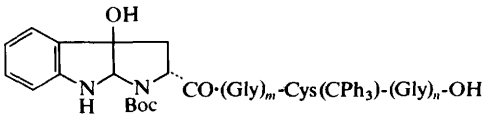


(41) $R^1 = OH$, $R^2 = H$, $R^3 = H$, $R^4 = H$
(42) $R^1 = OH$, $R^2 = OH$, $R^3 = H$, $R^4 = OH$
(43) $R^1 = OH$, $R^2 = OH$, $R^3 = OH$, $R^4 = OH$

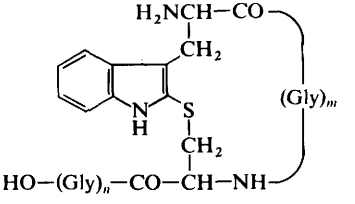


(44)

both of which are non-toxic.[67] In an alternative approach to the bicyclic tryptathionine peptides related to the phallotoxins and amatoxins, the linear peptide (45) has been constructed and shown to undergo, on deprotection with trifluoroacetic acid, an intramolecular thiolysis to monocyclic thioethers (46).



(45)



(46)

[66] E. Munekata, H. Faulstich, and T. Wieland, *Annalen*, 1978, 776.
[67] E. Munekata, H. Faulstich, and T. Wieland, *Annalen*, 1979, 1020.

These were subsequently employed to prepare bicyclic systems related to the toxins.[68] The full details of the crystallographic analysis of β-amanitin confirming all the structural and stereochemical assignments made on the basis of spectral and chemical evidence has appeared.[69] The noteworthy conformational features are the all-*trans* peptide bond geometry and the three intramolecular hydrogen bonds.

Antamanide (47), the non-toxic alkali metal-complexing cyclic decapeptide from *Amanita phalloides*, has also been the subject of an *X*-ray analysis.[70] The backbone

$$\begin{array}{l} \text{Pro}^8\text{—Phe}^9\text{—Phe}^{10}\text{—Val}^1\text{—Pro}^2 \\ \quad\quad\quad\

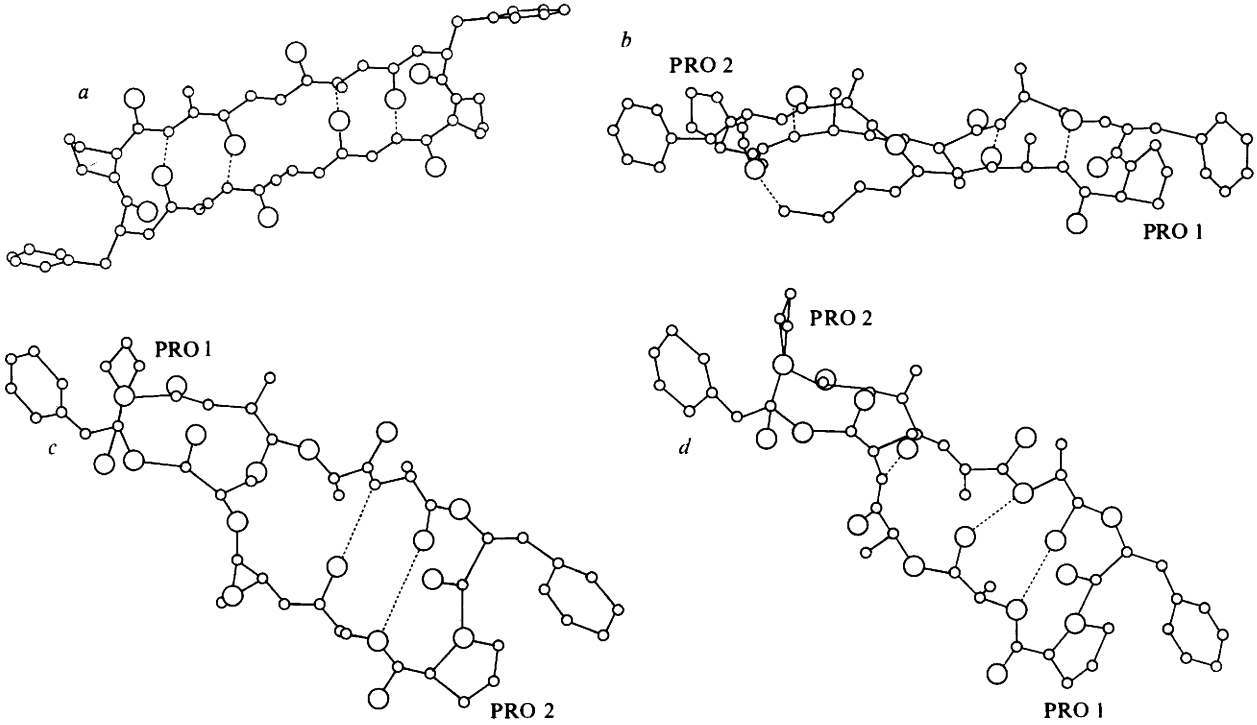


Figure 3 *The crystal structure of Gramicidin S–urea complex viewed from different positions to demonstrate the twist in the molecular backbone and the unpredicted hydrogen bond*
[Reproduced by permission from *Nature* (*London*), 1978, **275**, 206]

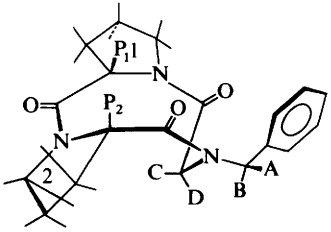


Figure 4 *The predominant boat conformer of* cyclo-(*Pro*$_2$-*NBz-Gly*) (Reproduced by permission from *J. Am. Chem. Soc.*, 1978, **100**, 2548)

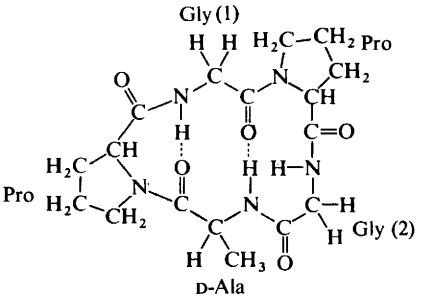
tetrapeptides were encompassed within the lowest energy subset.[84] Further energy calculations, but defining alternating *cis* and *trans* peptide bonds and restricting the structures to those with a symmetry-element-allowed prediction of preferred conformers with respect to side-chain and nitrogen substitution.[85] *Cyclo*-(L-Me·Tyr-L-Ile-L-Pro-L-Leu), an analogue of a phytoxin, clearly does not fit into this pattern, since n.m.r. evidence indicates a conformation with *trans–trans–cis–trans* peptide bonds.[86]

It is only in cyclic penta- and hexa-peptides that intramolecular hydrogen bonding becomes significant and these reflect the structural arrangements (reverse turns) found in protein. Two particular types of hydrogen bonding are now well-documented, the so-called β-turn (1 $\leftarrow$ 4 type) and γ-turn (1 $\leftarrow$ 3 type). A theoretical treatment of β-turns within *cyclo*-(Gly$_5$) together with the effects of introducing D- and L-alanines has been discussed in the light of experimental data.[87] On the more practical side, *cyclo*-(Gly-Pro-Gly-D-Ala-Pro) (50) was designed specifically so that the most likely conformation would contain both a β- and γ-turn.[88] *X*-Ray analysis[89] and n.m.r. studies[88,90] confirm that in both solution and the crystal the predominant conformer contains all-*trans* peptide bonds and the β- and γ-loops shown. Replacement of one of the glycine residues by serine leads to a significant change in the structure of the preferred conformer. Solution[91] and crystal analysis[92] of *cyclo*-(Gly-Pro-Ser-D-Ala-Pro) (51) revealed essentially the same backbone as (50), but with the amino-acid side-chains progressed by one peptide unit so that the β-turn encompasses a glycine and proline residue. A γ-loop was not observed in this case. Independent observations on *cyclo*-(Phe$_3$-Gly$_2$), *cyclo*-(Phe-Gly-Aib-Val-Ala),[93] and *cyclo*-(Phe-Gly-Leu-Val-Ala)[94] have provided evidence that the preferred conformers of these also contain both β- and γ-turns, and it would appear that the γ-loop is more favoured in small peptides than originally assumed. Some tentative general rules covering the relationship of β- and

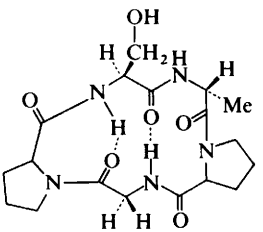
[84] D. N. J. White and C. Morrow, *Comput. Chem.* 1979, **3**, 33.
[85] G. Manjula and C. Ramakrishnan, *Biopolymers*, 1979, **18**, 591.
[86] A. Yasutake, S. Lee, H. Aoyagi, T. Kato, and N. Izumiya, *Chem. Lett.*, 1978, 875.
[87] G. Manjula and C. Ramakrishnan, *Int. J. Pept. Protein Res.*, 1979, **13**, 353.
[88] L. G. Pease and C. Watson, *J. Am. Chem. Soc.*, 1978, **100**, 1279.
[89] I. L. Karle, *J. Am. Chem. Soc.*, 1978, **100**, 1286.
[90] K. L. Williamson, L. G. Pease, and J. D. Roberts, *J. Am. Chem. Soc.*, 1979, **101**, 714.
[91] L. G. Pease, C. H. Niu and G. Zimmermann, *J. Am. Chem. Soc.*, 1979, **101**, 184.
[92] I. L. Karle, *J. Am. Chem. Soc.*, 1979, **101**, 181.
[93] Y. A. Bara, A. Friedrich, H. Kessler, and M. Molter, *Chem. Ber.*, 1978, **111**, 1045.

γ-turns in cyclic pentapeptides to the nature and sequence of the amino-acid residues have been outlined.[94]

Cyclic hexapeptides usually contain two intramolecular hydrogen bonds forming two β-turns. The C_2-symmetric peptides of the general structure *cyclo*-(X-Pro-Y)$_2$



(50)



(51)

have come under close scrutiny from both *X*-ray diffraction and n.m.r. methods. Comparison of n.m.r. results in solution with *X*-ray diffraction data from single crystals is beginning to show evidence for conformational differences associated with averaging on the n.m.r. time-scale. Compounds (52)[95] and (53)[96] have the same structure with C_2-symmetry and β-turn features in both solution and in the crystal. Whereas (54)[97] and (55)[98] both lose their C_2-symmetry on the n.m.r. time-scale on crystallizing and yield structures with only one transmolular hydrogen bond. In all of these peptides, proline has a *trans* peptide bond. Conformations containing both *cis*- and *trans*-Val-Pro peptide bonds were observed for the cyclic hexapeptide (56) in chloroform, while only the *cis*-conformer was detected in

(52) (Gly-Pro-D-Phe)$_2$
(53) (Ala-Pro-D-Phe)$_2$
(54) (Gly-Pro-Gly)$_2$
(55) (Gly-Pro-D-Ala)$_2$
(56) (Val-Pro-Gly)$_2$

water.[99] Samples of (54) labelled with [^{15}N]Gly have been prepared by established procedures. Unusually high ^{13}C–^{15}N spin–spin couplings were observed and these were attributed to the intramolecular hydrogen bonding.[100] Similar solution conformational averaging was observed for (57) and (58) and the evidence favoured a backbone with extended D-residues connecting Leu-Gly turns.[101] Considerable differences have been noted between the crystal conformations of the two

[94] H. Kessler and P. Konder, *Chem. Ber.*, 1979, **112**, 3538.
[95] J. N. Brown and C. H. Yang, *J. Am. Chem. Soc.*, 1979, **101**, 445.
[96] J. N. Brown and R. G. Teller, *J. Am. Chem. Soc.*, 1976, **98**, 7565.
[97] E. C. Kostansek, W. E. Thiessen, D. Schomburg, and W. N. Lipscomb, *J. Am. Chem. Soc.*, 1979, **101**, 5811.
[98] E. C. Kostansek, W. N. Lipscomb, and W. E. Thiessen, *J. Am. Chem. Soc.*, 1979, **101**, 834.
[99] M. A. Khaled, V. Renugopalakrishnan, H. Sugano, R. S. Rapaka, and D. W. Urry, *J. Phys. Chem.*, 1978, **25**, 2743.
[100] J. A. Walter and J. L. C. Wright, *Tetrahedron Lett.*, 1979, 3909.
[101] K. D. Kopple, A. Go, and T. J. Schamper, *J. Am. Chem. Soc.*, 1978, **100**, 4289.

cycloisomeric compounds (59) and (60). In both cases all the peptide bonds were *trans* and both possessed β-turns. However (59) has the unique feature of both type I and type II β-turns.[102] Although formally a cyclic tetrapeptide, *cyclo*-(Leu-Tyr-δ-Ava-δ-Ava) (δ-Ava = δ-aminovaleric acid) has the same ring size as the cyclic

(57) (Gly-D-Val-Leu)$_2$
(58) (Gly-D-Leu-Leu)$_2$

(59) ⌜Ala-Ala-Gly-Gly-Ala-Gly⌝

(60) ⌜Ala-Ala-Gly-Ala-Gly-Gly⌝

hexapeptides. An *X*-ray analysis of this effective inhibitor of chymotrypsin has established that the conformation is intrinsic to this particular molecule and has no intramolecular hydrogen bonds.[103] C.d. spectra of cyclic hexapeptides with firmly established conformations have been used to define two distinct types of spectra associated with two of the many possible turn geometries.[104, 105] Minimal-energy conformations of *cyclo*-(Gly$_6$) have been generated by a Monte-Carlo method which simulated the conformational changes of a molecule in solution. Many of those generated were similar to those observed for cyclic hexapeptides.[106]

Considerable activity still centres on the ion-binding properties of cyclic peptides and the concomitant conformational changes. The cyclic pentapeptide (50), already described, represents a new class of peptide which complexes with the divalent cations, Mg^{2+}, Mn^{2+}, Ca^{2+}, and Ba^{2+}. Preliminary data implicate a least two types of peptide–cation complex with 1 : 1 and 1 : 2 stoichemistry.[88] The strong intramolecular hydrogen bonds exhibited by most simple cyclic hexapeptides have been used to explain their lack of ion-complexing properties. These contrast with the corresponding cyclodepsipeptides of similar ring size, such as the enniatins. It is suggested that *cyclo*-(Pro-Gly)$_3$ and *cyclo*-(D-Ala-Pro-Gly)$_2$[107] exhibit ion-complexing properties because the side-chain interactions reduce the stabilization of the hydrogen bonds and favour the non-bonded conformations similar to those observed in depsipeptides. It was speculated that the bulky aromatic side-chains in the recently synthesized *cyclo*-[Tyr(OBzl)-Gly]$_3$ would have the same effect and evidence has been presented that this molecule does indeed complex with potassium.[108] The cyclic dodecapeptide analogues of valinomycin so far synthesized exhibit pronounced ion-complexing ability, but strikingly different ion-transporting properties from those of (61). Compound (62) has a 10^4 times greater affinity for K^+ than (61), yet is 10^3 times less potent in mediating ion transport across

(61) *cyclo*-(Lac-Val-D-Hyv-D-Val)$_3$
(62) *cyclo*-(Pro-Val-D-Pro-D-Val)$_3$
(63) *cyclo*-(Pro-Val-D-Ala-D-Val)$_3$
(64) *cyclo*-(Pro-Val-Gly-Gly)$_3$
(65) *cyclo*-(Ala-Pro-Gly-Val-Gly-Val)$_2$

[102] M. B. Hossain and D. van der Helm, *J. Am. Chem. Soc.*, 1978, 5191.
[103] I. L. Karle and J. L. Flippen-Anderson, *Acta Crystallogr.*, 1978, **B34**, 3237.
[104] C. A. Bush, S. K. Sarkar, and K. D. Kopple, *Biochemistry*, 1978, **17**, 4951.
[105] M. Iseli, G. Wagniere, J. G. Brahms, and S. Brahms, *Helv. Chim. Acta*, 1979, **62**, 921.
[106] N. Go and H. A. Scheraga, *Macromolecules*, 1978, **11**, 552.
[107] C. H. Niu, V. Madison, L. G. Pease, and E. R. Blout, *Biopolymers*, 1978, **17**, 2747.
[108] J. Al-Hassan and J. S. Davies, *Tetrahedron Lett.*, 1978, 3843.

lipid bilayers. The crystal structure [109] of (62) uncomplexed reveals β-turn features similar to (61) and n.m.r. studies on the cation complexes of (63) implicate a similar bracelet-like structure to those of valinomycin cation complexes. Attempts to account for the poor ion-transporting properties of synthetic peptides in terms of the dissociation kinetics of the complex at the water–membrane interface are discussed.[110] Similar observations with respect to the conformations of (64) and its ion complexes in acetonitrile have been reported, but in CD_2Cl_2 and $CDCl_3$ it adopts a C_3-symmetric conformer which does not complex with cations.[111] Compound (65) has been synthesized not as a model for (61) but in relation to conformational studies on tropoelastin, the soluble precursor protein of elastic fibre of arterial wall. N.m.r. studies show the presence of β- and γ-turns in a variety of polar solvents and these secondary features were compared with those observed in the corresponding linear polyhexapeptide.[112]

Synthesis of Cyclic Peptides.—The synthesis of naturally occurring cyclic peptides and their analogues, as well as synthetic peptides prepared for conformational studies, have been dealt with under the appropriate heading. General aspects relating to synthesis are covered in this section, and no attempt has been made to cover cyclic analogues of hormones.

An interesting paper [113] describing a general procedure for cyclic peptides employed the solid-phase for the synthesis of the linear intermediates. Cyclization of peptides of various sizes was achieved with little racemization using diphenylphosphonyl azide. In the case of the cyclic hexapeptide (66) markedly different yields were obtained from the cyclization of the three linear precursors (67)—(69),

H·D-*Phe*·Pro·Phe·Pro·Phe·Pro·OH
(67)

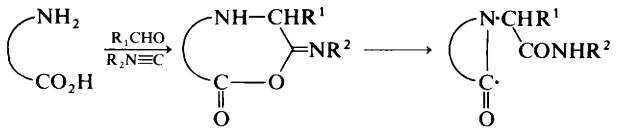
cyclo-(D-Phe·Pro·Phe·Pro·Phe·Pro)
(66)

H·Phe·Pro·D-*Phe*·Pro·Phe·Pro·OH
(68)

H·Phe·Pro·Phe·Pro·D-*Phe*·Pro·OH
(69)

only from (67) was an acceptable amount of monomer obtained. It is now well established that the propensity for cyclization is dependent on the amino-acid sequence of the linear intermediate and various explanations have been invoked for these observations. However, for the above case no satisfactory explanation could be deduced. The potential of the so-called four-component condensation reaction for the cyclic peptides has been explored (Scheme 2).[114] For triglycine the reaction

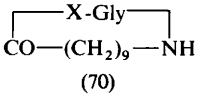
[109] J. A. Hamilton, M. N. Sabesan, B. F. Gisin, and L. K. Steinrauf, *Biochem. Biophys. Res. Commun.*, 1978, **80**, 949.
[110] D. G. Davis and B. F. Gisin, *J. Am. Chem. Soc.*, 1979, **101**, 3755.
[111] K. R. K. Easwaran, L. G. Pease, and E. R. Blout, *Biochemistry*, 1979, **18**, 61.
[112] M. A. Khaled, H. Sugano, and D. W. Urry, *Biochim. Biophys. Acta*, 1979, **577**, 273.
[113] S. F. Brady, S. L. Varga, R. M. Freidinger, D. A. Schwenk, M. Mendlowski, F. W. Holly, and D. F. Veber, *J. Org. Chem.*, 1979, **44**, 3101.
[114] A. Failli, H. Immer, and M. Gotz, *Can. J. Chem.*, 1979, **57**, 3257.



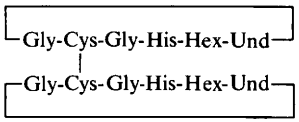
Scheme 2

failed to give the corresponding cyclic tripeptide, but afforded instead the substituted cyclic hexaglycine. With linear hexapeptides, the yield of cyclized material was comparable to those obtained by the standard methods. However, the resultant cyclic peptides possess a *N*-substituent containing a newly generated chiral centre. Consequently, the final product is a mixture of diastereoisomers. It is anticipated that by choosing an appropriate aldehyde it will be possible to remove this substituent after cyclization.

Although it should have rightly been included under the section on peptides from *Amanita phalloides*, the application of Ddz (3,5-dimethoxy-α,α-dimethylbenzyloxycarbonyl)-protected amino-acids mixed anhydride couplings is sufficiently noteworthy to be viewed from the synthetic standpoint.[115] The use of excess Ddz-amino-acid mixed anhydrides effectively suppressed side-reactions and the synthetic manipulations were rapid and the excess amino-acids were completely recycled. The procedure was applied to the synthesis of antamanide analogues. The synthesis of a number of cyclic systems (70), derived from the coupling of a dipeptide with ε-aminocaproic acid, have been described.[116] These were designed essentially as models for β-turns.



(70)



(71) Hex = 6-aminohexanoyl
Und = ω-aminoundecanoyl

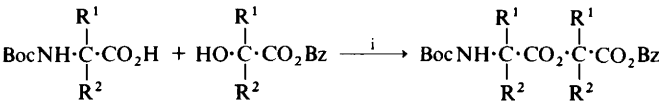
Previous attempts to construct cyclic peptides as simple models for hydrolytic enzymes have afforded disappointing results. The low efficiency of these models was ascribed to intramolecular hydrogen bonding. In order to overcome this problem, (71) which contains a large hydrocarbon residue was synthesized as a precursor for a papain model.[117] Unfortunately, the monomer was not obtained but (71) does exhibit some esterase activity and this has been evaluated.

3 Cyclic Depsipeptides and Cyclic Heterodetic Peptides

An alternative method for the synthesis of depsipeptides which may have implications with respect to cyclic depsipeptides has been reported. Distinct advantages over all other methods are claimed as regards yield, side-reactions, and

[115] C. Birr, M. Nassal, and R. Pipkorn, *Int. J. Pept. Protein Res.*, 1979, **13**, 287.
[116] R. Deslauriers, S. J. Leach, F. R. Maxfield, E. Minasian, J. R. McQuie, Y. C. Meinwald, G. Nemethy, M. S. Pottle, I. D. Rae, H. A. Scheraga, E. R. Stimson, and J. W. van Nispen, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2512.
[117] Y. Murakami, A. Nakano, K. Matsumoto, and K. Iwamoto, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 2690.

racemization. The procedure which is outlined (Scheme 3) is particularly effective for hindered carboxy- and hydroxy-components.[118]

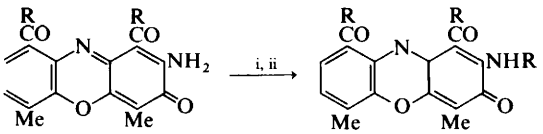


Reagent: i, DCC, 4-dialkylaminopyridine (CH_2Cl_2, 25 °C)

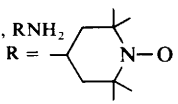
Scheme 3

A comprehensive mass spectrometric study on several naturally occurring cyclic depsipeptides of known structure has established that diagnostic information concerning sequence can be obtained from the fragmentation patterns observed on chemical ionization.[119]

Actinomycin.—An excellent review covering the synthesis and structure–activity relationships among semi-synthetic actinomycins provides a comprehensive coverage of the literature up until 1977.[120] The potential of actinomycin analogues for cancer chemotherapy continues to be the principal motivation for synthetic effort, and several studies [121–125] along standard lines have appeared in the period under review. A convenient route for the preparation of derivatives of actinomycin B spin-labelled on the chromophore has been outlined (Scheme 4) and these compounds



Reagents: i, $HCl/SOCl_2$; ii, RNH_2



and related derivatives

Scheme 4

employed to study DNA binding properties. They were less potent as antibacterial and antitumour agents than the parent antibiotic.[126] The chromophore has also been modified by simple condensation reactions with α-keto-acids. The resultant 1,4-oxazinone derivatives revert to actinomycin D under physiological conditions.

[118] C. Gilon, Y. Klausner, and A. Hassner, *Tetrahedron Lett.*, 1979, 3811.
[119] B. C. Das, P. Varenne, and A. Taylor, *J. Antibiotics*, 1979, **32**, 569.
[120] J. Meienhofer and E. Atherton in 'Structure–activity Relationships among Semi-synthetic Antibiotics', ed. D. Perlman, Academic Press, New York, 1977, p. 427.
[121] A. Chowdhury, J. R. Brown, and R. B. Longmore, *J. Med. Chem.*, 1978, **21**, 607.
[122] P. Cavalieri, P. De Santis, S. Morosetti, and M. Savino, *Gazzetta*, 1978, **108**, 509.
[123] G. P. Vlasov, V. N. Lashkov, and E. N. Glibin, *Zh. Org. Khim.*, 1979, **15**, 983.
[124] V. N. Lashkov and G. P. Vlasov, *Zh. Obshch. Khim.* 1978, **48**, 1875.
[125] G. P. Vlasov, V. N. Lashkov, S. V. Kulikov, and O. F. Ginzburg, *Zh. Org. Khim.*, 1978, **14**, 1961.
[126] B. K. Sinha, M. G. Cox, and C. F. Chignell, *J. Med. Chem.*, 1979, **22**, 1051.

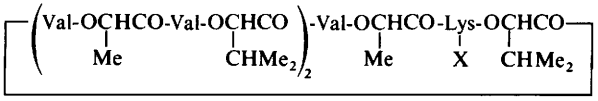
However, those containing lipophilic substituents demonstrated more pronounced antitumour activity and were less toxic than actinomycin D.[127] The role of complexation in the chemotherapy of actinomycin D in the presence of heterosteroid type substances is attracting increasing attention and a detailed n.m.r. study of a specific example examined the nature and orientation of complex formation.[128]

Valinomycin, Enniatins, and Related Compounds.—The Russian group are extending their already extensive programme on the synthesis of ionophoric analogues of valinomycin. The compounds (72)—(74) were prepared by standard step-wise peptide-coupling procedures and stability constants for the complexes with alkali metals were determined.[129] The lysine analogue and its derivatives (75)—(77) were prepared by solid-phase couplings of didepsipeptides followed by deblocking, resin-cleavage, and cyclization.[130] Compound (75) acts as a neutral ion carrier above pH = 7, but the presence of the bulky side-groups in (76) and (77) reduces their effectiveness as ion carriers.[131]

cyclo-(D-Ile-Lac-Ile-D-Hyi)$_n$
(72) n = 2—4

cyclo-(D-Val-Pro-Val-D-Hyi)$_n$
(73) n = 2—4

cyclo-(D-Val-Lac-Val-D-Pro)$_n$
(74) n = 2—4



(75) X = H
(76) X = *p*-nitrobenzyloxycarbonyl
(77) X = dansyl

cyclo-[-(D-Val-Hyi-Val-D-Hyi)$_3$]
(78)

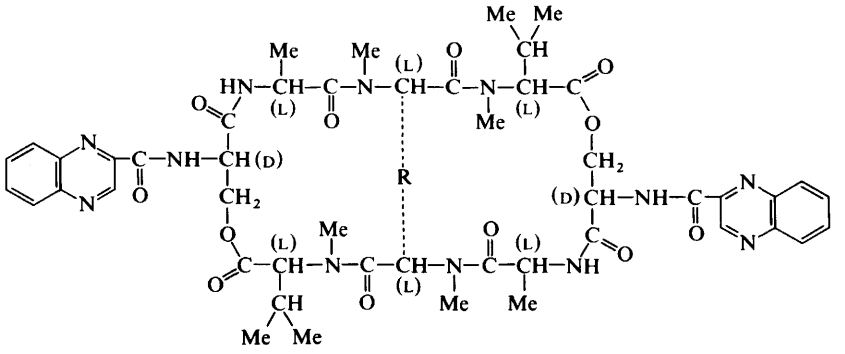
cyclo-(MeVal-D-Hyi-MeVal-Hyi-D-MeVal-Hyi)
(79)

The crystal structure of *meso*-valinomycin (78) shows a bracelet-type conformation stabilized by six identical intramolecular hydrogen bonds. The ester carbonyls are orientated towards the pseudo-axis to form the central cavity and the side-chain isopropyl groups are situated on the periphery of the molecule causing hydrophobic shielding. This differs from the crystal structure of valinomycin but is similar to that proposed for valinomycin in non-polar solvents.[132, 133]

[127] S. K. Sengupta, D. H. Trites, M. S. Madhavarao, and W. R. Beltz, *J. Med. Chem.*, 1979, **22**, 797.
[128] D. J. O'Donnell, K. Ramalingam, A. S. Radhakrishna, R. S. Fischer, N. N. Durham, and K. D. Berlin, *J. Org. Chem.*, 1978, **43**, 4542.
[129] G. Avotins, L. A. Fonina, V. T. Ivanov, and Yu. A. Ovchinnikov, *Bioorg. Khim.*, 1978, **4**, 581.
[130] B. F. Gisin and A. R. Dhundale, *Int. J. Pept. Protein Res.*, 1979, **14**, 356.
[131] G. Stark and B. F. Gisin, *Biophys. Struct. Mech.*, 1979, **6**, 39.
[132] V. Z. Pletnev, N. M. Galitskii, V. T. Ivanov, and Yu A. Ovchinnikov, *Biopolymers*, 1979, **18**, 2145.
[133] A. D. Vasilev, T. A. Shibanova, V. I. Andrianov, V. I. Simonov, A. A. Sanasaryan, V. T. Ivanov, and Yu. A. Ovchinnikov, *Bioorg. Khim.*, 1978, **4**, 1157.

The absence of a suitable isotope of potassium for n.m.r. studies has led to a consideration of the ^{205}Tl complex, and there are indications that n.m.r. data from this nucleus can be used to monitor complex formation and to give information on the groups which are acting as ligands.[134] The factors influencing the effect of valinomycin on lipid-bilayers [135] and model membranes [136, 137] have been discussed in some depth. Full details of the rubidium thioisocyanate complex of the enniatin analogue (79) have been reported. [138] The rubidium ion is complexed with five oxygens of carbonyl groups and the nitrogen of the thioisocyanate.

Quinoxaline Antibiotics.—The principal members of this important group of antibiotics are triostin A (80) and echinomycin (81). These cyclic octadepsipeptides are known to intercalate between the base pairs of double-stranded DNA and, in this general manner, they function *in vivo* as potent inhibitors of RNA synthesis. No new metabolites have been isolated in the period under review and advances have centred around solution conformation and synthesis. Full details of an extensive study on the solution structure of (80) in deuteriochloroform have now been published [139] and provide strong evidence that the molecule exists as an interconverting mixture of two symmetrical conformers resulting from the reversal of chirality of the disulphide bridge.

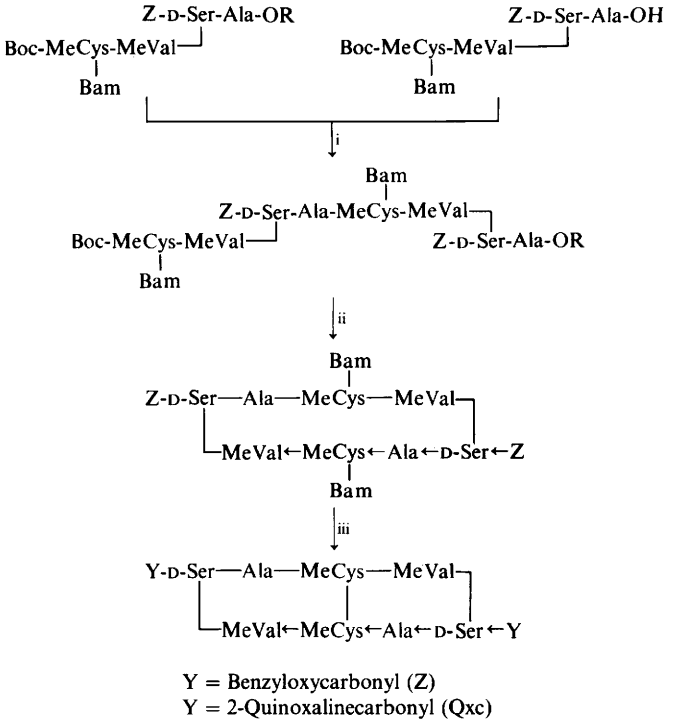


(80) Triostin A, R = $-CH_2-S-S-CH_2-$
(81) Echinomycin R = $-CH_2-S-\underset{\displaystyle SMe}{\underset{|}{CH}}-$

Des-*N*-tetramethyltriostin A was initially synthesized as a model for the total synthesis of (80).[140] The latter objective has now been achieved using a modification of the original route [141] (Scheme 5). The model compound together with the *bis*-L-serine analogue which has also been prepared are both capable of

[134] R. W. Briggs and J. F. Hunton, *J. Magn. Res.*, 1978, **32**, 155.
[135] O. A. Gracheva, A. E. Sokolova, P. L. Privalov, and A. A. Lev, *Dokl. Akad. Nauk. SSSR*, 1979, **246**, 986.
[136] J. Caspers, M. Landuyt-Caufriez, M. Deleers, and J. M. Ruysschaert, *Biochim. Biophys. Acta*, 1979, **554**, 23.
[137] K. Sundaram and R. S. Tyagi, *Int. J. Quantum Chem.* 1979, **15**, 149.
[138] G. N. Tishchenko and Z. Karimov, *Kristallografiya*, 1978, **23**, 729.
[139] J. R. Kalman, T. J. Blake, D. H. Williams, J. Feenety, and G. C. K. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1313.
[140] T. L. Ciardelli, P. K. Chakravarty, and R. K. Olsen, *J. Am. Chem. Soc.*, 1978, **100**, 7684.
[141] P. K. Chakravarty and R. K. Olsen, *Tetrahedron Lett.*, 1978, 1613.

binding to DNA. An alternative approach to the design of linear precursors has been reported.[142]

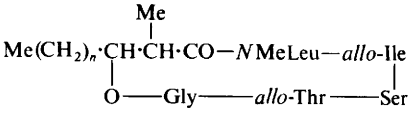


Reagents: i, isobutylchloroformate; ii, DCC, *N*-hydroxysuccinimide; iii, iodine

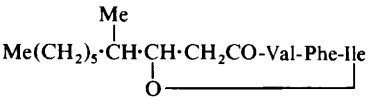
Scheme 5

Other Naturally Occurring Cyclic Depsipeptides.—The globomycins (82) and (83) are noteworthy novel peptidolipids in that they contain the L-*allo* diastereoisomers of the amino-acids isoleucine and threonine.[143, 144] These have not previously been observed in nature and their genesis is of considerable interest. The D-isomer of *allo*-isoleucine has been observed in a number of peptide antibiotics and more recently in beauverilide A (84) from the fungus *Beauveria bassiana*.[145] Similar cyclodepsipeptides have been isolated from a related fungus, but the chirality of the amino-acids was not established.[146] The above-mentioned fungus also affords the insecticidal bassianolide (85) and this compound along with two homologues has now been synthesized.[147] Permetin A (86), a broad-spectrum antibiotic produced

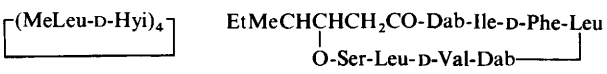
[142] M. Shin, K. Inouye, and H. Otsuka, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 1501.
[143] M. Nakajima, M. Inukai, T. Haneishi, A. Terahara, M. Arai, T. Kinoshita, and C. Tamura, *J. Antibiotics*, 1978, **31**, 426.
[144] S. Omoto, H. Suzuki, and S. Inouye, *J. Antibiotics*, 1979, **32**, 83.
[145] A. Isogai, M. Kanaoka, H. Matsuda, Y. Hori, and A. Suzuki, *Agric. Biol. Chem.*, 1978, **42**, 1797.
[146] F. Frappier, M. Pais, J. F. Elsworth, and J. F. Grove, *Phytochemistry*, 1978, **17**, 545.
[147] M. Kanaoka, A. Isogai, and A. Suzuki, *Agric. Biol. Chem.*, 1979, **43**, 1079.



(82) $n = 5$
(83) $n = 7$



(84)



(85) (86)

by a species of *Bacillus circulans*, is a further member of the polypeptin family of antibiotics.[148] These are related to the polymyxins in that they contain several diaminobutyric acid residues. A sequence for the amino-acids in the toxic cyclodepsipeptide reoseotoxin B has been assigned on the basis of the interpretation of the mass spectral fragmentation pattern.[149] The presence of β-alanine within the ring structure is unusual.

An excellent review covering all aspects related to structure, conformation, and biological activity of the virginamycin family offers an up to date coverage on this significant group of antibiotics.[150] The recently performed *X*-ray analysis on virginamycin S has confirmed the primary structure, previously determined by chemical methods. It also showed that the macrocyclic ring was constrained by transannular hydrogen bonds and only one of the seven peptide bonds possessed *cis* geometry.[151] The solution conformation appears to be similar to that adopted in the crystal lattice.[152] One of the major problems with reviewing this particular group of antibiotics concerns the plethora of synonyms which exist for the individual members of the group. Similar fermentation experiments aimed at producing congeners of viridogrisein[153] and etamycin[154] have been described. However, it is only on reading the papers in depth that it is apparent that these names refer to the same antibiotic.

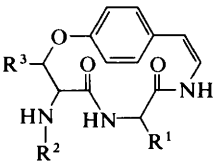
Synthetic endeavour relating to naturally occurring cyclodepsipeptides has been confined to the exploitation of already developed syntheses. These include the

[148] Y. Takeuchi, A. Murai, Y. Takahara, and M. Kainosho, *J. Antibiotics*, 1979, **32**, 121.
[149] G. W. Engstrom, *J. Agric. Food Chem.*, 1978, **26**, 1403.
[150] C. Coito, *Microbiol. Rev.*, 1979, **43**, 145.
[151] J. P. Declercq, G. Germain, M. van Meerssche, S. E. Hull, and M. J. Irwin, *Acta Crystallogr.*, 1978, **B34**, 3644.
[152] R. E. A. Callens and M. J. O. Anteunis, *Biochim. Biophys. Acta*, 1979, **577**, 337.
[153] C. Chopra, D. J. Hook, L. C. Vining, B. C. Das, S. Shimizu, A. Taylor, and J. C. L. Wright, *J. Antibiotics*, 1979, **32**, 392.
[154] Y. Okumura, T. Takei, M. Sakamoto, T. Ishikura, and Y. Fukagawa, *J. Antibiotics*, 1979, **32**, 584 and 1002.

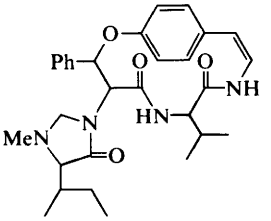
preparation of analogues and derivatives of AM toxin I and II,[155, 156] as well as a congener of monamycin.[157]

4 Cyclopeptide Alkaloids

The isolation of the new cyclopeptide alkaloids from species belonging to the family *Rhamnaceae* continues apace. All contain the 14-membered ring system and differ only in the constituent amino-acids and the degree of methylation of the side-chain amino-acid. Five new alkaloids (87)—(91) have been characterized as part of a systematic study of Californian *Ceanothus* species.[158, 159] Field desorption mass spectrometry played a prominent role in the initial identification. Bark extracts of *Scutia buxifolia* afforded several known scutiamines together with a new member of the series, scutianine H (92).[160] The bark of *Zizyphus sativa* yielded, in addition to several already described alkaloids, sativanine A (93) and sativanine B (94).[161] The latter is only the second peptide alkaloid to be isolated in which the terminal *N*-methylamino-acid unit is linked to the adjoining amide by a methylene bridge.



(87) $R^1 = CH_2$-β-indolyl, $R^2 = N$MeVal, $R^3 = Ph$
(88) $R^1 = CH_2$-β-indolyl, $R^2 = N$Me$_2$Val, $R^3 = CHMe_2$
(89) $R^1 = CH_2Ph$, $R^2 = N$Me$_2$Ile, $R^3 = Ph$
(90) $R^1 = CH_2CHMe_2$, $R_2 = N$MeIle, $R^3 = Ph$
(91) $R^1 = CHMeEt$, $R^2 = N$MePhe, $R^3 = CHMe_2$
(92) $R^1 = CHOHPh$, $R^2 = N$Me$_2$Ile, $R^3 = CHMe_2$
(93) $R^1 = CHMe_2$, $R^2 = N$Me$_2$Ile, $R^3 = Ph$

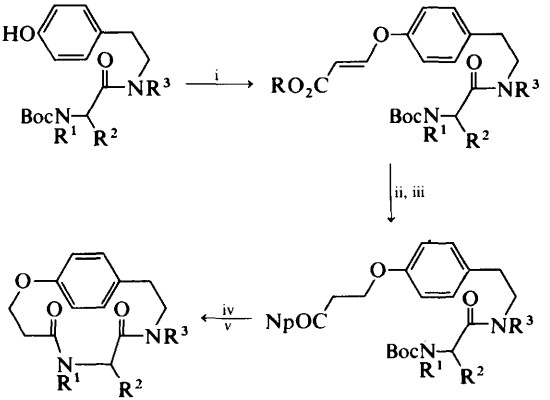


(94)

Although there are now some eighty or more cyclopeptide alkaloids known, no definitive pharmacological activity has been demonstrated for the individual members of this interesting group of natural products. In order to investigate fully

[155] J. Shimohigashi and N. Izumiya, *Int. J. Pept. Protein Res.*, 1978, **12**, 7.
[156] Y. Shimohigashi, S. Lee, T. Kato, and N. Izumiya, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 584.
[157] C. H. Hassall, W. H. Johnson, and C. J. Theobald, *J. Chem. Soc., Perkin Trans.* 1, 1979, 1451.
[158] J. C. Lagarias, D. Goff, F. K. Klein, and H. Rapoport, *J. Nat. Products*, 1979, **42**, 220.
[159] J. C. Lagarias, D. Goff, and H. Rapoport, *J. Nat. Products*, 1979, **42**, 663.
[160] A. F. Morel, R. van Fossen Bravo, F. A. M. Reis, and E. A. Ruveda, *Phytochemistry*, 1979, **18**, 473.
[161] R. Tschesche, A. H. Shah, and G. Eckhardt, *Phytochemistry*, 1979, **18**, 702.

the biological properties associated with these macrocyclic compounds, a general synthetic method has been developed [162] for the synthesis of model 14-membered *para*-bridged ring systems (Scheme 6). Cyclization of the linear precursor was achieved *via* an active ester cyclization. It is particularly noteworthy that the synthetic models bind monovalent and divalent cations, and similar properties have also been demonstrated for the naturally occurring ceanothine B. The same objective has been approached in a slightly different manner by a French group. Unfortunately to date they have not synthesized the 14-membered system but 15, 17, and 18 membered ansa-peptides were obtained.[163, 164] The lack of success with the 14-membered system was attributed to ring strain.



Reagents: i, $\equiv \cdot CO_2R$; ii, H_2 cat.; iii, $p\text{-}O_2N \cdot C_4H_6 \cdot OCOCF_3$–pyridine; iv, TFA; v, pyridine

Scheme 6

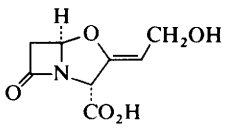
^{13}C N.m.r. analysis of representatives of several types of cyclopeptide alkaloids has led to chemical-shift assignments for certain characteristic types of structures.[165] *X*-Ray analysis had previously established the crystal structure and conformation of frangulanine. A recent ^1H and ^{13}C n.m.r. solution study has revealed solvent-induced conformational changes.[166]

5 Penicillins, Cephalosporins, and Related Antibiotics

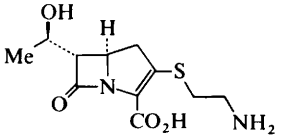
This area is now so prolific that it could easily warrant a chapter to itself, and as a consequence only the more significant developments are outlined. For readers with a casual interest, three valuable reviews [167–169] provide a basis from which this rapidly changing field can be followed.

[162] J. C. Lagarias, R. A. Houghten, and H. Rapoport, *J. Am. Chem. Soc.*, 1978, **100**, 8202.
[163] F. Rocchiccioli, F.-X. Jarreau, and M. Pais, *Tetrahedron*, 1978, **34**, 2911.
[164] F. Frappier, F. Rocchiccioli, F.-X. Jarreau, and M. Pais, *Tetrahedron*, 1978, **34**, 2917.
[165] M. Pais, F.-X. Jarreau, M. G. Sierra, O. A. Mascaretti, E. A. Ruveda, C.-J. Chang, E. W. Hagaman, and E. Wenkert, *Phytochemistry*, 1979, **18**, 1869.
[166] E. Haslinger, *Tetrahedron*, 1978, **34**, 685.
[167] G. Lowe in 'Comprehensive Organic Chemistry', ed. D. H. R. Barton and W. D. Ollis, Pergamon Press, Oxford, 1979, Vol. 6.
[168] W. J. Wheeler, *Lloydia*, 1977, **40**, 519.
[169] A. K. Mukerjee and A. K. Singh, *Tetrahedron*, 1978, **34**, 1731.

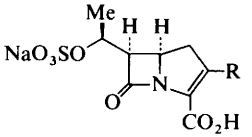
There is no doubt that the so called new generation of naturally occurring β-lactams in particular clavulanic acid (95), thienamycin (96), the olivanic acids (97), and the nocardicins, represented by nocardicin A (98), have occupied a prominent position in the past two years. The current interest in clavulanic acid stems from its potent action as an inhibitor of β-lactamases. The kinetics and chemistry associated with this inhibition for the β-lactamase from *E. coli* have been studied.[170] The enzyme is irreversibly inactivated by a complex series of reactions through which



(95)

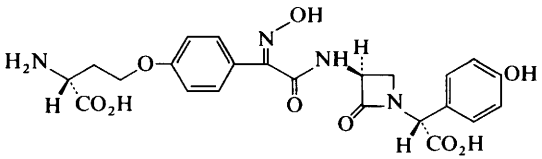


(96)



(97)

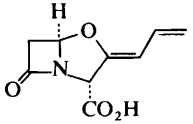
R = SO·CH:CH·NHCOMe
R = S·CH:CH·NHCOMe
R = S·CH_2·CH_2·NHCOMe



(98)

ring-opened forms of clavulanic acid become covalently bound to the protein. In view of the chemotherapeutic potential of (95) and its analogues synthetic studies have abounded. A further total synthesis employed the diene (99) which was itself prepared from the simple azetidinone (100). Subsequent selective ozonolysis and reduction of (99) afforded (95).[171] A wide variety of analogues and derivatives have also been prepared using simple azetidinones similar to (100) as starting materials.[172–175] In addition derivatives derived from clavulanic acid itself, which are claimed to possess improved β-lactamase inhibitory activity have been reported.[176, 177] The most noteworthy among these are (101) and (102). On treating with the dimethylacetal of DMF, (95) undergoes a clean dehydrative decarboxy-

[170] J. Fisher, R. L. Charnas, and J. R. Knowles, *Biochemistry*, 1978, **17**, 2180 and 2185.
[171] P. H. Bentley, G. Brooks, M. L. Gilpin, and E. Hunt, *Tetrahedron Lett.*, 1979, 1889.
[172] P. H. Bentley and E. Hunt, *J. Chem. Soc., Chem. Commun.*, 1978, 439.
[173] P. H. Bentley and E. Hunt, *J. Chem. Soc., Chem. Commun.*, 1978, 518.
[174] P. H. Bentley, G. Brooks, M. L. Gilpin, E. Hunt, and I. I. Zomaya, *Tetrahedron Lett.*, 1979, 391.
[175] P. Lombardi, G. Franceschi, and F. Arcamone, *Tetrahedron Lett.*, 1979, 3777.
[176] P. C. Cherry, G. I. Gregory, C. E. Newall, P. Ward, and N. S. Watson, *J. Chem. Soc., Chem. Commun.*, 1978, 467.
[177] P. C. Cherry, C. E. Newall, and N. S. Watson, *J. Chem. Soc., Chem. Commun.*, 1978, 469.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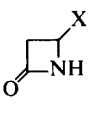
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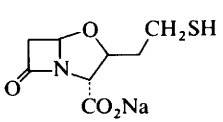
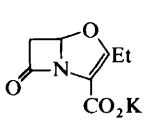
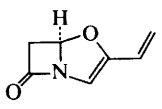
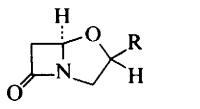

(101)

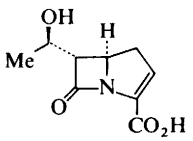

(102)

lation to give the diene (103) in high yield,[178] and this compound has been used in the preparation of a number of decarboxylated analogues of (95). Similar metabolites (104)—(106) have recently been isolated from culture filtrates of a clavulanic acid-producing strain of *Streptomyces clavuligerus*.[179] Their significance in relation to the biosynthesis of clavulanic acid remains to be determined. However, strong evidence that (95) is not derived from the classic β-lactam pathway, but from a glutamate precursor followed from incorporation studies.[180]

The related streptomyces products, thienamycin (96) and the olivanic acids (97), as well as possessing significant inhibitory action against β-lactamases, are also extremely potent antibacterial agents in their own right. The full details of the data relating to the assignment of both structure and stereochemistry of the olivanic acids [181–183] and thienamycin [184] are now available. It is of considerable interest to note the different relative stereochemistry at the centres 6 and 8. Several derivatives of thienamycin have been described,[185] including (107) which lacks the cysteamine


(103)

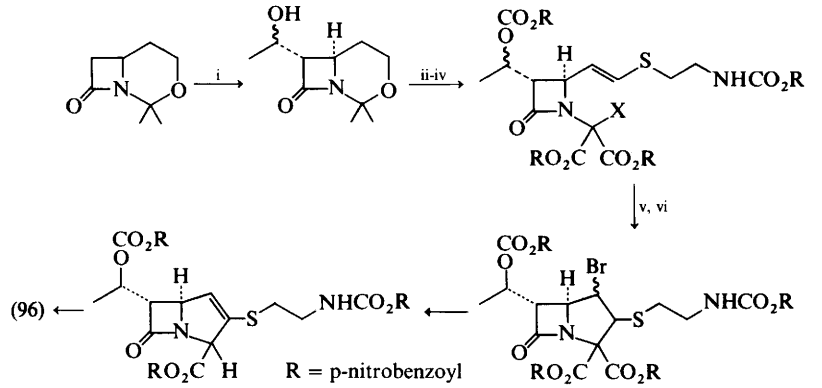

(104) R = CH_2OH
(105) R = CH_2OCHO
(106) R = CO_2H


(107)

side-chain.[186] This compound still retains substantial activity and is claimed to be the simplest active bicyclic β-lactam antibiotic. A considerable amount of synthetic endeavour has been directed towards the synthesis of the basic ring system [187–189] and two independent syntheses of descysteaminylthienamycin (107) and derivatives have been reported.[190, 191] With the considerable amount of work appearing in the whole of this area, it is very easy to overlook the fact that virtually all these molecules are extremely sensitive and all the chemical transformations require

[178] E. Hunt, *J. Chem. Soc., Chem. Commun.*, 1979, 686.
[179] D. Brown, J. R. Evans, and R. A. Fletton, *J. Chem. Soc., Chem. Commun.*, 1979, 282.
[180] S. W. Elson and R. S. Oliver, *J. Antibiotics*, 1978, **31**, 586.
[181] J. D. Hood, S. J. Box, and M. S. Verrall, *J. Antibiotics*, 1979, **32**, 295.
[182] S. J. Box, J. D. Hood, and S. R. Spear, *J. Antibiotics*, 1979, **32**, 1239.
[183] A. G. Brown, D. F. Corbett, A. J. Eglington, and T. T. Howarth, *J. Antibiotics*, 1979, **32**, 961.
[184] G. Alberts-Schonberg, B. H. Arison, O. D. Hensens, J. Hirschfield, K. Hoogsteen, E. A. Kaczka, R. E. Rhodes, J. S. Kahan, F. M. Kahan, R. W. Ratcliffe, E. Walton, L. J. Ruswinkle, R. B. Morin, and B. G. Christensen, *J. Am. Chem. Soc.*, 1978, **100**, 6491.
[185] D. H. Shih, J. Hannah, and B. G. Christensen, *J. Am. Chem. Soc.*, 1978, **100**, 8004.
[186] N. J. Leanza, K. J. Wildonger, T. W. Miller, and B. G. Christensen, *J. Med. Chem.*, 1979, **22**, 1435.
[187] T. Aida, R. Legault, D. Dugat, and T. Durst, *Tetrahedron Lett.*, 1979, 4993.
[188] J. J. Tufariello, G. E. Lee, P. A. Senaratne, and M. Al-Nuri, *Tetrahedron Lett.*, 1979, 4359.
[189] H. Onoue, M. Narisada, S. Uyeo, H. Matsumura, K. Okada, T. Yano, and W. Nagata, *Tetrahedron Lett.*, 1979, 3867.

careful choice of reagents and skilled manipulation. In this light, the synthetic achievements are at least spectacular, and none more so than the recent total synthesis of thienamycin. An outline of the synthesis along with the specific reagents and methods are shown in Scheme 7.[192] The final group of the new antibiotics,



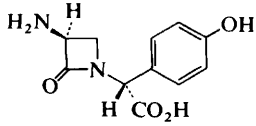
Reagents: i, MeCHO–LDA– –78 °C; ii, H^+, CrO_3–pyr.; iii, $HSCH_2CH_2NHCO_2R$–BF_3; iv, $OC{\cdot}(CO_2R)_2$; v, HCl–$SOCl_2$; vi, Br_2–ether; vii, AgF–pyridine; viii, collidine, 120 °C; ix, di-isopropylamine; x, Pd–C–H_2

Scheme 7

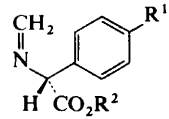
namely the nocardicins, have also been the subject of a number of elegant syntheses. Two groups have described the total synthesis of (98) through the intermediacy of 3-amino-nocardicinic acid (108) which was coupled with the appropriate side-chain.[193–196]

Different routes were chosen for the construction (108). The Japanese group adopted an acid chloride–imine cyclo-addition reaction on a *p*-hydroxyphenylglycine derivative (109), whereas the American group preferred an internal displacement reaction in a substituted thiazolidine (110). An alternative route to racemic (108) employing a cyclopropanone intermediate is claimed to be considerably more efficient than either of the initial methods.[197] Other approaches to the nocardicin skeleton as well as homocyclic analogues have appeared in the past two years.[198, 199] The chemical studies associated with the penicillins have to some extent reflected the developments that have arisen from the new generation of β-

[190] L. D. Cama and B. G. Christensen, *J. Am. Chem. Soc.*, 1978, **100**, 8006.
[191] A. J. G. Baxter, K. H. Dickinson, P. M. Roberts, T. C. Smale, and R. Southgate, *J. Chem. Soc., Chem. Commun.*, 1979, 236.
[192] D. B. R. Johnston, S. M. Schmitt, F. A. Bouffard, and B. G. Christensen, *J. Am. Chem. Soc.*, 1978, **100**, 313.
[193] R. D. G. Cooper, F. Jose, L. McShane, and G. A. Koppel, *Tetrahedron Lett.*, 1978, 2243.
[194] G. A. Koppel, I. McShane, F. Jose, and R. D. G. Cooper, *J. Am. Chem. Soc.*, 1978, **100**, 3933.
[195] T. Kamiya, T. Oku, O. Nakaguchi, H. Takeno, and M. Hashimoto, *Tetrahedron Lett.*, 1978, 5119.
[196] T. Kamiya, M. Hashimoto, O. Nakaguchi, and T. Oku, *Tetrahedron*, 1979, **35**, 323.
[197] H. H. Wasserman and D. J. Hlasta, *J. Am. Chem. Soc.*, 1978, **100**, 6780.
[198] M. Foglio, G. Franceschi, P. Lombardi, C. Scarafile, and F. Arcamone, *J. Chem. Soc., Chem. Commun.*, 1978, 1101.
[199] G. H. Hakimelahi and G. Just, *Can. J. Chem.*, 1979, **57**, 1932 and 1939.



(108)



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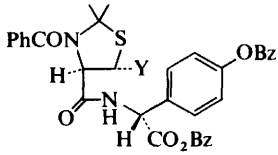
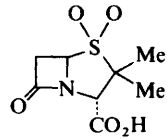
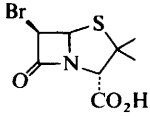


(110)

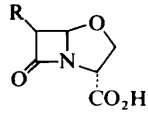


(111)

lactam antibiotics; (111)[200,201] and (112)[202] are interesting examples of compounds readily obtained from penicillins which possess notable β-lactamase inhibitory activity. The semi-synthesis of oxapenam derivatives of general type (113) with their obvious relationship to clavulanic acid has also attracted attention.[203-205] The penems (114) have been the subject of an intensive synthetic investigation by the Basle group over the past decade and some details have now been published.[206] The combination of the structural elements which appear to be associated with the biological activity of both penicillins and cephalosporins was the initial rationale for the study but the obvious structural relationships with (96) and (97) have stimulated further investigations.[207-209] Alternative approaches



(112)



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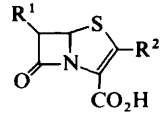

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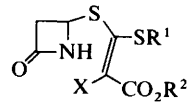


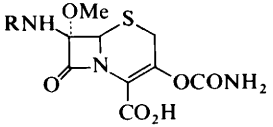
(115)

[200] A. R. English, J. A. Retsema, A. E. Girard, J. E. Lynch, and W. E. Barth, *Antimicrobial Agents Chemother.*, 1978, **14**, 414.
[201] A. R. English and H. C. Neu, *J. Antibiotics*, 1978, **31**, 1238.
[202] B. S. Orlek, P. G. Sammes, V. Knott-Hunziker, and S. G. Waley, *J. Chem. Soc., Chem. Commun.*, 1979, 962.
[203] S. Oida, A. Yoshida, and E. Ohk, *Chem. Pharm. Bull. Jpn.*, 1978, **26**, 448.
[204] T. Kobayashi, Y. Iwano, and K. Hirai, *Chem. Pharm. Bull. Jpn.*, 1978, **26**, 1761.
[205] L. D. Cama and B. G. Christensen, *Tetrahedron Lett.*, 1978, 4233.
[206] I. Ernest, J. Gosteli, C. W. Greengrass, W. Holick, D. E. Jackman, H. R. Pfaendler, and R. B. Woodward, *J. Am. Chem. Soc.*, 1978, **100**, 8214.
[207] M. Lang, K. Prasad, W. Holick, J. Gosteli, I. Ernest, and R. B. Woodward, *J. Am. Chem. Soc.*, 1979, **101**, 6296.
[208] I. Ernest, J. Gosteli, and R. B. Woodward, *J. Am. Chem. Soc.*, 1979, **101**, 6301.
[209] H. R. Pfaendler, J. Gosteli, and R. B. Woodward, *J. Am. Chem. Soc.*, 1979, **101**, 6306.

have involved a copper-mediated ring-closure on intermediates such as (115)[210] and semi-synthesis from clavulanic acid.[211]

The removal of the 6β-amino side-chain from the penicillin skeleton and subsequent synthesis of 6α- and 6β-analogues poses stereochemical problems which have been partially solved by selective removal of labile groups with tributyltin hydride.[212, 213] Further examples of the conversion of penicillins to monocyclic azetidinones which serve as building blocks in many synthetic programmes have been reported.[214–216] In the more traditional areas of penicillin chemistry, further exploration of the now well-established sulphoxide rearrangement,[217, 218] and the synthesis of new spiro[219, 220] and sulphoximine[221] analogues are worthy of mention.

Although the conversion of penicillins to cephem derivatives was demonstrated more than a decade ago, its significance to the production of clinically important cephems is still apparent.[222, 223] The synthesis of the recently isolated cephamycins (116) by the introduction of a 7-methoxy group into the cephalosporin nucleus is now established and further refinements have been added.[224] As with all the cephalosporins and cephamycins, the removal of the aminoadipoyl side-chain for the subsequent preparation of semisynthetic antibiotics can only be achieved chemically. In the case of cephamycin C (116), the standard phosphorus pentachloride method poses problems and an alternative procedure has been



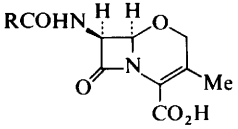
(116) R = aminoadipoyl
(117) R = $COCO_2H$
(118) R = H

developed.[225] The action of oxalyl chloride on the dibenzhydryl ester of (116) afforded the corresponding oxamic acid (117) which on treatment with diphenylcarbodi-imide readily yielded (118).

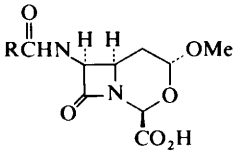
In the past two years major attention has focused on oxygen analogues of cephalosporins and much synthetic effort has been directed towards 1-oxacephems

[210] F. DiNinno, E. V. Linek, and B. G. Christensen, *J. Am. Chem. Soc.*, 1979, **101**, 2210.
[211] P. C. Cherry, C. E. Newall, and N. S. Watson, *J. Chem. Soc., Chem. Commun.*, 1979, 663.
[212] D. I. John, E. J. Thomas, and N. D. Tyrrell, *J. Chem. Soc., Chem. Commun.*, 1979, 345.
[213] J. A. Aimetti, E. S. Hamanaka, D. A. Johnson, and M. S. Kellogg, *Tetrahedron Lett.*, 1979, 4631.
[214] J. E. Baldwin and M. A. Christie, *J. Chem. Soc., Chem. Commun.*, 1978, 239.
[215] A. Suarato, P. Lombardi, C. Galliani, and G. Franceschi, *Tetrahedron Lett.*, 1978, 4059.
[216] A. C. Kaura and R. J. Stoodley, *J. Chem. Soc., Chem. Commun.*, 1979, 344.
[217] T. S. Chou, W. A. Spitzer, D. E. Dorman, S. Kakolja, I. G. Wright, N. D. Jones, and M. O. Chaney, *J. Org. Chem.*, 1978, **43**, 3835.
[218] A. G. M. Barrett, *J. Chem. Soc., Perkin Trans. 1*, 1979, 170.
[219] J. Leclercq, E. Cossement, R. Boydens, L. A. M. Rodriguez, L. Brouwers, F. DeLaveleye, and W. Libert, *J. Chem. Soc., Chem. Commun.*, 1978, 46.
[220] J. C. Sheehan, E. Chacko, Y. S. Lo, D. R. Ponzi, and E. Sato, *J. Org. Chem.*, 1978, **43**, 4856.
[221] J. E. G. Kemp, D. Ellis, and M. D. Closier, *Tetrahedron Lett.*, 1979, 3785.
[222] G. A. Koppel and L. J. Nummy, *Tetrahedron Lett.*, 1978, 25.
[223] G. A. Koppel and L. J. McShane, *J. Am. Chem. Soc.*, 1978, **100**, 289.
[224] T. Kobayashi, K. Iino, and T. Hiraoka, *Chem. Pharm. Bull. Jpn.*, 1979, **27**, 2727.
[225] M. Shiozaki, N. Ishida, K. Iino, and T. Hiraoko, *J. Chem. Soc., Chem. Commun.*, 1978, 517.

(119). Current evidence suggests that the biological activity of these compounds is at least comparable with their natural thia counterparts. Routes which stem from both cephalosporins [226, 227] and penicillins [228, 229] have been developed. The related analogues (120) and (121) which were derived by total synthesis [230] also possess comparable activity with the corresponding cephalosporin derivatives. Other transformations conducted on the cephalosporin skeleton, possibly of general interest, are the synthesis of 2-,[231-233] 3-, 4-,[234] and 7-[235] substituted derivatives.



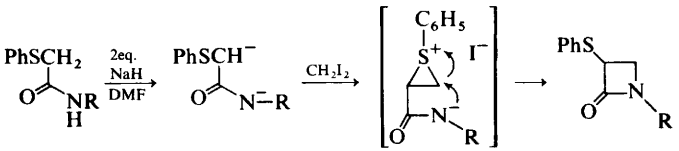
(119)



(120) R = CH_2OPh
(121) R = CH(OCHO)Ph

In the majority of the synthetic studies described above the β-lactam ring moiety was derived from a naturally occurring β-lactam. For those cases where total synthesis of an azetidinone ring was employed the cyclo-addition reaction was the principal method adopted. Re-examination of the cyclization of β-halogeno-propionamides suggests that under appropriate conditions competing elimination reactions can be reduced and yields of β-lactams dramatically improved.[236] This, and a completely novel approach using a 1,3-dianion and methylene iodide [237] (Scheme 8), offer convenient alternatives to this system.

Although the general principles concerning the mode of action of penicillins and cephalosporins on bacterial cell-wall synthesis were established some twenty years



Scheme 8

[226] C. U. Kim and D. N. McGregor, *Tetrahedron Lett.*, 1978, 409.
[227] Y. Hamashima, T. Kubota, K. Ishikura, and W. Nagata, *Tetrahedron Lett.*, 1979, 4943.
[228] M. Yoshioka, I. Kikkawa, T. Tsuji, Y. Nishitani, S. Mori, K. Okada, M. Murakami, F. Matsabará, M. Yamaguchi, and W. Nagata, *Tetrahedron Lett.*, 1979, 4287.
[229] S. Uyeo, I. Kikkawa, Y. Hamashima, H. Ona, Y. Nishitani, K. Okada, T. Kubota, K. Ishikura, Y. Ide, K. Nakano, and W. Nagata, *J. Am. Chem. Soc.*, 1979, **101**, 4403.
[230] J. G. Gleason, T. F. Buckley, K. G. Holden, D. Boles Bryan, and P. Siler, *J. Am. Chem. Soc.*, 1979, **101**, 4732.
[231] T. W. Doyle, T. T. Conway, M. Casey, and G. Lim, *Can. J. Chem.*, 1979, **57**, 222 and 227.
[232] H. Matsumura, T. Yano, M. Ueyama, K. Tori, and W. Nagata, *J. Chem. Soc., Chem. Commun.*, 1979, 485.
[233] T. Hashimoto, Y. Kawano, S. Natsume, T. Tanaka, T. Watanabe, M. Nagano, S. I. Sugawara, and T. Miyadera, *Chem. Pharm. Bull. Jpn.*, 1978, **26**, 1803.
[234] A. Balsamo, P. Crotti, B. Macchia, F. Macchia, A. Rosai, P. Domiano, and G. Nannini, *J. Org. Chem.*, 1978, **43**, 3036.
[235] A. Nadelman, F. Braun, E. Karoly, and R. C. Erikson, *J. Org. Chem.* 1978, **43**, 3788.
[236] H. H. Wasserman, D. J. Hlasta, A. W. Tremper, and J. S. Wu, *Tetrahedron Lett.*, 1979, 549.
[237] K. Hirai and Y. Iwano, *Tetrahedron Lett.*, 1979, 2031.

ago, the detailed mechanism has proved elusive. A renewed interest in the area [238] has arisen from the isolation of model enzymes related to those involved in cell-wall synthesis from various strains of streptomyces. An excellent survey covering the interaction of penicillins with these enzymes has appeared.[239] On the theoretical front, further correlations between the conformation of β-lactams and the transition states of dipeptide reactions involved in cell-wall synthesis are noteworthy.[240]

In spite of considerable effort, the details relating to the biosynthesis of the nuclei of penicillins and cephalosporins remains obscure. However, the involvement of the tripeptide L-α-amino-adipoyl-L-cysteinyl-D-valine as the immediate precursor is now firmly established. Further isolations [241, 242] of this significant peptide together with details of a synthesis [243] by classical procedures have been reported. Related peptides prepared from penicillins by a reaction sequence that is considered to be a formal reversion of the biosynthesis,[244] a biomimetic synthesis of β-lactams from an *O*-substituted hydroxylamine derivative,[245] and a model reaction for the carbon–sulphur bond formation [246] are described among papers providing chemical analogy for proposed biosynthetic pathways. The oxygen atoms of the 7-methoxy group and that linked to the exocyclic methylene group in cephamycin C (116) are derived from molecular oxygen [247] and accords with the view that these are introduced at a later stage in the biosynthesis.

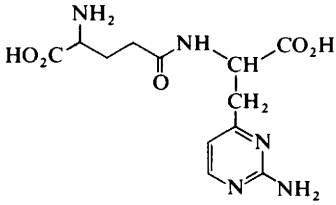
6 Linear Peptides Containing Unusual Structural Features

This section encompasses a variety of naturally occurring linear peptides ranging from dipeptides to nonadecapeptides and these are organized in order of increasing size. Finally some aspects relating to synthetic linear peptides containing unusual or significant features are reviewed.

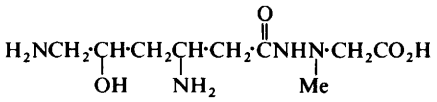
The widespread distribution of γ-glutamyl peptides and their significance has been noted in previous years. However, because of pressure imposed by the lack of space, only those containing an unusual amino-acid have been mentioned. The pyrimidine containing amino acid L-lathyrine is found in many species of *Lathyrus*, and has recently been isolated as its γ-glutamyl derivative (122).[248] The unique antibiotic negamycin (123) which just about qualifies as an unusual dipeptide offered an interesting synthetic challenge. The racemic antibiotic [249] as well as a number of analogues [250] have been prepared. The *N,O*-dibenzoyl reduced dipep-

[238] R. R. Yocum, D. J. Waxman, J. R. Rasmussen, and J. L. Strominger, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2730.
[239] J.-M.Ghysen and J. M. Frère, *Annu. Rev. Biochem.*, 1979, **48**, 73.
[240] D. B. Boyd, *J. Med. Chem.*, 1979, **22**, 533.
[241] H. Shirafuji, Y. Fujisawa, M. Kida, T. Kauzaki, and M. Yoneda, *Agric. Biol. Chem. Jpn.*, 1979, **43**, 155.
[242] L. A. Eriquez and M. A. Pisano, *Antimicrobial. Agents Chemother.* 1979, **16**, 392.
[243] S. Wolfe and M. G. Jokinen, *Can. J. Chem.*, 1979, **57**, 1388.
[244] J. E. Baldwin and M. Jung, *J. Chem. Soc., Chem. Commun.*, 1978, 609.
[245] P. G. Mattingly, J. F. Kerwin, and M. J. Miller, *J. Am. Chem. Soc.*, 1979, **101**, 3983.
[246] J. E. Baldwin and T. S. Wan, *J. Chem. Soc., Chem. Commun.*, 1979, 249.
[247] J. O'Sullivan, R. T. Alpin, C. M. Stevens, and E. P. Abraham, *Biochem. J.* 1979, **179**, 47.
[248] S. I. Hatanaka and S. Kaneko, *Phytochemistry*, 1978, **17**, 2027.
[249] W. Streicher, H. Reinshagen, and F. Turnowsky, *J. Antibiotics*, 1978, **31**, 725.
[250] W. V. Curran and J. H. Boothe, *J. Antibiotics*, 1978, **31**, 914.

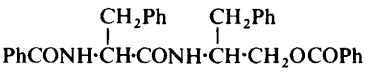
tide (124) is the major mycelial metabolite of *Penicillium canadense*,[251] whereas the isomeric depsipeptide (125) is a product of *Aspergillus flavipes*.[252] Both structures were confirmed by synthesis, but, as yet, no significant biological activity has been reported for either compound.



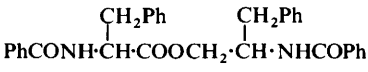
(122)



(123)



(124)



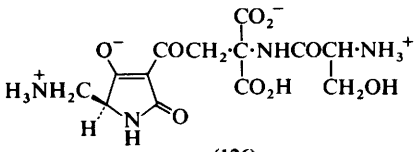
(125)

Malonomicin (126) is unique among peptides in containing an aminomalonic acid unit. The biosynthetic origin of this residue still remains a matter for conjecture, but the tetramic acid nucleus, as expected by analogy with other related microbial products, is derived from 2,3-diaminopropanoic acid and acetate.[253] The successful strategy adopted for the synthesis of this sensitive antibiotic involved the careful base-catalysed condensation of a suitably protected dipeptide entity with a pyrrolidine-2,4-dione derived from 2,3-diaminopropanoic acid.[254]

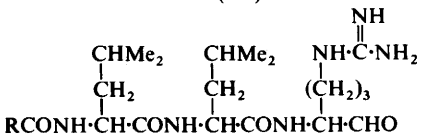
In the past decade, the screening of micro-organisms for specific inhibitors of proteinase enzymes has led to the isolation and characterization of a series of modified peptides. The leupeptins (127) were among the earliest to be recognized. Recent biosynthetic evidence has established that the peptide is built up from the *N*-terminal end and that the reduction of the carboxyl end group of the arginine to an aldehyde occurs after the peptide chain has been constructed.[255, 256] The presence of the aldehyde group in (127) is essential for inhibitory activity. A feature which is present is the newly characterized inhibitor (128) of thiol proteinases from *Streptomyces nigrescens*.[257] The molecule also contains a ureido-group linking the phenylalanine and arginine residue. This had previously been observed in antipain. Amastatin (129) inhibits aminopeptidase A and possesses a new β-amino-acid with

[251] N. J. McCorkindale, R. L. Baxter, T. P. Roy, H. S. Shields, R. M. Stewart, and S. A. Hutchinson, *Tetrahedron*, 1978, **34**, 2791.
[252] A. M. Clark and C. D. Hufford, *Phytochemistry*, 1978, **17**, 552.
[253] D. Schipper, J. L. van der Baan, and F. Bickelhaupt, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2017.
[254] J. L. Van der Baan, J. W. F. K. Barnick, and F. Bickelhaupt, *Tetrahedron*, 1978, **34**, 223.
[255] M. Hori, H. Hemmi, K. Suzukake, H. Hayashi, Y. Uehara, T. Takeuchi, and H. Umezawa, *J. Antibiotics.*, 1978, **31**, 95.
[256] K. Suzukakae, T. Fujiyama, H. Hayashi, M. Hori, and H. Umezawa, *J. Antibiotics*, 1979, **32**, 523.
[257] T. Watanabe, K. Fukuhara, and S. Murao, *Tetrahedron Lett.*, 1979, 625.

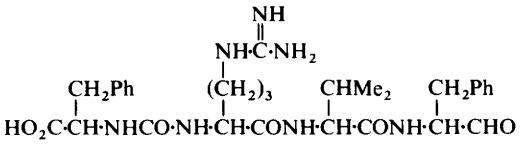
the chirality indicated. The amino-acid sequence was established by mass spectrometry and confirmed by total synthesis.[258]



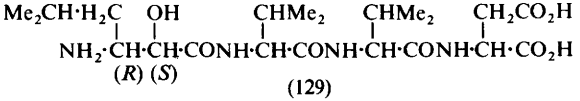
(126)



(127)



(128)

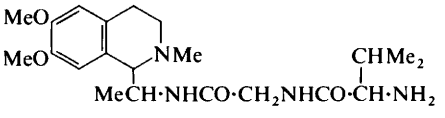


(129)

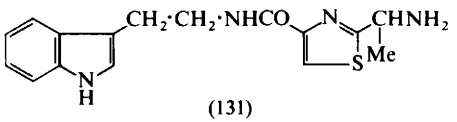
Synthesis of the structures proposed for the antibiotics bottromycins B_1 and B_2 afforded products which exhibited different spectral, physical, and biological properties from those of the natural materials.[259] On the basis of this evidence, it was concluded that the currently accepted structures are incorrect. No alternative structures have so far been put forward. The peptide alkaloids (130)[260] and (131),[261] derived respectively from plant and microbial sources, have been synthesized by relatively standard procedures confirming the original structural assignments.

The peptide responsible for inducing mating tube formation in *Rhodosporidium toruloides* is a normal undecapeptide (132) and the only notable feature to warrant its inclusion in this section is the presence of the *S*-farnesyl cysteine as the *C*-terminal residue.[262] The slightly larger tridecaptins (133)—(135) are broad spectrum antibiotics with a number of unusual features.[263, 264] The possession of

[258] H. Tobe, H. Morishima, H. Naganawa, T. Takita, T. Aoyagi, and H. Umezawa, *Agric. Biol. Chem.*, 1979, **43**, 591.
[259] T. Yamada, K. Takashima, K. Miyazawa, S. Kuwata, and H. Watanabe, *Bull. Chem. Soc. Jpn.*, 1978, **51**, 878.
[260] J. Koyama, Y. Suzuta, K. Kuriyama, H. Yajima, K. Koyama, and H. Irie, *Heterocycles*, 1978, **9**, 443.
[261] M. Onda and Y. Konda, *Chem. Pharm. Bull. Jpn.*, 1978, **26**, 2167.
[262] Y. Kamiya, A. Sakurai, S. Tamura, N. Takahashi, E. Tsuchiya, K. Abe, and S. Fukui, *Agric. Biol. Chem.*, 1979, **43**, 363.
[263] T. Kato, H. Hinoo, and J. Shoji, *J. Antiobiotics*, 1978, **31**, 652.
[264] T. Kato, R. Sakazaki, H. Hinoo, and J. Shoji, *J. Antibiotics*, 1979, **32**, 305.



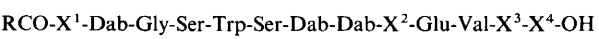
(130)



(131)

several diaminobutyric acid and D-amino-acid residues make these molecules close relatives of the cyclic polymyxins. However, they clearly lack the alternating sequence of L- and D-amino-acids found in gramicidin A (136). These result in the well defined right-handed helical structure which is responsible for the ion-conductance properties of this molecule in biological membranes. Spectral data [265] on (136) and *X*-ray data on a caesium complex [266] provide support for the

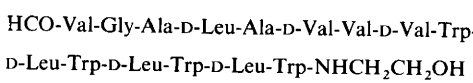
(132)



(133) Decapeptin A, RCO = anteisononanoyl
X^1 = D-Val, X^2 = Phe, X^3 = D-aIle, X^4 = Ala

(134) Decapeptin B, RCO = anteisononanoyl
X^1 = Gly, X^2 = Val, X^3 = D-aIle, X^4 = Ser
D-Val

(135) Decapeptin C, RCO = β-hydroxyanteisoundecanoyl
X^1 = D-Val, X^2 = Phe, X^3 = D-aIle, X^4 = Ser
D-Val



(136)

conformation of the native antibiotic and ^{13}C and ^{19}F n.m.r. studies have been employed to probe the conformation and nature of the helical dimer in phosphatidyl lipid vesicles.[267]

Related peptides with similar biological properties to gramicidin A, of which alamethicin (137) is the best known, are characterized by several α-amino isobutyric acid (α-methylalanine) units and a terminal amino-alcohol, usually phenylalaninol. However, in this group the amino-acids all have the L-configuration. Antiamoebin II (138), a newly characterized member, follows this general pattern.[268] Hypelcin A

[265] E. M. Popov and G. M. Lipkind, *Mol. Biol.*, 1979, **13**, 363.
[266] R. E. Koeppe, K. O. Hodgson, and L. Stryer, *J. Mol. Biol.*, 1978, **121**, 41.
[267] S. Weinstein, B. A. Wallace, E. R. Blout, J. S. Morrow, and W. Veatch, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4230.
[268] R. C. Pandey, J. C. Cook, jun., and K. L. Rinehart, jun., *J. Antibiotics*, 1978, **31**, 241.

(139) is also similar but the terminal phenylalaninol is replaced by leucinol.[269] The sequence of trichotoxin remains to be established, but clearly belongs to this class. The molecule apparently lacks the terminal amino-alcohol entity and possesses a carboxylic acid group.[270]

Ac-Aib-Pro-Aib-Ala-Aib-Ala-Gln-Aib-Val-Aib-Gly-
Leu-Aib-Pro-Val-Aib-Aib-Glu-Gln-PhOH
(137)

Ac-Phe-Aib-Aib-Aib-Iva-Gly-Leu-Aib-Aib-Hyp-
Gln-Iva-Pro-Aib-Pro-Pheol
(138)

Ac-Aib-Pro-Aib-Ala-Aib-Aib-Gln-Leu-Aib-Gly-
Aib-Aib-Aib-Pro-Val-Aib-Aib-Gln-Gln-Leuol
(139)

There is increasing evidence that these antibiotics adopt similar conformations to gramicidin A and that the mechanism of their biological activity is also similar. *X*-Ray data from protected tetrapeptides[271,272] with the *N*-terminal sequence of alamethicin demonstrate a helical conformation. Synthetic effort[273,274] in this area has culminated in the synthesis of the hexadecapeptide (140) and nonadecapeptide (141) analogues. The synthetic plan is outlined in Scheme 9.[275]

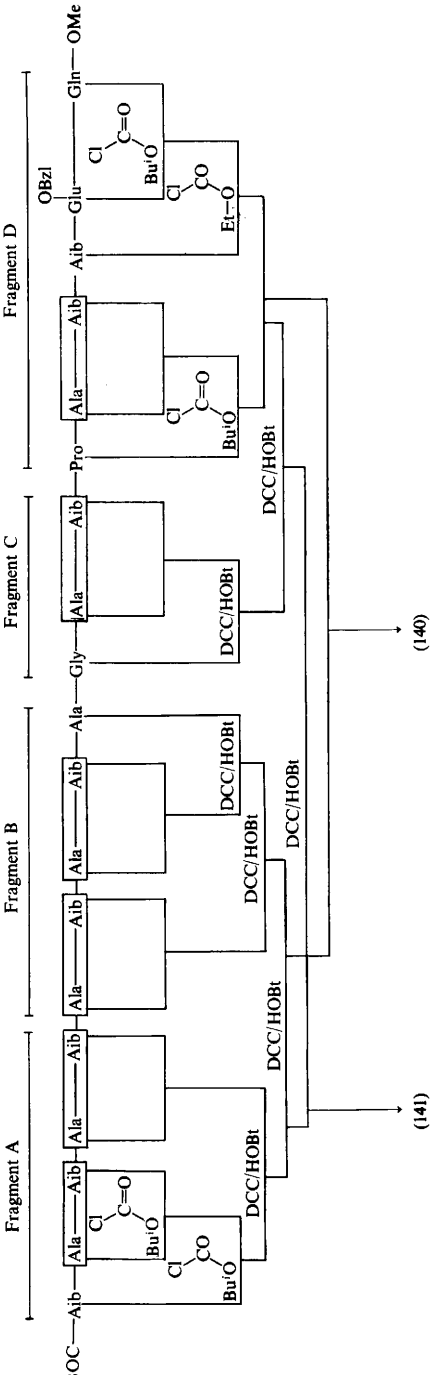
Boc-Aib-Ala-Aib-Ala-Aib-Ala-Aib-Ala-Aib-Ala-
Pro-Ala-Aib-Aib-Glu-Gln-OMe
OBzl
(140)

Boc-Aib-Ala-Aib-Ala-Aib-Ala-Aib-Ala-Aib-Ala-
Gly-Ala-Aib-Pro-Ala-Aib-Aib-Glu-Gln-OMe
OR
(141)

Compound (140) was biologically inactive, but (141) exhibited haemolytic activity comparable with alamethicin.

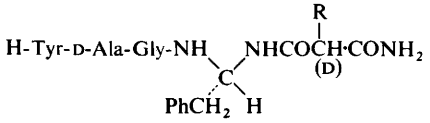
The concept of linear modified *retro*-peptides as potentially biologically active analogues of hormones and antibiotics has been clearly expounded in an interesting review article.[276] The problem of reversed end groups which is held to be responsible for the lack of activity for most of the *retro*-enantiomeric isomers of natural peptides so far synthesized, can be resolved by modifying the peptide with

[269] T. Fujita, Y. Takaishi, and T. Shiromoto, *J. Chem. Soc., Chem. Commun.*, 1979, 413.
[270] G. Irmscher, G. Bovermann, G. Boheim, and G. Jung, *Biochim. Biophys. Acta*, 1978, **507**, 470.
[271] N. Shamala, R. Nagaraj, and P. Balaram, *Biochem. Biophys. Res. Commun.*, 1977, **79**, 292.
[272] N. Shamala, R. Nagaraj, and P. Balaram, *J. Chem. Soc., Chem. Commun.*, 1978, 996.
[273] I. M. Shaw and A. Taylor, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1866.
[274] W. Mayr, R. Oekonomopulos, and G. Jung, *Biopolymers*, 1979, **18**, 425.
[275] R. Oekonomopulos and G. Jung, *Annalen*, 1979 1151.
[276] M. Goodman and M. Chorev, *Acc. Chem. Res.*, 1979, **12**, 1.

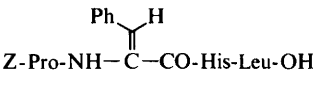

Fragment A
Fragment B
Fragment C
Fragment D
BOC—Aib—Ala—Aib—Ala—Aib—Ala—Aib—Ala—Aib—Ala—Gly—Pro—Aib—Glu—Gln—OMe
OBzl
DCC/HOBt
(140)
(141)

Scheme 9

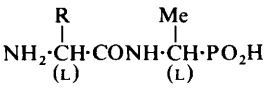
either an α,α diaminoalkyl- or malonyl-residue. In this way it is possible to produce compounds which are now termed 'end group modified *retro-inverso*-peptides'. The topological relationships within these molecules are essentially the same as those within the parent peptide. The enkephalinamide analogues (142) have recently been synthesized and are notable examples of the application of this principle.[227] These compounds exhibit *in vitro* and *in vivo* activity and are longer acting than any other enkephalin so far studied. However, caution has been recommended with respect to the whole of this approach. The basic premise that the side-chain topology of a peptide and its *retro*-enantiomer are very similar has been challenged and evidence presented that significant conformation differences result from the reversal of the peptide backbone.[278]



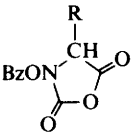
(142)



(143)



(144)



(145)

Alternative methods of modifying peptides with the same general objectives in view have continued along more traditional lines. The frequent occurrence of dehydroamino-acids in microbial peptides has led to increasing interest in the synthesis of dehydropeptides containing one or more of these units.[279–284] The dehydrophenylalanine containing peptide (143) was prepared as a potential inhibitor of angiotensin-converting enzyme and some inhibition of this enzyme by (143) was observed.[285] Valuable reviews covering the development of synthetic tri- and di-phosphonopeptides (144) as potent inhibitors of cell-wall biosynthesis describe their synthesis and mode of action.[286] The active component is the phosphonoalanine which is carried into the bacterial cell as the peptide and released by enzymatic hydrolysis. The Polish group who have also been active in this area

[277] M. Chorev, R. Shavitz, M. Goodman, S. Minick, and R. Guillemin, *Science*, 1979, **204**, 1210.
[278] R. M. Freidinger and D. F. Veber, *J. Am. Chem. Soc.*, 1979, **101**, 6129.
[279] G. C. Barrett, L. A. Chowdhury, and A. A. Usmani, *Tetrahedron Lett.*, 1978, 2063.
[280] S. Konno and C. H. Stammer, *Synthesis*, 1978, 598.
[281] M. L. English and C. H. Stammer, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 1464.
[282] C. Shin, Y. Yonezawa, K. Unoki and J. Yoshimura, *Tetrahedron Lett.*, 1979, 1049.
[283] C. S. Nomoto, A. Sano, and T. Shiba, *Tetrahedron Lett.*, 1979, 521.
[284] C. Shin, Y. Yonezawa and J. Yoshimura, *Tetrahedron Lett.*, 1979, 4085.
[285] C. H. Stammer, *Int. J. Pept. Protein Res.*, 1978, **12**, 222.
[286] F. R. Atherton, M. J. Hall, C. H. Hassall, R. W. Lambert, and R. S. Ringrose, *Antimicrob. Chemotherapy*, 1979, **15**, 677 and 696.

reported the synthesis of related phosphonic dipeptides.[287] Synthesis of peptide analogues which have *N*-hydroxyamide function in their sequence are of interest in relation to naturally occurring hydroxamic derivatives. A new method which is claimed to have general applicability employs the *N*-carboxyamino-acid anhydrides of benzyl-protected *N*-hydroxyamino-acids (145). These readily react with amino-acid esters to give dipeptides which can be extended by routine coupling reactions or the process repeated to add a further *N*-hydroxyamino-acid residues.[288]

On the basis of the extensive crystallographic data available, conformational properties and preferences of linear, as well as cyclic, peptides have been re-examined with particular reference to hydrogen-bonding.[289,290] The crystal structure of the simple peptide pivaloyl-D-Pro-Pro-Ala-NHMe has shown that it adopts a highly folded conformation with two consecutive β-turns [291] (Figure 5).

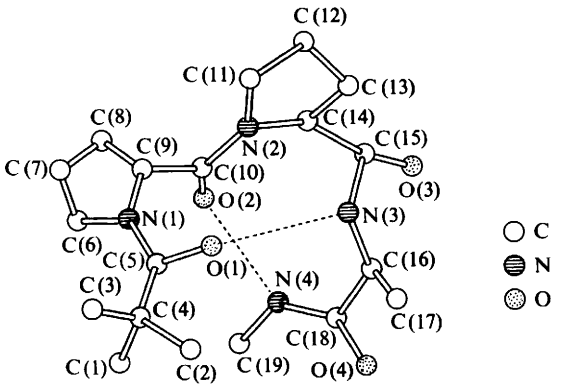


Figure 5 *Molecular conformation of pivaloyl-D-Pro-L-Pro-L-Ala-*N*-methylamide*

Although in general β-turns are widely observed in small peptides (see also Section 2) this conformation is rare. Solution studies on related tripeptides in which one of the prolines was replaced by alanine or sarcosine demonstrate that the favoured conformation with respect to the β-folding depends on both primary structure and chirality sequence.[292] The presence of β-alanine in simple tripeptide amides such as Pro-Leu-β-Ala-NHR markedly alters the conformational preferences and the intramolecular hydrogen bonding.[293] Two interesting applications of n.m.r. spectroscopy on ^{15}N-enriched peptides illustrates the subtle power of n.m.r. techniques. The *cis*- and *trans*-isomers of [^{15}N]-Ala-Pro can be monitored by the different ^{13}C–^{15}N coupling constants [294] and the diastereoisomers derived from the

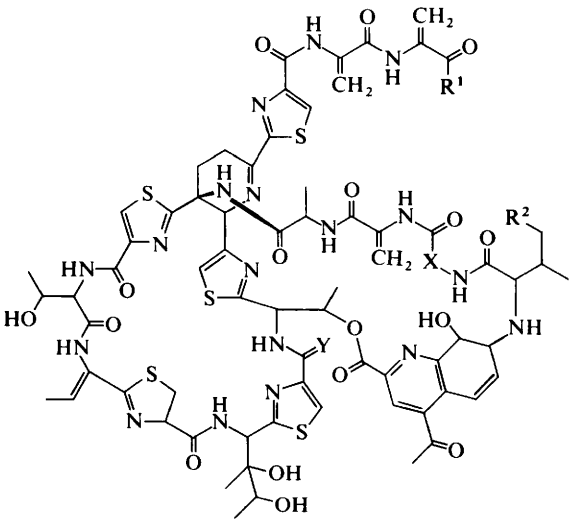
[287] A. Arendt, M. Hoffmann, A. Kolodziejczyk, A. Sobczak, T. Sokolowska, and C. Wasielewski, *Pol. J. Chem.*, 1979, **53**, 447.
[288] M. Akiyama, M. Hasegawa, H. Takeuchi, and K. Shimizu, *Tetrahedron Lett.*, 1979, 2599.
[289] G. N. Tishchenko, *Itogi Nauk. Tech. Kristallokhim.*, 1979, **13**, 189.
[290] C. S. Chen and R. Parthasarathy, *Int. J. Pept. Protein Res.*, 1978, **11**, 9.
[291] C. M. Nair, M. Vijayan, Y. V. Venkatachalapathi, and P. Balaram, *J. Chem. Soc., Chem. Commun.*, 1979, 1183.
[292] G. Boussard, M. Marraud, and A. Aubry, *Biopolymers*, 1979, **18**, 1297.
[293] P. Anagnostaras, P. Cordopatis, J. Matsoukas, G. Stravropoulos, and D. Theodoropoulos, *Tetrahedron*, 1978, **34**, 973.
[294] F. Blomberg, H. Roesterjans, K. Lintner, F. Torea, and S. Fermandjian, *Org. Magn. Reson.*, 1978, **11**, 598.

coupling of the Boc-DL-valine with DL-valine methyl ester can be distinguished by their ^{15}N.m.r. spectra.[295]

7 Large Highly Modified Peptides

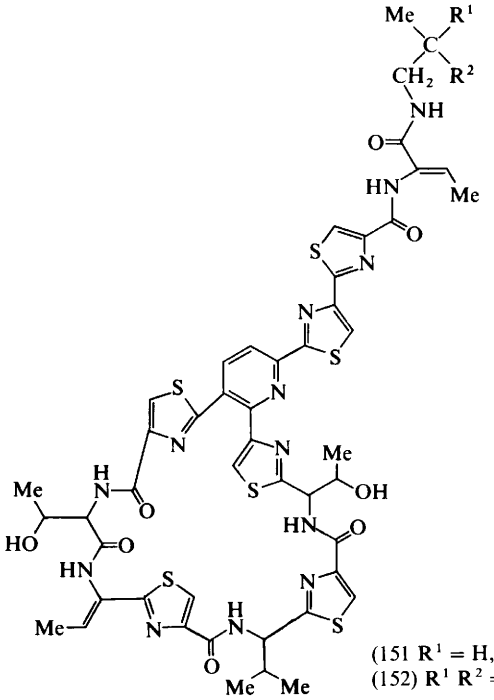
This section was introduced in an earlier volume in order to draw attention to the increasing number of relatively large peptide antibiotics, the structural features of which are dominated by extensive modification of many of the component amino-acids. Overlap with the preceding and following sections is clearly inevitable and the decision as to which area individual peptides belong is to some extent arbitrary. Those included here are extensively modified and are not conjugated with fatty acids or carbohydrates.

Thiostrepton (146) was the first of this group to be characterized nearly a decade ago. Since then several related antibiotics have been isolated from a variety of microbial sources and in the past two years a number have had their structures established. Siomycin A (147) is very closely related to thiostrepton and differs only in two adjacent amino-acid residues.[296, 297] The thiopeptin complex has found commercial use as a growth promotant for livestock and the structures of some of the components (148)—(150) have been unravelled.[298] They bear a close similarity



(146) $R^1 = NH_2$, $R^2 = Me$, X = >CHMe, Y = O
(147) $R^1 = NH_2$, $R^2 = H$, X = >C = CH_2, Y = O
(148) $R^1 = OH$, $R^2 = H$, X = >CHMe, Y = S
(149) $R^1 = OMe$, $R^2 = H$, X = >CHMe, Y = S
(150) $R^1 = NH_2$, $R^2 = H$, X = >CHMe, Y = S

[295] H. R. Kricheldorf and W. E. Hull, *Annalen.*, 1978, 1817.
[296] A. Olesker, L. Valente, L. Barate, G. Lukacs, W. E. Hull, K. Tori, K. Tokura, K. Okabe, M. Ebata, and H. Otsuka, *J. Chem. Soc., Chem. Commun.*, 1978, 577.
[297] K. Tori, K. Tokura, Y. Yoshimura, K. Okabe, H. Otsuka, F. Inagaki, and T. Miyazawa, *J. Antibiotics*, 1979, **32**, 1072.
[298] O. D. Hensens and G. Albers-Schonberg, *Tetrahedron Lett.*, 1978, 3649.



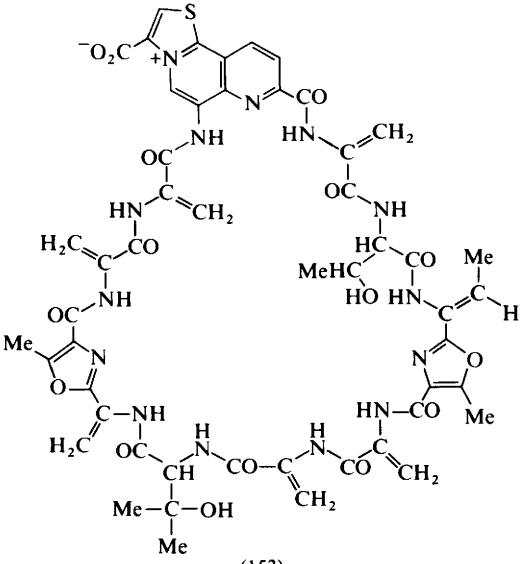
(151 R^1 = H, R^2 = OH
(152) R^1 R^2 = =O

to (146), but perhaps the most notable additional features are the thioamide linkages which are relatively rare in microbial peptides. In micrococcin P_1 and P_2 (151) and (152) the central tetrahydropyridine unit has been oxidized to a pyridine system [299] and the same feature is also present in nosiheptide which is identical with multhiomycin.[300] As yet there is little firm evidence to indicate at what stage in the biosynthesis the modifications occur. In berninamycin (153) the plethora of dehydroalanine units are derived from serine, but whether the dehydration occurs on a preformed peptide or at an earlier point remains to be resolved.[301] Formal structural analysis would suggest that the thiazoline and thiazole residues originate from cysteine. Elegant biomimetic syntheses of these systems from cysteine-containing peptides demonstrate the chemical feasibility of such transformations.[302]

8 Peptides Linked to Carbohydrates

Glycopeptide Antibiotics.—The possible clinical potential of the antitumour agents of the bleomycin family has led to a flurry of activity in this area. The most surprising outcome of which has been the revision of the structures of bleomycin (154), phleomycin (155),[303] and presumably all the related glycopeptide antibiotics.

[299] B. W. Bycroft and M. S. Gowland, *J. Chem. Soc., Chem. Commun.*, 1978, 256.
[300] T. Endo and H. Yonehara, *J. Antibiotics*, 1978, **31**, 623.
[301] C. J. Pearce and K. L. Rinehart, jun., *J. Am. Chem. Soc.*, 1979, **101**, 5069.
[302] D. K. Minster, U. Jordis, D. L. Evans, and S. M. Hecht, *J. Org. Chem.*, 1978, **43**, 1642.
[303] T. Takita, Y. Muraoka, T. Nakatani, A., Fujii, Y. Umezawa, H. Nagamawa, and H. Umezawa, *J. Antibiotics*, 1978, **31**, 801.



(153)

Although the structural changes are slight they do have considerable implications. The group were the largest peptides to contain a β-lactam entity. The evidence for this structural unit has been re-assessed and modified accordingly. The initial data for the change arose from biosynthetic studies which led to the isolation of several smaller peptides related to bleomycin. Among these was the peptide (156) designated P-3A. An *X*-ray analysis of the copper complex of this important precursor established the structure [304] (Figure 6) and provided basic information

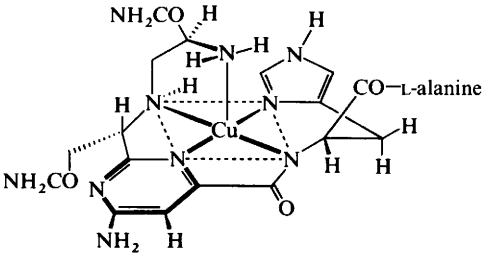
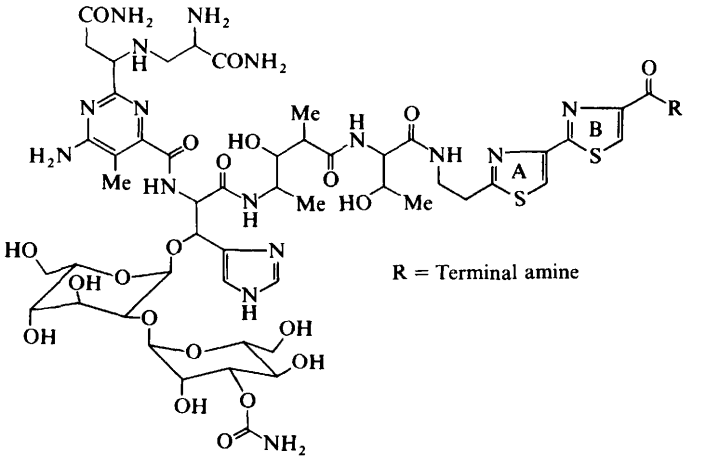


Figure 6 *The structure of the copper(II) complex of P-3A*
(Reproduced by permission from *J. Antibiotics*, 1978, **31**, 1070)

relating to the metal complexation of bleomycin itself.[305, 306] The presence of the extra nitrogen atom required by the new formulation was confirmed by ^{15}N.m.r. spectroscopy.[307]

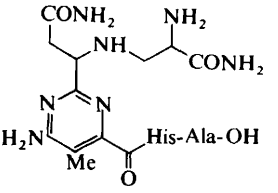
[304] Y. Iitaka, H. Nakamura, T. Nakatani, Y. Muraoka, A. Fujii, T. Takita, and H. Umezawa, *J. Antibiotics*, 1978, **31**, 1070.
[305] T. Takita, Y. Muraoka, T. Nakatani, A. Fujii, Y. Iitaka, and H. Umezawa, *J. Antibiotics*, 1978, **31**, 1073.
[306] Y. Sugiura, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 643.
[307] H. Nagamawa, T. Takita, and W. E. Hull, *J. Antibiotics*, 1979, **32**, 539.



R = Terminal amine

(154)

(155) Ring A Thiazoline



(156)

The current information concerning the conformation and possible mode of action of this important class of compounds is summarized in Figure 7. A considerable number of investigations in this general area have been reported, but only a selection of references relating more specifically to the chemical and conformational aspects are quoted.[308–311]

Vanomycin, risteocetin, risteomycin, actinoidin, and avoparcin are a class of complex water-soluble antibiotics with pronounced activity against Gram-positive organisms. Vanomycin is the only member of this group for which the structure is known with certainty. A modified derivative was the subject of an *X*-ray analysis [312] and the resulting structural data have served as a basis for much of the subsequent studies in this field. Risteocetin A has been the subject of an impressive series of chemical and spectroscopic studies [313–316] which have culminated in a proposal for

[308] J. C. Dabrowiak, F. T. Greenaway, and R. Grulich, *Biochemistry*, 1978, **17**, 4090.
[309] N. R. Krishna, J. L. Dallas, E. S. Mooberry, T. T. Sakai, R. C. Allen, G. C. Levy, and J. D. Glickson, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 363.
[310] L. W. Oberley and G. R. Buettner, *FEBS Lett.*, 1979, **97**, 47.
[311] Y. Sugiura, Y. Muraoka, A. Fujii, T. Takita, and H. Umezawa, *J. Antibiotics*, 1979, **32**, 756.
[312] G. M. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams, and G. A. Smith, *Nature (London)*, 1978, **271**, 223.
[313] C. M. Harris, J. J. Kibby, and T. M. Harris, *Tetrahedron Lett.*, 1978, 705.
[314] C. M. Harris, J. J. Kibby, J. R. Fehlner, A. B. Raabe, T. A. Barber, and T. M. Harris, *J. Am. Chem. Soc.*, 1979, **101**, 437.
[315] C. M. Harris and T. M. Harris, *Tetrahedron Lett.*, 1979, 3905.
[316] D. H. Williams, V. Rajananda, and J. R. Kalman, *J. Chem. Soc., Perkin Trans* 1, 1979, 787.

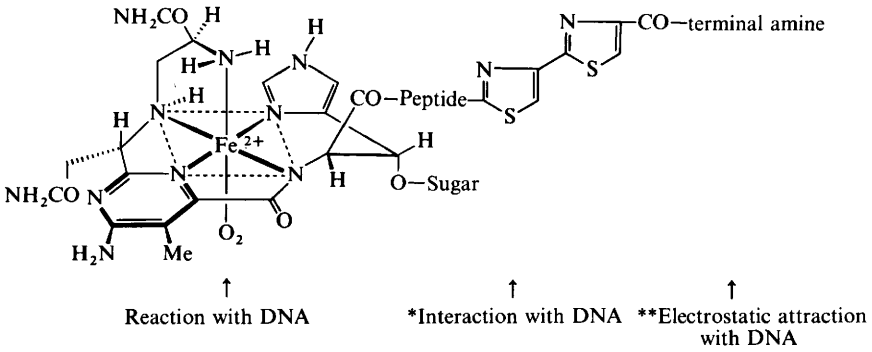


Figure 7 *Proposed mechanism of action of bleomycin*

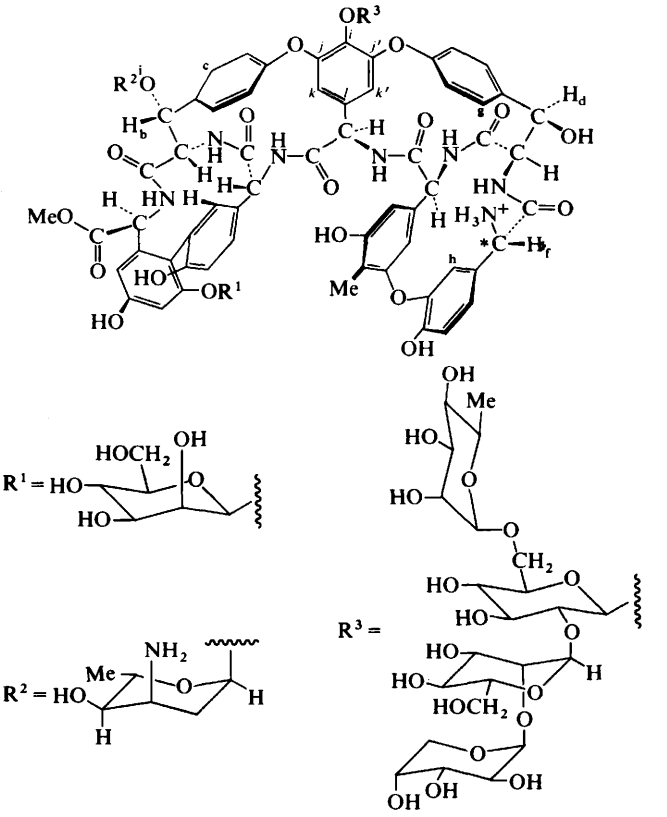
the total structure[317] (157). Ristomycin A and actinoidin are close relatives and similar investigations[318–320] have run in parallel to those on (157), but as yet no firm total structures have emerged. Avoparcin has commercial importance as a feed additive for livestock and once again contains an aglycone with biphenyl and phenolic ether cross-linkages. The structure (158) has been proposed for the rhamnoside of the aglycone.[321]

Glycopeptides from Bacterial Cell Walls.—As in previous years only those aspects relating directly to the peptide moiety are covered here. Antibiotics which inhibit cell-wall synthesis continue to provide valuable assistance in both structural and biosynthetic studies. The inhibition of cell-wall synthesizing enzymes by β-lactam[239] antibiotics and synthetic phosphono dipeptides[286] which were noted in earlier sections are providing detailed evidence concerning the specific enzymes involved in the peptide cross-linking. Treatment of bacteria with controlled amounts of penicillin frequently results in the excretion of peptidoglycan complexes. *Brevibacterium divaricatum* affords a peptidoglycan which possesses a linear uncross-linked structure made up of the repeating disaccharide pentapeptide (159).[322]

The peptidoglycan of *Cellulomonas catalyticum* is different from that of the other known species of *Cellulomonas*. Lysine replaces ornithine and the interpeptide bridge consists of D-Asp-D-Ser.[323] The same type of structure has been observed in species of *Brevibacterium*.

Extensive conformational energy calculations carried out on (160), as a model for the peptide unit of peptidoglycan, indicates that β-pleated sheet and helical structures are energetically unfavoured. A new model was proposed which

[317] D. H. Williams, V. Rajananda, G. Bojesen, and M. P. Williamson, *J. Chem. Soc., Chem. Commun.*, 1979, 906.
[318] F. Sztaricskai, C. M. Harris, and T. M. Harris, *J. Antibiotics*, 1979, **32**, 446.
[319] T. M. Harris, C. M. Harris, and J. R. Fehler, *J. Org. Chem.*, 1979, **44**, 1009.
[320] F. Sztaricskai, C. M. Harris, and T. M. Harris, *Tetrahedron Lett.*, 1979, 2861.
[321] W. J. McGahren, J. H. Martin, G. O. Morton, R. T. Hargreaves, R. A. Lease, F. M. Lovell, and G. A. Ellestad, *J. Am. Chem. Soc.*, 1979, **101**, 2237.
[322] D. Keglevic, B. Ladesic, J. Tomasic, Z. Valinger, and R. Naumski, *Biochim. Biophys. Acta*, 1979, **585**, 273.
[323] E. Stackebrandt, F. Fiedler, and O. Kandler, *Arch. Microbiol.*, 1978, **117**, 115.

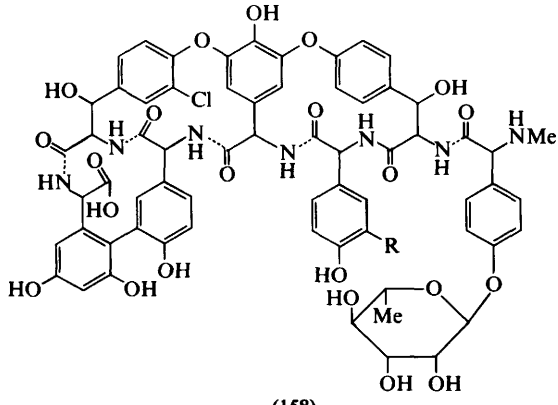


(157)

apparently bears no significant relationship to the solid-state conformation of β-lactam antibiotics.[324] Similar studies relating to the conformational effect of the fourth and fifth amino-acid residue on the cross-linking transpeptidase enzymes suggest certain amino-acid replacements which will adopt the same conformation as the native peptide. Whereas others, such as L-alanine and D-valine, would drastically reduce cross-linking due to the considerable conformational changes.[325]

N-Acetylmuramyl-L-alanyl-D-isoglutamine (161) is the minimum structure required for immuno-adjuvant activity and for this reason (161) has been the subject of considerable synthetic effort.[326-328] Specifically radio-labelled derivatives for assay purposes[329-330] and long-chain fatty acid ester (162) which exhibit

[324] G. Barnickel, H. Labischinski, H. Bradaczek, and P. Giesbrecht, *Eur. J. Biochem.*, 1979, **95**, 157.
[325] R. Virudachalam and V. S. R. Rao, *Biopolymers*, 1978, **17**, 2251.
[326] S. Kusumoto, K. Yamamoto, and T. Shiba, *Tetrahedron Lett.*, 1978, 4407.
[327] P. Lefrancier, M. Derrien, I. Lederman, F. Nief, J. Choay, and E. Lederer, *Int. J. Pept. Protein Res.*, 1978, **11**, 289.
[328] P. L. Durette, E. P. Meitzner, and T. Y. Shen, *Tetrahedron Lett.*, 1979, 4013.
[329] S. Kusumoto, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1177.
[330] P. L. Durette, A. Rosegay, M. A. R. Walsh, and T. Y. Shen, *Tetrahedron Lett.*, 1979, 291.



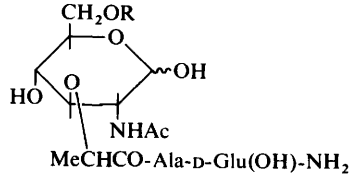
(158)

GlcNAc-Mur*N*Ac-Ala-D-Gln(*meso*-Dap-D-Ala-D-Ala)

(159)

Ac-Ala-D-γ-Glu-Lys-D-Ala-D-Ala

(160)



(161) R = H
(162) R = long chain fatty acid residue

significant anti-tumour activity [331] have been prepared. Structural variants of (161) in which L-alanine is replaced by glycine or serine and the carboxy group of glutamic acid is linked to other amino-acids are common to many bacteria which are adjuvant inactive. As part of a general investigation exploring structure–activity relationships a number of these have been synthesized by what are now standard procedures.[332]

[331] S. Kusumoto, M. Inage, T. Shiba, I. Azuma, and Y. Yamamura, *Tetrahedron Lett.*, 1978, 4899.
[332] S. Kusumoto, K. Ikenaka, and T. Shiba, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1665.

5
Chemical Structure and Biological Activity of Hormones and Related Compounds

With contributions by
D. BRANDENBURG, D. H. COY, D. GILLESSEN, S. GIRGIS, B. LAMMEK, M. MANNING, J. RAMACHANDRAN, P. D. ROY, D. SAUNDERS, W. H. SAWYER, P. W. SCHILLER, AND R. O. STUDER

1 Hypothalamic Releasing Hormones

Contributed by D. H. Coy

Introduction.—Several years ago, the discovery that somatostatin, initially considered to be purely a hypothalamic factor controlling pituitary secretion, was also present in, and able to act on, numerous peripheral areas came as a complete surprise. Subsequently, however, this situation was found to be the rule rather than an exception with many other peptides. Not only were thyrotrophin-releasing hormone (TRH) and luteinizing hormone-releasing hormone (LH-RH) found to have sites of action outside of the brain, but, conversely, many gastrointestinal peptides were found to be present in the central nervous system (CNS), including the hypothalamic area and, in some instances, even the pituitary. In addition, the classical hypothalamic-releasing hormones were found in many parts of the brain other than the hypothalamus and potential neurotransmitter candidates such as the enkephalins and substance P were found to be localized extensively in the hypothalamus. The results of these rather remarkable observations have been to make classifications difficult and misleading and to extend the realms of endocrinology into the disciplines of neuroscience and gastroenterology, thus encouraging the examination of CNS and peripheral activities of the three established hypothalamic peptide hormones. This, of course, is beginning to have profound effects on the design of analogues and is opening up many additional areas of considerable therapeutic potential.

Thyrotrophin-releasing Hormone.—There continues to be little published work on the endocrine aspects of TRH analogues. The CNS activity and the gastrointestinal and pancreatic effects of the tripeptide (Glp-His-Pro-NH_2) have claimed much of the recent attention and have been the subject of a recent review by Morley.[1]

Several analogues of TRH have been made[2] which have much increased activities in reserpine and hypothermia reversal tests, which are considered to be animal models for anti-depressant activity, together with lowered thyrotrophin- and prolactin-releasing activities. Modifications have mostly involved the Pro-NH_2 residue which has been replaced, *e.g.* by 3-methyl-Pro, 3,3-dimethyl-Pro, and

[1] J. S. Morley, *Life Sci.*, 1979, **25**, 1539.

[2] J. D. Bower, D. Brewster, P. W. Dettmar, G. Metcalf, B. A. Morgan, and M. J. Rance, *Peptides: Proceedings of the Sixth American Peptide Symposium*, ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, p. 909; R. F. Nutt, R. Hirschmann, and D. F. Veber, p. 913.

thiazolidine-4-carboxylic acid. Replacement of Glp by cyclized aminoadipic acid was also helpful.

In view of the success in dissociating the CNS and endocrine effects of TRH, it would seem that possibilities should exist for the design of analogues with specific actions on gastric and pancreatic secretions using a similar approach to that presently being used with somatostatin.

Luteinizing Hormone-releasing Hormone.—Several of the many analogues of LH-RH which have been developed over the past 8 years in order to be up to 100 times more active than the natural hormone are finally undergoing clinical trials. Their high and prolonged intranasal activity [3] has increased their therapeutic usefulness. In the induction of fertility, promising results have been obtained in both women [4, 5] and men [6] using either [D-Trp[6]]-LH-RH or [D-Ser(Bu^t)[6], des-Gly-NH_2[10]]-LH-RH ethylamide.

In contrast to the above results, recent studies have demonstrated anti-fertility or 'paradoxical' effects of relatively large doses of LH-RH and the highly active agonist analogues. These were initially attributed to the downgrading of ovarian LH-hCG receptors [7] caused by the massive release of LH. However, the fact that effects on the ovaries and a reduction in testicular LH receptors are still seen in hypophysectomized animals [8, 9] strongly suggests a direct effect of LH-RH on these organs. Observations of agonist effects on the uterus of hypophysectomized animals [10] indicates a direct effect on this tissue also. Thus, LH-RH, until now the 'purest' of the hypothalamic hormones in the sense of organ specificity, has joined the growing list of peptides with multiple peripheral sites of action. For these activities to be envisaged as anything more than pharmacological in character, local sites of synthesis and secretion must also be demonstrated.

Clinical use of the contraceptive properties of some of the agonist analogues has also begun. In a series of reports, Nillius *et al.* [11] were able to inhibit ovulation in normal women initially by daily injections of [D-Ser(Bu^t), des-Gly-NH_2]-LH-RH ethylamide and, subsequently, by its administration as a nasal spray. [12]

Reports on the synthesis of novel LH-RH agonist analogues have been quite limited during 1978—1979. Two novel approaches to analogue design utilizing peptide bond reversal [13] and substitution of a —CH_2S— group for —CONH— [14]

[3] J. A. H. Wass, G. M. Besser, A. Gomez-Pan, M. F. Scanlon, R. Hall, A. J. Kastin, D. H. Coy, and A. V. Schally, *Clin. Endocrinol.*, 1979, **10**, 419.

[4] C. Jaramillo-Jaramillo, A. Charro-Salgado, V. Perez-Infante, G. Lopez del Campo, J. Botella-Llusia, D. H. Coy, and A. V. Schally, *Fertil. Steril.*, 1978, **29**, 418.

[5] J. P. Hanker, H. G. Bohnet, M. Mohlenstedt, C. Nowack, and H. P. G. Schneider, *Acta Endocrinol.*, 1978, **89**, 625.

[6] C. Jaramillo-Jaramillo, A. Charro-Salgado, V. Perez-Infante, E. Bordiu-Obanzo, R. Cano-Iglesias, A. Fernandez-Cruz, D. H. Coy, and A. V. Schally, *Fertil. Steril.*, 1978, **30**, 430.

[7] G. S. Kledzik, L. Cusan, C. Auclair, P. A. Kelly, and F. Labrie, *Fertil. Steril.*, 1978, **30**, 348.

[8] S.-Y. Ying and R. Guillemin, *Nature (London)*, 1979, **280**, 593.

[9] A. Arimura, P. Serafini, S. Talbot, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 687.

[10] J. A. Vilchez-Martinez, E. Pedroza, A. Arimura, and A. V. Schally, *Fertil. Steril.*, 1979, **31**, 677.

[11] S. J. Nillius, C. H. Bergquist, and L. Wide, *Contraception*, 1978, **17**, 537.

[12] C. Bergquist, S. J. Nillius, and L. Wide, *Lancet*, 1979, 215.

[13] M. Chorev, N. Chaturvedi, P. Hoeprich, P. Pallai, and M. Goodman, *Peptides: Proceedings of the Sixth American Peptide Symposium*, ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, p. 909.

[14] A. F. Spatola, A. L. Bettay, K.-F. Fok, A. Saneii, and J. A. Yankeelov, *Peptides: Proceedings of the Sixth American Peptide Symposium*, ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, p. 273.

in several locations in the chain were described at the last American Peptide Symposium. So far, these approaches have given analogues with little gonadotropin-releasing activity, presumably due to conformational distortions.

Of late, the large majority of new analogue studies on the LH-RH decapeptide (Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2) have been devoted to the development of increasingly powerful antagonists for use as ovulation inhibitors by blockade of gonadotropin release from the pituitary. The synthetic chemistry involved continues to be almost completely of the solid-phase variety using techniques which have been perfected over a number of years. Coy and Branyas [15] reported the use of a standard LH-RH antagonist, [D-Phe^2, D-Trp^3, D-Phe^6]-LH-RH, as a model to test the feasibility of conducting its solid-phase synthesis without the use of protecting groups for the Ser and Tyr residues and HCl salt protection for the guanidine group of Arg. No dimunition in yields or increase in the degree of side-reactions was noted and it appeared that the general flexibility of the solid-phase method can be substantially increased by the use of the alternative minimal side-chain protection approach. Indeed, we have successfully extended the strategy in this laboratory to much larger peptides than LH-RH, for instance β-endorphin, and also to the use of unprotected Boc-Thr. Some problems have been encountered with Boc-Arg·HCl which, at times, can be harder to couple completely than covalently derivatized Arg compounds.

Advances in LH-RH antagonist design during the period covered by this report have been impressive and have led to at least a 20-fold increase in anti-ovulatory activity in the rat, the standard assay criterion for this family of peptides, or, in other words, a decrease in the effective dose from 500 to 25 μg and less. These improvements have principally emanated from new modifications to the first and second positions. *N*-Acetyl-D-amino-acids substituted in position 1 were found[16] to be a distinct improvement over other replacements for Glp; *N*-acetyl-aromatic D-amino-acid and D-Ala-analogues are particularly effective in completely blocking rat ovulation at around the 250 μg level. *N*-Acetyl-L-amino-acids appear to be substantially less effective.[17] The size and nature of the acylating group is not too critical, *e.g.*, it can be an additional amino-acid such as Glp. [16, 17]

Although the effects of D-*p*-Cl-Phe were not superior to the commonly used D-Phe in position 2 of antagonists containing either L- or D-Glp in position 1, its use in conjunction with the acylated D-amino-acids in position 1 produced dramatic improvements in anti-ovulatory activity.[18] Thus, [*N*-Ac-D-Phe^1, D-*p*-Cl-Phe^2, D-Trp^{3,6}]-LH-RH was *ca.* 10 times more active than its D-Phe^2-counterpart and was completely effective at doses down to 30 μg per animal. *p*-Br- and *p*-F-substituents in the Phe benzene ring were less effective in that order [18] and, to our knowledge, no other substitutions of halogens or different moieties in various positions on the ring

[15] D. H. Coy and N. Branyas, *Int. J. Pept. Protein Res.*, 1979, **14**, 339.

[16] K. Channabasavaiah and J. M. Stewart, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 1273; K. Channabasavaiah, E. Alvarez, and J. M. Stewart, *Peptides: Proceedings of the Sixth American Peptide Symposium*, ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, p. 803.

[17] C. Y. Bowers, J. Humphries, T. Wasiak, K. Folkers, G. A. Reynolds, and L. E. Reichert, *Endocrinology*, 1980, **106**, 674.

[18] D. H. Coy, I. Mezo, E. Pedroza, M. V. Nekola, J. Vilchez-Martinez, P. Piyachurawata, A. V. Schally, J. Seprodi, and I. Teplan, *Peptides: Proceedings of the Sixth American Peptide Symposium*, ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, p. 775.

have yet produced superior results to *p*-Cl. Recent investigations have shown that *N*-Ac-D-*p*-F- and D-*p*-Cl-Phe[1]-analogues have twice the activity of the corresponding *N*-acetyl-D-Phe[1]-analogues.

Some of the peptides which have marked the path towards increased inhibitory activity over the past 12 months are shown in Table 1 together with anti-ovulatory dose levels.

Table 1 *Minimal 100% effective anti-ovulatory dose levels in the rat of some of the most potent and recent LH-RH antagonists*

LH-RH analogue	*Dose*/μg
[D-Glp, D-*p*-Cl-Phe[2], D-Trp[3,6]]	500
[*N*-Ac-D-Phe[1], D-Phe[2], D-Trp[3,6]]	250
[*N*-Ac-D-Trp[1], D-Phe[2], D-Trp[3,6]]	250
[*N*-Ac-D-Ala[1], D-Phe[2], D-Trp[3,6]]	125
[*N*-Glp-D-Ala[1], D-Phe[2], D-Trp[3,6]]	125
[*N*-Ac-D-Phe[1], D-*p*-Cl-Phe[2], D-Trp[3,6]]	30
[*N*-Ac-D-*p*-Cl-Phe[1,2], D-Trp[3,6]]	15

Somatostatin.*—Size-fractionation of both hypothalamic and gastric tissue extracts has always suggested the presence of different molecular weight species of somatostatin which were assumed to result from the presence and breakdown of a pro-hormone form. Pradayrol *et al.*[19] recently reported the purification and sequencing of one of these species which they extracted from porcine intestine. It was found to be a 28-residue peptide (Figure 1) containing a *N*-terminal 12-amino-acid

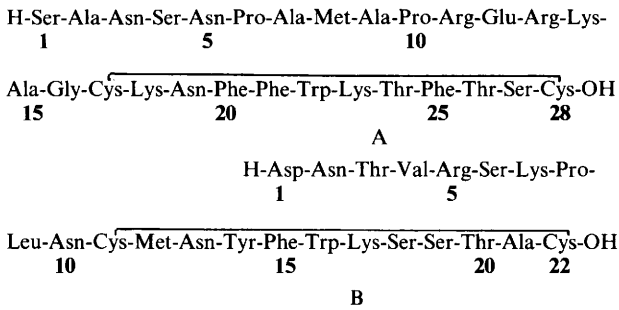


Figure 1 *Amino-acid sequences of* (A) *somatostatin*-28 (*of which the original somatostatin* 14-*peptide comprises residues* 15—28) *and* (B) *catfish pancreatic somatostatin I*

extension joined to the original somatostatin sequence by an Arg-Lys pair which must represent the type of activation site now being discovered in numerous peptide pro-hormones. There are already unpublished reports of both classical and solid-phase syntheses of this peptide.

A 22-amino-acid somatostatin peptide was also just isolated[20] from catfish pancreas and found to have perhaps a more intriguing sequence (Figure 1); there

[19] L. Pradayrol, J. A. Chayvialle, M. Carlquist, and V. Mutt, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 529; L. Pradayrol, H. Jornvall, V. Mutt, and A. Ribet, *FEBS Lett.*, 1980, **109**, 55.

[20] H. Oyama, R. A. Bradshaw, O. J. Bates, and A. Permutt, *J. Biol. Chem.*, 1980, **255**, 2251.

* See also Section 4 of this chapter.

are important differences in comparison to the mammalian type. Although the 12-residue ring unit is preserved, five amino-acids within it are changed. The *N*-terminal extension is also very different and does not contain the base-pair activating sequence for ready enzymatic production of 14-peptide.

Somatostatin isolated from pigeon pancreas was found [21] to be identical to the porcine and bovine variety.

One new large-scale synthesis of somatostatin itself, using a classical step-wise approach, was reported [22] during the present period. Protection of the carboxy-group of the *C*-terminal Cys-residue as the orange 2-[4-(phenylazo)-phenylsulphonyl]-ethyl ester enabled each new peptide to be readily examined analytically and also to be precipitated after every coupling reaction and, hence, partially purified by washing of the filtered solid. An overall yield of 5% was achieved.

The principal thrust of somatostatin-analogue studies continues to be the development of biologically selective and long-acting materials. There has been considerable success in the former endeavour and mixed success in the latter.

Veber and his co-workers have continued to develop their restricted-conformation approach to somatostatin analogue design. From model building and limited physico-chemical data,[23] it was concluded that the side-chains of Asn^5 and Thr^{12}, and Phe^6 and Phe^{11} were in close proximity to each other. A non-reducible, di-carba-analogue was chosen as the model peptide and Cys-residues were inserted in positions 5 and 12, and 6 and 7, respectively, allowing disulphide bridges to be formed between them.[24] The high activities of these restricted peptides supported the initial conformation proposed. Certain residues were then removed in order to obtain the smallest bicyclic peptide with high biological activity, *cyclo*-(-Aha-Cys-Phe-D-Trp-Lys-Thr-Cys-) (Aha = ω-aminoheptanoic acid). This hexapeptide was 1—3 times more active than somatostatin in inhibiting growth hormone (GH), insulin, and glucagon release. No significant dissociation was apparent between these actions, however, the di-carbapeptide and all bicyclic peptides had almost no gastric acid-release inhibiting activity. The hexapeptide also displayed slightly prolonged activity and was transiently active orally at high dose levels.

Another shortened, octapeptide analogue, [des-amino $acids^{1,2,3,4,5,12,13}$, D-Trp^8]somatostatin, gave [25] long-lasting suppression of hormones secreted in patients with pancreatic endocrine tumours after subcutaneous injection of high doses.

One potential drawback to the shortened analogue approach is its reduction of modification options for the production of selective compounds. Although several of the amino-acids within the somatostatin ring are not by any means critical to high overall activities, their side-chains can be readily manipulated to produce

[21] J. Spies, J. Rivier, J. A. Rodkey, C. D. Bennett, and W. Vale, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2974.

[22] J. Diaz, R. Guegon, M. Beaumont, J. Benoit, J. Clement, C. Fauchard, D. Gattier, J. Millan, C. Muneaux, Y. Muneaux, M. Vedel, and R. Schwyzer, *Bioorg. Chem.*, 1979, **8**, 429.

[23] B. H. Arison, R. Hirschmann, and D. F. Veber, *Bioorg. Chem.*, 1978, **7**, 447.

[24] D. F. Veber, F. W. Holly, R. F. Nutt, S. J. Bergstrand, S. F. Brady, R. Hirschmann, M. S. Glitzer, and R. Saperstein, *Nature (London)*, 1979, **280**, 512; D. Veber, *Peptides: Proceedings of the Sixth American Peptide Symposium*, ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, p. 409.

[25] R. G. Long, A. J. Barnes, T. E. Adrian, C. N. Mallinson, M. R. Brown, W. Vale, J. E. Rivier, N. D. Christopanides, and S. R. Bloom, *Lancet*, 1979, 764.

peptides with differing GH-, insulin-, and glucagon-release inhibiting activity. This is particularly true of positions 4 (Lys) and 5 (Asn). Replacement of Lys^4 by aromatic amino-acids [26] dramatically increases GH-release inhibiting activity both *in vivo* and *in vitro* whilst decreasing effects on insulin and glucagon release.[27] [$His^{4,5}$, D-Trp^8]somatostatin [28] was reported to be long-acting and a selective suppressor of GH and insulin release.

Reports continue to be published [29] on the selectivity of [D-Cys^{14}]-analogues which are GH and glucagon specific in most assay systems, although one [30] gave no selectivity on basal hormone release. It would appear that many of the selective peptides must be examined in greater depth and particularly in clinical situations before final conclusions concerning therapeutic value can be reached.

2 Anterior Pituitary Hormones

Contributed by J. Ramachandran

Introduction.—This review will be primarily concerned with the structure–activity relationships of the adenohypophyseal hormones. This subject was covered previously [1] in Vol. 9 of these reports. The literature for 1978 and 1979 is covered in this review. The nomenclature is according to the suggestions of the IUPAC–IUB commission of the Biochemical Nomenclature.[2]

The hormones of the anterior pituitary gland fall into three groups on the basis of structural similarities. Corticotropin (ACTH), the melanotropins (α- and β-MSH), lipotropin (LPH), and endorphin (β-EP) comprise the first group of closely related flexible polypeptides. The discovery that these five hormones are derived from a single common precursor is perhaps the most interesting development of the past 3 years and will be considered in this review. Somatotropin or growth hormone (GH) and prolactin (PRL) exhibit considerable structural homology and are compact globular proteins possessing characteristic three-dimensional structure. The placental hormone, chorionic somatomammotropin (CS), is closely related to this second group of pituitary hormones. The gonadotropins, lutropin (LH) and follitropin (FSH) as well as thyrotropin (TSH), are closely related glycoproteins forming the third group.

The glycoprotein hormones are composed of two non-identical subunits of comparable size. The α-subunit is common to the three pituitary glycoprotein hormones. Human chorionic gonadotropin (hCG) is also a glycoprotein composed of two subunits and is closely related to LH.

[26] C. A. Meyers, D. H. Coy, W. A. Murphy, T. W. Redding, A. Arimura and A. V. Schally, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 577.
[27] E. Lien and D. Sarantakis, *Diabetologia*, 1979, **17**, 59.
[28] V. M. Garsky, J. Bicksler, R. L. Fenichel, and E. L. Lien, *Peptides: Proceedings of the Sixth American Peptide Symposium*, ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Illinois, 1979, p. 547.
[29] N. R. Voyles, S. J. Bathena, L. Recant, C. A. Meyers, and D. H. Coy, *Proc. Soc. Exp. Biol. Med.*, 1979, **160**, 76.
[30] F. Marki, B. Kamber, H. Rink, and P. Sieber, *J. Endocrinol.*, 1979, **81**, 315.
[1] R. Schwyzer, in Vol. 9 of these Reports, p. 445.
[2] Reprinted in Vol. 8 of these Reports, p. 442.

Hormones of Group I.—*Corticotropin.* The amino-acid sequences of the hormone isolated from ostrich pituitary glands [3] and from three species of whale [4] have been elucidated. The primary structure of whale ACTH is identical to the structure of the human hormone. The first 26 residues of ostrich ACTH are also identical to the human sequence except for the replacement of lysine at position 15 by arginine in the avian hormone. There are six differences between ostrich and human ACTH in the region 27—39. These results further confirm that the biological activity of this hormone is derived from the highly conserved amino-terminal half of the molecule.[5]

The total synthesis of human,[6] porcine,[7] bovine,[8] and dogfish [9] corticotropins by solution methods have been described by Yajima and his co-workers. Synthetic dogfish ACTH had approximately 22% of the steroidogenic potency of synthetic human ACTH, thus confirming the low activity (15%) reported [10] for natural dogfish ACTH. The substitution of Met for Val in position-13 and Arg for Lys in position-15 in dogfish ACTH appears to decrease the affinity of the hormone for mammalian adrenocortical receptors.

^{1}H N.m.r. studies of aqueous solutions of ACTH-(1—32), ACTH-(1—24), ACTH-(15—32), ACTH-(20—32), and ACTH-(15—24) have enabled the assignment of Tyr-23 aromatic protons and Val-22 methyl protons.[11] These signals are sensitive to the geometry of Pro-24 and are useful in determining the ratio of *cis–trans* conformers. For peptides with *C*-terminal Pro the ratio is 1, whereas ACTH-(1—32) and ACTH-(15—32) with an internal Pro have 75% *trans*-form. The conformation of ACTH has also been studied by means of i.r. absorption measurements.[12] Solid-phase synthesis of analogues of human corticotropin containing 3,5-di-iodo-Tyr residues in place of Tyr have been described.[13] The synthetic products were carefully characterized and shown to be homogeneous by seven different analytical criteria. Analogues containing 3,5-di-iodo-Tyr in position-2 had very low steroidogenic activity (2.4% of the potency of human ACTH). Substitution of Tyr in position-23 by 3,5-di-iodo-Tyr was less damaging (64% as active as human ACTH). Analogues of ACTH-(1—19)-nonadecapeptide amide in which the Trp residue in position-9 was replaced by 1-Bu^t-Trp or 2,5,7-Bu$^t_3$-Trp have been prepared by conventional methods.[14] Both analogues were

[3] C. H. Li, D. Chung, W. Oelofsen and R. J. Naude, *Biochem. Biophys. Res. Commun.*, 1978, **81**, 900.
[4] H. Kawauchi, K. Muramoto, and J. Ramachandran, *Int. J. Pept. Protein Res.*, 1978, **12**, 318.
[5] J. Ramachandran, in 'Hormonal Proteins and Peptides', ed. C. H. Li, Academic Press, New York, 1973, Vol. 2, p. 1.
[6] K. Koyama, H. Watanabe, H. Kawatani, J. Iwai, and H. Yajima, *Chem. Pharm. Bull.* (*Tokyo*), 1976, **24**, 2558.
[7] H. Yajima, K. Koyama, Y. Kiso, A. Tamaka, and M. Nakamura, *Chem. Pharm. Bull.* (*Tokyo*), 1976, **24**, 492.
[8] K. Koyama, H. Kawatani, H. Yajima, M. Fujino, and O. Nishimura, *Chem. Pharm. Bull.* (*Tokyo*), 1976, **24**, 2106.
[9] S. Shinamura, K. Okamoto, M. Nakamura, A. Tamaka, and H. Yajima, *Int. J. Pept. Protein Res.*, 1978, **12**, 170.
[10] P. J. Lowry, H. P. J. Bennett, C. McMartin, and A. P. Scott, *Biochem. J.*, 1974, **141**, 427.
[11] F. Toma, S. Fermandjian, M. Low, and L. Kisfaludy, *Biochem. Biophys. Acta*, 1978, **534**, 112.
[12] E. Nabedryk-Viala, C. Thiery, P. Calvet, S. Fermandjian, L. Kisfaludy, and J. M. Thiery, *Biochem. Biophys. Acta*, 1978, **536**, 252.
[13] S. Lemaire, D. Yamashiro, C. Behrens, and C. H. Li, *J. Am. Chem. Soc.*, 1977, **99**, 1577.
[14] M. Low, L. Kisfaludy, and M. Sankozi, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 5.

active in stimulating steroidogenesis (60% and 40%, respectively, compared to ACTH-(1—19) amide). The synthesis of ACTH-(5—24), ACTH-(6—24), ACTH-(7—24), and ACTH-(8—24) has been described.[15] The biological properties of these peptides were summarized but no data were provided.

Three analogues of the *N*-terminal nonadecapeptide of ACTH containing cystine bridges between positions (5,10), (3,10), or (2,10) have been synthesized by the solid-phase method.[16] All three analogues had reduced steroidogenic activity (<3%). The peptide with the 5,10 cystine bridge also stimulated lipolysis in rat adipocytes (4.4%) whereas the other two did not.

The biological activities of ACTH analogues containing norarginine[17] or homoarginine in place of arginine at position-8 have been reported.[18] (Homoarginine-8)-ACTH-(1—24) and (norarginine-8)-ACTH-(1—24) were three and ten times less potent than ACTH-(1—24) in stimulating glycerol release from rat epididymal fat pads. Substitution of pentamethyl phenylalanine for Trp in position-9 resulted in loss of steroidogenic and rat lipolytic activities but increased the melanophore stimulating activity.[18] This is in agreement with previous findings that the Trp-residue in ACTH is crucial for productive interaction with ACTH receptors but unimportant for stimulating MSH receptors.[5]

The role of glutamic acid in position-5 of ACTH has been investigated.[19-21] Ethoxycarbonyl-[Lys-17]ACTH-(6—17)-4-aminobutylamide had the same steroidogenic activity as glutaroyl-[Lys-17]ACTH-(6—17)-4-aminobutylamide indicating that the γ-carboxyl group of Glu in position-5 is unimportant.[19] Replacement of Glu with Arg in position-5 in (2-δ-aminovaleric acid)-ACTH-(2—19) resulted in an increase in both steroidogenic and rat lipolytic potencies.[20] The melanotropic and rabbit lipolytic activities of the 5-arginine analogue were, however, significantly lower than the activities of the 5-glutamic acid analogue. These findings further support the concept that the rat adrenocortical cell receptor is very similar to the rat adipocyte receptor but quite different from the rabbit adipocyte receptor which appears to resemble the amphibian melanophore receptors in terms of the structural requirements for stimulation.[5] Another analogue [5-Arg]ACTH-(1—20) is reported[21] to stimulate steroidogenesis to 55% of the maximum effect caused by ACTH-(1—20). These authors also find that ACTH-(3—10) causes only 11% of the stimulation produced by ACTH-(1—24) whereas Schwyzer *et al.*[22] demonstrated some years ago that even ACTH-(4—10) stimulates corticosterone production to the same maximum as ACTH-(1—24). The reasons for this discrepancy are not clear. The synthesis and characterization of [5-Arg]ACTH(1—20) has not been described.

The lipolytic activities of a number of short-chain peptides related to the ACTH

[15] J. L. Fauchere and C. Petermann, *Helv. Chim. Acta*, 1978, **61**, 1186.
[16] J. Blake, A. J. Rao, and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, **13**, 346.
[17] J. W. Van Nispen, G. I. Tesser, and R. J. Nivard, *Int. J. Pept. Protein Res.*, 1977, **9**, 193.
[18] J. W. Van Nispen, G. I. Tesser, P. L. Barthe, R. Maier, and L. Schenkel-Hulliger, *Acta Endocrinol.*, 1977, **84**, 470.
[19] R. Geiger and J. Sandow, *Justus Liebigs Ann. Chem.*, 1977, 897.
[20] J. Blake and C. H. Li, *J. Med. Chem.*, 1976, **19**, 1252.
[21] E. Jean-Baptiste, M. W. Draper, and M. A. Rizack, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 4329.
[22] R. Schwyzer, P. Schiller, S. Seelig, and G. Sayers, *FEBS Lett.*, 1971, **19**, 229.

structure have been reported.[23, 24] The structural and functional organization of ACTH has been compared to that of wasp kinin.[25]

The preparation and characterization of specifically tritiated human ACTH has been described in detail.[26] Catalytic dehalogenation of synthetic human ACTH containing 3,5-di-iodotyrosine in position-23 yielded the tritiated hormone which was indistinguishable from synthetic human ACTH in all physiochemical and biological properties examined. The specific radioactivity of this preparation was 46 Ci $mmol^{-1}$. By the same procedure a tritiated product with a specific activity of 90 Ci $mmol^{-1}$ and full biological activity was obtained [27] using synthetic human ACTH substituted with 3,5-di-iodotyrosine at both positions-2 and -23. Tritiated human ACTH was also prepared [28] by catalytic dehalogenation of partially protected synthetic human ACTH containing 3,5-di-iodotyrosine in position-23. In this case the product had a specific radioactivity of 25.2 Ci $mmol^{-1}$.

Ovine ACTH was enzymatically methylated with purified calf brain methylase II and *S*-adenosyl-L-[*methyl*-^{14}C]methionine.[29] Analysis of a peptic digest of the methylated product suggested that Glu-28 of the hormone is the site of methyl esterification. An ACTH analogue containing biotin has been prepared.[30] [Biocytin-25]ACTH-(1—25) amide was as active as ACTH-(1—24) in stimulating steroidogenesis in bovine adrenocortical cells. A 1 : 1 complex of [biocytin-25]ACTH-(11—25) amide with avidin was 25% as active as the peptide alone.[31]

Corticotropin Inhibiting Peptide (*CIP*). Seventeen years ago, Pickering *et al.*[32] reported the isolation of a basic peptide from sheep pituitary extracts, the amino-acid composition of which corresponded to residues 7—38 of ACTH. This peptide was recently isolated from human pituitary glands and characterized.[33] The amino-acid sequence of this peptide was found to be identical to the sequence 7—38 of human ACTH. This peptide, named corticotropin inhibiting peptide (CIP), has been synthesized and found to be devoid of adrenal stimulating activity.[33, 34] CIP was found to act as a competitive inhibitor of the actions of ACTH on isolated rat adrenocortical cells.[34] The presence of CIP in the pituitary raises the possibility that enhanced conversion of ACTH or its precursor to CIP may be responsible for some clinical states associated with hypoadrenal function.

Melanotropins (*MSH*). The chemistry of melanotropins has been reviewed.[35] The isolation and characterization of α and β-melanotropins from salmon

[23] F. A. Opmeer, J. M. Van Ree, and D. DeWied, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1978, **302**, 31.
[24] E. A. Porunkevich, G. G. Kublis, A. A. Skuin'sh, and G. I. Chipens, *Biokhimiya*, 1977, **42**, 267.
[25] G. G. Kublis, E. A. Porunkevich, A. A. Skuin'sh, N. A. Makarova, I. P. Misin'sh, V. E. Klusha, and G. I. Chipins, *Biokhimiya*, 1977, **42**, 16.
[26] J. Ramachandran and C. Behrens, *Biochem. Biophys. Acta*, 1977, **496**, 321.
[27] J. Ramachandran, E. Canova-Davis, and C. Behrens, XIth Int. Cong. Biochem. Toronto, 1979, Abstract, p. 606.
[28] D. E. Brundish and R. Wade, *Biochem. J.*, 1977, **165**, 169.
[29] S. Kim and C. H. Li, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4355.
[30] K. Hofmann, F. M. Finn, and Y. Kiso, *J. Am. Chem. Soc.*, 1978, **100**, 3585.
[31] F. M. Finn, J. J. Kim, and K. Hofmann, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1153.
[32] B. T. Pickering, R. N. Andersen, P. Lohman, Y. Birk, and C. H. Li, *Biochem. Biophys. Acta*, 1963, **74**, 763.
[33] C. H. Li, D. Chung, D. Yamashiro, and C. Y. Lee, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 4306.
[34] C. Y. Lee, J. Ramachandran, and C. H. Li, *Endocrinology* Suppl. to vol. 102, 1978, p. 228.
[35] C. H. Li, in 'Hormonal Proteins and Peptides', ed. C. H. Li, Academic Press, New York, 1978, vol. 5. p. 1.

(*Onchorhynchus keta*) pituitaries has been reported.[36] The amino-acid sequences of the melanotropin isolated to date are shown in Figure 1. The structure of α-MSH is highly conserved. The two non-mammalian α-melanotropins from dogfish and

α-MSH	
Salmon	H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2
Dogfish	H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Met-NH_2/OH
Mammals	MeCO-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2
β-MSH	
Salmon	H-Asp-Gly-(G)-Ser-Tyr-Lys-Met-Asn-His-Phe-Arg-Trp-Ser-Gly-Pro-Pro-Ala-Ser-OH
Dogfish	H-Asp-Gly-Ile-Asp-Tyr-Lys-Met-Gly-His-Phe-Arg-Trp-Gly-Ala-Pro-Met-Asp-Lys-OH
Dogfish	H-Asp-Gly-Asp-Asp-Tyr-Lys-Phe-Gly-His-Phe-Arg-Trp-Ser-Val-Pro-Leu-OH
Macacus	H-Asp-Glu-Gly-Pro-Tyr-Arg-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH
Porcine	H-Asp-Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH
Equine	H-Asp-Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Arg-Lys-Asp-OH
Bovine	H-Asp-Ser-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH
Camel	H-Asp-Gly-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp-OH

Figure 1 *Amino-acid sequences of α- and β-melanotropins*

salmon have a free *N*-terminal whereas all mammalian α-MSH have the *N*-terminal acetylated. Since desacetyl-α-MSH is much less potent than α-MSH in amphibian melanophore assays, it would be of interest to examine the action of these peptides on fish melanophores. Five forms of MSH were detected in extracts of the neurointermediate lobe of the American bullfrog (*Rana catesbiana*) on polyacrylamide gels.[37] The principal secreted MSH appears to resemble mammalian β-MSH.

A structural variant of α-MSH containing an extra acetyl group on the serine residue in position-1 has been isolated from bovine pituitaries.[38] *N,O*-Diacetylserine-1-α-MSH has the same melanotropic and lipolytic activities as α-MSH. Replacement of lysine in position-11 by serine lowered the melanotropic activity to 43% of α-MSH.[39] A conjugate of α-MSH and fluoresceinisothiocyanate-labelled ferritin having 10% of the activity of α-MSH has been prepared.[40] α-MSH has been attached to tobacco mosaic virus for potential use in receptor detection and isolation.[40a]

Porcine β-MSH as well as several analogues of camel β-MSH have been synthesized by the solid-phase method.[41] β-MSH of two species of dogfish, *Squalus acanthias*[42] and *Scyliorhinus canicula*,[43] have been synthesized by conventional procedures. Both peptides were found to be 9% as potent as synthetic mammalian α-MSH. Contrary to other published reports, Heward *et al.*[44] found that iodinated

[36] H. Kawauchi and K. Muramoto, *Int. J. Pept. Protein Res.*, 1979, **14**, 373.
[37] W. W. Dickoff and C. S. Nicoll, *Gen. Comp. Endocrinol.*, 1979, **39**, 313.
[38] D. Rudman, R. K. Chawla, and B. M. Hollins, *J. Biol. Chem.*, 1979, **254**, 10102.
[39] R. Geiger, J. Sandow, and A. J. Kastin, *Hoppe-Seyler's Z. Physiol. Chem.*, 1977, **358**, 1474.
[40] A. Dipasquale, J. M. Varga, G. Moellmann, and J. McGuire, *Anal. Biochem.*, 1978, **84**, 37.
[40a] V. M. Kriwaczek, A. N. Eberle, M. Muller, and R. Schwyzer, *Helv. Chim. Acta*, 1978, **61**, 1232.
[41] S. Lemaire, D. Yamashiro, A. J. Rao, and C. H. Li, *J. Med. Chem.*, 1977, **20**, 155.
[42] H. Yajima, J. Iwai, K. Koyama, M. Nakamura, K. Miyata, and A. Tanaka, *Chem. Pharm. Bull. (Tokyo)*, 1978, **26**, 571.
[43] H. Yajima, J. Iwai, H. Watanabe, K. Koyama, M. Nakamura, K. Miyata, and A. Tanaka, *Chem. Pharm. Bull.*, 1977, **25**, 2048.
[44] C. B. Heward, Y. C. Yang, T. K. Sawyer, M. D. Bergman, B. B. Fuller, V. J. Hruby, and M. E. Hadley, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 266.

β-MSH is without biological activity as measured by frog skin bioassay, melanoma adenylate cyclase assay, or melanoma tyrosinase assay. Inactivation results in part from oxidation of the methionine residue by the chloramine T and sodium metabisulphite used in the iodination reaction. An analogue containing norleucine in place of methionine in β-MSH was more resistant to inactivation.

The pigmentary action of the melanotropins is clearly of importance in lower vertebrates. With the evolution of hair in the mammal, adaptive changes in skin colour are far less important and it is suspected that the melanotropins may have lost their significance as pigmentary hormones. There is no evidence that MSH has any physiological role as a pigmentary hormone in man. In fact, the adult human pituitary has apparently lost the ability to produce MSH.[45] For the past several years investigators have tried to elucidate the extrapigmentary actions of the melanotropins which may be physiologically relevant in mammals. Analysis of the structure–activity relationships of a number of peptide analogues of ACTH and MSH has indicated that α-MSH may function as a lipolytic hormone in rabbits.[46] Thody, Shuster, and their co-workers [47–49] have shown that α-MSH stimulates the sebaceous glands of mammals and propose that this may be an important function of the hormone.

During the past 3 years evidence has accumulated to suggest that α-MSH may play an important role during foetal life. Silman *et al.*[50] found immunoreactive α-MSH in large amounts in human foetal pituitary. Challis and Torosis [51] reported that α-MSH stimulated cortisol secretion by the foetal adrenal gland of the rabbit at a time when ACTH was ineffective, whereas in the newborn rabbit ACTH was potent and α-MSH was ineffective. Immunoreactive α-MSH has been detected in the pituitary [52] as well as in the plasma [53] of pregnant women. Significant amounts of α-MSH were detected in pituitary extracts of rhesus monkey foetuses using gel filtration and radioimmunoassay.[54] α-MSH was also reported to stimulate foetal growth in rats.[55] These results strongly suggest that α-MSH may regulate the development and function of the foetus.

Lipotropin (LPH). Lipotropin was discovered fortuitously during investigation of a simplified isolation procedure for ACTH.[56] Two peptides exhibiting lipolytic activity were isolated from ovine pituitaries and were designated β-LPH and γ-LPH. β-LPH is a linear polypeptide consisting of 91 amino-acid residues. The 58-residue γ-LPH is identical to the sequence region 1—58 of β-LPH. Residues 41—58 in LPH correspond to the sequence of β-MSH. The 51-residue peptide [57] of ovine β-

[45] P. J. Lowry and A. P. Scott, *Gen. Comp. Endocrinol.*, 1975, **26**, 16.
[46] J. Ramachandran, S. Farmer, S. Liles, and C. H. Li, *Biochem. Biophys. Acta*, 1976, **428**, 347.
[47] A. J. Thody and S. Schuster, *J. Endocrinol.*, 1975, **64**, 503.
[48] A. J. Thody, M. F. Cooper, P. E. Bowden, D. Meddis, and S. Schuster, *J. Endocrinol.*, 1976, **71**, 279.
[49] A. J. Thody, *Front Hormone Res.*, 1977, **4**, 117.
[50] R. E. Silman, T. Chard, P. J. Lowry, I. Smith, and I. M. Young, *Nature (London)*, 1976, **260**, 716.
[51] J. R. G. Challis and J. D. Torosis, *Nature (London)*, 1977, **269**, 818.
[52] M. Visser and D. F. Swaab, *Front. Hormone Res.*, 1977, **4**, 42.
[53] D. Clark, A. J. Thody, S. Schuster, and H. Bowers, *Nature (London)*, 1978, **273**, 163.
[54] R. E. Silman, D. Holland, T. Chard, P. J. Lowry, J. Hope, J. S. Robinson, and G. D. Thorburn, *Nature (London)*, 1978, **276**, 526.
[55] D. F. Swaab and M. Visser, *Front. Hormone Res.*, 1977, **4**, 170.
[56] Y. Birk and C. H. Li, *J. Biol. Chem.*, 1964, **239**, 1048.
[57] S. Lemaire, D. Yamashiro, and C. H. Li, *Int. J. Pept. Protein Res.*, 1978, **11**, 179.

LPH corresponding to residues 41—91 as well as the 91-residue peptide [58] have been synthesized by the solid-phase procedure. Synthetic ovine β-LPH was found to be identical to the natural peptide in all physicochemical and biological properties studied. The amino-acid sequence of fin whale lipotropin has been elucidated.[59]

The physiological role of LPH has remained obscure. Recent evidence strongly suggests that biological function of LPH may be that of a prohormone for the opioid peptide, β-endorphin. However, Liotta *et al.*[60] report that β-LPH is the predominant opioid-like peptide in the human pituitary and the anterior lobe of rat pituitary. The concentrations of β-LPH were comparable to that of ACTH on a molar basis. In human subjects, acute anterior pituitary stimulation using either insulin-induced hyperglycaemia or vasopressin administration was associated with increased levels of β-LPH and γ-LPH in the plasma of patients with disease of the pituitary–adrenal system (and with raised levels of ACTH). The presence of significant amounts of intact β-LPH in circulation raises the possibility that LPH may have some function besides that of a prohormone for β-endorphin and β-MSH.

Biosynthesis of ACTH-MSH-LPH-EP. The most significant development in the biochemistry of the Group I hormones in the past 3 years is the recognition that all the hormones of this group are derived from a single high molecular weight precursor. High molecular weight forms of ACTH were first detected in blood of patients with ACTH-producing tumours [62] and subsequently in rat and mouse pituitary extracts.[63] Gel filtration of extracts of mouse pituitaries or clonal mouse pituitary tumour cells (AtT-20/D-16v) under strongly denaturing conditions (6 M-guanidine hydrochloride) and analysis by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) revealed [64] precursors of ACTH with molecular weights 31 000 dalton (31 K) and 23 000 dalton. Subsequently, it was found [65] that the protein with molecule weight = 31 000 dalton is the precursor of both ACTH and β-LPH. When mRNA from AtT-20 cells is translated in a reticulocyte cell-free protein-synthesizing system and the immunoprecipitates obtained with ACTH or endorphin antisera are analysed by SDS–PAGE, only one labelled protein with molecular weight = 28 500 dalton is found.[66, 67] Similar results were obtained with beef pituitary mRNA.[68]

The common precursor of ACTH and EP has also been isolated from rat pituitaries and the name 'pro-opiocortin' has been proposed for this protein.[69] The structure of pro-opiocortin has been analysed in detail.[67, 70] The entire structure of

[58] D. Yamashiro and C. H. Li, *J. Am. Chem. Soc.*, 1978, **100**, 5174.
[59] H. Kawauchi, D. Chung, and C. H. Li, *Int. J. Pept. Protein Res.*, 1980, **15**, 171.
[60] A. S. Liotta, T. Suda, and D. T. Krieger, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 2950.
[61] W. J. Jeffcoate, L. H. Rees, P. J. Lowry, and G. M. Besser, *J. Endocrinol.*, 1978, **77**, 27.
[62] R. S. Yalow and S. A. Berson, *Biochem. Biophys. Res. Commun.*, 1971, **44**, 439.
[63] D. N. Orth, W. E. Nicholson, W. M. Mitchell, D. P. Island, M. Shapiro, and R. L. Byyny, *Endocrinology*, 1973, **92**, 385.
[64] R. E. Mains and B. A. Eipper, *J. Biol. Chem.*, 1976, **251**, 4121.
[65] R. E. Mains, B. A. Eipper, and N. Ling, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 3014.
[66] J. L. Roberts and E. Herbert, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 4826.
[67] J. L. Roberts and E. Herbert, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 5300.
[68] S. Nakanishi, A. Inoue, S. Taii, and S. Numa, *FEBS Lett.*, 1977, **84**, 105.
[69] M. Rubinstein, S. Stein, and S. Udenfriend, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 669.
[70] B. A. Eipper and R. E. Mains, *J. Biol. Chem.*, 1978, **253**, 5732.

pro-opiocortin can be accounted for by three smaller peptides: β-LPH, ACTH and a glycopeptide of unknown function (referred to as the 16 000 dalton fragment). β-LPH (and therefore β-EP) is located in the carboxyl terminal of the precursor, ACTH in the middle, and the 16 000 dalton fragment forms the amino-terminal. The complete amino-acid sequence of pro-opiocortin of bovine origin has now been deduced from the nucleotide sequence of a 1091-base pair cDNA insert coding for bovine pro-opiocortin determined by Nakanishi *et al.*[71] Inspection of the amino-acid sequence (265 residues) reveals a number of interesting features. The molecule appears to consist of four repetitive units based on the core sequence Met-Glu-His-Phe-Arg-Trp-Gly of ACTH and MSH. These units are separated by paired basic residues. ACTH and β-LPH exist as contiguous peptides at the carboxyl terminal and are separated by the Lys-Arg sequence. Thus, in common with other protein precursors (proinsulin, proPTH, *etc.*), the biologically active sequences in pro-opiocortin are flanked by paired basic residues at which proteolytic processing takes place. Nakanishi *et al.*[71] point out that the pro-opiocortin sequence contains a new MSH sequence flanked by paired basic residues in the amino-terminal region and have termed it γ-MSH. The arrangement of the various peptides in pro-opiocortin is shown schematically in Figure 2.

The steps in the processing of pro-opiocortin to ACTH and EP have been deduced from analysis of the precursor from AtT-20 cells in pulse-chase and continuous-labelling experiments using radioactive amino-acids and sugars.[72] The 31 000 dalton protein has been resolved into three glycoproteins of molecular weight 29 000, 32 000, and 34 000 dalton. The three forms have the same peptide backbone but differ from each other in the carbohydrate content. The nascent polypeptide of molecular weight 28 000 dalton is glycosylated to the 29 000 dalton form which is converted to 32 000 and 34 000 dalton forms by the addition of carbohydrate. The 29 000 dalton protein is also converted directly to ACTH (4500 dalton) and β-LPH (11 500 dalton). The 32 000 dalton form of pro-opiocortin is processed proteolytically to yield β-LPH and the 23 000 dalton ACTH intermediate, which is further processed to yield the glycosylated form of ACTH (13 000 dalton). In the anterior lobe of the pituitary, pro-opiocortin is converted primarily to ACTH and β-LPH. In the intermediate lobe, ACTH and β-LPH, formed from pro-opiocortin, are further processed by proteolysis at paired basic residues to yield α-MSH and corticotropin-like intermediate lobe peptide (CLIP) from ACTH, and β-MSH and β-EP from β-LPH.[72]

During the past year biosynthesis of ACTH, MSH, and LPH in the intermediate lobes of rat [73, 74] and toad [75] have been investigated. A comprehensive review of the biosynthesis and processing of pro-opiocortin has appeared.[76]

A new 39-residue glycopeptide has been isolated from porcine, bovine, and ovine pituitaries. The amino-acid sequence is strongly conserved, suggesting that the

[71] S. Nakanishi, A. Inoue, T. Kita, M. Nakamura, A. C. Y. Chang, S. N. Cohen, and S. Numa, *Nature (London)*, 1979, **278**, 423.
[72] J. L. Roberts, M. Phillips, P. A. Rosa, and E. Herbert, *Biochemistry*, 1978, **17**, 3609.
[73] R. E. Mains and B. A. Eipper, *J. Biol. Chem.*, 1979, **254**, 7885.
[74] P. Crine, F. Gossard, N. G. Seidah, L. Blanchette, M. Lis, and M. Chretien, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5085.
[75] P. Y. Loh, *Proc. Natl. Acad. Sci.*, 1979, **76**, 796.
[76] E. Herbert, J. Roberts, M. Phillips, R. Allen, M. Hinman, M. Budorf, P. Policastro, and P. Rosa, *Front. Neuroendocrinol.*, 1980, **6**, 67.

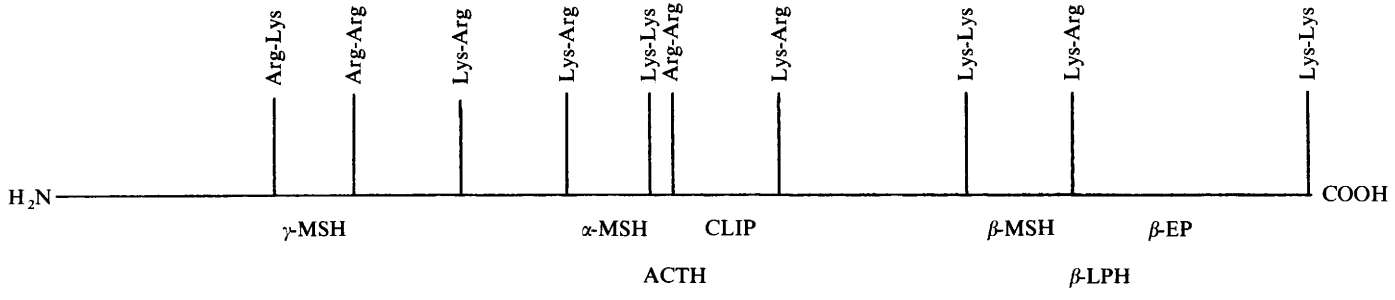


Figure 2 *Structure of pro-opiocortin. The Relative positions of the hormones of Group I are shown. The biologically active sequences are flanked by paired basic residues.* (Adapted from S. Nakanishi *et. al.*[71])

peptide may fulfil an important role. This peptide did not cross-react with antiserum raised against the 16 000 dalton fragment of rat pro-opiocortin.[77] An enzyme which acetylates the α-amino-group of the *N*-terminal residue of ACTH using acetyl-CoA has been detected in the rat pituitary.[78]

The steroidogenic actions of high molecular weight forms of ACTH produced by mouse pituitary tumour cells have been studied.[79] Pro-opiocortin (31 000 dalton), ACTH biosynthetic intermediate (23 000 dalton), and glycosylated ACTH (13 000 dalton) were isolated by gel filtration.[70] All three high molecular weight forms of ACTH were found to be capable of stimulating the same maximal level of steroidogenesis as synthetic human ACTH. Glycosylated ACTH was equipotent to ACTH whereas the 23 000 and 31 000 dalton forms were, respectively, 100- and 300-fold less potent than ACTH. Steroid production in response to all forms was linear in time. β-LPH and the 16 000 dalton amino-terminal fragment of pro-opiocortin[70] did not stimulate or interfere with steroidogenesis. Brief incubations of 31 000 and 23 000 dalton forms with trypsin generated lower molecular weight forms of ACTH and increased biological activity 50-fold. The decreased steroidogenic potency of these forms of ACTH is thought to be due to structural constraints on ACTH$^{1-39}$-like sequence in these larger precursor molecules.

The new MSH sequence flanked by paired basic residues noted in the *N*-terminal region of pro-opiocortin[71] is Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly. Four possible γ-MSH peptides derived from this sequence have been synthesized.[80] All of them had <0.1% of the melanotropic activity of α-MSH.

Hormones of Group II.—*Somatotropin (GH).* The regeneration of growth-promoting activity by the non-covalent recombination of inactive fragments of human GH has been one of the most exciting developments in the peptide hormone field. Following the observation that treatment with human plasmin does not cause any change in the biological properties of human GH, it was shown that plasmin removed a hexapeptide corresponding to residues 135—140 of human GH. The plasmin-modified hormone is held together by a disulphide bridge between residues 53 and 165. Reduction and alkylation of the plasmin-treated hormone results in the formation of two peptides corresponding to residues 1—134 and 141—191 of the native hormone. The purified fragments have very little biological activity. However, recombination of the two fragments gives the formation of a non-covalent complex which has full biological activity.[81] The recombinant molecule is identical to the plasmin-treated native hormone in its chromatography elution position, circular dichroism spectra, and immunochemical properties. These findings opened the way for the semi-synthesis of hGH. Combination of the synthetic peptide corresponding to the carboxyl terminal fragment (residues 141—191) with the natural 1—134 fragment gave the restoration of full biological activity and immunoreactivity.[82] Synthetic analogues containing norleucine in place of methionine and alanine in place of cysteine in the 141—191 fragment and the 145—

[77] D. G. Smyth and D. E. Massey, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1006.
[78] T. A. Woodford and J. E. Dixon, *J. Biol. Chem.*, 1979, **254**, 4993.
[79] J. Gasson, *Biochemistry*, 1979, **18**, 4215.
[80] N. Ling, S. Ling, S. Minick, and R. Guillemin, *Life Sci.*, 1979, **25**, 1773.
[81] C. H. Li and T. A. Bewley, *Proc. Natl. Acad. Sci. USA*, 1976, **73**, 1476.
[82] C. H. Li, T. A. Bewley, J. Blake, and T. Hayashida, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 1016.

191 fragment, complemented with natural 1—134 peptide to generate full growth-promoting activity.[83, 84] Plasmin digestion of hGH after reduction and alkylation of the hormone gives more extensive cleavage of the peptide chain compared to its action on native hGH.[85, 86] In addition to hGH-(1—134), hGH-(26—41), hGH-(95—134), and hGH-(42—134) are also formed.

Human chorionic somatomammotropin (hCS), the placental hormone, is very similar to hGH in its primary structure (95% homology), yet it has very low growth-promoting activity. Plasmin treatment of hCS gives the removal of a hepta-peptide from the hormone.[87] Reduction and alkylation of plasmin-treated hCS gave two fragments corresponding to residues 1—133 and 141—191 of hCS. The fragments were devoid of any biological or immunochemical activity. Complementation of the carboxyl-terminal fragment of hCS (residues 141—191) with the amino-terminal fragment of hGH (residues 1—134) yielded a product with 50% growth-promoting activity and nearly full immunoreactivity.[88] The other hybrid made up of hCS-(1—133) and hGH-(141—191) had very low growth-promoting activity ($<10\%$). The immunochemical and receptor-binding properties of the two hybrids have been analysed in greater detail.[89] It was concluded that the determinants for binding hGH to hepatic GH receptors as well as lactogenic receptors are present in the hGH-(1—134) fragment. The antigenic determinants for monospecific antisera to hCS and hGH also are on the amino-terminal fragments of their respective antigens.

The dissociation kinetics of reduced, alkylated, plasmin-modified hGH has been studied in detail.[90] The major products of the dissociation have been shown to be the monomeric form of hGH-(141—191) and a trimeric form of hGH-(1—134). The carboxyl-terminal fragment appears to be a random coil, whereas the amino-terminal fragment retains a considerable degree of secondary and tertiary structure.

The amino-terminal heterogeneity, which is generated in bovine GH during its extraction from mildly acidic pituitary homogenates, has been traced[91] to the action of a tripeptidyl amino-peptidase in the pituitary which is optimally active at pH = 4. This enzyme was found to be distinct from cathepsin D.

Semisynthesis of human GH analogues by complementation of the natural amino-terminal 134-amino-acid fragment of reduced carbamoylmethylated human GH (1) with synthetic carboxyl-terminal fragments of varying chain lengths has been reported.[92] Synthetic fragments of 57 (135—191) and 42 (150—191) amino-acid residues gave recombinants with full growth-promoting activity, whereas attempts to obtain a recombinant with the synthetic 38 amino-acid fragment (residues 154—191) were unsuccessful.

[83] J. Blake and C. H. Li, *Int. J. Pept. Protein Res.*, 1978, **11**, 315.
[84] C. H. Li, J. Blake, and T. Hayashida, *Biochem. Biophys. Res. Commun.*, 1978, **82**,217.
[85] J. B. Mills, J. L. Kostyo, M. H. Moseley, C. R. Reagan, and A. E. Wilhelmi, *Endocrinology*, 1978, **102**, 1366.
[86] C. R. Reagan, J. L. Kostyo, J. B. Mills, M. H. Moseley, and A. E. Wilhelmi, *Endocrinology*, 1978, **102**, 1377.
[87] C. H. Li and R. Houghten, *Int. J. Pept. Protein Res.*, 1978, **12**, 114.
[88] C. H. Li, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 1700.
[89] S. Burstein, M. M. Grumbach, S. L. Kaplan, and C. H. Li, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 5391.
[90] T. A. Bewley and C. H. Li, *Biochemistry*, 1978, **17**, 3315.
[91] T. W. Doebber, A. R. Divor, and E. Ellis, *Endocrinology*, 1978, **103**, 1794.
[92] C. H. Li and J. Blake, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6124.

A naturally occurring structural variant of human somatotropin that differed from the major form of the hormone has been reported.[93,94] The structural variant was shown[95] to be without residues 32—46 in the sequence of human GH. The results suggest that the variant hormone is the product of a deletion mutation in the human GH gene.

Two previously undetected forms of human GH have been found by the use of urea during electrophoretic analysis.[96] The newly recognized components were resolved at pH = 10 and 7.8 but not at pH = 4. They have molecular weights near 22 000 dalton and are neither electrophoretic artifacts nor proteolytically cleaved forms. Both forms had growth-promoting activity in the rat tibia line assay and both resembled human GH by peptide mapping and total amino-acid composition. A third form of human GH was detected when the gel electrophoresis was carried out at pH = 7.8 with or without urea. This form migrated faster than the major GH band, whereas the other two forms migrated slower. The fast-GH also had a molecular weight near 22 000 dalton, full growth-promoting activity, and great similarity to human GH as indicated by peptide mapping. These studies bring to five the number of intact (not proteolytically cleaved) forms of human GH that can be detected in currently available preparations of the hormone. Lewis *et al.*[96] suggest that human GH is a complex of substances and the multiple actions of GH may be in part a result of this heterogeneity.

Prolactin (PRL). The existence of prolactin as a distinct pituitary hormone in the human was established only at the beginning of this decade. Human PRL was isolated in 1972 and recently the complete amino-acid sequence was elucidated.[97] Only 16% of the hPRL sequence is identical with hGH sequence. Human PRL sequence-identity with porcine, ovine, and rat PRL was found to be 77, 73, and 60%, respectively. PRL has also been isolated from chinchilla,[98] salmon,[99] and fin whale[100] pituitaries. PRL isolated from chinchilla pituitaries maintained in organ culture was found to be similar to other mammalian prolactins with regard to lactogenic properties, molecular weight, and susceptibility to deamidation.[98] Salmon PRL also has a molecular weight similar to the mammalian prolactins.[99] The amino-acid composition and circular dichroism spectra of fin whale PRL were very similar to that of porcine PRL. The lactogenic potency of the whale hormone was 80% of that of porcine PRL.[100]

Ovine PRL was cleaved selectively between residues 53 and 54 by limited proteolysis with fibrinolysin.[101] The two fragments, PRL-(1—53) and PRL-(54—199), were isolated and characterized. The fragments were devoid of lactogenic and immunochemical activities. Recombination of the two fragments resulted in full restoration of immunoreactivity but not biological activity.[101] The physico-

[93] U. J. Lewis, J. T. Dunn, L. F. Bonewald, B. K. Seavey, and W. P. Vanderlaan, *J. Biol. Chem.*, 1978, **253**, 2679.
[94] L. G. Frigeri, S. M. Peteron, and U. J. Lewis, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 778.
[95] U. J. Lewis, L. F. Bonewald, and L. J. Lewis, *Biochem. Biophys. Res. Commun.*, 1979, **92**, 511.
[96] U. J. Lewis, R. N. Singh, L. F. Bonewald, L. J. Lewis, and W. P. Vanderlaan, *Endocrinology*, 1979, **104**, 1256.
[97] B. Shome and A. F. Parlow, *J. Clin. Endocrinol. Metab.*, 1977, **45**, 1112.
[98] M. Jibson and F. Talamantes, *Gen. Comp. Endocrinol.*, 1978, **34**, 402.
[99] D. R. Idler, K. M. Shamsuzzaman, and M. P. Burton, *Gen. Comp. Endocrinol.*, 1978, **35**, 469.
[100] H. Kawauchi and M. Tubokawa, *Int. J. Pept. Protein Res.*, 1979, **13**, 229.
[101] Y. Birk and C. H. Li, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 2155.

chemical properties of the recombinant molecule were very similar but not identical to that of the native hormone. Ovine PRL was cleaved at tryptophan residues 91 and 150 by reaction with 2-(2-nitrophenylsulphenyl)-3-methyl-3′-bromoindolemine.[102] A small fragment, PRL-(92—150) and a large fragment containing PRL-(1—91) and PRl-(151—191) linked by a disulphide bridge were obtained. Neither fragment had bioactivity or immunoreactivity.

Selective reduction of the disulphide bonds of ovine prolactin (PRL) has been reported.[103] Cleavage of all three disulphide bonds abolishes biological activity and denatures the hormone. Reduction and carbamoylmethylation of one or two of the disulphide bridges does not diminish the biological activities in the pigeon crop-sac and mouse mammary gland bioassays. However, cleavage of Cys-4—Cys-11 and Cys-191—Cys-199 bridges followed by carbamoylmethylation destroyed the biological activity of PRL in a teleost fish bioassay. In contrast, reduction of Cys-4—Cys-11 alone actually increased the teleost potency of this derivative compared to the intact hormone. Since teleost PRL appears not to have the homologue to the Cys-4—Cys-11 bridge of the ovine hormone, it is suggested that selective reduction of this bond in ovine PRL makes it more similar to the fish hormone.[103] The seven histidines of bovine PRL were modified with ethoxyformic anhydride.[104] Two classes of reactivity were apparent: five histidines were in the more reactive class and two were less reactive. When zero to five histidine residues were modified, there was no loss of acivity as measured by their ability to bind to PRL receptors from rabbit mammary gland. If all seven histidine residues were modified the derivative was unable to bind to the receptor preparation. No significant conformational differences could be detected in prolactins with two to seven residues modified. Modification of human GH and human chorionic somatomammotropin (hCS) with ethoxyformic anhydride gave a loss of ability of these lactogenic hormones to bind to the PRL receptor. For all three hormones, essentially full activity was recovered when the modifying group was removed by treatment with hydroxylamine. Sequence comparisons indicate that only two of the three histidines in hGH and two of the seven histidines in hCS were homologous with histidines in bovine PRL and that, in each case, they correspond to His-27 and His-30 in bovine PRL. It is postulated that these residues are present in the binding domain of bovine PRL.[104]

Biosynthesis of Somatotropin. Significant progress has been made towards the synthesis of somatotropin in bacteria by the use of recombinant DNA technology.[105-107] The direct expression in *E. coli* of a DNA sequence coding for human GH has been achieved[107] through a combination of cloning of complementary DNA (cDNA) prepared from pituitary mRNA with the cloning of

[102] R. Houghten and C. H. Li, *Int. J. Pept. Protein Res.*, 1978, **11**, 345.
[103] B. A. Doneen, T. A. Bewley, and C. H. Li, *Biochemistry*, 1979, **18**, 4851.
[104] T. T. Andersen and K. E. Abner, *J. Biol. Chem.*, 1979, **254**, 10995.
[105] P. H. Seeburg, J. Shine, J. A. Martial, J. D. Baxter, and H. M. Goodman, *Nature* (*London*), 1977, **270**, 486.
[106] P. H. Seeburg, J. Shine, J. A. Martial, R. D. Ivari, J. A. Morris, A. Ullrich, J. D. Baxter, and H. M. Goodman, *Nature* (*London*), 1978, **276**, 795.
[107] D. V. Goeddel, H. L. Heyneker, T. Hozumi, R. Arentzen, K. Itakura, D. G. Yansura, M. J. Ross, G. Miozzari, R. Crea, and P. H. Seeburg, *Nature* (*London*), 1979, **281**, 544.

chemically synthesized DNA. A DNA fragment of 551 base pairs which includes coding sequences for amino-acid residues 24—191 of human GH was obtained by restriction of endonuclease digestion of double stranded human GH cDNA. This fragment was combined with a chemically synthesized DNA adaptor fragment containing an initiation codon and the coding sequences for residues 1—23 of human GH. This hybrid gene was expressed in *E. coli* under the control of the *lac* promoter. A polypeptide with the size and immunological properties characteristic of mature human GH was produced.

Hormones of Group III.—*General.* A procedure for the isolation of six pituitary hormones including the three glycoprotein hormones from a single batch of human pituitaries has been described.[108] The isolation of bovine pituitary glycoprotein hormones has been improved by incorporating affinity chromatography on concanavalin A—sepharose in the purification procedure.[109] Immunochemical analysis of the unadsorbed fraction showed that <3% of the immunoreactive LH, FSH, and TSH are present as non-glycosylated forms in either crude pituitary extracts or concentrates.

Pregnant mare serum gonadotropin (PMSG) and its subunits have been further characterized.[110] The subunits were found to have <1% of the activity of the parent hormone. The gonadotropin in pregnant mare endometrial cups (PMEG) has been purified and compared to PMSG.[111] The amino-acid composition of PMEG is similar to that of PMSG, but PMEG preparation exhibited amino-terminal heterogeneity. The carbohydrate content of PMEG was considerably less than that of PMSG. In bioassay for LH and FSH activity *in vitro* and *in vivo*, PMEG was less active (11—54%) than PMSG.

The structure of the carbohydrate moieties of the α-subunits of human LH, FSH, and TSH have been studied.[112] A common structure of the carbohydrate moieties of three glycopeptides was deduced. These results suggest that the biosynthetic pathway for the carbohydrate chains is similar and may occur at the same site in the pituitary gland.

Lutropin (LH). LH has been isolated from several non-mammalian species and shown to resemble mammalian LH in possessing two chemically distinct, non-covalently linked subunits.[113] Hybrid LH molecules have been prepared by recombination of ovine LH subunits with sea turtle LH subunits.[114] Assay of the hybrid hormones in both mammalian and reptilian assay systems showed that the β-subunit is primarily responsible for determining species specificity in responses to LH. The isolation of lutropin from rabbit,[115] turkey,[116] and equine [117] pituitaries has been reported.

[108] M. R. Sairam, M. Chretien, and C. H. Li, *J. Clin. Endocrinol. Metab.*, 1978, **47**, 1002.
[109] G. A. Bloomfield, M. R. Faith, and J. G. Pierce, *Biochem. Biophys. Acta*, 1978, **533**, 371.
[110] H. Papkoff, T. A. Bewley, and J. Ramachandran, *Biochem. Biophys. Acta*, 1978, **532**, 185.
[111] H. Papkoff, S. W. Farmer, and H. H. Cole, *Proc. Soc. Exp. Biol. Med.*, 1978, **158**, 373.
[112] K. Hara, P. Rathnam, and B. B. Saxena, *J. Biol. Chem.*, 1978, **253**, 1582.
[113] P. Licht, H. Papkoff, S. W. Farmer, C. H. Miller, H. W. Tsui, and D. Crews, *Rec. Prog. Hormone Res.*, 1977, **33**, 169.
[114] P. Licht, S. W. Farmer, and H. Papkoff, *Gen. Comp. Endocrinol.*, 1978, **35**, 289.
[115] D. N. Ward, C. Desjardins, W. T. Moore, jun., and H. S. Nahm, *Int. J. Pept. Protein Res.*, 1979, **13**, 62.
[116] W. H. Burke, P. Licht, H. Papkoff, and A. B. Gallo, *Gen. Comp. Endocrinol.*, 1979, **37**, 508.
[117] P. Licht, A. B. Gallo, B. B. Aggarwal, S. W. Farmer, J. B. Castelino, and H. Papkoff, *J. Endocrinol.*, 1979, **83**, 311.

The interaction of the α- and β-subunits of ovine lutropin has been investigated by photochemical cross-linking [118] and with the aid of fluorescent probes.[119] The β-subunit of ovine LH was treated with 4-fluoro-3-nitrophenyl azide under select conditions to yield a product in which Lys-20 was modified.[118] This derivative recombined stoicheiometrically with LH-α to form a modified LH. Photolysis of the modified LH yielded a covalently coupled product in which the subunits are no longer dissociable. The modified LH was not attached significantly to rat testis homogenate preparations upon photolysis. These results suggest Lys-20 in LH-α is located in the region of the binding interface between the two subunits. From measurements of the fluorescence of tyrosyl residues of ovine LH and the subunits it was inferred that most of the conformation changes which accompany dissociation of LH and recombination of the subunits occur in the α-subunit.[117]

The modification of histidine residues of ovine pituitary LH by Rose Bengal sensitized photo-oxidation has been studied.[120] The alteration of an average of one histidine out of six gave 90% loss of biological activity as measured by the ability to stimulate testosterone production in rat Leydig cells *in vitro*. Further modification of an average of two to three histidine residues reduced the biological activity to <1% of the native hormone. The photo-oxidized LH did not inhibit the steroidogenic action of native LH. No dissociation of the subunits occurred as a result of the modification.[120]

The α- and β-subunits of ovine LH prepared by acid dissociation and salt precipitation have been further characterized.[121] No evidence of internal peptide cleavage was found in the α-subunit. The subunits had <1% activity but recombined effectively to generate a complex that had full receptor-binding activity and *in vitro* steroidogenic activity. The α-subunit prepared by the salt-precipitation method could be completely reduced and re-oxidized in the absence of denaturants. The re-oxidized α-subunit combined with the native α-subunit to generate full activity. However, this recombined hormone tended to lose activity with time, suggesting that re-oxidation may not fully restore the native structure of the reduced α-subunit. Native LH α-combined effectively with FSH-β to generate complete FSH activity.

Chemical deglycosylation of ovine pituitary LH with anhydrous HF was studied.[122] Treatment of the hormone for 75 min at 0 °C removed nearly two-thirds of the carbohydrate moiety. Deglycosylation altered the gel filtration and electrophoretic behaviour of the hormone. Carbohydrate removal also gave the dissociation into subunits to the extent of 20%. Immunological activity was fully retained but the steroidogenic activity of the deglycosylated LH in the isolated Leydig cell assay was <3% of native LH. The deglycosylated LH effectively antagonized the steroidogenic activity of native LH in rat Leydig cells.

Pierce *et al.*[123] have studied the properties of complexes between LH and monovalent antibodies raised against its α-subunit. The complex between bovine LH and monovalent antibody (Fab-α) retained only 13% receptor-binding activity

[118] B. D. Burleigh, W. K. Lin, and D. N. Ward, *J. Biol. Chem.*, 1978, **253**, 7179.
[119] K. C. Ingham and C. Bolotin, *Arch. Biochem. Biophys.*, 1978, **191**, 134.
[120] B. B. Aggarwal and H. Papkoff, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 169.
[121] M. R. Sairam, *Int. J. Pept. Protein Res.*, 1979, **14**, 153.
[122] M. R. Sairam and P. W. Schiller, *Arch. Biochem. Biophys.*, 1979, **197**, 294.
[123] J. G. Pierce, G. A. Bloomfield, and T. F. Parsons, *Int. J. Pept. Protein Res.*, 1979, **13**, 54.

as compared to LH in a radioligand receptor assay. Complex formation does not lead to dissociation of the LH into its subunits. Complexing of LH with Fab-α also caused little or no change in the affinity of the β-subunit of the hormone for anti-LH β-antibodies.

Follitropin (FSH). Follitropin and its subunit have been isolated from bovine [124, 125] and porcine [126, 127] pituitaries and characterized. The amino-acid sequence of the β-subunits of porcine FSH has been elucidated.[128] Porcine β-FSH is shorter than the human β-FSH both at the amino- and carboxy-ends. On the basis of the sequence homologies between the α- and β-chains of the glycoprotein hormones and that of the three β-chains, it is suggested that the divergence of the α- and β-chains preceded the divergence of the β-chains, which led to the three different hormones.[128] Circular dichroism studies of mammalian follitropins showed that the tryptophan residue in FSH is not affected by *N*-bromosuccinimide treatment of the intact hormone suggesting that the domain containing the tryptophan residue may be in, or near, a region of subunit–subunit contact.[129]

The functional role of the amino-groups of ovine [130] and human follitropin [131] have been investigated. The free amino-groups of ovine LH and the α- and β-subunits undergo nearly complete modification when treated with maleic anhydride or potassium cyanate. Acylation of the amino-groups in FSH alters the conformation but does not disrupt the quaternary structure. The carbamylated or maleylated β-subunit retained its ability to interact with the native α-subunit. Carbamylated FSH-α, but not maleylated FSH-α, was able to recombine with native FSH-β. The native hormone is completely inactivated by both modifications. The results suggest that the ε-amino-groups of FSH-α play a very important role in determining the biological activity of the hormone. Similar results were obtained in studies with purified human FSH and its subunits.

Thyrotropin. The isolation and characterization of human thyrotropin and its subunits has been described.[132] The complete amino-acid sequences of both subunits have been elucidated.[133] The chemistry of human thyrotropin has been reviewed.[134] Biosynthesis of thyrotropin subunits has been studied.[135–137] The α- and β-subunits appear to be translated from separate mRNA. Analysis of cell-free translation products of mRNA for thyrotropin showed that the glycoprotein hormone subunits are also synthesized with *N*-terminal hydrophobic extensions in accord with the signal hypothesis for secreted proteins.

[124] J. F. Beckers, J. Closset, J. Maghuin-Rogister, and G. Hennen, *Biochimie*, 1977, **59**, 825.
[125] K. W. Cheng, *Biochem. J.*, 1978, **175**, 29.
[126] R. J. Whitley, H. T. Keutman, and R. J. Ryan, *Endocrinology*, 1978, **102**, 1974.
[127] J. Closset and G. Hennen, *Eur. J. Biochem.*, 1978, **86**, 105.
[128] J. Closset, G. Maghuin-Rogister, G. Hennen, and A. D. Strosberg, *Eur. J. Biochem.*, 1978, **86**, 115.
[129] L. C. Giudice, J. G. Pierce, K. W. Cheng, R. Whitley, and R. J. Ryan, *Biochem. Biophys. Res. Commun.*, 1978, **81**, 725.
[130] M. R. Sairam, *Arch. Biochem. Biophys.*, 1979, **194**, 79.
[131] M. R. Sairam and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, **13**, 394.
[132] M. R. Sairam and C. H. Li, *Can. J. Biochem.*, 1977, **55**, 747.
[133] M. R. Sairam and C. H. Li, *Can. J. Biochem.*, 1977, **55**, 755.
[134] M. R. Sairam and C. H. Li, in 'Hormonal Proteins and Peptides', ed. C. H. Li, Academic Press, New York, 1978, vol. 6, p. 1.
[135] I. A. Kourides and B. D. Weintraub, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 298.
[136] L. C. Giudice, M. J. Waxdal, and B. D. Weintraub, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 4798.
[137] L. C. Giudice and B. D. Weintraub, *J. Biol. Chem.*, 1979, **254**, 12679.

3 Posterior Pituitary Hormones

Contributed by M. Manning, B. Lammek, and W. H. Sawyer

This Report covers the literature from early 1979 to early 1980. During this period close to 60 new synthetic analogues of oxytocin and vasopressin have been reported. Rather than putting all of these analogues in one table, we have followed the format utilized in our previous two reports (see Vol. 10, p. 418 and Vol. 11, p. 391) of placing them in tables under the following categories.

1A. Reversible antagonists of *in vitro* and *in vivo* oxytocic responses to oxytocin.
1B. Irreversible antagonists of *in vitro* oxytocic responses to oxytocin.
2. Antagonists of the vasopressor response to arginine vasopressin (AVP).
3. Agonistic analogues of oxytocin.
4. Agonistic analogues of AVP.
5. Analogues of oxytocin and AVP prepared for conformational studies.
6. Tritiated and ^{13}C enriched analogues of oxytocin and AVP.
7. New syntheses of known analogues of oxytocin and AVP.

The assignments for the most part are self-selective. However some of the assignments are rather arbitrary. Thus, the analogues in Table 5 could also be placed in Tables 3 or 4. Also, dual assignment has been necessary for the analogues in Tables 1 and 2.

Many of the new analogues have very interesting properties, however, space does not permit discussion of the pharmacological data for all of these analogues. We have chosen to mention only a few analogues from each table simply to illustrate some of the advances which have been made in each category during the past year.

Reversible Antagonists of *in vitro* and *in vivo* Oxytocic Responses to Oxytocin (Table 1A).—Six new analogues of the oxytocin antagonist [1-deaminopenicillamine]oxytocin (dPOT) [1] (see Vol. 8, p. 381; Vol. 10, p. 423; and Vol. 11, p. 394) have been prepared and tested for their abilities to inhibit responses to oxytocin by the isolated rat uterus in the absence and in the presence of Mg^{2+}, by the rat uterus *in situ* and by the rat mammary gland *in situ*. These studies are part of an ongoing programme to explore the effects of (i) individual substitutions and (ii) various combinations of these substitutions in dPOT on antagonistic potencies with a view to developing a potent effective *in vivo* antagonist for possible use in the prevention of premature labour.

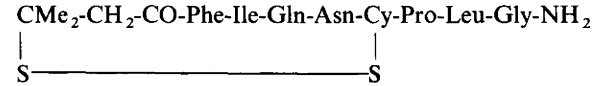
Previous reports [2, 3] have dealt with the effects of Thr[4] and *O*-methyltyrosine[2] substitutions alone and in combination in dPOT (see Vol. 11, p. 392).

The analogues of dPOT in Table 1 have the following: (i) individual substitutions: Phe[2]; and Orn[8] and (ii) combined substitutions: Phe[2], Thr[4]; Phe[2], Orn[8]; Phe[2], Asn[4], Orn[8]; and Tyr(Me)[2], Orn[8]. All six analogues are more potent antagonists of oxytocic responses *in vitro* and *in vivo* than dPOT (pA_2, 7.14 $\pm$ 0.05 (no Mg^{2+}); 5.63 $\pm$ 0.17 (0.5 mM Mg^{2+}); mixed agonist/antagonist *in vivo* [1a]).

The substitution of Orn[8] in dPOT brought about a striking enhancement in antagonism of all uterine responses particularly *in vivo*. The combination of the

[1] H. Schulz and V. du Vigneaud, *J. Med. Chem.*, 1966, **9**, 647.
[2] M. Manning, J. Lowbridge, J. Seto, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, 1978, **21**, 977.
[3] J. Lowbridge, M. Manning, J. Seto, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, 1979, **22**, 565.
[4] J. J. Nestor, jun., M. F. Ferger, and V. du Vigneaud, *J. Med. Chem.*, 1975, **18**, 284.

Table 1A *Reversible antagonists of* in vitro *and* in vivo *oxytocic responses to oxytocin*



[1-deaminopenicillamine, 2-phenylalanine]oxytocin (dPϕOT)

Compound number	*Peptide*[a]	*Isolated rat uterus (O)* pA_2[a] *No* Mg^{2+}	*Isolated rat uterus (O)* pA_2[a] 0.5 mM Mg^{2+}	*Rat uterus* in situ *Effective dose* ($nmol\ kg^{-1}$)	*Rat uterus* in situ *Estimated* pA_2[b]	*Rat milk ejection* (M.E.) *Effective dose* ($nmol\ kg^{-1}$)	*Rat milk ejection* (M.E.) *Estimated* pA_2[b]	*Ref.*
1	dPϕOT	7.78 ± 0.12	7.01 ± 0.11	62 ± 10	6.07 ± 0.08	Agonist		1*Aa*
2	dPϕTOT	7.49 ± 0.05	7.58 ± 0.06	61 ± 14	6.10 ± 0.12	10 ± 1	6.82 ±0.04	1*Aa*
3	dPϕOVT	7.20 ± 0.08	7.21 ± 0.07	16 ± 3	6.71 ± 0.11	5 ± 1	6.65 ± 0.02	1*Aa*
4	dPϕAOVT	7.98 ± 0.11	7.55 ± 0.13	14 ± 3	6.75 ± 0.09	16 ± 2	6.63 ± 0.06	1*Aa*
5	dPOVT	7.89 ± 0.11	6.97 ± 0.11	6.2 ± 0.9	7.06 ± 0.06	Agonist		1*Aa*
6	dPOMeOVT	7.70 ± 0.11	7.99 ± 0.05	5.9 ± 1.0	7.15 ± 0.09	4.9 ± 0.5	7.17 ± 0.04	1*Aa*
7	$d(CH_2)_5AVP$	8.15 ± 0.20	7.19 ± 0.08	17 ± 7	6.79 ± 0.19			1*Ab*
8	$d(CH_2)_5Tyr(Me)AVP$	8.19 ± 0.10	7.72 ±0.14	17 ± 3	6.62 ± 0.07			1*Ab*

[a] Abbreviations and their full names are as follows: dPϕOT = [deaminopenicillamine, 2-phenylalanine]oxytocin; dPϕTOT = [1-deaminopenicillamine, 2-phenylalanine, 4-threonine]oxytocin; dPϕOVT = [1-deaminopenicillamine, 2-phenylalanine, 8-ornithine]vasotocin; dPϕAOVT = [1-deaminopenicillamine, 2-phenylalanine, 4-asparagine, 8-ornithine]vasotocin; dPOVT = [1-deaminopenicillamine, 8-ornithine]vasotocin; dPOMeOVT = [1-deaminopenicillamine, 2-*O*-methyl-tyrosine, 8-ornithine]vasotocin; $d(CH_2)_5AVP$ = [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid]arginine vasopressin; $d(CH_2)_5Tyr(Me)AVP$ = [1-β-mercapto-β,β-cyclopentamethylenepropionic acid, 2-*O*-methyltyrosine]arginine vasopressin.

[b] pA_2; represents the negative logarithm to the base 10 of the average molar concentration (M) of an antagonist which will reduce the specific biological response to 2 × units of an agonist to the level of response to × units of the agonist.

1*Aa* W. H. Sawyer, J. Haldar, D. Gazis, J. Seto, K. Bankowski, J. Lowbridge, A. Turan, and M. Manning, *Endocrinology*, 1980, **106**, 81.
1*Ab* M. Kruszynski, B. Lammek, M. Manning, J. Seto, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, 1980, **23**, 364.

Tyr(Me)[2] and Orn[8] substitutions led to a further modest increase in antagonistic potency *in vivo*. Thus two of these six analogues, *i.e.* [1-deaminopenicillamine-8-ornithine]oxytocin (dPOVT) and [1-deaminopenicillamine, 2-*O*-methyltyrosine, 8-ornithine]oxytocin (dPOMe OVT) appear to be the most effective antagonists of oxytocin *in vivo* yet reported. The two analogues of arginine vasopressin (AVP) shown in Table 2, *i.e.* $d(CH_2)_5$ AVP and $d(CH_2)_5$ Tyr(Me)AVP,[2a] are of interest primarily because of their potent antivasopressor properties (see below and Table 2). Nonetheless, they warrant mention in this section also because of their strikingly high antioxytocic potencies *in vitro* in the absence of Mg^{2+}. These potencies are diminished in the presence of Mg^{2+} and *in vivo*. Curiously, $d(CH_2)_5$AVP and the analogous oxytocin analogue, *i.e.* $d(CH_2)_5$OT[4], exhibit almost identical antagonistic oxytocic potencies *in vivo*. Their pA_2 values are 6.79 $\pm$ 0.19 [2a] and 6.65 $\pm$ 0.06, respectively.[3] It should also be noted that all of the oxytocin antagonists in Table 1 are also antagonists, albeit for the most part relatively weak ones, of the vasopressor response to AVP (see Table 2).

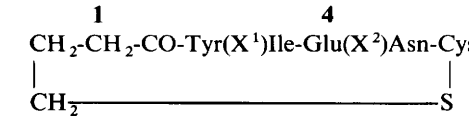
Irreversible Antagonists of *in vitro* Oxytocic Responses to Oxytocin (Table 1B).—Specific irreversible inhibitors of uterine responses to oxytocin would be very useful tools for the study of peptide receptor interactions and for receptor isolation and characterization studies. With these goals in mind, the ten analogues shown in Table 1B were synthesized and evaluated for agonistic and antagonistic properties on the isolated rat uterus *in vitro* in the absence of Mg^{2+}. Eight of the analogues are derivatives of deamino-[1-carba-4-glutamic acid]-oxytocin [5] modified at position 4 with highly reactive groups. The two remaining analogues contain *O*-methyltyrosine in place of tyrosine at position 2. All except one of the analogues examined had intrinsic oxytocic activity and they also behaved as irreversible inhibitors of the action of oxytocin. Unfortunately no data are given for their antagonistic potencies, although the statement is made that the active ester derivatives of deamino[1-carba-4-glutamic acid]oxytocin and its Tyr(Me) [2] analogue are the most effective inhibitors. These latter analogues containing suitable labels could thus be useful for studies on the uterine oxytocic receptors.

Antagonists of the Vasopressor Response to Arginine Vasopressin (AVP) (Table 2).—Vasopressor antagonists are proving to be valuable tools in studies on the role(s) of AVP in regulating blood pressure under normal and patho-physiological conditions [6, 7] (see Vol. 10, p. 423 and Vol. 11, p. 395).

Two of the analogues in Table 2, *i.e.* $d(CH_2)_5$Tyr(Me)AVP and $d(CH_2)_5$AVP, are the most potent antagonists of the vasopressor response to AVP yet reported. Deamino-AVP [8] has a vasopressor potency of 346 units mg^{-1}.[9] Replacement of the two hydrogens on the β-carbon at position one by two methyl groups gave [1-deaminopenicillamine]arginine vasopressin (dPAVP) with an antivasopressor pA_2 = 7.45.[10] Replacement of these two methyl groups with a cyclopentamethylene

[5] M. Lebl and K. Jost, *Collect. Czech. Chem. Commun.*, 1978, **43**, 523.
[6] C. C. Y. Pang, J. R. McNeill, W. C. Wilcox, M. Manning, and W. H. Sawyer, *Proc. Soc. Exp. Biol. Med.*, 1979, **161**, 41.
[7] A. W. Cowley, jun., S. J. Switzer, and W. M. Guinn, *Circulation Res.*, 1980, **46**, 58.
[8] R. L. Huguenin and R. A. Boissonnas, *Helv. Chim. Acta*, 1966, **49**, 695.
[9] M. Manning, L. Balaspiri, J. Moehring, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, 1976, **19**, 842.
[10] K. Bankowski, M. Manning, J. Haldar, and W. H. Sawyer, *J. Med. Chem.*, 1978, **21**, 850.

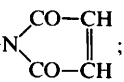
Table 1B *Irreversible antagonists of the oxytocic response to oxytocin*



[4-Glutamic acid-γ-*p*-nitrophenyl ester]deamino-1-carba-oxytocin (15)
X^1 = H; X^2 = O-C_6H_4-NO_2-*p*

Compound number	*Peptide*	*Isolated rat uterus*[a] /I.U. mg^{-1}	*Ref.*
9	[4-γ-Glutamoyl-cysteine methyl ester] deamino-1-carba-oxytocin[b]	1.8	1*Ba*
10	[4-γ-Glutamic s-butyl carbonic anhydride] deamino-1-carba-oxytocin[c]	16.0	1*Ba*
11	[4-γ-Glutamoyl-(*N'*-maleoylglycine) Hydrazide]deamino-1-carba-oxytocin[d]	5.0	1*Ba*
12	[4-γ-Glutamoyl-*N'*-bromoacetyohydrazide]deamino-1-carba-oxytocin[e]	2.0	1*Ba*
13	[4-γ-Glutamoyl azide]deamino-1-carba-oxytocin[f]	0.6	1*Ba*
14	[4-Glutamic acid-γ-1-hydroxybenzotriazole ester]deamino-1-carba-oxytocin[g]	160	1*Ba*
15	[4-Glutamic acid-γ-*p*-nitrophenyl ester] deamino-1-carba-oxytocin[h]	5.0	1*Ba*
16	[4-γ-Glutamoyl-ξ-aminocaproic acid *p*-nitrophenyl ester]deamino-1-carba-oxytocin[i]	not determined	1*Ba*
17	[2-*O*-methyltyrosine, 4-glutamic acid-γ-1-hydroxybenzotriazole ester]deamino-1-carba-oxytocin[j]	0	1*Ba*
18	[2-*O*-Methyltyrosine, 4-glutamic acid-γ-*p*-nitrophenyl ester]deamino-1-carba-oxytocin[k]	not determined	1*Ba*

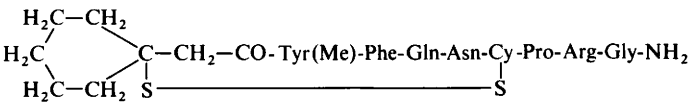
[a] The assay was performed using an isolated rat uterus.[1*Bb*,1*Bc*] The response of the uterus to oxytocin was determined before and after the action of the inhibitor. First, the dependence of the response on the log of oxytocin doses (cumulative doses) was determined until the maximum response was achieved. Oxytocin was then removed from the bath by repeated washing and the compound that was expected to have inhibitory properties was added in a dose of 10–100 μg (6 ml)$^{-1}$ (bath volume). After 5 min the uterine strip was thoroughly washed until all contractions stopped. Afterwards, the dependence of the response of the uterus to the log dose of oxytocin was determined again.

X^1 and X^2 are as follows: [b] X^1 = H, X^2 = NH·CH(CH_2SH)·COOMe; [c] X^1 = H, X^2 = O·COO·CH(MeEt); [d] X^1 = H, X^2 = NH·NH·CO·CH_2  ; [e] X^1 = H, X^2 = NH·NH·CO·CH_2·Br; [f] X^1 = H, X^2 = N_3; [g] X^1 = H, X^2 = O·C_6H_4·N_3; [h] X^1 = H, X^2 = O·C_6H_4·NO_2-*p*; [i] X^1 = H, X^2 = NH·$(CH_2)_5$·COO·C_6H_4·NO_2-*p*; [j] X^1 = Me_3, X^2 = O·C_6H_4·N_3; [k] X^1 = Me_3, X^2 = O·C_6H_4·NO_2-*p*.

1*Ba* M. Lebl, V. Bojanovska, T. Barth, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2573.
1*Bb* P. Holton, *Brit. J. Pharmacol.*, 1948, **3**, 328.
1*Bc* R. A. Munsick, *Endocrinology*, 1960, **66**, 451.

group [$(CH_2)_5$] to give d$(CH_2)_5$AVP brought about a nine-fold increase in antivasopressor potency and $pA_2 = 8.35$. The same substitution in the antagonist dPTyr(Me)AVP [10] to give d$(CH_2)_5$Tyr(Me)AVP increased pA_2 from 7.96 to 8.67 – a five-fold increase in antagonistic potency. d$(CH_2)_5$Tyr(Me)AVP also exhibited a protracted antivasopressor effect. The enhancement of antivasopressor potency of d$(CH_2)_5$AVP and d$(CH_2)_5$Tyr(Me)AVP, coupled with their low antidiuretic activities (0.033 and 0.31 units mg^{-1} respectively), endow these peptides with high antivasopressor/antidiuretic specificity. Thus, both antagonists are valuable additions to the small number of previously reported vasopressor antagonists.

Table 2 *Antagonists of the vasopressor response to arginine vasopressin*



[1-(β-mercapto-β,β-cyclopentamethylenepropionic acid]-2-*O*-methyl-tyrosine]-arginine vasopressin (d$(CH_2)_5$Tyr(Me)AVP)

Compound number	*Peptide*	*Antivasopressor* pA_2[a]	*Antidiuretic act.* /units mg^{-1}	*Ref.*
19	d$(CH_2)_5$ AVP	8.35 ± 0.09	0.033 ± 0.005	2*a*
20	d$(CH_2)_5$ Tyr(Me)AVP	8.67 ± 0.02	0.31 ± 0.07	2*a*
21	dPφOT	6.57 ± 0.08	0.09	2*b*
22	dPφTOT	6.63 ± 0.06	0.01	2*b*
23	dPφOVT	6.57 ± 0.08	0.04	2*b*
24	dPφAOVT	7.39 ± 0.07	0.014	2*b*
25	dPOVT	6.95 ± 0.06	0.87	2*b*
26	dPOMeOVT	7.62 ± 0.06	0.08	2*b*

[a] As in Table 1; the abbreviations and their full names are as in Table 1.
2*a* Ref. 1*b* from Table 1.
2*b* Ref 1*a* from Table 1.

Agonistic Analogues of Oxytocin (Table 3).—Although none of the analogues in Table 3 exhibit potent oxytocic activities, they all contribute to a better understanding of structure–activity relationships. Also two of them have high milk-ejecting potencies and selectivities. In addition, the analogues with free glutamic acid at position-4 are potentially useful as illustrated above (Table 1B) for conversion to irreversible antagonists of oxytocin. [7-(Azetidine-2-carboxylic)]-oxytocin [3a] is a useful addition to the small number of 7-substituted analogues of oxytocin. This analogue exhibits an interesting selectivity in its activities on the mammary gland *in situ* (353 units mg^{-1}) and on the isolated mammary strip (66 units mg^{-1}). The authors speculate that the higher *in vivo* activity may be due to the effect of Mg^{2+} ions. 1-Deamino-[1-carba-4-glutamic acid methyl ester]oxytocin [3c] has a milk-ejecting potency *in vivo* of 1307 units mg^{-1} compared with an activity of only 26 units mg^{-1} on the rat uterus in the absence of Mg^{2+}. This is thus the most potent and selective milk-ejecting analogue reported to date. It would be of much interest to compare the milk-ejecting potencies *in vivo* of [4-

Table 3 *Agonistic analogues of oxytocin*

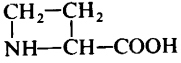
Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

Oxytocin

Compound number	*Peptide*	*Biological activities*[a] *Rat uterus oxytocic (O)*	*Rat milk ejection (M.E.)*	*Rat antidiuretic (A)*	*Rat pressor (P)*	*Ref.*
27	[7-(Azetidine-2-carboxylic acid)]oxytocin[b]	43	353[c]	<0.25	0.1	3*a*
28	[4-Glutamic acid α-methyl ester]oxytocin	10.5			no response	3*b*
29	[1-β-Mercaptopropionic acid, 4-glutamic acid α-methyl ester]oxytocin	21.4			no response	3*b*
30	[4-Glutamic acid methyl ester] deamino-1-carba-oxytocin	26.0	1307	0.045	0.1	3*c*
31	[4-Glutamic acid ethyl ester] deamino-1-carba-oxytocin	5.7	132.7			3*c*
32	Methylsuphonium salt deamino-1-carba-oxytocin	20.0	47.0			3*c*
33	[2-*O*-Methyltyrosine, 4-glutamic acid]deamino-1-carba-oxytocin	0.1	0.5			3*c*
34	[2-*O*-Methyltyrosine, 4-glutamic acid methyl ester]deamino-1-carba-oxytocin	0.2	2.0			3*c*
35	Sulphoxide of [4-glutamic acid methyl ester]deamino-1-carba-oxytocin	0.2	11.3			3*c*
36	[4-Glutamic acid-γ-dimethylamide] deamino-1-carba-oxytocin	4.26	8.1			3*d*
37	[4-Glutamic acid-γ-adamantylamide] deamino-1-carba-oxytocin	9.2	0.89			3*d*

38	[4-Glutamic acid-γ-(*p*-toluidide)] deamino-1-carba-oxytocin	21.2	4.7	3*d*
39	[4-Glutamic acid-γ-N-BOC-hydrazide]deamino 1-carba-oxytocin	3.24	6.7	3*d*
40	[4-Glutamic acid-γ-hydrazide] deamino-1-carba-oxytocin	19.4	0.71	3*d*
41	[4-γ-Glutamoyl-glycin amide]-deamino-1-carba-oxytocin	5.3		3*d*
42	[4-γ-Glutamoyl-glycin methyl ester]deamino-1-carba-oxytocin	10.2	5.6	3*d*
43	[4-γ-Glutamoyl-prolyl-leucyl glycin amide]deamino-1-carba-oxytocin	0.15	1.9	3*d*
44	[4-γ-Glutamoyl-ε-aminocaproic acid-t-bulytester]deamino-1-carba-oxytocin	2.36	9.85	3*d*
45	[4-γ-Glutamoyl-ε-aminocaproic acid]deamino-1-carba-oxytocin	4.78	8.90	3*d*

[a] Activities expressed in I.U. $mg^{-1} \pm$S.E.

[b] Azetidine-2-carboxylic acid = 

[c] Isolated strip 66 I.U. mg^{-1}.

3*a* M. Barber, J. H. Jones, A. V. Stachulski, G. W. Bisset, H. S. Chowdrey, and A. L. Hudson, *Int. J. Pept. Protein Res.*, 1979, **14**, 247.
3*b* I. Photaki, Ch. Tzougraki, and Ch. Kotsira-Engenopoulos, *Int. J. Pept. Protein Res.*, 1979, **13**, 426.
3*c* M. Lebl, T. Barth, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2563.
3*d* M. Lebl, A. Dimeli, V. Bojanovska, J. Slaninova, T. Barth, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2556.

glutamic acid-γ-methyl ester]oxytocin[3b] and its deamino-derivative[3b] with those of this highly potent deamino-1-carba-analogue. It is not unlikely that they might also exhibit potent and selective *in vivo* milk-ejecting potencies.

Agonistic Analogues of Arginine Vasopressin (Table 4).—Several highly potent and in many instances highly selective analogues of AVP are listed in Table 4. The 7-dehydroproline (Δ^3Pro^7) analogues have been discussed previously (see Vol. 10, p. 422). They are included again here because more definitive data for their pharmacological properties were reported in the past year.[4h] The Δ^3Pro^7 substitution in analogues of AVP is one of the most interesting and potentially useful discoveries in structure–activity studies of AVP. It brought about an enhancement in antidiuretic potency in all of the analogues studied to date while at the same time greatly diminishing pressor potency. This substitution could thus be extremely valuable for the design of antagonists of the antidiuretic response to AVP.

The quantitative meaning of the activity ratios for the two analogues [Mpr^1, DapG^8]VP and [Mpr^1, D-DapG^8]VP[4d] is not clear since the antidiuretic assays were performed by an unconventional method and activities translated into units based on similar assays of dDAVP against LVP. There is no accepted unit for the antidiuretic activity of dDAVP since the slopes of the dose-response curves for this analogue differ markedly from those to vasopressin-containing standards.[18]

Of the remaining analogues in Table 4 [1-(β-mercaptopropionic acid), 8-norarginine]vasopressin [Mpr^1, Narg^8]VP is by far the most potent pressor analogue of AVP yet reported. Thus, the deletion of a methylene group in the side-chain of the arginine residue of 1-deaminoarginine vasopressin has increased the pressor potency from *ca.* 360[9] to *ca.* 1500 units mg^{-1}.[4f] Unfortunately, the antidiuretic potency has also been increased but only by *ca.* 25—30%. Thus, this substitution, *i.e.* 8-L-norarginine, may point the way towards the design of analogues of AVP possessing greater pressor/antidiuretic selectivity than any currently available.

Analogues Prepared for Conformational Studies (Table 5).—The asparagine residue has been assigned an important role in the maintenance of the conformation of oxytocin in solution.[11] It has been proposed that a hydrogen bond between the peptide NH of asparagine and the C=O of tyrosine stabilizes a β-turn in the cyclic part of oxytocin and that the C=O of the asparagine carboxamide may be hydrogen bonded to the peptide NH of leucine, thus stabilizing the relationship of this β-turn to a second COOH terminal β-turn in the molecule composed of the sequence -Cys-Pro-Leu-Gly-NH_2.[11,12] Two of the analogues in Table 5 deamino-[8-*N*-Me-leucine]oxytocin and deamino-[8-α-hydroxyisocaproic acid]oxytocin cannot form hydrogen bonds with the carboxamide carbonyl of asparagine and yet they retain the ability to evoke the characteristic biological responses to oxytocin.[5d] From these data and from the work of Hlaveck *et al.*[13] it is concluded that the proposed hydrogen-bond requirement is invalid. A number of other reports dealing

[11] R. Walter, I. L. Schwartz, J. H. Darnell, and D. W. Urry, *Proc. Natl. Acad. Sci. USA*, 1971, **68**, 1355.
[12] R. Walter, *Proc. Fed. Am. Soc. Exp. Biol.*, 1977, **36**, 1872.
[13] J. Hlavack, K. Poduska, K. Jost, I. Fric, T. Barth, J. H. Cort, K. Blaha, and F. Sorm, *Collect. Czech. Chem. Commun.*, 1977, **42**, 1233.

Table 4 *Agonistic analogues of arginine vasopressin (AVP)*

Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2

AVP

Compound number	*Peptide*	*Biological activities*[a] *Rat uterus oxytocic (O)*	*Rat milk ejection (M.E.)*	*Rat antidiuretic (A)*	*Rat pressor (P)*	*A/P*	*Ref.*
46	[1-β-Mercaptopropionic acid, 8-α-amino-β-glycylaminopropionic acid]vasopressin [Mpr[1],Dap(Gly)[8]]VP	0.2—0.3	no data	435	5.75	76	4*a*
47	[3-D-Phenylalanine, 8-α,γ-diaminobutyric acid]vasopressin [D-Phe[3]Dab[8]]VP	0.005	no data	*ca.* 30	13	2.3	4*b*
48	[1-β-Mercaptopropionic acid, 8-α,β-diaminopropionic acid]-vasopressin [Mpr[1]Dap[8]]VP	0.9	no data	1079	14.0	77	4*c*
49	[1-β-Mercaptopropionic acid 8-D-α,β-diaminopropionic acid] vasopressin [Mpr[1], D-Dap[8]]VP	1.72	no data	1000	22.8	42	4*c*
50	[1-β-Mercaptopropionic acid, 8-α-amino-β-guanidinopropionic acid] vasopressin [Mpr[1],DapG[8]]VP	1.5	no data	10% dDAVP	49.5	102	4*d*
51	[1-β-Mercaptopropionic acid, 8-D-α-amino-β-guanidinopropionic acid]vasopressin[Mpr[1], D-DapG[8]]VP	0.4	no data	87 ± 8% dDAVP	2.7	16200	4*d*
52	[1-β-Mercaptopropionic acid, 2-*O*-methyltyrosine, 8-D-arginine] vasopressin [Mpr[1],Tyr(Me)[2], D-Arg[8]]VP	without effect *in vitro*	no data	10	without effect *in vitro*		4*e*
53	[1-β-Mercaptopropionic acid, 8-norarginine]vasopressin [Mpr[1],Nar[8]]VP	7.4	no data	~200% DDAVP	~1500		4*f*

Table 4 *Cont.*

Compound number	*Peptide*	*Biological activities*[a] *Rat uterus oxytocic (O)*	*rat milk ejection (M.E.)*	*Rat antidiuretic (A)*	*Rat pressor (P)*	*A/P*	*Ref.*
54	[1-β-Mercaptopropionic acid 8-D-norarginine]vasopressin [Mpr[1],D-Nar[8]]VP	16.4	no data	~20% DDAVP	16.3		4*f*
55	[7-(Azetidine-2-carboxylic acid]-lysine-vasopressin	2.0		80	27	3	4*g*
56	[1-β-Mercaptopropionic acid, 2-phenylalanine, 7-(3,4-dehydroproline)]arginine-vasopressin [β-Mpr[1],Phe[2],Δ[3]Pro]AVP	6.0 ± 0.6		13000 ± 1250			4*h*
57	[1-β-Mercaptopropionic acid 7-(3,4-dehydroproline)]arginine-vasopressin[β-Mpr[1],Δ[3]Pro[7]]AVP	69 ± 5		4134 ± 306	240 ± 4.5	17	4*h*
58	[2-Phenylalanine, 7-(3,4-dehydroproline)]arginine-vasopressin [Phe[2],Δ[3]Pro[7]]AVP	0.80 ± 0.11		1541 ± 336	63.8 ± 39	24	4*h*
59	[7-(3,4-Dehydroproline)]arginine-vasopressin [Δ[3]Pro[7]]AVP	40.6 ± 4.7		1260 ± 126	255 ± 23	5	4*h*

[a] Activities expressed I.U. mg^{-1} ± S.E.

4*a* M. Zaoral, W. W. Krchnak, J. Skopkova, and A. Machova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 3133.
4*b* M. Zaoral, D. Konopinska, I. Blaha, V. Krchnak, J. Skopkova, and A. Machova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 3128.
4*c* V. Krchnak, M. Zaoral, and A. Machova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2161.
4*d* M. Zaoral, V. Krchnak, F. Brtnik, A. Machova, and J. Skopkova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2447.
4*e* V. Krchnak and M. Zaoral, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1642.
4*f* M. Zaoral, F. Brtnik, M. Flegel, T. Barth, and A. Machova, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1179.
4*g* Ref. 3a from Table 3.
4*h* C. R. Botos, C. W. Smith, Yun-Lai Chan, and R. Walter, *J. Med. Chem.*, 1979, **22**, 926.

Table 5 *Analogues prepared for conformational studies*

Compound number	*Peptide*	*Biological activity*				*Ref.*
		Rat uterus oxytocic (O)	*Rat milk ejection (M.E.)*	*Rat antidiuretic (A)*	*Rat pressor (P)*	
60	[3-(-1,4-Cyclohexadienyl)-L-alanine 8-lysine]vasopressin	6.3 ± 0.4		137 ± 11	137 ± 9	5*a*
61	[4-(N^5,N^5-Dimethylglutamine)] oxytocin	3.01 ± 0.14				5*b*
62	[4-(N^5,N^5-di-n-propylglutamine)] oxytocin	<0.1		<0.05	<0.05	5*b*
63	[4-(N^5,N^5-dimethylglutamine)] lysine-vasopressin	<0.05		1.88 ± 0.04	1.27 ± 0.03	5*b*
64	[5-(N^4,N^4-dimethylasparagine),8-lysine]vasopressin	<0.05		5.5 ± 0.3	2.55 ± 0.05	5*c*
65	Deamino-[8-*N*-methyl-leucine]oxytocin	48 ± 7		3.15 ± 1.5		5*d*
66	Deamino-[8-α-hydroxyisocaproic acid] oxytocin	134 ± 12		9.60 ±3.0		5*d*

5*a* S. N. Banerjee, L. Diamond, C. Ressler, and W. H. Sawyer, *J. Med. Chem.*, 1979, **22**, 1487.
5*b* G. L. Stahl, C. W. Smith, R. Walter, T. Tsengenidis, G. Stavropoulos, P. Cordopatis, and D. Theodorpoulos, *J. Med. Chem.*, 1980, **23**, 213.
5*c* C. W. Smith, R. Walter, G. Stavropoulos, and D. Theodorpoulos, *J. Med. Chem.*, 1980, **23**, 217.
5*d* J. Roy, U. Roy, D. C. Gazis, and I. L. Schwartz, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3309.

with conformational aspects of oxytocin, vasopressin and some of their known analogues have also appeared.[14-17]

Tritiated, Deuteriated, and C-Enriched Analogues of Oxytocin and AVP (Table 6).—Four specifically labelled (90% ^{13}C-enriched) oxytocin derivatives were prepared and used to study the interactions of the neurohypophyseal hormones with bovine neurophysins as functions of pH and temperature. Under all conditions, whether high or low pH, the chemical shifts of the labelled carbon atoms of the bound

Table 6 *Tritiated, deuteriated, and ^{13}C enriched analogues of oxytocin and vasopressin*

Compound number	*Peptide*	*Ref.*
67	[MeCO-^{3}H][2-*O*-methyltyrosine]oxytocin	6*a*
68	[1-hemi[1-^{13}C]cystine]oxytocin	6*b*
69	[1-hemi[2-^{13}C]cystine]oxytocin[a]	6*b*
70	[2-[2-^{13}C]tyrosine]oxytocin[b]	6*b*
71	[3-[2-^{13}C]leucine]oxytocin[c]	6*b*
72	[2-DL-[3′,5′-$^{13}C_2$]tyrosine]oxytocin[c]	6*c*
73	[2-DL-[3′,5′-$^{13}C_2$]tyrosine, 8-arginine]vasopressin	6*c*
74	^{3}H-oxytocin	6*d*
75	[2-DL-(α,β,β-$^{2}H_3$)tyrosine]oxytocin	6*e*
76	[2-DL-[α-^{2}H,]tyrosine, 8-arginine]vasopressin[d]	6*e*

[a] M. Blumenstein and V. J. Hruby, *Biochemistry*, 1977, **16**, 5169.
[b] M. Blumenstein, V. J. Hruby, and D. M. Yamamoto, *Biochemistry*, 1978, **17**, 4971.
[c] V. Viswanatha, B. Larsen, and V. J. Hruby, *Tetrahedron*, 1979, **35**, 1575.
[d] D. M. Yamamoto, D. A. Upton, D. K. Linn, and V. J. Hruby, *J. Am. Chem. Soc.*, 1977, **99**, 1564.

6*a* V. Bojanovska, T. Barth, B. Cerny, K. Hauzer, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, **44**, 2702.
6*b* M. Blumenstein, V. J. Hruby, and V. Viswanatha, *Biochemistry*, 1979, **18**, 3552.
6*c* V. Viswanatha and V. J. Hruby, *J. Org. Chem.*, 1979, **44**, 2892.
6*d* H. Sakamoto, K. Den, K. Yamamoto, T. Arai, S. Kawai, Y. Oyama, T. Yoshida, and S. Takagi, *Endocrinol.Jpn.*, 1979, **26**, 515.
6*e* V. J. Hruby, D. A. Upson, D. M. Yamamoto, C. W. Smith, and R. Walter, *J. Am. Chem. Soc.*, 1979, **101**, 2717.

hormones were the same, but shifted significantly from their positions in the free hormone. These results indicate that the interactions of the side-chain and disulphide moieties of the hormone with the neurophysins do not change as a function of pH. At neutral pH and 20—35 °C, the labelled atoms of the hormone are in slow exchange with the nerophysins for the above hormone derivatives, but at low pH they are in intermediate or fast exchange depending upon pH and temperature. At high pH the decrease in binding affinity is due solely to the deprotonation of the α-amino-group of the free hormone.

Reference 5*c* contains a very elegant and efficient synthesis of labelled tyrosine. The enantiomers were resolved by enzymatic methods. Also, labelled derivatives of

[14] N. R. Krishna, D. H. Huang, J. D. Glickson, R. Rowan, and R. Walter, *Biophys. J.*, 1979, **26**, 345.
[15] G. V. Nikiforovich, V. I. Leonova, S. G. Galaktionov, and G. J. Chipens, *Int. J. Pept. Protein Res.*, 1979, **13**, 363.
[16] A. T. Tu, J. Lee, K. K. Deb, and V. J. Hruby, *J. Biol. Chem.*, 1979, **254**, 3272.
[17] H. R. Wyssbrod, A. J. Fischman, D. H. Live, V. J. Hruby, N. S. Agarwal, and D. A. Upson, *J. Am. Chem. Soc.*, 1979, **101**, 4037.
[18] I. Vavra, A. Machova, and I. Krejci, *J. Pharmacol. Expt. Ther.*, 1974, **188**, 241.

DL-tyrosine were used for the synthesis of specifically labelled oxytocin and vasopressin derivatives. The diastereoisomers were separated from each other by partition chromatography on Sephadex G-25 followed by gel filtration.

New Syntheses of Known Analogues of Oxytocin and AVP (Table 7).—These analogues served as models in a number of interesting investigations of synthetic and purification methods. Hruby and his co-workers,[7d] using reverse phase h.p.l.c., developed methods for the synthesis, separation, and purification of ^{13}C-labelled

Table 7 *New syntheses of known analogues of oxytocin and AVP*

Compound number	*Peptide*	*Ref.*
77	[7-(*U*-^{13}C)proline]oxytocin	7*a*
78	Des-9-glycine-oxytocin	7*a*
79	[9-Glycine methyl ester]oxytocin	7*a*
80	Oxytocin	7*b*
81	[1-β-Mercaptopropionic acid, 8-D-arginine]vasopressin(DDAVP)	7*c*
82	[3-DL-[2-^{13}C]Leucine]oxytocin	7*d*
83	[8-DL-[2-^{13}C]Leucine]oxytocin	7*d*

7*a* J. Hlavacek, T. Barth, K. Blaha, and K. Jost, *Collect. Czech. Chem. Commun.*, 1979, **44**, 275.
7*b* G. Fluoret, S. Terada, T. Kato, R. Guaitieri, and A. Lipkowski, *Int. Pept. Protein Res.*, 1979, **13**, 137.
7*c* V. Krchnak and M. Zaoral, *Collect. Czech. Chem. Commun.*, 1979, **44**, 1173.
7*d* V. Viswanatha, B. Larsen, and V. J. Hruby, *Tetrahedron*, 1979, **35**, 1575.
7*e* F. Nachtmann, *J. Chromatogr.*, 1979, **176**, 391.

hormone diastereoisomeric analogues. The general procedure, with appropriate solvent systems, column packing, or other modifications, should prove very useful in preparing various labelled derivatives (^{13}C, ^{3}H, ^{2}H, ^{125}I, *etc.*) of peptide hormones without the need to resolve precious enantiomeric amino-acid derivatives before incorporating them into the growing chain.

A high performance liquid chromatographic method was introduced for the determination of peptides formed during the synthesis of oxytocin.[7e]

Fluoret *et al.*[7b] reported synthetic and chromatographic modifications which allow the synthesis of oxytocin, in amounts more than ten-fold higher than obtained by usual methods.

For the synthesis of oxytocin analogues modified at the carboxy-terminal part of the molecule, a method based on the condensation of a protected amino-terminal hexapeptide with tripeptides by the action of DCC and pentaflurophenol in the presence of 1-hydroxybenzotriazole was devised.[7a]

Other studies.—Synthetic analogues of the posterior pituitary hormones continue to be useful for characterizing the several types of receptors mediating responses to these hormones. Such studies have been extended to include responses other than the classical antidiuretic, vasopressor, oxytocic, and milk-ejection responses. Keppens and de Wulf,[19] for example, use a series of 16 agonistic analogues to characterize the receptors on isolated rat-hepatocytes that mediate their glycogenolytic response to vasopressin. Glycogenolytic and vasopressor activities of these peptides were closely correlated over a 10000-fold range of potencies in both

[19] S. Keppens and H. de Wulf, *Biochim. Biophys. Acta*, 1979, **588**, 63.

assays. There was no correlation between glycogenolytic and antidiuretic activities. the pA_2 values of five antagonistic analogues in the *in vitro* hepatocyte assay were also strikingly similar to the 'pA_2' values that had been estimated from *in vivo* vasopressor assays. These results indicate that the hepatocyte receptors mediating glycogenolysis and the vasopressor receptors have essentially identical pharmacological properties. The results with the antagonists are also reassuring in that they suggest that 'pA_2' values for vasopressor antagonists estimated from *in vivo* assays may be reasonable approximations of true pA_2 values despite the fact that their estimation requires the use of many assumptions concerning distribution and elimination of the antagonists in order to estimate their molar concentrations at the receptors.

Considerable recent attention has focused on the behavioural effects of neurohypophysial hormones and their analogues. These effects include those on memory,[20] spontaneous behaviour,[21] and the development of physical dependence on morphine.[22] A particularly interesting recent report [23] shows that rather large doses of oxytocin injected into the lateral cerebral ventricles of virgin rats can evoke clear maternal behaviour, while vasopressin is ineffective. Oxytocin is most effective in oestrous or oestrogen-primed rats and its effect persists for at least 10 days. This should be a rewarding model in which to exploit synthetic analogues to characterize the nature of the receptors involved.

4 Pancreatic Hormones

Contributed by D. Brandenburg and D. Saunders

Introduction.—The Proceedings of the 15th European [1] and the 5th American [2] Peptide Symposium contain several papers on the synthesis of insulin chains, on the synthesis and semisynthesis of insulin and analogues, including structure–function studies,[3-7] as well as on semisynthetic studies with pro- and pre-proinsulin.[8] The Proceedings of the Symposium 'Proinsulin, insulin, and C-peptide'[9] contain sections on the chemistry of insulin, proinsulin, and C-peptide, and on biochemistry, but they concentrate on biological and clinical aspects.[10-12] The

[20] D. de Wield and D. H. G. Versteeg, *Fed. Proc.*, 1979, **38**, 2348.
[21] R. L. Delanoy, A. J. Dunn, and R. Walter, *Life Sci.*, 1979, **24**, 579.
[22] R. Walter, R. F. Ritzmann, H. N. Bhargava, and L. B. Flexner, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 518.
[23] C. A. Pedersen and A. J. Prange, jun., *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6661.
[1] 'Peptides 1978', Proc. 15th Eur. Peptide Symp., Poland, Sept. 1978, ed. I. Z. Siemion and G. Kupryszewski, Wroclaw University Press, Wroclaw, Poland, 1979.
[2] 'Peptides, Structure and Biological Function', Proc. 6th American Peptide Symp., June 1979, ed. E. Gross and J. Meienhofer, Pierce Chem. Co., Rockford, Ill., USA, 1979.
[3] C. Birr and R. Pipkorn, ref. 1, p. 625
[4] P. Thamm and D. Brandenburg, ref. 1, p. 639.
[5] H. J. Wieneke, W. Danho, E. Büllesbach, H.-G. Gattner, and H. Zahn, ref. 2, p. 515.
[6] K. Morihara, T. Oka, H. Tsuzuki, K. Inouye, and S. Sakakibara, ref. 2, p. 617.
[7] H. J. Friesen, D. J. Saunders, V. K. Naithani, and C. Diaconescu, ref. 2, p. 511.
[8] V. K. Naithani, H.-G. Gattner, E. Büllesbach, J. Föhles, and H. Zahn, ref. 2, p. 571.
[9] 'Proinsulin, Insulin, C-Peptide', Proc. Symp. on Proinsulin, Insulin, C-Peptide, July 1978, Tokushima, Japan, ed. S. Baba, T. Kaneko, and N. Yanaihara, Excerpta Medica, Amsterdam, Oxford, 1979.
[10] J. Markussen, ref. 9, p. 50.
[11] N. Sakabe, K. Sakabe, and K. Sasaki, ref. 9, p. 73.
[12] R. E. Chance, M. A. Root, M. G. Johnson, T. M. Wolfe, N. Yanaihara, T. Hashimoto, M. Sakagami, and C. Yanaihara, ref. 9, p. 81

contribution of peptide chemistry to our knowledge of insulin and diabetes,[13] clinical application of insulin derivatives,[14] and structure–function relationships of the hormone[15] have been discussed. A review on conformation and molecular biology of insulin, insulin-like growth factor, and relaxin has appeared.[16] Reports on the European Workshop on insulin structure[17] and the Second International Insulin Symposium[18] have been published. The Proceedings[19] of the latter have appeared, and will be discussed in Volume 13.

Hormone–receptor interactions, receptor-mediated endocytosis, and hormonal regulation of peptide receptors and target cell responses are discussed in three recent reviews.[20–22] Insulin receptors and receptor antibodies are the topic of another review.[23] The molecular basis of insulin action is discussed.[24] The Proceedings of the First International Symposium on Endocrine Pancreas and Diabetes contain further information on biological aspects and action of insulin and glucagon.[25] Historical aspects of insulin and diabetes are brought up in two papers[26,27] dedicated to the memory of the late Charles H. Best.

Insulin.—*Isolation, Synthesis, Semisynthesis, and Chemical Modification.* Advances in the development of methods for the synthesis of insulin in bacterial cells have been reported. A purified A-chain and partially purified B-chain of human insulin have been obtained from synthetic genes for A- and B-chains separately cloned in plasmids. Co-oxidation of the reduced chains led to the formation of insulin which was detected by radioimmunoassay.[28] An overview of the strategy of the 'two-chain approach' has been given.[29] Yields of insulin chains are *ca.* $1\,mg\,l^{-1}$ of culture, and chains have been obtained by purification of milligram amounts. The cultured insulin was purified by h.p.l.c. and was biologically active. Biological activity and immunoreactivity have also been demonstrated for material obtained through expression of a fused penicillase (24—182)-$(Gly)_6$-rat proinsulin I (4—86) gen.[30] Another approach aims at the synthesis of the gene for a proinsulin with short connecting peptide. 17 Desoxyribonucleotide fragments corresponding to the

[13] R. Geiger and R. Obermeier, ref. 9, p. 62.
[14] R. Geiger and F. Enzmann, ref. 9, p. 306.
[15] E. J. Dodson, G. G. Dodson, D. C. Hodgkin, and C. D. Reynolds, *Can. J. Biochem.*, 1979, **57**, 469.
[16] T. L. Blundell, *Trends Biochem. Sci.*, 1979, **4**, 51.
[17] P. Newmark, *Nature (London)*, 1979, **277**, 431.
[18] P. H. Sönksen, *Nature (London)*, 1979, **282**, 13.
[19] 'Insulin – Chemistry, Structure and Function of Insulin and Related Hormones', Proc. 2nd Int. Insulin Symp., Sept. 1979, Aachen, F.R.G., ed. D. Brandenburg and A. Wollmer, Walter de Gruyter, Berlin, New York, 1980.
[20] P. Gorden, *Diabetes*, 1979, **28**, 8.
[21] J. L. Goldstein, R. G. W. Anderson, and M. S. Brown, *Nature (London)*, 1979, **279**, 679.
[22] K. J. Catt, J. P. Harwood, G. Aguilera, and M. L. Daufau, *Nature (London)*, 1979, **280**, 109.
[23] C. R. Kahn, *Proc. Soc. Exp. Biol. Med.*, 1979, **162**, 13.
[24] C. R. Kahn, *Trends Biochem. Sci.*, 1979, **4**, 263.
[25] 'Endocrine Pancreas and Diabetes', Proc. 1st Int. Symp. on Endocrine Pancreas and Diabetes, June 1977, Caracas, ed. J. Pierluissi, Excerpta Medica, Amsterdam, 1979.
[26] R. Levine, *Can. J. Biochem.*, 1979, **57**, 447.
[27] T. H. Jukes, *Can. J. Biochem.*, 1979, **57**, 455.
[28] D. V. Goeddel, D. C. Kleid, F. Bolivar, H. L. Heyneker, D. G. Yansura, R. Crea, T. Hirose, A. Kraszewski, K. Itakura, and A. D. Riggs, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 106.
[29] A. D. Riggs, K. Itakura, T. Hirose, A. Kraszewski, D. Goedell, D. Kleid, D. Yansura, F. Bolivar, and H. Heyneker, ref. 2, p. 985.
[30] S. Naber, L. Villa-Komaroff, S. Broome, W. Gilbert, and W. Chick, *Diabetes*, 1979, **28**, 393.

amino-acid sequence B(1—30)-Arg-Arg-Lys-Leu-Lys-Arg-A(1—3) have been synthesized.[31] A summary of recent findings has been given.[32]

Plasmin has been suggested for use as a more specific converting enzyme, and the construction of a gene coding for the sequence -Lys-(B1—29)-(A1—21)- has been proposed.[10] In a semisynthetic test A-chain was coupled to 1,29-$(Boc)_2$(B1—29) with DCC/HOBt. The resulting mixture of products was reduced, oxidized, and the desired 'miniproinsulin' isolated on a column with immobilized antibodies. After purification, cleavability by plasmin could be demonstrated.

A considerable number of new analogues and derivatives have been prepared, and new data have also been obtained with known insulin derivatives (Table 1). The preparation of 17 analogues by total synthesis is particularly noteworthy.[48]

Table 1 *Analogues and derivatives of insulin*[a]

Compound number	*Structure*[b]	*Species*[c]	*% Biological activity*[d] in vivo	in vitro	*Test*[e]	*Ref.*
1a	Insulin	a		+	I	33
1b	Insulin[f]	b		100	C, F	3, 34
1c	Insulin	c		400	F	35
				90	J	35
				+	D, F	36
				380	F	37
					i	38
1d	Insulin	cd				
1e	Insulin	g	100		F	36
1f	Insulin	gp	2		F	35
					J	35
				~3	F	37
1g	Insulin	hg			*x*	39
				5	D	37
				5—10	F	37
				5—10	H	37
				3.8	C	40
				4.4	E	40
1h	Insulin[h]	h	100		B	6, 41
	Insulin[h]		100		B	42
	Insulin[f]		100		B[j]	43
	Insulin[f]				*i*	44
					i	38

[31] W. L. Sung, H. M. Hsiung, R. Brousseau, J. Michniewicz, R. Wu, and S. A. Narang, *Nucleic Acids Research*, 1979, **7**, 2199.
[32] M. J. Gait, *Nature (London)*, 1979, **277**, 429.
[33] M. H. Cobb and W. N. Scott, ref. 2, p. 881.
[34] C. Birr and R. Pipkorn, *Angew. Chem.*, 1979, **91**, 571.
[35] A. C. Santora, F. B. Wheeler, R. L. De Haan, and L. J. Elsaas, jun., *Endocrinology*, 1979, **104**, 1059.
[36] J. Simon, *Biochem. Biophys. Acta*, 1979, **555**, 563.
[37] M. Muggeo, E. Van Obberghen, C. R. Kahn, J. Roth, B. H. Ginsberg, P. De Meyts, S. O. Emdin, and S. Falkmer, *Diabetes*, 1979, **28**, 175.
[38] P. J. Hollins and M. M. Nathan, *Clin. Exp. Immunol.*, 1979, **38**, 127.
[39] J. F. Cutfield, S. M. Cutfield, E. J. Dodson, G. G. Dodson, S. F. Emdin, and C. D. Reynolds, *J. Mol. Biol.*, 1979, **132**, 85.
[40] S. H. L. Thomas, M. H. Wisher, D. Brandenburg, and P. H. Sönksen, *Biochem. J.*, 1979, **184**, 355.
[41] K. Morihara, T. Oka, and H. Tsuzuki, *Nature (London)*, 1979, **280**, 412.
[42] K. Inouye, K. Watanabe, K. Morihara, Y. Tochino, T. Kanaya, J. Emura, and S. Sakakibara, *J. Am. Chem. Soc.*, 1979, **101**, 751.
[43] A. Teuscher, *Schweizer. Mediz. Wochenschrift*, 1979, **109**, 743.
[44] P. Diem and A. Teuscher, *Schweizer. Mediz. Wochenschrift*, 1979, **46**, 1814.

Table 1 (*cont.*)

Compound number	*Structure*[b]	*Species*[c]	*% Biological activity*[d] in vivo	in vitro	*Test*[e]	*Ref.*
1i	Insulin	o			[x]	45
1j	Insulin	p			[x]	15
1k	Insulin	po	70		A[i]	46
1l	Insulin	r			[i]	38
1m	Insulin	s	83		A[i]	46
A-chain modification						
2	Acetoacetyl A1			25 31	C E	40 40
3	Apa A1	b				4
4	Pht A1				[i]	38
5	Tan A1		60	76 81	A, C F	47 47
*6	[D-Allo-Ile^{A2}]	h		0.2	D[i]	48
*7	[Pro^{A2}]	h		1.7 1.5	D[i] E	48 48
*8	[Ala^{A5}]	h	105 87	38 49	B, D E[i] B[i]	48 48 48
*9	[D-Cys^{A6}]	h		0.23	D[i]	48, 49
*10	[D-Cys$^{A6, A11}$]	h		0.03	D[i]	48, 49
*11	[D-Cys^{A7}]	h		0.16	D	48, 49
*12	[His^{A8}]	h		246 299	D E	48 48
*13	[Lys^{A8}]	h	66	64 153	B, D E[i]	48 48
*14	[Phe^{A8}]	h	87	80 107	B, D E[i]	48 48
*15	[D-Cys^{A11}]			0.73	D[i]	48, 49
*16	[^{125}I-Tyr^{A14}]			100	C, F	50
*17	*N*-Methyl-pyridinium A14	b	72	35	A, C	51
*18	Tan A14	b	52		A	52
*19a	[I-Tyr^{A19}]	p		24	C	5
*19b	[^{125}I-Tyr^{A19}]	p		50 100	C, F C[i]	50 53

[45] S. M. Cutfield, G. G. Dodson, E. Schwertner, and H. Zahn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 783.
[46] Y. I. Rusakov and V. M. Bondareva, *Zh. Evol. Biokhim. Fiziol.*, 1979, **15**, 136.
[47] M. Lesniak, J. Gliemann, J. Roth, B. R. D. Easter, D. A. Sutton, and S. E. Drewes, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 467.
[48] F. Märki, M. De Gasparo, K. Eisler, B. Kamber, B. Riniker, W. Rittel, and P. Sieber, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1619.
[49] K. Eisler, B. Kamber, B. Riniker, W. Rittel, P. Sieber, M. De Gasparo, and F. Märki, *Bioorg. Chem.*, 1979, **8**, 443.
[50] J. Gliemann, O. Sonne, S. Linde, and B. Hansen, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1183.
[51] S. E. Drewes, H. M. Robinson, and J. Gliemann, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 987.
[52] B. R. D. Easter and S. E. Drewes, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1335.
[53] L. Citti, L. Battini, P. Cecchetti, and R. Navalesi, *J. Nuclear Med. Allied Sci.*, 1977, **21**, 152.

Table 1 (*cont.*)

Compound number	*Structure*[b]	*Species*[c]	*% Biological activity*[d] in vivo	in vitro	*Test*[e]	*Ref.*
*20	[Arg^{A21}]	o	48	40	A, F[t]	54
B-chain modification						
*21	Ala^{BO}	b				8
*22	Arg^{BO}	b		38	C[i]	55
				43	F[p]	55
23a	Lys^{BO}	b		78	C[i]	55
				69	F[p]	55
23b			50		B	56
24	$(Lys)_4{}^{BO}$		30		B	56
*25	Met^{BO}	b		63.5	C[i]	55
				54	F[p]	55
26	Acetoacetyl B1			89	C	40
				95	E	40
*27	Apa B1	b				4
*28	Boc B1			106	C	7
29	Carbamoyl B1			87	C	7
30	Pht B1				[i]	38
31	[^{3}H-Phe^{B1}]	p	+		[k]	57, 58
*32	[$Phe(N_3)^{B1}$]	b				4
33	des-Phe^{B1}	b[b,p]	+		[j]	14
*34	[Ala^{B5}]	h	101	21	B, D	48
				31	E[i]	48
*35	[D-Cys^{B7}]	h		1.2	D[i]	48, 49
*36	[Gln^{B13}]	h		18	D[i]	48
37	[D-Tyr^{B16}]	h	85	17	B, D	48
				28	E[i]	48
*38	des-Tyr^{B16}	h		0.066	D	48
				0.075	E[i]	48
*39	endo-Tyr^{B16a}	h		0.84	D	48
				0.89	E[i]	48
*40	[D-Arg^{B22},D-Ala^{B23}]-des(B25—30)		~5		A	59
*41	[Asp^{B22}, D-Ala^{B23}]-des(B25—30)		20—40		A	59
*42	[Gly^{B22}, D-Ala^{B23}]-des(B25—30)		~2		A	59
*43	[Lys^{B22}, D-Ala^{B23}]-des(B25—30)		~20		A	59
*44	[Val^{B22}, D-Ala^{B23}]-des(B25—30)		~10		A	59

[54] N. Ferderigos, A. Cosmatos, A. Ferderigos, and P. G. Katsoyannis, *Int. J. Pept. Protein Res.*, 1979, **13**, 43.
[55] C. W. T. Yeung, M. L. Moule, and C. C. Yip, *J. Biol. Chem.*, 1979, **254**, 9453.
[56] G. P. Vlasov, O. V. Glinskaya, N. L. Izvarina, N. G. Illarionova, and G. I. Shelykh, *Biokhimiya (Moskow)*, 1979, **44**, 1468.
[57] M. Berger, P. A. Halban, J. Seydoux, R. E. Offord, and A. E. Renold, *Diabetologia*, 1979, **17**, 97.
[58] M. Berger, P. A. Halban, J. P. Assal, R. E. Offord, M. Vranic, and A. E. Renold, *Diabetes*, 1979, **28**, 53.
[59] S.-C. Chu, T.-F. Li, C.-P. Tsao, and Y.-S. Chang, *Acta Biochim. Biophys. Sinica*, 1979, **11**, 159.

Table 1 (*cont.*)

Compound number	*Structure*[b]	*Species*[c]	% *Biological activity*[d] in vivo	in vitro	*Test*[e]	*Ref.*
*45	[Arg(D-(DHCD))^{B22}]-des-ala^{B30}	p				6
*46	Endo-Glu^{B22a}-Arg^{B22b}		10—20		B	13
*47	[D-Ala^{B23}]		50		B	13
48	[D-Ala^{B23}]-des(B25—30)		20		A	59
49	des(B23—30)			+		60
				~4	F	37
				+	F	61, 62
				+	[l]	63, 64
*50	[Leu^{B24,25}][m]	h		12	D	65
				11	H	65
					F	65
	Purified			1—5	D, F	66
*51	[Phe-NH$_2^{B25}$]-des(B26—30)	b	44		A[i]	67
*52	[Tyr-NH$_2^{B26}$]-des(B27—30)	b	70		A[i]	67
			30		B	13
53	Ac			89	C	40
	B29			92	E	40
*54	Apa	b				4
	B29					
*55	[Arg-NH$_2^{B29}$]des-Thr^{B30}		100		B	13
*56	[Thr^{B30}]	b				6, 41
*57	[Thr-*O*Bu^{tB30}]	h				6
58a	des-Ala^{B30}				F[l]	6
						64
58b		b	100			10
58c		p			[i,j]	68
58d	des-Ala^{B30}[^{125}I]	p			[i]	68
59	Arg^{B31}, Arg^{B32}				[l]	64
Modifications in A- and B-chains; di- and tri-substitued						
60	Arg^{AO}, des-Ala^{B30}	b	61			10
61	Boc, Tan	b				52
	A1, A14					
*62	(Gua)$_2$			75—100	C	7
	A1, B29			100	F	7
*63	(Gua)$_2$, Boc			101	C	7
	A1, B29, B1					
*64	(Gua)$_2$, carbamoyl			100	C	7
	A1, B29, B1					
*65	(Msc)$_2$	b		28	C	69
	A1, B1					

[60] J. Larner, K. Kikuchi, R. Freer, and H. Morris, *Diabetes*, 1979, **28**, 390.
[61] J. M. Olefsky and H. Chang, *Endocrinology*, 1979, **104**, 462.
[62] M. Van Houten, B. I. Posner, B. M. Kopriwa, and J. R. Brawer, *Endocrinology*, 1979, **105**, 666.
[63] H. A. Pershadsingh and J. M. McDonald, *Diabetes*, 1979, **28**, 366.
[64] P. Luly and M. Shinitzky, *Biochemistry*, 1979, **18**, 445.
[65] H. Tager, B. Given, D. Baldwin, M. Mako, J. Markese, A. Rubenstein, J. Olefsky, M. Kobazashi, O. Kolterman, and R. Poucher, *Nature (London)*, 1979, **281**, 122.
[66] J. Olefsky, M. Kobayashi, M. Saekow, M. Mako, B. Givens, A. Rubenstein, H. Tager, and R. Poucher, *Diabetes*, 1979, **28**, 364.
[67] A. Cosmatos, N. Ferderigos, and P. Katsoyannis, *Int. J. Pept. Protein Res.*, 1979, **14**, 457.
[68] D. Kumar, *Diabetes*, 1979, **28**, 994.
[69] A. Schüttler and D. Brandenburg, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1721.

Table 1 (*cont.*)

Compound number	*Structure*[b]	*Species*[c]	*% Biological activity*[d] in vivo	in vitro	*Test*[e]	*Ref.*
66	$(Msc)_2$ A1, B29	b		20	C	69
67	$(Pht)_3$ A1, B1, B29		15—70[n]	10—52[n]	F	70
				10, 70[n]	C	70
					[i]	38
*68	[Cys(Acm)$^{A7, B7}$]	h	0.43	0.29	B, D	48
				0.37	E[i]	48
69	des-Asn^{A21}, des-Ala^{B30}				F	61
70	$(Msc)_2$ B1, B29			54	C	69
Homo-crosslinked, proinsulin, preproinsulin						
71	A_2Sub				I	33
*72	Mini-pro(des-Ala^{B30})-insulin	b			[i]	10
73	Proinsulin-(54—55 bond split)	p			[i]	12
74	des(55—63)proinsulin	p			[i]	12
75	des(62—63)proinsulin	p			[i]	12
*76	des(1—13)preproinsulin[h]	r/b				8
*77	des(1—21)preproinsulin[h]	r/b				8, 71
78	Sub$(insulin)_2$ B1—B29			41	C	40
				46	E	40
79	Sub$(insulin)_2$ B29—B29			5	C	40
				4.5	E	40
Undetermined structure; not homogeneous						
80	Dalanated	p			[i]	68
81	Glycosylated	b		de-creased	C, D, E[i]	72
82	Glycosylated	p	~90		B	73
83	3-Maleimidobenzoyl	b, p				74
84	2-Nitro,4-azido-phenyl[^{125}I]	p			F	75
Undetermined structure; hetero-cross-linked						
85	Dextran-coupled	p		~10	O[i]	76
86	Invertase-coupled				[i]	77
87	Sepharose-coupled					78

[a] New compounds are marked by an asterisk. [b] Abbreviations: A_2Sub = LL-diamino-suberoyl, Apa = 4-azidophenylacetyl, Gua = guanyl, DHCH = 1,2-dihydroxycyclohex-1,2-ylene, Pht = phthaloyl, Tan = 2-nitro-4-trimethylammoniophenyl. [c] Anglerfish = a, beef = b, chicken = c, cod = cd, goose = g, guinea-pig = gp, hagfish = hg, human = h, sheep = o, pork = p, pollock = po, rat = r, salmon = s. [d] % potency based on insulin (porcine, bovine). [e] The following tests are indicated: A, mouse convulsion assay; B, *in vivo* blood glucose assay; C, lipogenesis in isolated fat cells; D, *in vitro* glucose oxidation; E, antilipolysis in isolated fat cells; F, receptor binding; G, enzymatic degradation; H, glucose transport; I, sodium transport; J, amino-acid transport; [f] Synthetic. [g] Crystalline. [h] Semisynthetic. [i] Immunological studies. [j] Clinical studies. [k] Degradation studies. [l] Cell membrane interactions studied. [m] Mixture with 40% insulin. [n] Different species. [o] Enzyme induction. [p] C.d. studies. [x] *X*-ray studies.

[70] R. Horuk, P. Goodwin, K. O'Connor, R. W. J. Neville, and N. R. Lazarus, *Nature (London)*, 1979, **279**, 439.
[71] V. K. Naithani, E. Büllesbach, and H. Zahn, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 1363.
[72] R. Dolhofer and O. H. Wieland, *FEBS Lett.*, 1979, **103**, 282.
[73] M. Brownlee and A. Cerami, *Science*, 1979, **206**, 1190.

A structurally abnormal, almost inactive, insulin has been isolated from the pancreas of a diabetic patient.[65] It occurs in a 60 : 40 mixture with normal insulin and could be purified on a micro-scale by differential receptor binding.[66] The analogue contains leucine in position B24 or B25. Similar to guinea-pig insulin, the hormone from casiragua, another hystricomorph, deviates highly in its sequence (21 differences) from bovine insulin and exhibits low potency *in vitro* and *in vivo*.[70] The isolation of two fish insulins (1k, 1m) with somewhat reduced potency has been described.[46] Material with insulin immunoreactivity and biological activity has been isolated from the brain of the blowfly *Calliphora vomitoria*.[78]

All syntheses of insulin analogues reported in 1979 have been carried out in solution. Applying the principle of stepwise selective formation of disulphide bonds, 17 analogues of human insulin with variations in the *N*-terminal region of the A-chain (positions 2—11) and the B-chain (positions 5—16) have been synthesized (compounds 6—15, 34—39, 68).[48] The analogues with A-chain variations were obtained by coupling ten different tridecapeptides A(1—13) containing a pre-formed intrachenar (6—11) disulphide ring to a common intermediate A(14—21)/B(1—30). The analogues with modified positions B5, B7, and B13 were prepared using the intermediate A(1—21)/B(17—30) as amino-component. Coupling with the pentadecapeptide B(1—15) gave the des-Tyr^{B16} analogue, while elongation by Tyr(Bu^t) and subsequent condensation with B(1—16) yielded the endo-Tyr^{B16a} insulin (39). The derivative (68) which contains only two disulphide bonds was prepared by deblocking the precursor of unmodified human insulin only at *N*- and *O*-functions. The analogues were mostly purified by counter-current distribution, and all were thoroughly characterized by physicochemical and biological data. Details of the insulins containing D-Cys are given in ref. 49.

An A-chain with Arg replacing Asn^{21}, and two B-chains with shortened *C*-termini, have been synthesized using benzyl[54] or diphenylmethy[67] groups for sulphur protection. From these, analogues 20, 51, and 52 have been obtained following previously described routes. For the unequivocal preparation of iodo-insulins, two A-chains with 3-iodo- and 3,5-di-iodo-Tyr have been synthesized with S-trityl groups at the four cysteines.[5] The iodo-Tyr moieties were not affected during the conversion to *S*-sulphonate chains. Combination of the mono-iodo-chain with natural B-chain gave the derivative (19a).

Solid-phase synthesis of insulin employing differential sulphur protection is being explored, and has yielded half-synthetic, active insulin.[3, 34] The A-chain fragments 1—14 and 15—21 have been synthesized on solid supports and coupled in solution.

An important step forward in the semisynthesis of insulin analogues is the finding that trypsin can be used for peptide couplings at Arg^{B22} and Lys^{B29}. So far, human insulin has been obtained in 49% yield by enzyme-assisted coupling of synthetic ε-Boc-octapeptide to A1,B1-(Boc)$_2$-des-octapeptide (B23—30) porcine insulin in 50% DMF in a molar ratio of 10 : 1 at pH 6.5.[42] Similarly, Thr-OBu^t was coupled to des-Ala^{B30}-insulin to give a conversion yield of 41% for human from porcine

[74] J. A. Schroer, J. K. Inman, J. W. Thomas, and A. S. Rosenthal, *J. Immunol.*, 1979, **123**, 670.
[75] S. Jacobs, E. Hazum, Y. Scechter, and P. Cuatrecasas, *Proc. Natl. Acad. Sci., USA*, 1979 **76**, 4918.
[76] Y. Sakamoto and T. Kuzuya, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 37.
[77] S. Pillai and B. K. Bachhawat, *FEBS Lett.*, 1979, **101**, 301.
[78] H. Duve, A. Thorpe, and N. R. Lazarus, *Biochem. J.*, 1979, **184**, 221.

insulin, and a yield of 60% of analogue (56) from bovine insulin.[6, 41] Protection of Arg^{B22} by cyclohexane-1,2-dione (45) has initially been employed, but it was found that tryptic cleavage of the B22—23 bond does not occur even with free arginine.[42]

A series of six analogues of des-hexapeptide (B25—30)insulin containing D-Ala in place of Gly^{B23} was prepared from $(Boc)_2$-des-nonapeptide(B21—30)insulin pentamethylester by condensation with X-D-Ala-Phe-OMe [X = Gly, D-Arg, Val, Arg, Lys(Boc), Asp(OBu^t) followed by removal of Boc-groups and saponification.[59]

Chemical semi-synthesis has been extended: Porcine Arg-A-chain-*S*-sulphonate has been successively coupled with the anhydrides of four synthetic *C*-peptide fragments to give the proinsulin sequence 39—86.[8] Bovine proinsulin could be preferentially acylated at pH = 6 at the *N*-terminus with the rat prepeptide sequences 22—24 and 14—24 using mixed anhydrides (see below) to give compounds (76) and (77).[8, 71]

Several new derivatives and analogues have been obtained by partial substitution of unprotected insulin or by specific acylation of protected intermediates. Msc- and Boc-groups play the most important role as reversible blocking groups. A1, $B1(Msc)_2$-insulin (65) can be obtained in 60% yield by acylation of insulin with the *N*-hydroxysuccinimide ester at pH = 5.8.[69] The derivative (70) was prepared *via* A1-citraconyl insulin. Preferential introduction of the Boc-group into position B1 can be achieved by reaction with di-t-butyl carbonate at pH = 6 [compound (28)].[7] Mixed anhydrides formed from Boc-amino-acids and isobutylchloroformate are very effective acylating reagents in 80% aq. DMF or DMSO.[8] At pH = 6 or 7, reaction at B1 is favoured. The intermediate (66) was quantitatively acylated with Boc-Ala-OH to give the analogue (21), but the lysine amino-group of (65) was less reactive.[8]

The three analogues (22), (23a), and (25) have been prepared from A1, $B29\text{-}(Boc)_2$-insulin by acylation with Boc-Met-ONp, Boc-Arg(NO_2)-ONSu, or Boc-Lys(Boc)-ONp, respectively.[55] Photoreactive insulins [(27), (54)] carrying a 4-azidophenylacetyl group in positions B1 or B29 have been synthesized *via* the Msc-protected intermediates (66) and (65).[4] The A1-derivative (3) resulted from partial acylation of insulin and fractionation.[4]

The replacement of B1-phenylalanine by 4-azido-Phe was accomplished by semisynthesis using Msc-groups for protection of des-Phe-insulin and the amino-acid.[4] The analogue (32) crystallizes like insulin. The guanyl residue has been used as a permanent protecting group for insulin amino-groups.[7] In aprotic solvents (DMF, DMSO) in the presence of base, guanyldimethylpyrazole reacts preferentially with residues A1 and B29. Compound (63) has been obtained from B1-Boc-insulin, (64) from crude (62).[7] Analytical investigations on the reactivity of insulin amino-groups have been performed using competitive labelling with [^{3}H]- and [^{14}C]acetic anhydride.[79]

Two derivatives each with one modified tyrosine residue [(18), (17)] were prepared by reaction of insulin at pH = 8—8.5 with 2-chloro- or 2-iodo-*N*-methylpyridinium iodide[51] or 1-fluoro-2-nitro-4-trimethylammoniobenzene iodide.[52] A lower yield of (17) was obtained in the presence of zinc ions, a higher

[79] M. G. Sheffer and H. Kaplan, *Can. J. Biochem.*, 1979, **57**, 489.

yield *via* A1-Boc-insulin. Mono-iodo-insulin could be separated into the A14 and A19 isomers by disc electrophoresis.[50] According to another report,[53] lactoperoxidase-catalysed iodination gives almost exclusively A19-iodo-insulin (90%).

Des-octapeptide insulin (49) has been extensively purified by isoelectric focusing and h.p.l.c. under conditions which separate 0.1% of contaminating insulin (no details).[60] The crystalline analogues (58) and (60) have been obtained by cleavage of proinsulin with plasmin, which is more specific than other enzymes.[10]

Unprotected insulin has been partially glycosylated by incubation with glucose, mannose, and other oligosaccharides at pH = 7.4—8 at 37 °C,[72, 73] treated with 3-maleimidobenzoyl-*N*-hydroxysuccinimide ester for further coupling to sheep erythrocytes,[74] and with a photo-label for receptor studies.[75] The hormone has been linked to invertase by means of glutardialdehyde, using succinyl-Con A-Sepharose as an affinity matrix to improve the stoicheiometry of coupling.[77] In these and other cases [compounds (81)—(87)] purification was not attempted.

Structure, Receptor Binding, and Biological Activity. The crystal structure of porcine 2-zinc insulin has been refined on the basis of 1.5 Å data [15] and even further using 1.2 Å resolution data.[11] A detailed comparison between the two individual molecules present in the dimer has been made, and the interactions between monomers, dimers, and hexamers have been described. The arrangement of the main chains in molecules (I) and (II) is very similar except for the *N*-terminus of the A-chain (helix 2—8) and residues 22, 25, 29, and 30 at the *C*-terminus of the B-chain. At 1.2 Å resolution, 80% of the water molecules hydrogen bonded to insulin and disordered side-chains could be determined, and information on local flexibility been obtained.[11] Structure–function relationships of insulin in the light of the high resolution structures have been discussed in considerable detail (ref. 15, also ref. 11).

Further arguments are provided by the *X*-ray analysis of hagfish insulin.[39] Although the resolution is limited to 3.1 Å, it allows a number of valuable observations and shows clearly that the two monomers of the symmetrical dimer correspond much more to porcine monomer (II) than monomer (I). Different conformations are seen at the *N*- and *C*-termini of the B-chain. Residues B30 and B31 partially cover the *N*-terminus of the A-chain, but this structural feature does not cause the low potency. The first crystallographic study on a half-synthetic insulin has been reported.[45] This sheep insulin crystallized unexpectedly with four zinc ions per hexamer. Cell dimensions and the intensities of the *X*-ray reflections correspond to those of porcine 4-zinc insulin (6.0 Å spacing). Further studies in the solid state include electron diffraction of crystalline porcine 2-zinc insulin [80] and an investigation using electron paramagnetic resonance on insulin hexamers containing copper(II) ions.[81]

Conformational data in solution have been obtained by high-resolution ^{1}H n.m.r. spectroscopy at pH = 9.[82] The conformation of the 2-zinc hexamer appears to be fixed by the first zinc ion. Addition of SCN^- ions to the zinc hexamer causes marked changes of the spectrum indicative of major conformational changes

[80] R. H. Lange, J. Blödorn, G. Magdowski, and H. J. Trampisch, *J. Ultrastruct. Res.*, 1979, **68**, 81.
[81] J. C. Evans, P. H. Morgan, M. Mahbouba, and H. J. Smith, *J. Inorg. Biochem.*, 1979, **11**, 129.
[82] K. L. Williamson and R. J. P. Williams, *Biochemistry*, 1979, **18**, 5966.

leading to a new hexamer which binds two equivalents of rhodanide.[82] The interactions of insulin with zinc, cobalt, and cadmium ions, but not with calcium or magnesium ions, give rise to perturbations of u.v.-spectra in the region of Tyr absorption.[83] Binding of glucose to insulin has been studied by equilibrium dialysis, and discussed with respect to biological fluids.[84]

Further conclusions on the structure of insulin in solution have been drawn on the basis of antibody-binding [38] and competitive-labelling experiments.[79] From the latter it is concluded that the amino-groups, even that of lysine, are deprotonated under physiological conditions. Correlations between crystalline and solution state of insulin may be possible with photoreactive analogues. Preliminary irradiation experiments with B1-Apa-insulin (27) show cross-linking of B-chains in the crystalline and dissolved hexamer.[4]

Biological Consequences of A-chain Modification. In contrast to uncharged substituents [(acetoacetyl, Msc; compounds (2), (65), (66)] at the amino-group of A1-glycine, positively charged groups, such as Arg [in (60)], the guanyl residue (62—64), or even the bulky aromatic group in (5) cause only a small or even no reduction of potency. Replacement of the invariant Ile^{A2} [(6), (7)] or of the half-cystines A6 or A7 by the D-enantiomer has a very strong effect on bioactivity, although the ability of these analogues to form 2-zinc rhombohedral crystals, and high immunological cross-reactivity indicate extensive conservation of the spatial insulin structure. On the other hand, replacement of the likewise invariant Gln^{A5} has only a minor effect. According to the insulin model D-Cys residues in positions 6 or 7 are acceptable without major structural changes. Absence of the A7—B7 bond results in an analogue (68) of very low potency.

The potency of the crystallizable His^{A8} analogue (12) proves that the high activity of chicken/turkey insulin is due to this replacement. But this activity cannot be due to a positively charged or bulky side-chain, as apparent from the properties of (13) and (14). Interestingly, (12) is also the only analogue with enhanced immunoreactivity. The presence of D-Cys in position A11, and particularly of the enantiomorphic A(6—11)D-cystine ring [(15), (10)] causes a very strong loss of activity. Low potency was in most cases linked to marked increase of hydrophobicity, as shown by an increase of R_F-values in t.l.c. and high K-values in countercurrent distribution.[48]

Substitution of the A14-tyrosine side-chain, which is on the surface of the monomer, by iodine has no consequences for activity, and only a moderate inactivation occurs upon substitution with large, positively charged, substituents [(17), (18)], which still can be accommodated in the crystal.[51, 52] In agreement with the crystal structure, modifications at Tyr^{A19} are deleterious. Both the synthetic and the isolated mono-iodo-insulin exhibit impaired activity and receptor binding, which is an important observation with respect to application as tracer in binding, and particularly metabolic studies. Findings in another report [53] are contradictory. Replacement of the invariant Asn^{A21} by Arg results in a relatively small reduction of potency, confirming that the side-chain of Asn plays no critical role.[54]

Biological Consequences of B-chain Modification. A partial reduction of potency

[83] J. Hudeček, P. Anzenbacher, and V. Kalous, *FEBS Lett.*, 1979, **100**, 374.
[84] V. Kalous and P. Anzenbacher, *Acta Diabetol. Latina*, 1979, **16**, 169.

and receptor binding concomitant with changes of c.d.-spectra upon elongation of the B-chain with α-amino-acids [55] is in agreement with earlier findings. The attenuation of the 222 nm ellipticity has been assigned to a loss of β-structure.[55] The chemical changes would thus be inferred to affect a remote part of the molecule. Uncharged substituents of different size (Boc, carbamoyl) have, in contrast, no effect [compounds (28), (29), (63), (64)].

Replacement of His^{B5} confirms that histidine is important for potency, but not essential. A moderate loss of activity is also observed with analogues (36) and (37), but D-Cys in position B7 causes a large reduction. Deletion from, or insertion into, the α-helical B-chain region of a tyrosine residue has serious consequences for the conformation of the hormone. Evidence for marked structural changes are, besides high loss of antibody binding, very pronounced increases of hydrophobicity for the D-Cys analogue and even more for (38) and (39).

Further evidence against a critical role of Arg^{B22} comes from the data of a series of analogues (40—44) of [D-Ala^{B23}]- des-hexapeptide insulin.[59] While the Asp^{B22} analogue is equipotent, a gradual decrease in *in vivo* potency is observed: Arg/Asp > Lys > Val > D-Arg > Gly. The possibility of replacing Gly^{B23} by D-amino-acids had already been demonstrated earlier by the Chinese group (see Volume 8 in this series). It is surprising to note that the insertion of two amino-acids which causes an 'outward' shift of the sequence B(24—26), results in an analogue (46) with still marked activity (*in vivo*). For a long time, the small residual activity of des-octapeptide insulin preparations has been a point of uncertainty. It has now been shown that the highly purified analogue (49) has indeed intrinsic activity and is able to elicit maximal response in fat cells.[60] A very interesting analogue is the abnormal insulin (50) with Leu in position B24 or 25. While the purified preparation is almost inactive,[66] the mixture is less active than expected on the basis of its composition (60 : 40). This indicates that the abnormal insulin has an inhibitory effect on the action of the hormone. Omission of the *C*-terminal tetra- and penta-peptide causes a partial inactivation, also when the carboxyl group is missing (51), (52).[67, 13]

Correlation Between Receptor Binding and Biological Activity. As data have usually been obtained in a single assay or a limited number of assays, complete correlations are not possible. But, whenever studied, experiments show a good correlation between receptor binding and biological activity *in vitro*. Isolated fat cells are the standard system, and identical results are obtained by monitoring either conversion of labelled glucose into either CO_2 or lipids. Relative potencies obtained in assays of deoxyglucose transport agree well with these data. The dose–response relationships of insulin stimulation of lipogenesis [40] as well as glucose oxidation,[48] and of inhibition of adrenaline-induced lipolysis in rat adipocytes correspond well. The sensitivity of both responses are the same for insulins modified at one of the three amino-groups [(2), (26), (53)], for dimers [(78), (79)], the synthetic analogues [48] [(*e.g.* (10), (12)] and hagfish insulin,[40] which vary in potency between 0.03 and 250%. It has therefore been concluded that both effects are mediated through the same set of receptors.[40, 48] All modified insulins assayed so far are full agonists and produce maximal insulin effects, even in those cases where extremely low potencies have been found.

The biological effect of modified insulins *in vivo* is naturally of primary interest,

but, as this is a complex phenomenon, it is difficult to interpret with respect to structure–function relationships. Potencies obtained from measurements of blood sugar depression may correspond to *in vitro* data, but large differences have been observed.

Glucagon.—Very little new work on the structure–function relationships of glucagon has appeared. The role of glucagon in diabetes has been reviewed,[85] as has the relation between its crystal structure, storage forms, and receptor binding.[86] A spectroscopic study indicated that in fresh acid solutions the hormone assumes mostly an α-configuration,[87] but that the structure in basic solution was different. The thermodynamics of aggregation have been investigated by studying the heats of dilution of concentrated glucagon solutions.[88] A similar conclusion to that of another study[89] was drawn, namely that the negative heat capacity change indicates that a hydrophobic interaction is involved in aggregation. However, it is emphasized that the measurable proton uptake on aggregation[90] will make a significant, but as yet undetermined contribution to the heat capacity change, so that the interpretations based on heat capacities should be approached with caution.

The synthesis of pancreatic proglucagon and its subsequent processing has been studied.[91] Proglucagon seems to have a molecular weight of *ca.* 18 000 dalton. The size increases slightly before immunoreactive species of mol. wt. 13 000, 4500, and finally 3500 dalton (glucagon) are produced. A large peptide (10 000 dalton) is released; the possibility of this producing secondary hormones is discussed, as GIP,[92,93] CCK,[94] and even β-endorphin[95] have been detected in the A-cell by immunohistochemical techniques. Gut glucagons have also been studied. At least two of the glucagon-like immunoreactive (GLI) forms from rabbit intestine (12 000, 8000 dalton) are similar to materials isolated from the pancreas,[96] but it is probable that the post-translational processing differs in the two tissues. Another study[97] reports at least four GLI species from porcine colon (12 000, 8000, 5000, and 3000 dalton). All are bound tightly to high molecular weight proteins. Only the 8000 and 3000 dalton species are homogeneous by isoelectric focusing. However, none of the species have significant lipolytic activities. A relation between the larger forms of glucagon in gut and pancreas was also deduced by immunohistochemical methods.[98] Different GLIs have also been detected in nerve tissue:[99] a neuro-modulator role may be possible.

[85] P. J. Lefebvre and A. S. Luyckx, *Diabetologia*, 1979, **16**, 347.
[86] T. Blundell, *Trends Biochem. Sci.*, 1979, **4**, 80.
[87] T. C. Jao and G. Marquez, *Arch. Biochem. Biophys.*, 1979, **197**, 342.
[88] R. E. Johnson, V. J. Hruby, and J. A. Rupley, *Biochemistry*, 1979, **18**, 1176.
[89] S. Formisano, M. L. Johnson, and H. Edelhoch, *Proc. Natl. Acad. Sci. USA*, 1979, **74**, 3340.
[90] W. B. Gratzer and G. H. Beaven, *J. Biol. Chem.*, 1969, **244**, 6675.
[91] C. Patzelt, H. S. Tager, R. J. Carroll, and D. F. Steiner, *Nature (London)*, 1979, **282**, 260.
[92] P. H. Smith, F. W. Merchant, D. G. Johnson, W. Y. Fujimoto, and R. H. Williams, *Analyt. Rec.*, 1977, **149**, 585.
[93] J. Alumets, R. Håkanson, T. O'Dorisio, K. Sjölund, and F. Sundler, *Histochemistry*, 1978, **58**, 253.
[94] D. Grube, V. Maier, S. Raptis, and W. Schlegel, *Histochemistry*, 1979, **56**, 13.
[95] D. Grube, K. H. Voigt, and E. Weber, *Histochemistry*, 1978, **59**, 75.
[96] H. S. Tager and J. Markese, *J. Biol. Chem.*, 1979, **254**, 2229.
[97] J. M. Conlon, R. F. Murphy, and K. D. Buchanan, *Biochim. Biophys. Acta.*, 1979, **577**, 229.
[98] M. Ravazzola, A. Siperstein, A. J. Moody, F. Sundby, H. Jacobsen, and L. Orci, *Life Sci.*, 1979, **25**, 287.
[99] J. M. Conlon, W. K. Samson, R. E. Dobbs, L. Orci, and R. H. Unger, *Diabetes*, 1979, **28**, 700.

Reappraisals of three glucagon derivatives have been published. Specific reaction of Lys-12 with *O*-methylisourea [100] was achieved by converting the *N*-terminal histidine into its copper chelate. Several spectroscopic methods were used to show that the helix content of the derivative was very similar to glucagon. However, [12-Homoarg]glucagon was only 15% active in a lipolysis assay, indicating that Lys-12 is very important in receptor binding. A curious side-reaction, namely copper ion-catalysed cleavage on the *N*-terminal side of Tyr-10 and Tyr-13, was observed.

Des[His^1]glucagon has been reported to be a full antagonist,[101] although the product of a solid-state Edman degradation was a partial agonist.[102] The reaction of glucagon with PITC has been re-investigated [103] and shown to be pH-dependent, with the amount of reaction at Lys-12 increasing with higher pH as expected. After treatment with TFA and purification, it could be shown that des[His^1]glucagon was a partial antagonist while des[His^1], [N^{ε}-PTC^{12}]-glucagon was essentially inactive, although having a binding affinity of *ca.* 5%. This, and the previous study,[100] demonstrates that Lys-12 is important both for receptor binding and biological activity.

The third reappraisal is of the *N*-terminal hexapeptide of glucagon.[104] The retention of activity, albeit only 0.1%, of the fragment (1—21) [105] was reported last year. Now it seems that the synthetic hexapeptide (1—6) retains 0.001% binding potency, and is a partial agonist having a maximum stimulation in a cyclase assay of 75% that of glucagon. The closely related peptides VIP (1—7) and secretin (1—6) NH_2 showed no effect either in inhibiting glucagon binding or in activating adrenylate cyclase.[106] It would seem therefore that nearly all of the requirements for receptor activation lie in the *N*-terminus of the hormone. Presumably the role of the *C*-terminus is either to enhance the binding *per se* or to induce a more favourable conformation in the *N*-terminal region. It is interesting that glucagon (13—29) may inhibit 50% of glucagon binding at 5×10^{-6} M (0.5% affinity of glucagon) but has no biological activity.[106] Obviously many exciting results can be awaited especially if it proves possible to enhance the binding potency without increasing the biological activity.

An improved synthesis of mammalian glucagon has been disclosed,[107] as has synthesis of the fragment (15—29) for antibody production and immunoassay.[108] The synthetic *C*-terminal octapeptide (22—29) was shown to have a slight inhibitory effect (18%) on GPDH activity *in vitro* at a concentration of 0.5 mg ml^{-1}; the fragment (1—14) had no effect.[109]

Somatostatin.*—A very nice review of many physiological and structural aspects of somatostatin has appeared,[110] as has another which concentrates mainly on the

[100] J. B. Alexander Ross, K. W. Rousslang, C. De Haën, V. R. Lavis, and D. A. Deranleau, *Biochim. Biophys. Acta*, 1979, **576**, 372.
[101] M. Rodbell, L. Birnbaumer, S. L. Pohl, and F. Sundby, *Proc. Natl. Acad. Sci. USA*, 1971, **68**, 909.
[102] M. C. Lin, D. E. Wright, V. J. Hruby, and M. Rodbell, *Biochemistry*, 1975, **14**, 1559.
[103] M. D. Bregman and V. J. Hruby, *FEBS Lett.*, 1979, **101**, 191.
[104] D. E. Wright and M. Rodbell, *J. Biol. Chem.*, 1979, **254**, 268.
[105] D. E. Wright, V. J. Hruby, and M. Rodbell, *J. Biol. Chem.*, 1978, **253**, 6338.
[106] D. E. Wright and M. Rodbell, ref. 2, p. 1029.
[107] J. D. Mee, *Research Disclosure*, 1979, 246.
[108] M. Fujino, M. Wakimasu, and O. Akira, *Ger. Offen.*, 1979, 2 832 090.
[109] T. Abiko, M. Kumikawa, and H. Sekino, *Chem. Pharm. Bull.*, 1979, **27**, 2827.
[110] D. F. Veber and R. Saperstein, *Annual Reports in Medicinal Chemistry*, 1979, **14**, 209.

* See also Section 1 of this chapter.

physiological aspects.[111] Somatostatin-containing cells in the gut seem to have extended cytoplasmic processes,[112] similar to dendrites, which interface with, and thereby possibly directly control, many surrounding cells which produce gastrin or hydrochloric acid.

Details have appeared of the study[113] in which [D-Cys[14]]somatostatin analogues were shown not to have the selective inhibition of glucagon secretion reported previously.[114] It is obvious that the test system is very difficult – whereas no differences can be detected between analogue and native hormone when maximal suppression is used as the parameter – some differences exist in later stages of the action curve. This makes a comparison between effects on different *in vivo* and *in vitro* systems with different analogues virtually impossible. Differential binding of analogues to specific cell types would be easier to interpret, but the development of a suitable iodinated tracer has also been difficult. Iodinated [Tyr[0]]-somatostatin[115] is claimed to give a more stable tracer than the [Tyr[1]]-analogue,[116] and a ten-fold increase in sensitivity. [Tyr[11]]somatostatin gives an even more stable tracer,[117] and may be more acceptable to antibodies raised against somatostatin. It is obvious that somatostatin analogues react with widely varying affinities with anti-somatostatin antibodies.[117]

Rather few new analogues have been reported this year. Two glycosylated derivatives, substituted on Asn[5], have been synthesized.[118] However, the fascinating reduction in the size of bioactive analogues continues with a report from Veber's laboratory[119] on a highly potent bicyclic analogue containing only residues (7—10) of the original tetradecapeptide. This derivative, *cyclo*[-ω-aminohexanoyl-Cys-Phe-D-Trp-Lys-Thr-Cys-], is very long acting *in vivo* (> 2 h) and very potent in suppressing the secretion of insulin, glucagon, growth hormone, and gastric fluids. The stability could be ascribed to a high resistance towards enzymatic degradation, and the activity to the constraining of the residues to the configuration required for a β-turn, as described for other derivatives.[120] This was supported by a high-field shift in the n.m.r. resonance for the γ-CH_2 of Lys-9 which is probably due to the proximity of the ring of Trp-8. Future developments can be awaited with interest.

It seems unlikely that animals ever biosynthesize a peptide as small as 14 residues directly. Indeed the recent work on preprohormones seems to prescribe a minimum size of 50—70 residues before processing.[121] Evidence is now accumulating for the

[111] M. T. McQuillan, *Somatostatin*, vol. 1, 1977, Eden Press, Montreal, 1979.
[112] L.-I. Larsson, N. Goltermann, L. De Magistris, J. F. Rehfeld, and T. W. Schwartz, *Science*, 1979, **205**, 1393.
[113] F. Märki, B. Kamber, H. Rink, and P. Sieber, *J. Endocrinol.*, 1979, **81**, 315.
[114] M. Brown, J. Rivier, and W. Vale, *Science*, 1977, **196**, 1467.
[115] A. Dupont, D. H. Coy, G. Alvarado-Urbina, J. Coté, C. A. Meyers, J. McManus, N. Barden, A. De Léan, and F. Labrie, *Clin. Endocrinol.*, 1979, **10**, 47.
[116] A. Arimura, H. Sato, D. H. Coy, and A. V. Schally, *Proc. Soc. Exp. Biol. Med.*, 1975, **148**, 784.
[117] J. Gerich, K. Greene, M. Hara, R. Rizza, and G. Patton, *J. Lab. Clin. Med.*, 1979, **93**, 1009.
[118] S. Lavielle, N. Ling, P. Brazeau, R. Benoit, R. Buillemin, T. Wasada, and R. Unger, ref. 2, p. 543.
[119] D. F. Veber, F. W. Holly, R. F. Nutt, S. J. Bergstrand, S. F. Brady, R. Hirschmann, M. S. Glitzer, and R. Saperstein, *Nature* (*London*), 1979, **280**, 512.
[120] D. F. Veber, F. W. Holly, W. J. Paleveda, R. F. Nutt, S. J. Bergstrand, M. Torchiana, M. S. Glitzer, R. Saperstein, and R. Hirschmann, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 2636.
[121] A. Lernmark, S. J. Chan, R. Choy, A. Nathans, R. Carroll, H. S. Tager, A. H. Rubenstein, H. W. Swift, and D. Steiner, in 'Polypeptide Hormones: molecular and cellular aspects', Ciba Foundation Symposium, 1976, **41**, 7.

existence of prosomatostatin. Chromatographic analysis of pulse-chase experiments, using anglerfish islets and [^{3}H]Trp as a specific marker, indicates that material in the range 8000—15 000 daltons fulfils the requirements of somatostatin precursors, and can be converted apparently to native somatostatin by trypsin.[122] The large immunoreactive species was not reduced in size by treatment with urea and thioglycollic acid. A large immunoreactive species (11 000—12 500 daltons) was seen during the isolation of pigeon pancreatic somatostatin by h.p.l.c.,[123] but this was almost totally converted to native somatostatin by urea and thioglycol. A similar species was also observed in another study.[91]

5 Gastrointestinal Hormones

Contributed by D. Gillessen and R. O. Studer

Introduction.—In the reporting period the proceedings of the second international symposium on 'Hormonal Receptors in Digestive Tract Physiology'[1] and of a symposium on the effects of gastrointestinal hormones on maldigestion and malabsorption [2] as well as special journal issues on gastrointestinal hormones [3] and gastrointestinal physiology [4] have appeared. In addition a number of reviews were published dealing with gastrointestinal hormones in general;[5-10] their chemical and biochemical aspects;[11-13] their occurrence in gut and brain;[14-17] their evolution [18, 19] and heterogeneity;[20] their clinical significance;[21-23] their function

[122] B. D. Noe, D. J. Fletcher, and J. Spiess, *Diabetes*, 1979, **28**, 724.
[123] J. Spiess, J. E. Rivier, J. A. Rodkey, C. D. Bennett, and W. Vale, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 2974.
[1] 'Hormone Receptors in Digestion and Nutrition', ed. G. Rosselin, P. Fromageot, and S. Bonfils, Elsevier, Amsterdam, 1979.
[2] 'Intestinale Hormone' Maldigestion-Malabsorption, ed. L. Wannagat, Georg Thieme, Stuttgart, 1979.
[3] 'Clinics in Endocrinology and Metabolism', Vol. 8, No. 2, Gastrointestinal Hormones, ed. K. D. Buchanan, W. B. Saunders Co. Ltd., London, 1979.
[4] 'Gastrointestinal Physiology III', International Review of Physiology, Vol. 19, ed. R. K. Crane, series ed. A. C. Guyton, University Park Press, Baltimore, 1979.
[5] S. R. Bloom and J. M. Polak, *Proc. Nutr. Soc.*, 1978, **37**, 259.
[6] S. R. Bloom, *Eur. J. Clin. Invest.*, 1979, **9**, 111.
[7] S. R. Bloom, in 'Hormones in Blood', 3rd edn., series 2, ed. C. H. Gray and V. H. T. James, Academic Press, London, 1979, p. 321.
[8] G. J. Dockray, ref. 7, p. 357.
[9] G. E. Feurle, *Med. Klin. (Munich)*, 1979, **74**, 121.
[10] R. Klapdor, *Med. Monatsschr. Pharm.*, 1978, **1**, 353; *Med. Lab.*, 1979, **32**, 71 and 116.
[11] V. Mutt, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 2309.
[12] J. D. Gardner, *World J. Surg.*, 1979, **3**, 407.
[13] G. J. Dockray, *Annu. Rev. Physiol.*, 1979, **41**, 83.
[14] A. G. E. Pearse, in 'Centrally Acting Peptides', ed. J. Hughes, Macmillan, London, 1978, p. 49.
[15] S. R. Bloom and J. M. Polak, in 'Brain Peptides', ed. A. M. Gotto, jun., E. J. Peck, jun., and A. E. Boyd, tert., Elsevier, Amsterdam, 1979, p. 103.
[16] J. M. Polak and S. R. Bloom, in 'Gastrins and the Vagus', ed. J. F. Rehfeld and E. Amdrup, Academic Press, London, 1979, p. 15.
[17] N. Yauchihara and S. Kubota, *Medicina (Tokyo)*, 1979, **16**, 2235.
[18] G. J. Dockray, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 2295.
[19] S. van Noorden and J. M. Polak, in *'Horm. Evol.'*, series 2, ed. E. J. W. Barrington, Academic Press, London, 1979, p. 791.
[20] J. F. Rehfeld, *World J. Surg.*, 1979, **3**, 415.
[21] R. Arnold, *Acta Med. Austriaca*, 1979, **6**, 77.
[22] S. R. Bloom and J. M. Polak, *Acta Gastro-Enterol. Belg.*, 1978, **41**, 371.
[23] J. Girodet and S. Bonfils, *Ann. Endocrinol.*, 1979, **40**, 235.

in the intestinal phase;[24,25] their effects on the pancreas,[26,27] on gastric juice secretion,[28] and on gastrointestinal motility;[29] and their use for functional diagnosis.[30] The peptidergic [31] and neuroendocrine [32] system of the gut, receptors [33] and radioimmunoassays [34] for gastrointestinal hormones as well as the effects of vagotomy [35,36] and renal failure [37] on these hormones are topics of further reviews.

Since some investigations are concerned with several gastrointestinal hormones, they will be reported here. The role and importance of the 20 peptides isolated from the alimentary tract and the pancreas are discussed. Five of the 12 completely sequenced peptides (gastrin, secretin, GIP, insulin, and glucagon) are well established hormones and evidence for a hormonal status of CCK, motilin, and pancreatic polypeptide is suggestive whereas the physiological role of the remaining 12 remains to be determined.[38] The distribution of the gastrointestinal hormones has been determined in the gut of baboons and monkeys by gel chromatography and radioimmunoassay. Only motilin and VIP were single components, while CCK, GIP, pancreatic and enteroglucagon occurred in multiple forms and gastrin could not be determined.[39] The receptors,[40] the intracellular mechanism involved in stimulus secretion coupling in pancreatic acinar cells,[41] and the effects of external calcium on amylase release from these cells [42] were investigated. The influence of different gastrointestinal hormones on cAMP accumulation and parathyroid hormone release in dispersed bovine parathyroid cells,[43] on the levels of cAMP of hybrid and cultured mouse brain cells [44] and on ATP levels, amylase release, fatty-acid metabolism, and net lipolysis in rat pancreas *in vitro* [45] were studied. The insulin- and glucagon-releasing activities of CCK, CCK-8, secretin, GIP, and pancreatic polypeptide were tested in dogs,[46] in isolated perfused rat pancreas,[47] and in islet cultures.[48] Actions of gastrointestinal hormones on the isolated

[24] M. J. Orloff, P. V. B. Hyde, L. D. Kosta, R. C. L. Guillemin, and R. H. Bell jun., *World J. Surg.*, 1979, **3**, 523.
[25] S. J. Konturek, *World J. Surg.*, 1979, **3**, 537.
[26] J. F. Rehfeld, *Dev. Endocrinol.*, 1979, **4** (Lipoprotein Metab. Endocr. Regul.), p. 75.
[27] T. Sugano, *Medicina (Tokyo)*, 1979, **16**, 2249.
[28] Y. Ohe and A. Miyoshi, *Medicina (Tokyo)*, 1979, **16**, 2246.
[29] P. A. Thomas, O. E. Akwari, and K. A. Kelly, *World J. Surg.*, 1979, **3**, 545.
[30] T. U. Hausamen, W. P. Fritsch, and T. Scholten, ref. 2, p. 77.
[31] R. Haakanson and F. Sundler, *Verh. Dtsch. Ges. Inn. Med.*, 1979, **85**, 1525.
[32] P. U. Heitz, *Pathol. Res. Pract.*, 1979, **165**, 333.
[33] J. D. Gardner, *Gastroenterology*, 1979, **76**, 202.
[34] P. L. Rayford, K. Hejtmancik, and J. C. Thompson, *World J. Surg.*, 1979, **3**, 423.
[35] H. D. Becker, H. W. Boerger, and A. Schafmayer, *World J. Surg.*, 1979, **3**, 615.
[36] H. T. Debas, *World J. Surg.*, 1979, **3**, 621.
[37] J. Hansky, *World J. Surg.*, 1979, **3**, 463.
[38] M. I. Grossman, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 2341.
[39] M. G. Bryant and S. R. Bloom, *Gut*, 1979, **20**, 653.
[40] R. T. Jensen and J. D. Gardner, ref. 1, p. 167.
[41] J. Christophe, M. Svoboda, P. Calderon, and M. Lambert, ref. 1, p. 177.
[42] J. D. Gardner, C. L. Costenbader, and E. R. Uhlemann, *Am. J. Physiol.*, 1979, **236**, E 754.
[43] R. Windeck, E. M. Brown, D. G. Gardner, and G. D. Aurbach, *Endocrinology (Philadelphia)*, 1978, **103**, 2020.
[44] F. Propst, D. Van Calker, L. Moroder, E. Wünsch, and B. Hamprecht, ref. 1, p. 475.
[45] P. Calderon, J. Furnelle, and J. Christophe, *Biochem. Biophys. Acta*, 1979, **574**, 391.
[46] R. H. Williams and J. Champagne, *Life Sci.*, 1979, **25**, 947.
[47] R. A. Pedersen and J. C. Brown, *Can. J. Physiol. Pharmacol.*, 1979, **57**, 1233.
[48] W. Y. Fujimoto, R. H. Williams, and J. W. Ensinck, *Proc. Soc. Exp. Biol. Med.*, 1979, **160**, 349.

amphibian spinal cord [49] and the central nervous system [50, 51] were described. The effects of a bombesin infusion,[52] a waterload,[53] a test meal,[54] duodenal acidification,[55] and vagal stimulation [56] on release and plasma levels of the various hormones were determined. The influence of different pathological conditions in man [57–59] or of hyperglycaemia in genetically obese mice [60] on plasma hormone levels or on the number of hormone-containing cells was investigated. A new method for bile collection in the guinea-pig was proposed for the testing of gastrointestinal hormones.[61]

Gastrins.—(Figure 1). Valuable reviews on the different aspects of the gastrins have been published during 1979. An account of the history, distribution, and chemistry of the several forms and fragments which have been identified in gastric, intestinal, nervous, and tumour tissues and in circulation,[62] as well as aspects of the structure of gastrin and gastrin-like forms and fragments in gut and brain,[63] peptides of the gastrin cell,[64] and gastrin[65] have appeared. The action of gastrin on gastrointestinal tract secretion, mobility, and absorption;[66] the growth stimulating actions;[67] the interactions between gastrin and the exocrine function of the pancreas;[68] the physiology and pathology of gastrin and hypergastrinaemias;[69] the neurovegetative regulation of gastrin secretion;[70] and the possible physiological role as neurotransmitter in central and peripheral nerves [71] have been reviewed. The radioimmunological methods for gastrin and gastrin-related peptides [72] and the use

[49] J. W. Phillis and J. R. Kirkpatrick, *Can. J. Physiol. Pharmacol.*, 1979, **57**, 877.
[50] K. Fuxe, K. Andersson, T. Hoekfelt, V. Mutt, L. Ferland, L. F. Agnati, D. Ganten, S. Said, P. Eneroth, and J. A. Gustafsson, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 2333.
[51] S. Ishibashi, Y. Oomura, T. Okajima, and S. Shibata, *Physiol. Behav.*, 1979, **23**, 401.
[52] S. R. Bloom, M. A. Ghatei, N. D. Christofides, A. M. Blackburn, T. E. Adrian, E. Lezoche, N. Basso, F. Carlei, and F. Speranzo, *J. Endocrinol.*, 1979, **83**, 51P.
[53] N. D. Christofides, D. L. Sarson, R. H. Albuquerque, T. E. Adrian, M. A. Ghatei, I. M. Modlin, and S. R. Bloom, *Experientia*, 1979, **35**, 1521.
[54] P. G. Burhol, H. L. Waldum, R. Jorde, and I. Lygren, *Scand. J. Gastroenterol.*, 1979, **14**, 939.
[55] S. R. Bloom, S. J. Mitchell, G. R. Greenberg, N. Christofides, W. Domschke, S. Domschke, P. Mitznegg, and L. Demling, *Acta Hepato-Gastroenterol.*, 1978, **25**, 365.
[56] S. Guzman, J. A. Chayviálle, W. A. Banks, P. L. Rayford, and J. C. Thompson, *Surgery* (*St. Louis*), 1979, **86**, 329.
[57] C. Owyang, L. J. Miller, E. P. DiMagno, L. A. Brennan, jun., and V. L. W. Go, *Mayo Clin. Proc.*, 1979, **54**, 769.
[58] H. S. Besterman, G. C. Cook, D. L. Sarson, N. D. Christofides, M. G. Bryant, M. Gregor, and S. R. Bloom, *Br. Med. J.*, 1979, **2**, 1252.
[59] K. Sjoelund, J. Alumets, N. O. Berg, R. Haakanson, and F. Sundler, *Gut*, 1979, **20**, 547.
[60] T. E. Adrian, M. G. Bryant, and S. R. Bloom, *J. Endocrinol.*, 1979, **81**, 156 P.
[61] G. Perret, M. L. Gelin, M. Vagne, V. Mutt, M. Faivre, and A. Desvigne, *Gastroenterol. Clin. Biol.*, 1978, **2**, 817.
[62] R. A. Gregory, *Bioorg. Chem.*, 1979, **8**, 497.
[63] R. A. Gregory, ref. 16, p. 47.
[64] L. I. Larsson, ref. 16, p. 5.
[65] V. Guarner, *Gac. Med. Mex.*, 1979, **115**, 215.
[66] H. Ketterer, ref. 2, p. 112.
[67] L. R. Johnson, K. Takeuchi, and A. B. Dembinski, ref. 16, p. 139.
[68] H. Shapiro, F. E. Rosato, N. J. Jackson, and D. A. Dreiling, *Am. J. Gastroenterol.*, 1979, **71**, 53.
[69] M. J. Treffot and A. Monges, *Mediterr. Med.*, 1979, **7**, 41, 45, 49, and 55.
[70] N. Elicio, N. Boletto, P. Pronzato, R. Sturaro, and B. Vatteroni, *Arch. Sci. Med.*, 1979, **136**, 523.
[71] J. F. Rehfeld, N. Goltermann, L. I. Larsson, P. M. Emson, and C. M. Lee, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 2325.
[72] B. M. Jaffe and J. H. Walsh, in 'Methods Hormone Radioimmunoassay' (2nd Ed.), ed. B. M. Jaffe and H. R. Behrman, Academic Press, London, 1979, p. 455.

G–34	Pyr-Leu-Gly-Pro-Gln-Gly-His-Pro-Ser-Leu-Val-Ala-Asp-Pro-Ser-Lys-
CCK–33	Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile -Lys-Asn-Leu-Gln-Ser-
CCK–39	Tyr-Ile -Gln-Gln-Ala-Arg-Lys-Ala-Pro-Ser-Gly-Arg-Val-Ser-Met-Ile -Lys-Asn-Leu-Gln-Ser-
G–14	Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2
G–17	Pyr-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2
G–34	-Lys-Gln-Gly-Pro-Trp-Leu-Glu-Glu-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH_2
CCK–33	-Leu-Asp-Pro-Ser-His-Arg-Ile -Ser-Asp-Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2
CCK–39	-Leu-Asp-Pro-Ser-His-Arg-Ile -Ser-Asp-Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH_2
Caerulein	Pyr-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH_2

Figure 1 *Sequences of human gastrins,*[a,b] *porcine cholecystokinin-pancreocymins (CCK),*[c] *and caerulein*[a,c]

of region-specific antisera have also been reviewed.[73] A nomenclature system based on the number of residues has been proposed.[74]

Final proof that minigastrin is the *C*-terminal tetradecapeptide of gastrin has been obtained by sequence studies at the *N*-terminus, by total synthesis, and by equal potency with G-17 in stimulating gastric acid secretion.[75] Fragments of the revised sequence of the *N*-terminal region of porcine big gastrin (G-34), including the entire sequence of the *N*-terminal tryptic fragment 1—19, which differs from the original sequence in the substitution of Pro for His at position 7 and His for Pro at position 9 have been synthesized [76] and proven to have the full immunoreactivity compared with natural porcine G-34.[76, 77] A new component with gastrin activity has been isolated from hog intestine and found identical with the common *C*-terminal tetrapeptide amide of gastrin and CCK by immunochemical methods and chromatography.[78] The isolation of a heptacosapeptide with potent gastrin releasing activity and its tentative structure have been reported.[79] A striking homology in the *C*-terminal region with bombesin, which exerts similar biological activity, can be observed. Its function as a physiological releasing factor is yet unknown, however, its potent bioactivities and presence in the mammalian gut suggest an important functional role. The *C*-terminal tetrapeptide having L-2-aminoxy-3-phenylpropionic acid in place of Phe has been synthesized and found devoid of agonistic and antagonistic activity.[80] The structure–activity relationship of a number of pentagastrin analogues containing α-aminohydroxy-acids has been reviewed.[81] Several analogues of tetragastrin including dimerized forms of Leu-tetragastrin with muconic acid and adipic acid have been prepared;[82] while the latter had normal activities when tested in rats with Lawrence's conductometric method the former showed 750% of the pentagastrin activity. Slightly modified *N*-terminal and *C*-terminal fragments and their coupling to proteins have been described.[83] Biological testing indicated that introduction of additional ionic groups in the *C*-terminal region decreased the ability to stimulate acid secretion. Tetragastrin, the corresponding tripeptide and related compounds with [^{13}C]-enriched methionine have been prepared by solution synthesis.[84] The physiological and biological activity of the tetrapeptide was proved to be identical to tetragastrin.

High-pressure liquid chromatography (h.p.l.c.) was used to separate un-sulphated human G-13, G-17, G-34, and their corresponding Leu-analogues with

[73] G. J. Dockray, ref. 16, p. 73.
[74] G. J. Dockray, J. F. Rehfeld, and J. H. Walsh, ref. 16, p. 95.
[75] R. A. Gregory, H. J. Tracey, J. I. Harris, M. J. Runswick, S. Moore, G. W. Kenner, and R. Ramage, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 73.
[76] A. M. Choudhury, K. Y. Chu, G. W. Kenner, S. Moore, K. L. Ramachandran, and R. Ramage, *Bioorg. Chem.*, 1979, **8**, 471.
[77] G. J. Dockray, R. A. Gregory, L. Hood, and M. Hunkapiller, *Bioorg. Chem.*, 1979, **8**, 465.
[78] J. F. Rehfeld and L. I. Larsson, ref. 16, p. 85.
[79] T. J. McDonald, H. Joernvall, G. Nilsson, M. Vagne, M. Ghatei, S. R. Bloom, and V. Mutt, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 227.
[80] M. T. Briggs and J. S. Morley, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2138.
[81] L. Kisfaludy, M. Low, I. Schön, and O. Nyeki, *Acta Chim. Acad. Sci. Hung.*, 1979, **100**, 101 (*Chem. Abs.*, 1979, **91**, 186898).
[82] B. Penke, M. Zarandi, K. Kovacs, J. Nafrady, V. Varro, M. Szuecs, G. Toth, M. Wollemann, A. Seprodi, and I. Teplan, ref. 1, p. 151.
[83] A. N. Prusakov, M. A. Samartsev, and V. F. Martynov, *Bioorg. Khim.*, 1979, **5**, 497 (*Chem. Abs.*, 1979, **91**, 175708).
[84] S. Yamada, *Kyoto-furitsu Ika Daigaku Zasshi*, 1979, **88**, 583 (*Chem. Abs.*, 1979, **91**, 135814).

good success [85] and criteria for purity and identity have been discussed.[86] Field desorption mass spectra, using the *C*-terminal gastrin-tetrapeptide, has revealed sufficient molecular ions and fragmentation to obtain partial sequence information.[87]

Further efforts have been undertaken to trace the sources for the heterogeneity of the gastrins by studying different gastrointestinal endocrine cell types,[88-92] biosynthesis in different species,[93] and through different life stages,[94-96] by detection and partial characterization of gastrin mRNA,[97,98] by investigating tumour tissues,[99] by comparison of food responses in normal and ulcer patients [100] and by further studies of the metabolism of endogenous [101] and exogenous [102,103] gastrin, as well as of *C*-terminal penta-peptides.[104,105] Since all these factors contribute to the diversity of the molecular forms in the gastrin family and since in addition the well known overlap with CCK in the *C*-terminal region exists, there is still a number of conflicting results and interpretations. From the size of the gastrin mRNA isolated from preparations of hog antrum the existence of a gastrin precursor peptide containing 110—140 amino-acids is proposed [97,98] which would be processed at both ends into the known gastrins.

I.v. infusion of equimolar doses of the unsulphated human G-14 and G-17 produced equimolar increases over the basal level of serum immunoreactive gastrin and no statistical differences in gastric acid output. There was also no significant difference in half-times for disappearance, clearance rates, calculated volumes of distribution, or mean plateau serum levels.[106] Sulphated and unsulphated human gastrin were found to have similar potencies, whereas for the hexapeptide CCK-8 and caerulein the sulphated forms were more potent than the unsulphated forms.[107] All forms from G-4 to G-34 stimulated the gastric mucosal protein synthesis.[108]

[85] W. Goehring, L. Moroder, and E. Wünsch, ref. 1, p. 127.
[86] E. Wünsch, ref. 1, p. 115.
[87] M. Przybylski, I. Luederwald, E. Kraas, W. Voelter, and S. D. Nelson, *Z. Naturforsch., B: Anorg. Chem., Org. Chem.*, 1979, **34**, 736.
[88] L. I. Larsson and J. F. Rehfeld, *Nature (London)*, 1979, **277**, 575.
[89] L. I. Larsson and J. F. Rehfeld, *Histochemistry*, 1978, **58**, 23.
[90] J. F. Rehfeld and L. I. Larsson, *Acta Physiol. Scand.*, 1979, **105**, 117.
[91] A. M. J. Buchan, J. M. Polak, E. Solcia, and A. G. E. Pearse, *Nature (London)*, 1979, **277**, 138.
[92] C. Vaillant, G. Dockray, and C. R. Hopkins, *J. Histochem. Cytochem.*, 1979, **27**, 932.
[93] J. F. Rehfeld and K. Uvnaes-Wallensten, *J. Physiol. (London)*, 1978, **283**, 379.
[94] T. Lehy, L. Gres, and E. Ferreira de Castro, *Cell Tissue Res.*, 1979, **198**, 325.
[95] N. S. Track, C. Creutzfeldt, J. Litzenberger, C. Neuhoff, R. Arnold, and W. Creutzfeldt, *Digestion*, 1979, **19**, 292.
[96] T. Yabana, Y. Kakumoto, K. Watanabe, N. Sekiyama, S. Maeda, Y. Narasaki, S. Tsunoda, T. Kondo, S. Takasu, *et al.*, *Gastroenterol. Jpn.*, 1978, **13**, 333 (*Chem. Abs.*, 1979, **90**, 69983).
[97] B. E. Noyes, M. Mevarech, R. Stein, and K. L. Agarwal, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1770.
[98] M. Mevarech, B. E. Noyes, and K. L. Agarwal, *J. Biol. Chem.*, 1979, **254**, 7472.
[99] R. M. Bolman, III, C. W. Cooper, J. F. Obie, and S. A. Wells, jun., *Surg. Forum*, 1978, **29**, 386.
[100] I. L. Taylor, G. J. Dockray, J. Calam, and R. J. Walker, *Gut*, 1979, **20**, 957.
[101] M. Sugiyama, K. Yamanaka, T. Takizawa, K. Watanabe, S. Eto, S. Shi, T. Fukushima, and S. Tsuchiya, *Saishin Igaku*, 1979, **34**, 1339 (*Chem. Abs.*, 1979, **91**, 121 176).
[102] J. C. Thompson, O. L. Llanos, R. K. Teichmann, A. Schafmayer, and P. L. Rayford, *World J. Surg.*, 1979, **3**, 469.
[103] P. Saksoe, J. F. Rehfeld, and J. Christiansen, ref. 16, p. 263.
[104] L. Varga, M. Sasvari, B. Penke, L. Balaspiri, and V. Varro, *Acta Hepato-Gastroenterol.*, 1979, **26**, 499.
[105] P. Pham Van Chuong, J. L. Morgat, P. Fromageot, M. Dubrasquet, L. Ory-Lavollee, and S. Bonfils, ref. 1, p. 157.
[106] D. C. Carter, I. L. Taylor, J. Elashoff, and M. I. Grossman, *Gut*, 1979, **20**, 705.

Gastrin *C*-terminal immunoreactive substances have been identified in the hypothalamus and in the anterior preoptic area of an amphibian.[109] Similar activities were found in the hypothalamus, the median eminence, and the neurohypophysis of rats, whereas in most other parts of the brain CCK-8 has been observed.[110] The existence of mucosal gastrin receptors has been further demonstrated by isolating membrane preparations from rat gastric mucosa specifically binding gastrin.[111] Since trypsin treatment abolished the specific binding, a protein nature of the gastrin receptor has been proposed.[112, 113] On the other hand the existence of gastrin receptor sites in porcine gastric mucosa could not be demonstrated unambiguously.[82]

Serum levels of gastrin have been determined by radioimmunoassays in a number of conditions, *e.g.* in healthy subjects of various ages [114, 115] and various blood groups,[116] in fasting subjects,[117] in healthy men and patients with chronic duodenal ulcer,[118] in uraemia,[119] and in pregnancy.[120] No correlation was found between concentrations of gastrin in human serum and in cerebrospinal fluid, an indication that gastrin is synthesized in central neurons rather than transported from peripheral blood.[121]

The pentagastrin-stimulated gastric acid secretion is inhibited by i.v. application of TRH in man [122] and competitively by somatostatin in dogs.[123] Somatostatin antiserum stimulates gastrin secretion from the perfused rat stomach, a fact attributed to neutralization of endogenous somatostatin which exerts a continuous restraint on gastrin secretion.[124] The inhibitory actions of cimetidine [125–129] and an analogue of prostaglandin [130] have been further investigated.

[107] B. H. Hirst, J. D. Reed, B. Shaw, L. A. Smeaton, E. L. Blair, and R. De Castiglione, ref. 1, p. 413.
[108] N. Goltermann and A. P. Nandi Majumdar, *Horm. Res.*, 1979, **11**, 142.
[109] J. Doerr-Schott, J. C. Garaud, and R. O. Clauss, *Cell Tissue Res.*, 1979, **203**, 65.
[110] I. Loren, J. Alumets, R. Haakanson, and F. Sundler, *Histochemistry*, 1979, **59**, 249.
[111] K. Takeuchi, G. R. Speir, and L. R. Johnson, *Am. J. Physiol.*, 1979, **237**, E284.
[112] K. Takeuchi, G. R. Speir, and L. R. Johnson, *Am. J. Physiol.*, 1979, **237**, E295.
[113] L. R. Johnson, K. Takeuchi, and G. R. Speir, ref. 1, p. 401.
[114] A. Archimandritis, G. Alegakis, G. Theodoropoulos, A. Kalos, G. Drivas, and K. Melissinos, *Acta Hepato-Gastroenterol.*, 1979, **26**, 58.
[115] D. J. Sanders P. K. Lund, and S. Zahedi-Asl, *Lancet*, 1979, *i*, 1193.
[116] K. Melissinos, G. Alegakis, A. J. Archimandritis, and G. Theodoropoulos, *Acta Hepato-Gastroenterol.*, 1978, **25**, 482.
[117] N. S. Track, L. M. Watters, and J. Gauldie, *Clin. Biochem. (Ottawa)*, 1979, **12**, 109.
[118] R. J. Dash, S. L. Broor, V. Mehta, S. R. Naik, and S. K. Mehta, *Bull. Postgrad. Inst. Med. Educ. Res., Chandigarh*, 1979, **13**, 97.
[119] R. Haellgren, J. Landelius, K. E. Fjellstroem, and G. Lundqvist, *Gut*, 1979, **20**, 763.
[120] A. Francavilla, C. Panella, D. Sansonno, L. Polimeno, O. Albano, G. Mollica, A. Restaino, L. Selvaggi, and B. Del Salvatore, *Clin. Exp. Obstet. Gynecol.*, 1979, **6**, 222.
[121] C. Kruse-Larsen and J. F. Rehfeld, *Brain Res.*, 1979, **176**, 189.
[122] L. O. Dolva, K. F. Hanssen, and A. Berstad, *Scand. J. Gastroenterol.*, 1979, **14**, 33.
[123] G. O. Barbezat, E. R. Kaplan, F. S. B. Bowey, M. Berelowitz, and R. Van Hoorn-Hickman, *Eur. J. Pharmacol.*, 1979, **53**, 255.
[124] B. Saffouri, G. Weir, K. Bitar, and G. Makhlouf, *Life Sci.*, 1979, **25**, 1749.
[125] E. Aadland and A. Berstad, *Int. Congr. Ser.—Excerpta Med.*, 1978, **443** (Cimetidine), p. 47.
[126] F. Halter, L. Witzel, W. H. Haecki, A. J. Olah, P. Heitz, and L. Varga, *Int. Congr. Ser.– Excerpta Med.*, 1978, **443** (Cimetidine), p. 108.
[127] B. I. Hirschowitz and R. G. Gibson, *Am. J. Gastroenterol.*, 1978, **70**, 437.
[128] P. Vezzadini, G. Bonora, P. Tomassetti, and G. Labo, *Acta Gastro-Enterol. Belg.*, 1978, **41**, 463.
[129] R. F. A. Logan, J. A. H. Forrest, G. P. McLoughlin, G. Lidgard, and R. C. Heading, *Digestion*, 1978, **18**, 220.
[130] D. G. Colton, D. R. Driskili, E. L. Phillips, P. Poy, and E. Z. Dajani, *Arch. Int. Pharmacodyn. Ther.*, 1978, **236**, 86.

Vagal stimulation led to a significant increase of blood levels of gastrin as well as other gastrointestinal hormones;[56] however, the difficulties in standardizing an experimental model to study the many vagal effects on acid secretion have been emphasized.[131]

Indications for the existence of labile complexes between gastrin and serum proteins, especially ceruloplasmin [132] and an albumin population,[133] as means for selective transportation of gastrin have been presented.

Further evidence for the limited physiological action of gastrin as a trophic hormone has been published.[134]

Cholecystokinin-Pancreozymin (CCK).—(Figure1). The chemistry,[135] the physiology and pharmacology [136] of CCK, and the pharmacology of sincalide, a synthetic CCK-8 preparation [137] have been reviewed. Other articles deal with the presence of CCK and the *C*-terminal octapeptide of CCK (CCK-8) in cerebral cortex and its relation to appetite regulation by neurons,[138] the occurrence of CCK and gastrin in central and peripheral neurons,[71] and the characterization of molecular forms of CCK, VIP, and bombesin-like immunoreactivity in nerves and endocrine cells.[139] The development and application of region-specific antisera against CCK and gastrin [73] and a critical evaluation of the radioimmunoassay methods for CCK [140] are topics of further reviews.

A new nomenclature system based on the number of residues in the gastrin and CCK peptides was proposed in order to cope with the multiplicity of molecular forms in which these hormones occur in biological sources.[74] The use of Z-Tyr($SO_3Ba_{\frac{1}{2}}$)$OBa_{\frac{1}{2}}$ or otherwise suitably protected tyrosine-*O*-sulphate esters was suggested for the introduction of the *O*-sulphate moiety with the intention of preventing the undesired side-reactions usually occurring during the sulphatation of larger peptide sequences. This concept was exemplified in the synthesis of [Thr[28], Leu[31]]-CCK_{25-33} in which Z-Tyr($SO_3Ba_{\frac{1}{2}}$)$OBa_{\frac{1}{2}}$ was coupled in good yield *via* dicyclohexylcarbodi-imide/hydroxybenzotriazole activation. The purified CCK analogue possessed *ca.* 20% of the gallbladder-contracting activity of CCK-8.[141] Eight analogues of CCK-8 and the corresponding *C*-terminal tetrapeptides in which the tryptophan residue has been replaced by benzofuryl-L-alanine, benzothienyl-L-alanine, N^{in}-methyl-L-tryptophan, 5-fluoro-, 6-fluoro-, and 4,5,6,7-tetrafluoro-L-tryptophan have been prepared by step-wise elongation using different active esters for activation. When the biological potencies of these analogues were determined with regard to pancreatic secretion and stimulation of the adenylate

[131] P. K. Lund and D. J. Sanders, ref. 16, p. 157.
[132] M. V. Polosatov, P. K. Klimov, C. G. Masevich, M. A. Samartsev, and E. Wünsch, *Acta Hepato-Gastroenterol.*, 1979, **26**, 154.
[133] P. K. Klimov and M. V. Polosatov, *Z. Med. Laboratoriumsdiagn.*, 1979, **20**, 187.
[134] J. Oscarson, R. Haakanson, G. Liedberg, G. Lundqvist, F. Sundler, and J. Thorell, *Acta Physiol. Scand., Suppl.*, 1979, **475**, 27.
[135] V. Mutt, ref. 16, p. 57.
[136] J. Plessier, *Med. Chir. Dig.*, 1979, **8**, 519.
[137] S. L. Engel, M. A. Ondetti, and B. Rubin, *Pharmacol. Biochem. Prop. Drug Subst.*, 1979, **2**, 516.
[138] E. Straus and R. S. Yalow, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1979, **38**, 2320.
[139] G. J. Dockray, C. Vaillant, R. Dimaline, J. B. Hutchison, and R. A. Gregory, ref. 1, p. 501.
[140] R. F. Harvey, ref. 72, p. 495.
[141] L. Moroder, L. Wilschowitz, E. Jaeger, S. Knof, P. Thamm, and E. Wünsch, *Hoppe-Seyler's Z. Physiol. Chem.*, 1979, **360**, 787, and ref. 1, p. 129.

cyclase system, it became evident that the charge-transfer capacity which varied greatly between the tryptophan analogues plays an important role in the expression of biological activity but that hydrogen-bonding may be at least equally important.[142] CCK-8 and three of its analogues in which the methionine residue in position 3 and/or 6 was exchanged against L-methoxinine (γ-methoxy-L-α-aminobutyric acid) were synthesized by classical fragment-condensation procedures. The biological potencies of these compounds as measured by their gallbladder-contracting activity *in vitro* showed that methionine in position 6 cannot be replaced even by its oxygen analogue without considerable loss of potency, whereas the same change in position 3 has no effect.[143] Reverse-phase h.p.l.c. has been used for controlling the purity and for purifying peptides of the CCK family.[141, 144] The cholekinetic and choleretic activities of desamino-CCK-8 have been compared with those of a commercial CCK-preparation and found to be almost the same.[145] A radioactive derivative of CCK with good stability, full biological activity, and good cross-reactivity with *N*- and *C*-terminal-specific antisera resulted from the reaction of CCK-33 with 3′-^{125}I-phenylpropionic acid hydroxysuccinimide ester (Bolten–Hunter reagent).[146] CCK-8, caerulein, and some of their modified fragments were tested both in *O*-sulphated and unsulphated form in comparison with gastrins for acid- and pepsin-releasing activity in cats, and it was found that the CCK compounds were more active with regard to acid secretion in the sulphated than in the unsulphated form. Pepsin release, however, was not affected in the same way. Caerulein inhibited stimulation of pepsin secretion by unsulphated CCK-8 or pentagastrin.[107]

The cause for the different chromatographic and electrophoretic behaviour of two peptides isolated from sheep brain, both of which have the same amino-acid sequence as CCK-8, is yet unknown.[147] A fraction with CCK-8-like immunoreactivity and CCK-like biological activity was also obtained during the isolation of substance P from brain.[148]

The distribution of gastrin and CCK-like peptides in the rat brain has been studied by immunocytochemical techniques.[110, 149] Immunocytochemical and radioimmunological studies demonstrated that CCK is present in five molecular forms in both the central and peripheral nervous systems.[150] The probability that CCK plays a role in synaptic function was further strengthened by the localization of CCK-like immunoreactivity in isolated nerve terminals[151] and by its release from synaptosome-enriched fractions of rat cerebral cortex in potassium- and

[142] H. M. Rajh, M. J. Smyth, G. I. Tesser, J. J. H. H. M. de Pont, R. J. F. Nivard, and S. L. Bonting, ref. 1, p. 145.
[143] D. Gillessen, A. Trzeciak, R. K. M. Müller, and R. O. Studer, *Int. J. Pept. Protein Res.*, 1979, **13**, 130.
[144] L. Pradayrol, N. Vaysse, J. Cassigneul, and A. Ribet, ref. 1, p. 95.
[145] M. Roenfanz, E. Morgenstern, A. Hagen, G. Meineke, and E. Goeres, *Pharmazie*, 1979, **34**, 346.
[146] H. Sankran, C. W. Deveney, I. D. Goldfine, and J. A. Williams, *J. Biol. Chem.*, 1979, **254**, 9349.
[147] G. J. Dockray, R. A. Gregory, J. B. Hutchison, J. I. Harris, and M. J. Runswick, *Nature (London)*, 1978, **274**, 711.
[148] G. Zetler, D. Cannon, D. Powell, P. Skrabanek, and J. J. Vanderhaeghen, *Arch. Int. Pharmacodyn. Ther.*, 1979, **238**, 28, and *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1978, **305**, 189.
[149] R. B. Innis, F. M. A. Correa, G. R. Uhl, B. Schneider, and S. H. Snyder, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 521.
[150] L. I. Larsson and J. F. Rehfeld, *Brain Res.*, 1979, **165**, 201.
[151] M. Pinget, E. Straus, and R. S. Yalow, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 6324.

calcium-containing solutions.[152] The presence of two CCK-like peptides which resemble the *N*- and *C*-terminal tetrapeptide fragments of CCK-8 was detected in hog cortical extracts by radioimmunological analysis and the *C*-terminal tetrapeptide appeared to occur in amounts larger than those of CCK-8.[153] Radioimmunoassay and immunocytochemical investigations showed that a peptide corresponding to the *C*-terminal tetrapeptide of gastrin and CCK is the predominating molecular form of these hormones in the gut.[78, 90] The innervation of the pancreas by gastrin/CCK- and VIP-immunoreactive nerves was revealed by immunohistochemistry.[154, 155] The distribution of three distinct types of cells which store *C*-terminal gastrin/CCK immunoreactivity was determined in the gastrointestinal tract of rats by radioimmunoassay and immunocytochemistry.[89] Extracts of turkey brain and jejunum contained a factor closely resembling CCK-8. Antral extracts, however, contained factors similar to but distinguishable from the mammalian forms of CCK and gastrin.[156] The detection of two factors from brain and gut of the lamprey, the chromatographic and immunoreactive behaviour of which quite closely resembled that of *C*-terminal CCK/gastrin peptides but not that of the entire gastrin, demonstrates that the *C*-terminal region of CCK/gastrin has been strongly conserved during evolution.[157]

The technique of adsorption of basic peptides to surfaces of silica or talc has been used in the course of radioimmunoassay studies to separate CCK-39 and CCK-33 from CCK-12 and CCK-8. The larger molecular forms were completely eluted from silica but not from talc with dilute hydrochloric acid.[158] A sensitive bioassay which lends itself to automation has been developed for the quantitative determination of CCK in serum samples in order to circumvent the problems associated with the radioimmunological analysis of such specimens.[159]

A nontrypsin CCK-converting enzyme which splits CCK into smaller immunoreactive fragments, but not big gastrin into gastrin, was isolated from canine and porcine cerebral cortex and partially purified.[160] Furthermore enzymatic activity which splits CCK-8 was detected in various canine organ homogenates; its concentration was highest in renal cortex. Preliminary characterization indicates mitochondrial origin.[161, 162] The secretion and clearance rate of CCK was studied in man and a half-life of 2.46 min and a calculated clearance rate of $9.13\,ml\,kg^{-1}\,min^{-1}$ was found.[163]

The role of CCK as satiety signal was further investigated. One group [164] found a remarkable constancy in the CCK and CCK-8 content of brain extracts of rats and

[152] M. Pinget, E. Straus, and R. S. Yalow, *Life Sci.*, 1979, **25**, 339.
[153] J. F. Rehfeld and N. R. Goltermann, *J. Neurochem.*, 1979, **32**, 1339.
[154] L. I. Larsson and J. F. Rehfeld, *Scand. J. Gastroenterol.*, 1979, **14**, 433.
[155] L. I. Larsson, *J. Histochem. Cytochem.*, 1979, **27**, 1283.
[156] G. J. Dockray, *Experientia*, 1979, **35**, 628.
[157] A. L. Holmquist, G. J. Dockray, G. L. Rosenquist, and J. H. Walsh, *Gen. Comp. Endocrinol.*, 1979, **37**, 474.
[158] E. Straus, A. Malesci, M. Pinget, and R. S. Yalow, *Life Sci.*, 1979, **25**, 343.
[159] C. E. Marshall, E. H. Egberts, and A. G. Johnson, *J. Endocrinol.*, 1978, **79**, 17.
[160] E. Straus, A. Malesci, and R. S. Yalow, *Proc. Natl. Acad. Sci. USA*, 1978, **75**, 5711.
[161] J. Lonovics, F. Hajnal, E. Fodor, I. Szabo, and V. Varro, *Kiserl. Orvostud.*, 1979, **31**, 386 (*Chem. Abs.*, 1979, **91**, 170 521).
[162] J. Lonovics, F. Hajnal, P. Mara, I. Szabo, and V. Varro, *Acta Hepato-Gastroenterol.*, 1979, **26**, 222.
[163] R. F. Harvey, A. Ederle, I. Vantini, L. A. Scuro, J. M. Oliver, and A. E. Read, *Ital. J. Gastroenterol.*, 1978, **10**, 100.
[164] B. S. Schneider, J. W. Monahan, and J. Hirsch, *J. Clin. Invest.*, 1979, **64**, 1348.

mice despite extreme variations of the nutritional status including genetic obesity, whereas another group [165] found significantly less CCK-8 immunoreactivity in cerebral cortex extracts of genetically obese mice with hyperphagia than in those of the non-obese littermates. Experiments with CCK or CCK-8 infusions or injections in rats proved that CCK provoked a consistent reduction in food intake independent of the duration of fasting time;[166] that the reduction of food intake is dose-dependent although the very first meal after deprivation is always taken;[167] that impure CCK was five times as potent as CCK-8 on a weight basis and that gastrin, secretin, and GIP has no effect;[168] that doses of CCK which reduced the meal size did not slow gastric emptying;[169] that the effect on suckling time and milk intake was age-dependent;[170] and that fat rats were less sensitive to CCK than normal ones.[171] CCK or CCK-8 also reduced food intake in sheep [172] and monkeys,[173] decreased appetite in man, and created a mental situation similar to postprandial satiety and deactivation;[174] CCK injected i.p., however, had no effect on the daily food consumption of the domestic fowl.[175]

A great number of *in vitro* and *in vivo* studies deal with the various actions of CCK or CCK-8 on different organs and cell preparations. The main effort was concentrated on the effect of CCK on enzyme synthesis [176] and secretion [176–182] from the exocrine pancreas, on insulin and glucagon content [183] and release [181, 184] from the endocrine pancreas and on the influence of insulin,[185] VIP,[186] somatostatin,[187] and bile salts [188] on this secretion. In the course of these studies it was discovered that dibutyryl-cGMP acts as a reversible, competitive antagonist of CCK and CCK-like peptides with regard to amylase release from pancreatic acini. This antagonism is specific to the extent that it does not affect the secretion of amylase stimulated by secretin or VIP.[189] With the use of this

[165] E. Straus and R. S. Yalow, *Science*, 1979, **203**, 68.
[166] K. Mueller and S. Hsiao, *Physiol. Behav.*, 1979, **22**, 809.
[167] S. Hsiao, C. H. Wang, and T. Schallert, *Physiol. Behav.*, 1979, **23**, 909.
[168] D. N. Lorenz, G. Kreielsheimer, and G. P. Smith, *Physiol. Behav.*, 1979, **23**, 1065.
[169] K. A. Houpt and T. R. Houpt, *Physiol. Behav.*, 1979, **23**, 925.
[170] E. M. Blass, W. Beardsley, and W. G. Hall, *Am. J. Physiol.*, 1979, **236**, E567.
[171] C. L. McLoughlin and C. A. Baile, *Pharmacol. Biochem. Behav.*, 1979, **10**, 87.
[172] M. A. Della-Fera and C. A. Baile, *Science*, 1979, **206**, 471, and *Ann. Rech. Vet.*, 1979, **10**, 234.
[173] J. D. Falasco, G. P. Smith, and J. Gibbs, *Physiol. Behav.*, 1979, **23**, 887.
[174] G. Stacher, H. Bauer, and H. Steinringer, *Physiol. Behav.*, 1979, **23**, 325.
[175] N. Snapir and Z. Glick, *Physiol. Behav.*, 1978, **21**, 1051.
[176] B. Carlsoo and A. Danielsson, *Acta Hepato-Gastroenterol.*, 1979, **26**, 37.
[177] M. V. Singer and H. Sarles, *Scand. J. Gastroenterol.*, 1978, **13**, 969.
[178] J. C. Dagorn and A. Estival, *J. Physiol.* (*London*), 1979, **290**, 51.
[179] P. C. Lee, *Digestion*, 1979, **19**, 6.
[180] S. Moss, G. R. Birch, R. W. Lobley, and R. Holmes, *Scand. J. Gastroenterol.*, 1979, **14**, 1001.
[181] M. Otsuki, C. Sakamoto, H. Yuu, M. Maeda, S. Morita, A. Ohki, N. Kobayashi, K. Terashi, K. Okano, *et al.*, *J. Clin. Invest.*, 1979, **63**, 478.
[182] M. L. Steer and T. Manabe, *J. Biol. Chem.*, 1979, **254**, 7228.
[183] T. Fujita, Y. Matsunari, K. Sato, M. Hayashi, and Y. Koga, *Endocrinol. Jpn.*, 1979, **26**, 35 (*Chem. Abs.*, 1979, **91**, 14303).
[184] A. Kopylow, Z. Kaszuba, A. Stachowski, and K. Marlicz, *Pol. Arch. Med. Wewn*, 1979, **61**, 127 (*Chem. Abs.*, 1979, **91**, 50155).
[185] T. Kanno and A. Saito, *Int. Congr. Ser. – Excerpta Med.*, 1979, **468** (Proinsulin, Insulin, C-Peptide), p. 148.
[186] S. R. Peikin, A. J. Rottman, S. Batzri, and J. D. Gardner, *Am. J. Physiol.*, 1978, **235**, E743.
[187] T. A. Miller, F. S. Tepperman, W.-F. Fang, and E. D. Jacobson, *J. Surg. Res.*, 1979, **26**, 488.
[188] M. Otte, W. Schmiedt, M. M. Forell, and G. R. Thurmayr, *Verh. Dtsch. Ges. Inn. Med.*, 1979, **85**, 596.
[189] S. R. Peikin, C. L. Costenbader, and J. D. Gardner, *J. Biol. Chem.*, 1979, **254**, 5321.

antagonist bombesin was shown to act at other sites on the pancreatic acinar cell than peptides of the CCK family.[190] Further studies dealt with the effects of CCK on GTPase activity of pancreatic plasma membranes;[191] cGMP concentration in pancreatic duct fragments;[192] secretion of cyclic nucleotides, calcium, and bicarbonate from pancreatic tissue;[193] and on lipid metabolism,[194] the glucose transport system,[195] cell to cell coupling,[196] and the ultrastructure of intracellular junctions [197] in the same tissue as well as on the calcium requirements in the action of CCK on acinar cells.[198]

Besides their well known effects on pancreatic tissue, CCK-peptides also stimulated the production and release of a myocardial depressant factor from pancreatic slices,[199] the release of certain intestinal hydrolases,[200] of corticosterone from adrenal cortex,[201] and of growth hormone from anterior pituitary quarters and cultured GH_3 pituitary tumour cells.[202] Intraventricular injections of CCK increased prolactin and growth hormone levels, decreased those of LH and TSH, but had no effect on FSH concentration.[203] CCK-8 and CCK-4 were also excitatory on certain neurons from rat hippocampal slices.[204] Unsulphated but not sulphated CCK-7 bound to the opiate receptor in rat brain [205] while opioid peptides antagonized the CCK-8-induced concentrations of ileal strips.[206]

The effects of CCK on motility and haemodynamics of the gastrointestinal tract;[207] on myoelectric activity of the isolated colon;[208] the interaction of gastrin, secretin, and CCK on antral smooth muscle;[209] and of CCK-8 and acetylcholine on dispersed smooth muscle cells [210] were studied. CCK-8 given by the i.v., s.c., or intranasal routes increased intestinal motility in man.[211] The potency of caerulein with regard to pancreatic secretion was compared with that of CCK in man [212] and the pressure response to CCK-8 to that of 20% pure CCK in the antrum and fundus of isolated guinea-pig stomachs.[213] The dose-dependence of the pancreatic

[190] H. G. Philpott and O. H. Petersen, *Pfluegers Arch.*, 1979, **382**, 263.
[191] M. Lambert, M. Svoboda, and J. Christophe, *FEBS Lett.*, 1979, **99**, 303.
[192] U. R. Foelsch, M. Nose, and W. Creutzfeld, ref. 1, p. 209.
[193] H. L. Cailla, H. Sarles, and M. V. Singer, *Can. J. Physiol. Pharmacol.*, 1979, **57**, 541.
[194] P. Calderon, J. Furnelle, and J. Christophe, *Biochim. Biophys. Acta*, 1979, **574**, 404.
[195] M. Korc, J. A. Williams, and I. D. Goldfine, *J. Biol. Chem.*, 1979, **254**, 7624.
[196] O. H. Petersen and N. Iwatsuki, ref. 1, p. 191.
[197] K. Tsukiyama, *Arch. Histol. Jpn.*, 1979, **42**, 141 (*Chem. Abs.*, 1979, **91**, 102647).
[198] M. Singh, *Life Sci.*, 1979, **25**, 247.
[199] N. Savion and Z. Selinger, *J. Physiol. (London)*, 1978, **285**, 27 P.
[200] F. Senegas-Balas, D. Balas, L. Pradayrol, J. Laval, and A. Ribet, *Acta Hepato-Gastroenterol.*, 1979, **26**, 486.
[201] S. Itoh, R. Hirota, G. Katsuura, and K. Odaguchi, *Life Sci.*, 1979, **25**, 1725.
[202] J. E. Morley, S. Melmed, J. Briggs, H. E. Carlson, J. M. Hershman, T. E. Solomon, C. Lamers, and D. A. Damassa, *Life Sci.*, 1979, **25**, 1201.
[203] E. Vijayan, W. K. Samson, and S. M. McCann, *Brain Res.*, 1979, **172**, 295.
[204] J. Dodd and J. S. Kelly, *J. Physiol. (London)*, 1979, **295**, 61 P.
[205] P. W. Schiller, A. Lipton, D. F. Horrobin, and M. Bodanszky, *Biochem. Biophys. Res. Commun.*, 1978, **85**, 1332.
[206] G. Zetler, *Eur. J. Pharmacol.*, 1979, **60**, 67.
[207] J. A. J. Schuurkes and G. A. Charbon, *Arch. Int. Pharmacodyn. Ther.*, 1978, **236**, 214.
[208] W. J. Snape, jun. and S. Cohen, *Am. J. Physiol.*, 1979, **236**, E458.
[209] J. W. Fara, M. Praissman, and J. M. Berkowitz, *Am. J. Physiol.*, 1979, **236**, E39.
[210] K. N. Bitar, A. M. Zfass, and G. M. Makhlouf, *Am. J. Physiol.*, 1979, **237**, E172.
[211] J. Lonovics, G. Narai, and V. Varro, *Orv. Hetil.*, 1979, **120**, 3029 (*Chem. Abs.*, 1980, **92**, 158226).
[212] M. Otte and M. M. Forell, *Klin. Wochenschr.*, 1979, **57**, 789.
[213] T. Gerner, *Scand. J. Gastroenterol.*, 1979, **14**, 73.

response to CCK was compared with that of the gallbladder [214] and the effect of substance P on CCK-induced choleresis [215] and of CCK on gastrin-stimulated acid secretion [216] were investigated. Although CCK stimulated the release of lipase from the intestine of the hagfish it had no effect on gallbladder and intestinal muscle contraction.[217]

Secretin.—(Figure 2). Secretin analogues have been reviewed.[218, 219] In a comparison in humans, synthetic and natural secretin increased the volume of pancreatic

	1	5	10
Secretin	His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-	-Glu-Leu-Ser-	-Arg-Leu-Arg-
VIP	His-Ser-Asp-Ala-Val-Phe-Thr-Asp-	-Asn-Tyr-Thr-	-Arg-Leu-Arg-
Glucagon	His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-	-Asp-Tyr-Ser-	-Lys-Tyr-Leu-
GIP	Tyr-Ala-Glu-Gly-Thr-Phe-Ile-	-Ser-Asp-Tyr-Ser-	-Ile-Ala-Met-

	15	20	25
Secretin	Asp-Ser-Ala-	-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-	$-NH_2$
VIP	Lys-Gln-Met-Ala-Val-Lys-Lys-	-Tyr-Leu-Asn-Ser-Ile-	-Leu-Asn-NH_2
Glucagon	Asp-Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr		
GIP	Asp-Lys-Ile-	-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-	-Gln-

	30	35	40
GIP	Gln-Lys-Gly-Lys-Lys-	-Ser-Asp-Trp-Lys-His-	-Asn-Ile-Thr-Gln

Figure 2 *The secretin family (porcine sequences)*

secretion as well as the content of bicarbonate, amylase, and trypsin in a similar but not fully identical way.[220] The synthetic hormone had a greater effect on secretory volume and bicarbonate secretion than the natural hormone after pancreozymin pretreatment, and lipase secretion after administration of synthetic secretin was smaller than after administration of natural secretin. The increase in volume, bicarbonate, and enzyme secretion was slower and reached higher levels after infusion than after bolus injection.

Circular dichroism studies on secretin and some of its analogues modified in the *N*-terminal hexapeptide were performed in order to evaluate a possible correlation between conformational behaviour and biological and immunological properties.[221] Except for the VIP-secretin, where an additional increase of ordered structure attributed to an elongation of the α-helix into the sequence portion 5—6 parallelled enhanced activites, drastic differences in activity are not reflected in the circular dichroism.

[214] L. I. Geller and O. S. Bulgakova, *Fiziol. Chel.*, 1979, **5**, 567 (*Chem. Abs.*, 1979, **91**, 69 112).
[215] I. M. Holm and L. Thulin, *Acta Physiol. Scand.*, 1979, **106**, 293.
[216] E. Corazziari, T. E. Solomon, and M. I. Grossman, *Gastroenterology*, 1979, **77**, 91.
[217] S. R. Vigna and A. Gorbman, *Gen. Comp. Endocrinol.*, 1979, **38**, 356.
[218] W. Koenig, M. Bickel, H. Wissmann, R. Uhmann, and R. Geiger, ref. 1, p. 137.
[219] V. J. Hruby, Report 1978, *Gov. Rep. Announce Index (U.S.)*, 1979, **79**, 29.
[220] P. G. Lankisch, B. Hoppe, W. P. Fritsch, W. Rick, and W. Creutzfeldt, *Verh. Dtsch. Ges. Inn. Med.*, 1979, **85**, 581.
[221] E. Jaeger, B. Filippi, S. Knof, P. Lehnert, L. Moroder, and E. Wünsch, ref. 1, p. 25.

An active principle in porcine brain extracts, which stimulates exocrine pancreas secretion in a secretin-like fashion, behaved during purification like secretin.[222] The *C*-terminal Val-NH_2 and the fact that one of the tryptic fragments had the same chromatographic mobility as the *C*-terminal tryptic fragment of secretin indicate a close relationship with secretin.

VIP-secretin and secretin were successfully separated by h.p.l.c.[223] Secretin was used as model substance for high resolution structure determination by electron microscopy.[224] The evidence reported, that secretin is adsorbed on to glass[225] as well as to plastic [226] surfaces and that this adsorption is overcome by addition of bovine serum albumin, should be taken into consideration when designing future studies.

The determination of secretin by radioimmunological methods has been reviewed [227] and inherent problems have been pointed out.[227, 228] Additional evaluations of radioimmunoassays have been reported.[229–232] Immunoreactive secretin levels in plasma are increased following intraduodenal HCl infusion in humans.[55, 232–235] The secretin response to a brief acidification of the first 4—6 cm of the duodenum was estimated to be sufficient to explain the physiological variations in the concentration of secretin in plasma.[235] Continuous recording of duodenal pH parallelled by short interval measurement of plasma secretin after a liquid test meal demonstrated significant release of secretin in subjects showing variations of the duodenal pH $\leqslant 4.0$.[236] These and other results [237] support the hypothesis that secretin is involved in the bicarbonate response to postprandial duodenal acidification in man, although they do not provide a precise estimate of this contribution. No significant rise however has been reported for fasting healthy young males after a test meal [54] while an increase in plasma levels of immunoreactive secretin during starvation was depressed after re-feeding.[238, 239] Plasma secretin levels in several disease states have been reported.[230, 231, 240] The release of secretin is unlikely to be under vagal control.[56, 241]

[222] V. Mutt, M. Carlquist, and K. Tatemoto, *Life Sci.*, 1979, **25**, 1703.
[223] D. Bataille, J. Besson, C. Gespach, and G. Rosselin, ref. 1, p. 79.
[224] A. P. Korn, *Diss. Abstr. Int. B*, 1979, **40**, 1036.
[225] J. Ogino, K. Noguchi, and K. Terato, *Chem. Pharm. Bull.*, 1979, **27**, 3160.
[226] K. N. Bitar, A. M. Zfass, and G. M. Makhlouf, *Gastroenterology*, 1978, **75**, 1080.
[227] G. Boden and R. M. Wilson, ref. 72, p. 479.
[228] P. G. Burhol and H. L. Waldum, *Acta Hepato-Gastroenterol.*, 1978, **25**, 474.
[229] A. Ishimori, F. Koizumi, K. Tsuda, T. Kawamura, M. Koizumi, M. Hongo, S. Nakame, T. Endo, T. Sato, *et al.*, *Horumon to Rinsho*, 1979, **27**, 457 (*Chem. Abs.*, 1979, **91**, 52166).
[230] Y. Takahara, Y. Sasaki, and Y. Yonahara, *Kaku Igaku*, 1979, **16**, 235 (*Chem. Abs.*, 1979, **91**, 2113).
[231] Y. Takahara, Y. Sasaki, C. Suzuki, and Y. Yonahara, *Iryo*, 1978, **32**, 1201 (*Chem. Abs.*, 1979, **90**, 182686).
[232] P. Ho and J. Hansky, *Aust. J. Exp. Biol. Med. Sci.*, 1979, **57**, 95.
[233] D. J. Byrnes, J. Marjason, L. Henderson, and L. Lazarus, *Aust. N.Z. J. Med.*, 1979, **9**, 151.
[234] M. Otsuki, C. Sakamoto, M. Maeda, H. Yuu, T. Yamasaki, K. Okano, and S. Baba, *Nippon Naibumpi Gakkai Zasshi*, 1978, **54**, 1116 (*Chem. Abs.*, 1979, **90**, 67034).
[235] O. B. Schaffalitzky de Muckadell, J. Fahrenkrug, and S. J. Rune, *Scand. J. Gastroenterol.*, 1979, **14**, 79.
[236] M. J. Pelletier, J. A. Chayvialle, and Y. Minaire, *Gastroenterology*, 1978, **75**, 1124.
[237] W. Y. Chey, Y. H. Lee, J. G. Hendricks, R. A. Rhodes, and H.-H. Tai, *Am. J. Dig. Dis.*, 1978, **23**, 981.
[238] R. W. Henry, R. W. Stout, and K. D. Buchanan, *Diabete Metab.*, 1979, **5**, 21.
[239] J. C. Mason, R. F. Murphy, R. W. Henry, and K. D. Buchanan, *Biochim. Biophys. Acta*, 1979, **582**, 322.
[240] M. Kawanaka, T. Matsuoka, K. Nagai, A. Kido, and M. Fukuchi, *Hyogo Ika Daigaku Igakkai Zasshi*, 1979, **4**, 71 (*Chem. Abs.*, 1980, **92**, 69726).
[241] W. Y. Chey, M. S. Kim, and K. Y. Lee, *J. Physiol. (London)*, 1979, **293**, 435.

Administration of secretin in quantities which led to increasing physiological levels of plasma secretin gave a parallel increase of pancreatic bicarbonate output and duodenal concentration,[242] indicating that the physiological concentrations influence the pancreatic bicarbonate secretion. The response of the exocrine pancreas secretion to secretin is decreased and delayed in older persons,[243, 244] necessitating double administration to overcome the age-dependent changes. Secretin stimulation leads to increased levels of amylase and its isoenzymes in serum and urine;[245] increased secretion from pancreatic acinar cells;[179] increased concentrations of trypsinogen and trypsin inhibitor in pancreatic juice;[246] increased serum levels of group I pepsinogens;[247] and elevated concentrations of enterokinase in human duodenal fluid.[180] No variations in enzymic proportions by pancreozymic stimulation was found in the rat.[248] The potentiation of the pepsin response to secretin by gastrins was attributed to a non-specific increase in gastric mucosal blood flow.[249] Adenylate cyclase secretion from pancreatic fragments was found to be species dependent.[250] An obviously acquired higher enzyme output was found in European than in North African adults.[251] A potent stimulating effect on the gallbladder [252] and a preferentially increased blood flow in the pancreatoduodenal arteries [253] by secretin have been reported. Little effect on hypothalamic neurons [51] and on insulin release [46, 48] were found. The pancreatic stimulatory effect of secretin could be inhibited by the administration of the corresponding antiserum [254, 255] and by i.v. infusion of pancreatic polypeptide.[256] Secretin had an inhibitory effect on bombesin-[257] and meat-induced[258], but not on histamine-induced, gastric acid secretion.[258]

The secretin-CCK/caerulein test has been compared with a modified Lundh test [259] and its value for the diagnosis of pancreatic disorders [259, 260] has been further investigated. The mean serum half-life (2.7 min) of secretin in cirrhotic patients, which respond to secretin with hypersecretion, does not differ significantly from normal values.[261] Inactivation of secretin appears not to be a specific process but occurs on transit of all capillary beds.[102]

[242] O. B. Schaffalitzky de Muckadell, J. Fahrenkrug, P. Matzen, S. J. Rune, and H. Worning, *Scand. J. Gastroenterol.*, 1979, **14**, 85.
[243] O. V. Korkushko and V. P. Tereshchenko, *Z. Alternsforsch.*, 1978, **33**, 379.
[244] O. V. Korkushko, F. Dietze, V. P. Tereshchenko, and H. J. Schulz, *Z. Alternsforsch.*, 1979, **34**, 357.
[245] N. Derugin and I. L. MacGregor, *Am. J. Gastroenterol.*, 1979, **72**, 416.
[246] A. Eddeland and L. Wehlin, *Hoppe-Seyler's Z. Physiol. Chem.*, 1978, **359**, 1653.
[247] H. L. Waldum, P. G. Burhol, and B. K. Straume, *Scand. J. Gastroenterol.*, 1979, **14**, 761.
[248] J. C. Dagorn, R. Laugier, P. Demol, C. Figarella, and H. Sarles, *Gastroenterol. Clin. Biol.*, 1978, **2**, 915.
[249] F. B. Beswick, J. M. Braganza, and H. T. Howat, *J. Physiol. (London)*, 1979, **289**, 1.
[250] J. J. H. H. M. De Pont, D. Luyben, and S. L. Bonting, *Biochim. Biophys. Acta*, 1979, **584**, 33.
[251] G. Palasciano, J. F. Saunieres, R. Laugier, and H. Sarles, *Gut*, 1979, **20**, 1063.
[252] R. Jansson, G. Steen, and J. Svanvik, *Scand. J. Gastroenterol.*, 1978, **13**, 919.
[253] H. J. M. Beijer, P. F. Hulstaert, F. A. S. Brouwer, and G. A. Charbon, *Arch. Int. Pharmacodyn. Ther.*, 1979, **240**, 269.
[254] W. Y. Chey, M. S. Kim, K. Y. Lee, and T.-M. Chang, *Gastroenterology*, 1979, **77**, 1268.
[255] L. Kayasseh, K. Gyr, J. B. Baumann, and J. Girard, *Experientia*, 1979, **35**, 1596.
[256] T. E. Adrian, H. S. Besterman, C. N. Mallinson, G. R. Greenberg, and S. R. Bloom, *Gut*, 1979, **20**, 37.
[257] I. Kisfalvi, *Digestion*, 1979, **19**, 315.
[258] Y. Matsuo, A. Seki, H. Mori, and T. Kitamura, *Igaku no Ayumi*, 1978, **107**, 567 (*Chem. Abs.*, 1979, **90**, 97 799).
[259] P. Rolny and R. Jagenburg, *Scand. J. Gastroenterol.*, 1978, **13**, 927.
[260] I. Triandaf, C. Stanciu, G. Russu, M. Stoian, I. Croitoru, and F. Serban, *Rev. Med.-Chir.*, 1979, **83**, 525 (*Chem. Abs.*, 1980, **92**, 142 767).
[261] P. G. Justus, S. G. Wheeldon, and B. E. Kolts, *Proc. Soc. Exp. Biol. Med.*, 1979, **162**, 351.

Another indication for a trophic action has been reported [262] but the question is still open. Modifications in the technique for the preparation of dispersed pancreatic acinar cells resulted in a 80- to 100-fold higher release of cAMP and a five—six-fold higher release of amylase by secretin or VIP than reported previously.[263] In this system the dose–response curves for the action of secretin and VIP on cAMP and amylase release as well as the pattern of effects of secretin fragments (Secretin$_{5-27}$, [Asn^{15}]secretin$_{5-27}$ and [Lys^{15}]secretin$_{5-27}$) on these actions indicated that the increase in amylase secretion is mediated exclusively by the VIP-preferring receptors.

Vasoactive Intestinal Peptide (VIP).—(Figure 2). Various recent reviews deal with VIP in general;[264, 265] its occurrence in different molecular forms in nerves and endocrine cells;[139] its measurement,[266] distribution, and putative neurotransmitter function;[267] its relation to neural and neuroendocrine function;[268] its effect on adenylate cyclase;[269] its possible role in watery diarrhoea;[270, 271] and the distribution of VIP receptors in the intestinal tract.[272]

The primary structure of bovine VIP has been elucidated and found to be identical with that of VIP of porcine origin.[273] The bovine VIP used in this study was isolated in much better yield than in earlier work with porcine VIP by use of an improved process with h.p.l.c. as the final purification step. The high separation power of h.p.l.c. for VIP peptides was also demonstrated by separating porcine VIP from chicken VIP.[223] These peptides differ only in four of the 28 amino-acid residues. Two groups reported syntheses of chicken VIP ([Ser^{11}, Phe^{13}, Val^{26}, Thr^{28}]porcine VIP) in short communications. The first group [274] employed the '*in situ*' technique using active esters of Boc-amino-acids and stepwise elongation. The carboxy- and hydroxy-side-functions were protected by benzyl groups, the ε-amino-groups of the lysine residues by 2-chlorobenzyloxycarbonyl groups and the guanido-groups of the arginine residues by *p*-methoxybenzenesulphonyl groups. Treatment with HBr in a mixture of trifluoroactic acid, phenol, and *p*-cresol with acetylmethionine butyl ester as scavenger was used for final deprotection. The resulting free peptide showed almost the same biological potency as the natural hormone after purification by counter-current distribution and chromatography on Sephadex LH-20 and G-25. In the other synthesis [275] the sequence was built up from six fragments by azide coupling. The individual fragments were prepared by

[262] H. Petersen, T. E. Solomon, and M. I. Grossman, *Gastroenterology*, 1979, **76**, 790.
[263] J. D. Gardner, A. J. Rottman, S. Natarajan, and M. Bodanszky, *Biochim. Biophys. Acta*, 1979, **583**, 491.
[264] J. Kachelhoffer, J. Marescaux, F. Michel, and J. F. Grenier, *Gastroenterol. Clin. Biol.*, 1979, **3**, 381.
[265] T. Scratcherd, *Clin. Sci.*, 1979, **56**, 1.
[266] S. R. Bloom, ref. 72, p. 553.
[267] J. Fahrenkrug, *Digestion*, 1979, **19**, 149.
[268] S. I. Said, ref. 1, p. 439.
[269] B. Simon and H. Kather, ref. 1, p. 419.
[270] B. M. Jaffe, *Gastroenterology*, 1979, **76**, 417.
[271] S. I. Said, *World J. Surg.*, 1979, **3**, 559.
[272] M. Laburthe, J. Prieto, B. Amiranoff, C. Dupont, J. Broyart, D. Hui Bon Hoa, Y. Broer, and G. Rosselin, ref. 1, p. 241.
[273] M. Carlquist, V. Mutt, and H. Joernvall, *FEBS Lett.*, 1979, **108**, 457.
[274] M. Bodanszky, A. Bodanszky, and S. I. Said, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 1978, **37**, 1829, No. 3071.
[275] H. Yajima, M. Takeyama, K. Koyama, T. Tobe, K. Inoue, T. Kawano, and H. Adachi, *Chem. Pharm. Bull.*, 1979, **27**, 3199.

standard methods. Their α-amino-groups were protected by methoxybenzyloxycarbonyl groups, the ε-amino-groups by benzyloxycarbonyl groups, the guanido-groups by mesitylene-2-sulphonyl groups, and the hydroxy-group of serine in position 2 by a benzyl group. The methionine residue was introduced in the form of its sulphoxide derivative. Final deprotection was effected after reduction of the sulphoxide with thiophenol by treatment with HF in the presence of *m*-cresol. The free peptide was purified by gel filtration, ion exchange chromatography on CM-cellulose, and isolectric focusing and showed essentially full biological potency. The chicken VIP_{16-28} fragment with a pyroglutamyl instead of the glutamine residue in the *N*-terminal position has also been synthesized in connection with the evaluation of the hypothesis that VIP is a prohormone yielding biologically active partial sequences upon enzymatic cleavage.[276] The synthesis was carried out by step-wise elongation by use of active esters of Z-amino-acids with the exception of the last two residues which were incorporated as dipeptide Boc-Gln-Met-OH. The side-chain functions were protected by t-butyl or t-butyloxycarbonyl groups. Final deprotection was achieved by trifluoroacetic acid treatment and the glutamine residue was converted to a pyroglutamyl moiety by storing the free peptide in 2 M-acetic acid at room temperature for six days. The tridecapeptide was purified by counter-current distribution and is being tested in various pharmacological systems for biological activity in order to test the prohormone hypothesis.

The widespread occurrence of VIP, especially in neural structures, has been intensively studied and its possible role as neurotransmitter or modulator was further strengthened by these investigations. Immunohistochemical techniques usually in combination with radioimmunoassay have been used to determine the presence of VIP in various organs such as the posterior pituitary,[277] the carotid body,[278] the salivary glands,[279, 280] and the female reproductive tract [281] or to study its distribution in substructures, for instance in various parts of brain of human,[282, 283] porcine,[284] rat,[285-287] and mouse [286] origin. With the same methods it was also shown that VIP-immunoreactive nerves innervate the pancreas [154, 155] and the pylorus;[288] that the vagus nerve contains VIP-immuno-

[276] M. Bodanszky, A. Bodanszky, S. S. Deshmane, J. Matinez, and S. I. Said, *Bioorg. Chem.*, 1979, **8**, 399.
[277] S. Van Noorden, J. M. Polak, S. R. Bloom, and M. G. Bryant, *Neuropathol. Appl. Neurobiol.*, 1979, **5**, 149.
[278] J. M. Lundberg, T. Hoekfelt, J. Fahrenkrug, G. Nilsson, and L. Terenius, *Acta Physiol. Scand.*, 1979, **107**, 279.
[279] S. R. Bloom, M. G. Bryant, J. M. Polak, S. Van Noorden, and J. Wharton, *J. Physiol. (London)*, 1979, **289**, 23P.
[280] J. Wharton, J. M. Polak, M. G. Bryant, S. Van Noorden, S. R. Bloom, and A. G. E. Pearse, *Life Sci.*, 1979, **25**, 273.
[281] J. E. Goodnough, T. M. O'Dorisio, C. I. Friedman, and M. H. Kim, *Am. J. Obstet. Gynecol.*, 1979, **134**, 579.
[282] W. K. Samson, S. I. Said, J. W. Graham, and S. M. McCann, *Lancet*, 1978, *ii*, 901, and *IRCS Med. Sci.: Libr. Compend.*, 1979, **7**, 13.
[283] P. C. Emson, J. Fahrenkrug, and E. G. S. Spokes, *Brain Res.*, 1979, **173**, 174.
[284] J. Fahrenkrug and O. B. Schaffalitzky de Muckadell, *J. Neurochem.*, 1978, **31**, 1445.
[285] J. Besson, W. Rotsztejn, M. Laburthe, J. Epelbaum, A. Beaudet, C. Kordon, and G. Rosselin, *Brain Res.*, 1979, **165**, 79.
[286] I. Loren, P. C. Emson, J. Fahrenkrug, A. Bjoerklund, J. Alumets, R. Haakanson, and F. Sundler, *Neuroscience*, 1979, **4**, 1953.
[287] W. K. Samson, S. I. Said, and S. M. McCann, *Neurosci. Lett.*, 1979, **12**, 265.
[288] R. Edin, J. M. Lundberg, H. Ahlman, A. Dahlstroem, J. Fahrenkrug, T. Hoekfelt, and J. Kewenter, *Acta Physiol. Scand.*, 1979, **107**, 185.

reactive fibres;[289] that sphincters are especially rich in VIP-nerves as compared to the adjacent regions;[290] and that VIP occurs in differentiating and mature ganglion cells of normal and neoplastic neural tissue.[291] Immunohistochemical investigations utilizing anti-VIP and anti-acetylcholinesterase sera on the same structures proved that VIP-immunoreactivity is present in certain cholinergic neurons.[292]

Extracts of porcine intestinal muscle tissue contained predominantly one molecular species of VIP which was compatible in its chromatographic behaviour with the standard VIP octacosapeptide. Intestinal mucosa extracts, however, contained significant amounts of two additional VIP-immunoreactive compounds which emerged on CM-Sephadex before and on Sephadex G-50 in a position similar to that of standard VIP. From these results it was concluded that the molecular form of VIP in nerves, which are the main VIP source in the muscle extracts, is standard VIP, while the two other forms originate in the endocrine cells located in the mucosa. Furthermore the predominant form of VIP-like immunoreactivity observed in brain corresponded to standard VIP.[293] The difference in the staining pattern with different anti-VIP sera between nerves and endocrine cells in the intestinal tract also led to the conclusion that true VIP is a neuronal peptide and that endocrine cells store peptides resembling, but not identical with, VIP.[294, 295] A compound with VIP-like immunoreactivity and an apparent molecular weight larger than 5000 dalton was produced besides standard VIP in rat cell sap by placental ribosomes, indicating that VIP might be synthesized *via* a high molecular weight precursor.[296]

Two specific and sensitive radioimmunoassays for VIP have been described in the reporting period; one method measures directly in plasma samples[297] while the other needs prior elimination of interfering substances by adsorption to a resin.[298] The average plasma VIP level of fasting subjects determined by either method was of the order of a few pmole l^{-1}. An increase of plasma VIP concentration was found after prolonged fasting and exercise.[299] The concentration of immunoreactive VIP in portal hypophysial blood was *ca.* 19 times higher than in arterial blood of rats, indicating release of VIP from the hypothalamic–hypophysial complex in the portal circulation.[300] With the use of a radioimmunoassay it was also shown that VIP is transported along the sciatic nerve in a proximal to distal direction.[301]

[289] J. M. Lundberg, T. Hoekfelt, J. Kewenter, G. Pettersson, H. Ahlman, R. Edin, A. Dahlstroem, G. Nilsson, L. Terenius, *et al.*, *Gastroenterology*, 1979, **77**, 468.
[290] J. Alumets, J. Fahrenkrug, R. Haakanson, O. Schaffalitzky de Muckadell, F. Sundler, and R. Uddman, *Nature* (*London*), 1979, **280**, 155.
[291] G. Mendelsohn, J. C. Eggleston, J. L. Olson, S. I. Said, and S. B. Baylin, *Lab. Invest.*, 1979, **41**, 144.
[292] J. M. Lundberg, T. Hoekfelt, M. Schultzberg, K. Uvnaes-Wallensten, C. Koehler, and S. I. Said, *Neuroscience*, 1979, **4**, 1539.
[293] R. Dimaline and G. J. Dockrey, *J. Physiol.* (*London*), 1978, **285**, 39P; *Life Sci.*, 1979, **25**, 1893.
[294] L. I. Larsson, J. M. Polak, R. Buffa, F. Sundler, and E. Solcia, *J. Histochem. Cytochem.*, 1979, **27**, 936.
[295] F. Sundler, J. Alumets, J. Fahrenkrug, R. Haakanson, and O. B. Schaffalitzky de Muckadell, *Cell Tissue Res.*, 1979, **196**, 193.
[296] C. Humphrey, H. Munro, and J. E. Fischer, *Surg. Forum*, 1979, **30**, 355.
[297] S. J. Mitchell and S. R. Bloom, *Gut*, 1978, **19**, 1043.
[298] T.-M. Chang, F. L. Roth, jun., H.-H. Tai, and W. Y. Chey, *Anal. Biochem.*, 1979, **97**, 286.
[299] H. Galbo, J. Hilsted, J. Fahrenkrug, and O. B. Schaffalitzky de Muckadell, *Acta Physiol. Scand.*, 1979, **105**, 374.
[300] S. I. Said and J. C. Porter, *Life Sci.*, 1979, **24**, 227.
[301] A. Giachetti and S. I. Said, *Nature* (*London*), 1979, **281**, 574.

The distribution, disappearance half-time, and clearance of VIP were determined by radioimmunoassay in man and found to be $14\,ml\,kg^{-1}$, 1 min, and $9\,ml\,kg^{-1}\,min^{-1}$, respectively.[302] When ^{125}I-labelled VIP was injected into the portal or systemic circulation of rats most of the radioactivity accumulated in the liver. In contrast, if the liver was bypassed, radioactivity was maximal in the lung. Analysis of various tissue extracts showed the presence of a number of fragments smaller than VIP.[303]

Release of VIP from the gastrointestinal tract was caused by nervous stimulation,[304, 305] intraduodenal infusion of fat,[306–308] glucose or amino-acids,[306] and from the pancreas by bile or sodium chloride given *via* the pancreatic duct or by intraparenchymal injection.[309] Infusions of esterase inhibitors liberated VIP in the central nervous system [310] whereas histamine liberators stimulated the release of VIP from peritoneal mast cells.[311]

VIP-sensitive adenylate cyclase systems have been localized in brain,[312–314] pituitary,[315, 316] cardiac,[317, 318] pancreatic acinar,[319] and intestinal epithelial cell membranes.[320] Furthermore, the receptors by which VIP stimulates these adenylate cyclase systems have been detected or even characterized by studies utilizing binding of ^{125}I-labelled VIP to brain,[321, 322] pituitary,[323] prolactin-secreting pituitary tumour,[324] pancreatic acinar,[325] intestinal epithelial,[326, 327] and adrenal

[302] S. Domschke, W. Domschke, S. R. Bloom, P. Mitznegg, S. J. Mitchell, G. Lux, and U. Strunz, *Gut*, 1978, **19**, 1049.
[303] C. S. Humphrey, P. Murray, A. M. Ebeid, and J. E. Fischer, *Gastroenterology*, 1979, **77**, 55.
[304] J. Fahrenkrug, U. Haglund, M. Iodal, O. Lundgren, L. Olbe, and O. B. Schaffalitzky de Muckadell, *J. Physiol. (London)*, 1978, **284**, 291.
[305] S. R. Bloom, A. V. Edwards, and S. J. Mitchell, *J. Physiol. (London)*, 1979, **289**, 47P.
[306] A. Ohneda, J. Nihei, and K. Watanabe, *Rinsho Kagaku Shimpojumu*, 1978, **17**, 17 (*Chem. Abs.*, 1979, **90**, 133 245).
[307] J. Christiansen, A. Bech, J. Fahrenkrug, J. J. Holst, K. Lauritsen, A. J. Moody, and O. B. Schaffalitzky de Muckadell, *Scand. J. Gastroenterol.*, 1979, **14**, 161.
[308] T. Hossdorf, M. Burger, M. Burger, and H. Wagner, *Verh. Dtsch. Ges. Inn. Med.*, 1979, **85**, 167.
[309] I. M. Modlin, A. Shulkes, C. Lamers, and J. H. Walsh, *Surg. Forum*, 1979, **30**, 405.
[310] A. M. Ebeid, R. R. Attia, P. Sundaram, and J. E. Fischer, *Am. J. Surg.*, 1979, **137**, 123.
[311] E. Cutz, W. Chan, N. S. Track, A. Goth, and S. I. Said, *Nature (London)*, 1978, **275**, 661.
[312] M. Quik, L. L. Iversen, and S. R. Bloom, *Biochem. Pharmacol.*, 1978, **27**, 2209.
[313] C. Borghi, S. Nicosia, A. Giachetti, and S. I. Said, *Life Sci.*, 1979, **24**, 65.
[314] P. Robberecht, M. Deschodt-Lanckman, P. De Neef, and J. Christophe, *Life Sci.*, 1979, **25**, 1001.
[315] C. Borghi, S. Nicosia, A. Giachetti, and S. I. Said, *FEBS Lett.*, 1979, **108**, 403.
[316] P. Robberecht, M. Deschodt-Lanckman, J. C. Camus, P. De Neef, M. Lambert, and J. Christophe, *FEBS Lett.*, 1979, **103**, 229.
[317] P. Chatelain, M. Deschodt-Lanckman, P. De Neef, J. Christophe, and P. Robberecht, *Arch. Int. Physiol. Biochim.*, 1979, **87**, 783.
[318] P. Chatelain, P. Robberecht, P. De Neef, M. Claeys, and J. Christophe, *FEBS Lett.*, 1979, **107**, 86.
[319] E. J. Olinger and J. D. Gardner, *Gastroenterology*, 1979, **77**, 704.
[320] B. Amiranoff, M. Laburthe, C. Dupont, and G. Rosselin, *Biochim. Biophys. Acta*, 1978, **544**, 474.
[321] P. Robberecht, W. Koenig, M. Deschodt-Lanckman, P. De Neef, and J. Christophe, *Life Sci.*, 1979, **25**, 879.
[322] D. P. Taylor and C. B. Pert, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 660.
[323] P. Robberecht, M. Deschodt-Lanckman, P. De Neef, J. C. Camus, and J. Christophe, ref. 1, p. 447.
[324] D. Bataille, F. Peillon, J. Besson, and G. Rosselin, *C. R. Hebd. Scéances Acad. Sci. Ser. D*, 1979, **288**, 1315.
[325] E. R. Uhlemann, P. Robberecht, and J. D. Gardner, *Gastroenterology*, 1979, **76**, 917.
[326] M. Laburthe, J. C. Prieto, B. Amiranoff, C. Dupont, D. Hui Bon Hoa, and G. Rosselin, *Eur. J. Biochem.*, 1979, **96**, 239.
[327] J. C. Prieto, M. Laburthe, and G. Rosselin, *Eur. J. Biochem.*, 1979, **96**, 229.

cells.[328] Interestingly enough, chicken VIP exhibited a six–seven times higher affinity for intestinal epithelial cells of rats than porcine VIP. The affinity of secretin was *ca.* 100 times lower than that of porcine VIP in the same system and it was concluded from the affinity of some secretin analogues that binding ability is partially related to the presence of hydrophobic residues in the *N*-terminal hexapeptide of VIP.[326, 327] On the other hand it was also observed that although secretin, [Val[5]]secretin, and [Gln[9], Asn[15]]secretin$_{5-27}$ inhibited binding of ^{125}I-labelled VIP to guinea-pig brain, only the former two compounds stimulated adenylate cyclase. The secretin fragment, although inactive *per se*, inhibited VIP-induced stimulation.[321] Ethanol did not alter the binding of ^{125}I-labelled VIP to pancreatic acinar cells but potentiated the increase in adenylate cyclase activity while inhibiting the VIP-induced increase of amylase release.[325] In the mouse adrenal cell-line, VIP stimulated both steroid and cAMP production and was not replaced by ACTH.[328] Even a transfer of a VIP receptor from one cell to another by cell fusion has been reported.[329] Besides adenylate cyclase, VIP also stimulated a cAMP-dependent protein kinase in isolated intestinal epithelial cells from rats.[330] The effect of different neuron-destroying manipulations on the formation of VIP-stimulated cAMP was studied in slices of various brain structures.[331]

The effects of VIP on the pancreas were studied and it was found that chicken VIP was twice as potent as porcine VIP and 100—150 times as potent as porcine or chicken secretin in stimulating the flow of pancreatic juice in turkeys; porcine secretin, however, was 20—50 times more active in this regard than chicken secretin or either VIP in rats. None of the four hormones stimulated protein output in either species.[332] It was found that VIP seems to mediate the vagally induced, atropine-resistant secretion of fluid and bicarbonate from the pancreas;[333] that it stimulates the release of insulin and glucagon following intrapancreatic infusion;[334] and that it enhances glycogenolysis in isolated rat liver cells.[335] The kinetics of VIP-induced amylase release and its interaction with CCK have also been studied.[186]

Besides its aforementioned effects on the pancreas, VIP also acts on brain tissue and is, for example, excitatory to certain neurons in rat hippocampal slice preparations[336] and able to release certain anterior pituitary hormones. One group,[337] however, observed the release of prolactin, growth hormone, and LH only in *in vivo* and postulated therefore a hypothalamic site of action for VIP, while other groups attained a VIP-induced *in vitro* release of prolactin from hemi-

[328] A. M. Morera, A. M. Cathiard, M. Laburthe, and J. M. Saez, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 78.
[329] M. Laburthe, G. Rosselin, M. Rousset, A. Zweibaum, M. Korner, Z. Selinger, and M. Schramm, *FEBS Lett.*, 1979, **98**, 41.
[330] M. Laburthe, P. Mangeat, G. Marchis-Mouren, and G. Rosselin, *Life Sci.*, 1979, **25**, 1931.
[331] M. Quik, P. C. Emson, J. Fahrenkrug, and L. L. Iversen, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 1979, **306**, 281.
[332] R. Dimaline and G. J. Dockray, *J. Physiol. (London)*, 1979, **294**, 153.
[333] J. Fahrenkrug, O. B. Schaffalitzky de Muckadell, J. J. Holst, and S. Lindkaer Jensen, *Am. J. Physiol.*, 1979, **237**, E535, and ref. 16, p. 123.
[334] T. Kaneko, H. Oka, H. Yasuda, T. Oda, A. Kaneto, K. Kosaka, S. Nakaya, and N. Yanaihara, *Rinsho Kagaku Shimpojumu*, 1978, **17**, 12 (*Chem. Abs.*, 1979, **90**, 133 244).
[335] M. Matsumura, H. Akiyoshi, S. Saito, and H. Mori, *Endocrinol. Jpn.*, 1979, **26**, 233 (*Chem. Abs.*, 1979, **91**, 117 700).
[336] J. Dodd, J. S. Kelly, and S. I. Said, *Br. J. Pharmacol.*, 1979, **66**, 125P.
[337] E. Vijayan, W. K. Samson, S. I. Said, and S. M. McCann, *Endocrinology (Philadelphia)*, 1979, **104**, 53.

pituitaries [338, 339] and from a pituitary cell line.[340, 341] VIP was found to release prolactin in a dose-dependent manner and, at high concentrations, also growth hormone,[338] to be more potent in stimulating prolactin release than TRH,[340] and to inhibit the release of somatostatin from hypothalamic slices but not from preparations of other brain areas.[342] VIP caused a dilatation of pial arterioles when injected perivascularly and increased cerebral tissue perfusion and cerebral metabolic activity following intracarotid infusion but only after hypotonic opening of the blood–brain barrier.[343, 344] In other experiments it was also shown that VIP apparently mediates the atropine-resistant vasodilation in the salivary gland.[345, 346]

The following additional effects of VIP on various muscles or muscle preparations have been reported: contraction and relaxation of the lower oesophageal sphincter;[347] decrease of the resting and pentagastrin-induced lower oesophageal sphincter pressure;[348] decrease of CCK-8-stimulated contraction of gallbladder strips;[349, 350] relaxation of the taenia coli;[349] inhibition of oxytocin-induced activity in the myometrium;[351] and inhibition of the electrically induced contractions of the isolated vas deferens.[352] Other reports describe the influence of VIP on villous microcirculation and superior mesenteric artery blood flow;[353] on blood flow, motility, and fluid transport in the gastrointestinal tract;[354] and on ileal, colonic water, and electrolyte transport.[355]

Gastric Inhibitory Peptide (GIP).—(Figure 2). The physiological actions of GIP;[265, 356] its role in carbohydrate metabolism;[357] in obesity, diabetes, and hyperlipoproteinemia;[356, 358] its radioimmunological determination;[356, 359] and the results of clinical studies [360] have been reviewed.

[338] J. Besson, W. Rotsztejn, and M. Ruberg, ref. 1, p. 457.
[339] C. J. Shaar, J. A. Clemens, and N. B. Dininger, *Life Sci.*, 1979, **25**, 2071.
[340] D. Bataille, D. Gourdji, M. Maletti, N. Vauclin, D. Grouselle, A. Tixier-Vidal, and G. Rosselin, ref. 1, p. 465.
[341] D. Gourdji, D. Bataille, N. Vauclin, D. Grouselle, G. Rosselin, and A. Tixier-Vidal, *FEBS Lett.*, 1979, **104**, 165.
[342] J. Epelbaum, L. Tapia-Arancibia, J. Besson, W. H. Rotsztejn, and C. Kordon, *Eur. J. Pharmacol.*, 1979, **58**, 493.
[343] L. Edvinsson and J. McCulloch, *J. Physiol. (London)*, 1979, **291**, 42P.
[344] J. McCulloch and L. Edvinsson, *Acta Neurol. Scand., Suppl.*, 1979, **72** (Cereb. Blood Flow Metab.), p. 128.
[345] S. R. Bloom and A. V. Edwards, *J. Physiol. (London)*, 1979, **295**, 35P.
[346] T. Shimizu and N. Taira, *Br. J. Pharmacol.*, 1979, **65**, 683.
[347] J. Behar, S. Field, and C. Marin, *Gastroenterology*, 1979, **77**, 1001.
[348] S. R. Siegel, F. C. Brown, D. O. Castell, F. Lawrence, and S. I. Said, *Dig. Dis. Sci.*, 1979, **24**, 345.
[349] A. Johns, *Can. J. Physiol. Pharmacol.*, 1979, **57**, 106.
[350] J. Lonovics, P. Devitt, P. L. Rayford, and J. C. Thompson, *Surg. Forum*, 1979, **30**, 407.
[351] B. Ottesen, H. Ulrichsen, G. Wagner, and J. Fahrenkrug, *Acta Physiol. Scand.*, 1979, **107**, 285.
[352] A. J. Kastin, D. H. Coy, A. V. Schally, and C. A. Meyers, *Pharmacol., Biochem. Behav.*, 1978, **9**, 673.
[353] C. Holliger, M. Radzyner, A. Villiger, M. Anliker, and M. Knoblauch, *Bibl. Anat.*, 1979, **18** (Curr. Adv. Basic Clin. Microcirc. Res.), p. 129.
[354] S. Eklund, M. Jodal, O. Lundgren, and A. Sjoeqvist, *Acta Physiol. Scand.*, 1979, **105**, 461.
[355] Z. C. Wu, T. M. O'Dorisio, S. Cataland, H. S. Mekhjian, and T. S. Gaginella, *Dig. Dis. Sci.*, 1979, **24**, 625.
[356] R. Ebert and U. Finke, *Z. Gastroenterol.*, 1978, **16**, 311.
[357] L. M. Morgan, *Ann. Clin. Biochem.*, 1979, **16**, 6.
[358] W. Creutzfeldt and R. Ebert, *Dev. Endocrinol.*, 1979, **4** (Lipoprotein Metab. Endocr. Regul.), p. 65.
[359] J. C. Brown and J. R. Dryburgh, ref. 72, p. 541.
[360] J. C. Brown and S. Otte, *World J. Surg.*, 1979, **3**, 553.

No new synthetic efforts in the GIP field have been published. It was, however, reported that a synthetic homologue corresponding to fragment GIP_{1-38} exhibited full insulin-releasing but no inhibitory activity.[86] Based on this result and on the lack of activity of the previously reported synthetic GIP preparations the question was raised as to whether the published primary structure of GIP is correct or, if not, whether the correct sequence contains one glutamine residue less.[86]

The distribution of GIP in the rat duodenum and jejuno-ileum was determined at different developmental stages by radioimmunoassay with results which led to the conclusion that GIP is involved in the endocrine regulation of metabolism.[361]

A large number of studies deal with the release of GIP by carbohydrates and lipids and with the insulin-releasing activity of GIP, as measured by radioimmunoassay. It has been found that oral ingestion of glucose and galactose gives a similar increment of plasma GIP levels, whereas mannose or fructose cause no significant GIP release,[362] but that the response to galactose is diminished in a period of hyperglycaemia.[363] It has also been demonstrated that various carbohydrates which are not absorbed or transported have no GIP-releasing activity [364] and that unabsorbable carbohydrates like those which occur in guar flour decrease postprandial GIP secretion.[365] Intraduodenal infusions of glucose to dogs equipped with Heidenhain pouches and Mann–Bollman fistulae increased plasma GIP levels and inhibited gastric acid secretion. Mannitol infusions, although without effect on GIP release, nevertheless inhibited acid secretion.[366] A similar result, namely that glucose but not mannitol releases GIP, was also obtained in an *in vitro* system using hamster small intestine.[367] Patients with morbid obesity reacted with a GIP release very similar to that of controls to a standard breakfast but following jejunal-ileal bypass surgery their GIP response was markedly reduced.[368] The GIP response to an oral glucose load was likewise impaired during late pregnancy as compared to *post partum*.[369]

Intrajejunal infusion of fat increased plasma GIP levels.[307] Such an increase was also observed after an oral fat load but it was suppressed in healthy volunteers by a simultaneous infusion of glucose. This suppression was less marked in obese subjects and absent in obese patients with glucose intolerance.[370] Intraduodenal acidification was found to be a further stimulus to GIP release.[371] Infusion of growth hormone decreased plasma GIP levels in dogs and reduced the oral glucose-induced increment. Fasting also decreased GIP concentration but did not affect the increment. Infusion of insulin or glucagon had no effects on either. It was the aim of this study, which also used some other compounds, to show that the ability of these

[361] C. Gespach, D. Bataille, C. Jarrousse, and G. Rosselin, *Acta Endocrinol. (Copenhagen)*, 1979, **90**, 307.
[362] O. P. Ganda, J. S. Soeldner, R. E. Gleason, I. G. M. Cleator, and C. Reynolds, *J. Clin. Endocrinol. Metab.*, 1979, **49**, 616.
[363] L. M. Morgan, J. W. Wright, and V. Marks, *Diabetologia*, 1979, **16**, 235.
[364] K. R. Sirinek, T. M. O'Dorisio, B. A. Levine, and S. Cataland, *Surg. Forum*, 1979, **30**, 358.
[365] L. M. Morgan, T. J. Goulder, D. Tsiolakis, V. Marks, K. G. M. M. Alberti, *Diabetologia*, 1979, **17**, 85.
[366] T. M. O'Dorisio, J. T. Spaeth, E. W. Martin, jun., K. R. Sirinek, N. R. Thomford, E. L. Mazzaferri, and S. Cataland, *Am. J. Dig. Dis.*, 1978, **23**, 1079.
[367] D. A. Lewis, T. S. Gaginella, T. M. O'Dorisio, and S. Cataland, *Life Sci.*, 1979, **25**, 673.
[368] D. L. Sarson, H. S. Besterman, and S. R. Bloom, *J. Endocrinol.*, 1979, **81**, 155P.
[369] P. J. Hornnes, C. Kuhl, and K. B. Lauritsen, *J. Clin. Endocrinol. Metab.*, 1979, **48**, 506.
[370] R. Ebert, H. Frerichs, and W. Creutzfeldt, *Eur. J. Clin. Invest.*, 1979, **9**, 129.
[371] R. Ebert, K. Illmer, and W. Creutzfeldt, *Gastroenterology*, 1979, **76**, 515.

substances to increase plasma insulin concentration is not correlated with their effect on GIP release.[372]

Studies in healthy volunteers revealed that GIP is a gastrointestinal mediator of insulin release, but that its effect is dependent on the ambiant glucose level, and that its potency varies directly with the glucose concentration.[373] Infusion of GIP into the pancreas caused release of insulin and glucagon [334] and in isolated pancreatic islets GIP stimulated not only insulin release but also the biosynthesis of proinsulin.[374]

GIP was a poor inhibitor of pentagastrin-induced acid secretion in the isolated innervated canine stomach [375] and had no effect either on the concentrating activity and motility of the gallbladder or the pancreatic secretion in the cat.[252] GIP was also inactive with regard to decreasing food intake in rats;[168] however, it enhanced lipoprotein lipase activity in cultured preadipocytes.[376]

Motilin.—(Figure 3). The effect and mode of action of motilin,[377] its determination by radioimmunoassay,[378] its localization, and possible molecular heterogeneity [379]

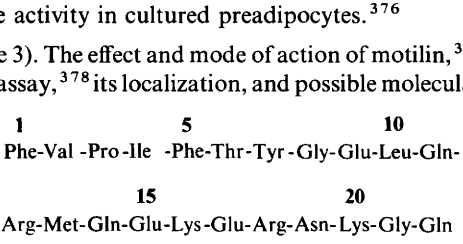


Figure 3 *The sequence of porcine motilin*

have been reviewed. A patent describes the synthesis of the fragment [Met-(O)[13]]motilin$_{1-15}$ by fragment condensation.[380] Motilin was localized in endocrine non-enterochromaffin cells of the small intestine of humans and monkeys by immunohistochemical procedures utilizing two antisera raised against synthetic motilin and [Nle[13]]motilin. In contrast to earlier findings, motilin could not be detected in enterochromaffin cells of human tissue. The anti-[Nle[13]]motilin serum, however, reacted very weakly at low dilution with cells of this type in monkey tissue.[381] The discrepancy between the results of the various groups was explained by different reactivities of the antisera used and by the possible existence of a family of motilin peptides.[379, 381] The distribution of motilin-like immunoreactivity was determined in the human, canine, monkey, and tupaia gastrointestinal tract and other tissues and species differences were observed. The concentration was very low in the canine brain and higher in the anterior than in the posterior pituitary lobe.[382]

[372] R. H. Williams, *Proc. Soc. Exp. Biol. Med.*, 1979, **162**, 157.
[373] D. Elahi, D. K. Andersen, J. C. Brown, H. T. Debas, R. J. Hershcopf, G. S. Raizes, J. D. Tobin, and R. Andres, *Am. J. Physiol.*, 1979, **237**, E185.
[374] R. Schaefer and H. Schatz, *Acta Endocrinol. (Copenhagen)*, 1979, **91**, 493.
[375] P. Soon-Shiong, H. T. Debas, and J. C. Brown, *J. Surg. Res.*, 1979, **26**, 681.
[376] R. H. Eckel, W. Y. Fujimoto, and J. D. Brunzell, *Diabetes*, 1979, **28**, 1141.
[377] W. Domschke, ref. 2, p. 106.
[378] J. R. Dryburgh and J. C. Brown, ref. 72, p. 567.
[379] J. M. Polak and A. M. J. Buchan, *Gastroenterology*, 1979, **76**, 1065.
[380] M. Fujino and S. Shinagawa, *Jpn. Kokai Tokkyo Koho*, 79 09267 (*Chem. Abs.*, 1979, **91**, 39 878).
[381] V. Helmstaedter, W. Kreppein, W. Domschke, P. Mitznegg, N. Yanaihara, E. Wünsch, and W. G. Forssmann, *Gastroenterology*, 1979, **76**, 897.
[382] C. Yanaihara, H. Sato, N. Yanaihara, K. Yamaguchi, and K. Abe, *Rinsho Kagaku Shimpojumu*, 1978, **17**, 21 (*Chem. Abs.*, 1979, **90**, 135 470).

Motilin nevertheless excited neurons in the cerebral cortex of the rat and in the spinal cord of the toad.[383]

Plasma motilin levels were influenced neither by age nor by sex in man [117] and were also unchanged in dogs suffering from pancreatic atrophy.[384] Studies in dogs and man revealed that plasma motilin levels were elevated when the gastric contractile activity was in the interdigestive state and that motilin concentration decreased following ingestion of food and stayed low as long as gastric motor activity showed the digestive pattern. However, plasma concentrations returned to high levels again by the time gastric motor activity was in the interdigestive state. A similar fluctuation of plasma motilin levels parallel to the digestive states was also observed after prolonged fasting.[385] In another study, however, it was observed that the composition of the food determines the motilin response since ingestion or infusion of fat increased, whereas that of glucose or amino-acids decreased, plasma motilin concentration.[386]

Infusion of motilin increased the rate of gastric emptying after a standard breakfast and the postprandial insulin release, but had no effect on various other gastrointestinal or pancreatic hormones.[387] In dogs, motilin increased the frequency of interdigestive cycles;[388] promoted gastric motility in a pattern different from that induced by tetragastrin;[389] stimulated motility of the lower oesophageal sphincter, stomach, and duodenum; accelerated gastric emptying and interdigestive, but not digestive, motility; and stimulated secretion of water, electrolytes, and enzymes from the stomach and pancreas.[390] In humans, however, only enzyme secretion from the stomach was enhanced and gastric emptying was inhibited.[390] The latter observation contradicts the results reported earlier in this paragraph.[387] Motilin was also found to be one of the factors involved in production of the activity front of the migrating motor complex in man.[391]

Urogastrone.—(Figure 4). Human urogastrone and mouse epidermal growth factor (mEGF) have 37 of their 53 amino-acid residues in common. Based on this homology it has been speculated that human urogastrone and human epidermal growth factor (hEGF) are probably identical.[392] However, from the large number of publications on EGF only those which refer to urogastrone in their title will be mentioned here.

Specific radioimmunoassays for urogastrone and mEGF, demonstrating *ca.* 0.1% cross-reactivity between these structurally related peptides, have been

[383] J. W. Phillis and J. R. Kirkpatrick, *Eur. J. Pharmacol.*, 1979, **58**, 469.
[384] V. B. Troitskaya and N. S. Track, *Fiziol. Zh. SSSR im. I. M. Sechenova*, 1979, **65**, 306 (*Chem. Abs.*, 1979, **91**, 3442).
[385] Y. Seino, K. Mori, S. Matsukura, Z. Itoh, S. Takeuchi, I. Takahashi, H. Imura, and N. Yanaihara, *Rinsho Kagaku Shimpojumu*, 1978, **17**, 27 (*Chem. Abs.*, 1979, **90**, 135664).
[386] N. D. Christofides, S. R. Bloom, H. S. Besterman, T. E. Adrian, and M. A. Ghatei, *Gut*, 1979, **20**, 102.
[387] N. D. Christofides, I. M. Modlin, M. L. Fitzpatrick, and S. R. Bloom, *Gastroenterology*, 1979, **76**, 903.
[388] P. A. Thomas, K. A. Kelly, and V. L. W. Go, *Dig. Dis. Sci.*, 1979, **24**, 577.
[389] M. Tani, K. Sugahara, M. Manabe, M. Kato, Y. Akazaka, and K. Kawai, *Nippon Heikatsukin Gakkai Zasshi*, 1978, **14**, 247 (*Chem. Abs.*, 1979, **91**, 134638).
[390] S. Harasawa, N. Tani, S. Nomiyama, R. Sakita, M. Miwa, S. Suzuki, and T. Miwa, *Horumon to Rinsho*, 1979, **27**, 175 (*Chem. Abs.*, 1979, **90**, 180689).
[391] G. Vantrappen, J. Janssens, T. L. Peeters, S. R. Bloom, N. D. Christofides, and J. Hellemans, *Dig. Dis. Sci.*, 1979, **24**, 497.
[392] G. Carpenter and S. Cohen, *Annu. Rev. Biochem.*, 1979, **48**, 193.

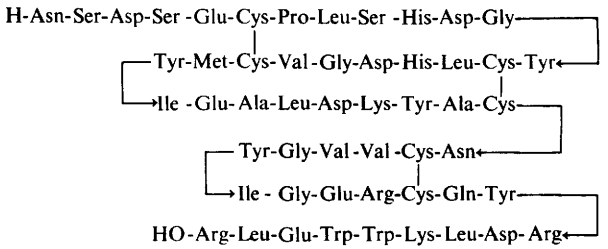


Figure 4 *The amino-acid sequence of β-urogastrone*

developed and used for measurements in urine and serum.[393] When this assay was used to locate immunoreactive urogastrone in serum, saliva, and gastric juice samples after chromatography on Sephadex G-200 and G-50, it became evident that the major component in serum had a molecular weight of 100 000–200 000 dalton, whereas the predominant forms in both saliva and gastric juice appeared in the same position as pure urogastrone. The high molecular weight compound, however, was cleaved by trypsin to yield a small species which eluted in the urogastrone position. All compounds with molecular weights similar to urogastrone showed mitogenic activity. It was concluded from these results that urogastrone-like molecules can be released enzymatically from a high molecular weight serum precursor.[394] The presence of relatively low amounts of immunoreactive hEGF–urogastrone was also detected in extracts of adult submandibular gland, thyroid gland, duodenum, jejunum, and kidney but not in foetal tissue by a hEGF-specific radioimmunoassay after ion exchange and immune affinity chromatography. These compounds were similar in size to the standard hEGF as judged from their elution profile on Sephadex G-50.[395] Compounds with similar molecular weights and hEGF–urogastrone-like immunoreactivity were also found in unextracted saliva and milk, while urine contained a high molecular weight component with low receptor-binding activity in addition.[396] This high molecular weight species was cleaved by a specific arginine esterase, the EGF-binding protein from mouse submandibular glands, to yield a molecular form very similar to hEGF with regard to chromatographic behaviour and biological activity. Trypsin cleavage also transformed this species into a small molecular weight hEGF with similar immunoreactivity but much less biological activity.[397]

Other investigations deal with the receptors of urogastrone–EGF. When, for example the EGF receptors in human placental membranes were cross-linked with ^{125}I-labelled EGF–urogastrone, solubilized, and analysed by polyacrylamide electrophoresis, two major constituents with apparent molecular weights of 180 000 and 160 000 dalton could be identified by autoradiography. Since these components were adsorbable to lectin–agarose columns and desorbable with appropriate lectin-specific sugars, they are probably glycoproteins. The adsorption of these constituents to an anti-EGF antibody–agarose column was also possible and offers a

[393] H. Gregory, J. E. Holmes, and I. R. Willshire, ref. 72, p. 927.
[394] H. Gregory, S. Walsh, and C. R. Hopkins, *Gastroenterology*, 1979, **77**, 313.
[395] Y. Hirata and D. N. Orth, *J. Clin. Endocrinol. Metab.*, 1979, **48**, 667.
[396] Y. Hirata and D. N. Orth, *J. Clin. Endocrinol. Metab.*, 1979, **48**, 673.
[397] Y. Hirata and D. N. Orth, *J. Clin. Endocrinol. Metab.*, 1979, **49**, 481.

good procedure for the isolation of such solubilized receptors, which lose their ligand-binding capacity upon solubilization and are therefore not adsorbed to ligand-affinity columns.[398] The characterization and purification of the human placental receptor for EGF–urogastrone have also been the topic of a dissertation.[399] It has been demonstrated further that the solubilized EGF receptor regains its ligand-binding ability upon immobilization to lectin–agarose particles.[400] It was also observed that chemically transformed tumorigenic cell lines exhibited decreased EGF–urogastrone binding compared with the parent cell strain, but that no direct relationship existed between this effect and cellular tumorigenesis,[401] and that the binding characteristics of adult human fibroblast do not change with age.[402]

Possible Gastrointestinal Hormone Candidates.—A novel peptide, named PIHIA, having a *N*-terminal histidine residue and *C*-terminal isoleucine-amide has been isolated from porcine duodenum. This peptide exhibits VIP-like biological effects and probably belongs to the secretin–glucagon family.[403,404]

The present concept of the effects of gut hormones on the endocrine pancreas, the 'incretin' effect,[405] as well as the problems in its evaluation [26] have been reviewed. The secretion of an insulinotropic factor which is different from GIP, CCK-8, secretin, and gastrin from isolated perfused rat intestine,[406] of a duodenal insulin-releasing activity (DIRA), which seems to be of peptidic nature but not identical with GIP, and of a duodenal insulin release-inhibiting activity [407] have been described.

Pancreotone, a polypeptide which inhibited the secretin- and CCK-stimulated secretion of the pancreas and the CCK-induced contraction of the gallbladder, has been extracted from ileal and colonic mucosa. These actions of pancreotone resemble those caused by intraileal and intracolonic infusion of oleic acid in cats, suggesting that pancreotone may be the humoral mediator of these inhibitory effects of fatty acids.[408] The present status of a peptide with similar activities and the same origin but named 'anticholecystokinin' (ACP) has also been reviewed.[409]

The properties and purification of a urinary glycoprotein with gastric secretion-inhibiting activity have been reviewed [410] and the isolation of a polypeptide with a molecular weight of *ca.* 69 000 dalton and gastric juice-inhibiting activity from vaccinia virus-infected animal tissue has been claimed in a patent.[411]

[398] R. A. Hock, E. Nexo, and M. D. Hollenberg, *Nature* (*London*), 1979, **277**, 403.
[399] R. A. Hock, *Diss. Abstr. Int. B*, 1979, **40**, 2105.
[400] E. Nexo, R. A. Hock, and M. D. Hollenberg, *J. Biol. Chem.*, 1979, **254**, 8740.
[401] M. D. Hollenberg, J. C. Barrett, P. O. P. Ts'o, and P. Berhanu, *Cancer Res.*, 1979, **39**, 4166.
[402] M. D. Hollenberg and E. L. Schneider, *Mech. Ageing Dev.*, 1979, **11**, 37.
[403] K. Tatemoto and V. Mutt, *Scand. J. Gastroenterol., Suppl.*, 1978, **49**, 181.
[404] D. Bataille, M. Laburthe, C. Dupont, K. Tatemoto, N. Vauclin, G. Rosselin, and V. Mutt, *Scand. J. Gastroenterol., Suppl.*, 1978, **49**, 13.
[405] W. Creutzfeldt, *Diabetologia*, 1979, **16**, 75.
[406] S. R. Levin, M. Z. Pehlevanian, A. E. Lavee, and R. I. Adachi, *Am. J. Physiol.*, 1979, **236**, E710.
[407] A. Zermatten and J. P. Felber, *Diabete Metab.*, 1978, **4**, 151.
[408] A. A. Harper, A. J. C. Hood, J. Muschens, and J. R. Smy, *J. Physiol.* (*London*), 1979, **292**, 455.
[409] H. Sarles, G. Hage, R. Laugier, P. Demol, and D. Bataille, *Digestion*, 1979, **19**, 73.
[410] G. Lugaro, G. Carrea, P. Pasta, M. M. Casellato, E. Manera, P. Vecchini, and R. Niada, *Perspect. Inherited Metab. Dis.*, 1979, **2** (Glycoproteins, Glycosaminoglycans Health Dis.), p. 73.
[411] S. Aonuma, *Jpn. Kokai Tokkyo Koho* 79 49313 (*Chem. Abs.*, 1979, **91**, 78 895).

6 Calcitonin and Parathyroid Hormone

Contributed by S. Girgis

Introduction.—This part of Chapter 5 reviews the literature on calcitonin and parathyroid hormone published in the main journals during 1979, and papers relevant to the relationship between chemical structure and biological activity since the last review of this subject in Vol. 8 by Schafer and Szelke.

A number of books and reviews covering the area of this review have appeared including the proceedings of three international symposia,[1-3] and a book on hormones in blood.[4]

Calcitonin.—*Isolation and Chemical Structure.* The isolation and sequence determination of both eel[5,6] and rat calcitonin[7-9] were accomplished in 1976. Rat calcitonin differs from human calcitonin M[10] in only two positions: Leu[16] and Ser[26] in the rat, *versus* Phe[16] and Ala[26] in human calcitonin. Eel calcitonin differs in only three positions from salmon calcitonin I,[11] the major form of salmon calcitonin: Asp[26], Val[27], and Ala[29] in eel calcitonin correspond to Asn[26], Thr[27], and Ser[29] in salmon calcitonin I. The amino-acid sequences of the calcitonins from the various species are shown in Figure 1.

Synthesis. Eel calcitonin has been synthesized.[12] Its hypocalcaemic potency in rats was found to be comparable to that of the native hormone and to that of salmon calcitonin I (4300 MRC units mg^{-1}). Furthermore, the duration of hypocalcaemic effect of eel calcitonin in rats was found to be more persistent than that of porcine calcitonin and similar to that of salmon calcitonin I.[13] Eel calcitonin was more stable than either porcine or salmon calcitonin I when incubated with either rat or human plasma *in vitro*.

Structure-Activity Relationship. [Asu[1,7]]eel calcitonin (Asu = 1-aminosuberic acid) has been synthesized. In this analogue the *N*-terminal amino-group is replaced by hydrogen and the disulphide bond between residues 1—7 by an ethylene linkage.[12] The hypocalcaemic potency of this analogue in rats is *ca.* 75% of that of eel calcitonin. [Asu[1,7]]eel calcitonin shows stability similar to that of eel calcitonin

[1] 'Molecular Endocrinology', ed. I. MacIntyre and M. Szelke, Elsevier/North-Holland Biomedical Press, Amsterdam, 1977.

[2] 'Molecular Endocrinology', Vol. 1, ed. I. MacIntyre and M. Szelke, Elsevier/North-Holland Biomedical Press, Amsterdam, 1979.

[3] 'Human Calcitonin and Paget's Disease', Proceedings of an International Workshop, London, April 1976, ed. I. MacIntyre, Hans Huber Publishers, Bern, 1977.

[4] 'Hormones in Blood', ed. C. H. Gray and V. H. T. James, Vol. 2, Academic Press, New York, 1979.

[5] M. Otani, H. Yamanchi, T. Meguro, S. Ktazawa, S. Watanabe, and H. Orimo, *J. Biochem. (Tokyo)*, 1976, **79**, 345.

[6] T. Noda and K. Narita, *J. Biochem. (Tokyo)*, 1976, **79**, 353.

[7] P. G. H. Byfield, E. W. Matthews, J. N. M. Heersche, G. A. Boorman, S. I. Girgis, and I. MacIntyre, *FEBS Lett.*, 1976, **65**, 238.

[8] P. G. H. Byfield, J. L. McLoughlin, E. W. Matthews, and I. MacIntyre, *FEBS Lett.*, 1976, **65**, 242.

[9] D. Raulais, J. Hagaman, D. A. Ontjes, R. L. Lumdblad, and H. S. Kingdon, *Eur. J. Biochem.*, 1976, **64**, 607.

[10] R. Neher, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, 1968, **51**, 1900.

[11] H. D. Niall, H. T. Keutmann, D. H. Copp, and J. T. Potts, jun., *Proc. Natl. Acad. Sci. USA*, 1969, **64**, 771.

[12] T. Morikawa, E. Munekata, S. Sakakibara, T. Noda, and M. Otani, *Experientia*, 1976, **32**, 1104.

[13] M. Otani, S. Kitazawa, H. Yamanchi, T. Meguro, and H. Orimo, *Horm. Metabol. Res.*, 1978, **10**, 252.

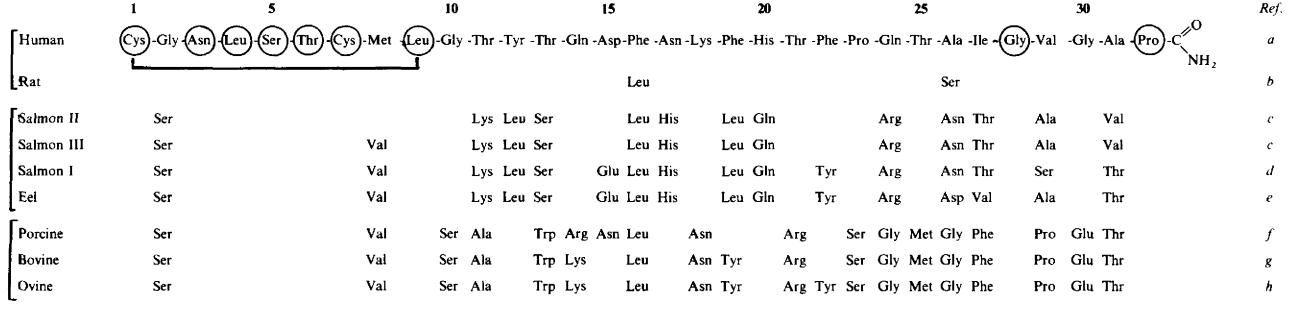


Figure 1 *Amino-acid sequences of the calcitonins arranged in order of their similarity to human calcitonin. Residues identical with human calcitonin are not shown*

(*a*) R. Neher, B. Riniker, W. Rittel, and H. Zuber, *Helv. Chim. Acta*, 1968, **51**, 1900. (*b*) D. Raulais, J. Hagaman, D. A. Ontjes, R. L. Lundblad, and H. S. Kingdom, *Eur. J. Biochem.*, 1976, **64**, 607. (*c*) H. T. Keutmann, R. M. Lequin, J. F. Habener, F. R. Singer, H. D. Niall, and J. T. Potts, jun., 'Endocrinology 1971', Proceedings of the 3rd International Symposium, 1972, p. 316, ed. S. Taylor, Heinemann Medical Books, London. (*d*) H. D. Niall, H. T. Keutmann, D. H. Copp, and J. T. Potts, jun., *Proc. Natl. Acad. Sci., USA*, 1969, **64**, 771. (*e*) T. Noda and K. Narita, *J. Biochem. (Tokyo)*, 1976, **79**, 353. (*f*) J. T. Potts, jun., H. D. Niall, H. T. Keutmann, H. B. Brewer, jun., and L. J. Deftos, *Proc. Natl. Acad. Sci. USA*, 1968, **59**, 1321. (*g*) H. B. Brewer, jun. and R. Ronan, *Proc. Natl. Acad. Sci. USA*, 1969, **63**, 940. (*h*) J. T. Potts, jun., H. D. Niall, H. T. Keutmann, L. J. Deftos, and J. A. Parsons, in 'Calcitonin 1969', Proceedings of 2nd International Symposium, 1970, p. 59, ed. S. Taylor, Heinemann Medical Books, London.

when incubated with human plasma [14] and is more stable than eel calcitonin when kept at 65 °C. It is considerably less expensive to synthesize than eel calcitonin. The high biological potency of [Asu[1,7]]eel calcitonin indicates that the role of the disulphide bridge in eel calcitonin is a stereochemical one. However, an open-chain analogue of human calcitonin has been synthesized and found to be inactive.[15]

Continued attempts have been made to explore the reasons for the greater biological potency of fish calcitonins as compared with mammalian ones. Further analogues of human calcitonin (hCT) have been synthesized with substitutions of salmon calcitonin-like basic amino-acids at positions 11 and 24.[16] While [Lys[11]]hCT is three times more potent than hCT in producing hypocalcaemia in rats, [Arg[24]]hCT has only 10% of the activity of hCT. Gains in potency resulting from multiple modifications are not additive. It seems unlikely therefore that the greater biological activity of salmon calcitonin is related to the number or position of basic amino-acids present in the molecule.

Structure–activity relationship in human calcitonin was assessed by examining the hypocalcaemic activity of eight analogues of hCT with shortened or terminally modified peptide chains.[15] Table 1 shows hCT analogues with shortened or terminally modified peptide chains, together with their hypocalcaemic potencies in the rat:

Table 1 *Hypocalcaemic potency of hCT analogues*

Analogues	*Relative hypocalcaemic activity in rat assay*[a]
hCT	100
(1) hCT-(1—32)-OH	0.06
(2) hCT-(1—32)-OMe (*C*-terminal methyl ester)	0.13
(3) hCT-(1—32)-NH_2 (*C*-terminal amide)	0.12
(4) [Pro[29]]hCT-(1—29)-NH_2	0.02
(5) [Ser[2], des-Gly[10]]hCT	0.12
(6) [Mpa[1]]hCT (desamino-Cys[1]-hCT)	120
(7) [*N*-Acetyl-Cys[1]]hCT	150
(8) [*N*met[1,7]]hCT(1,7-dinormet-hCT, open-chain derivative)	0.18

[a] W. Rittel, R. Maier, M. Brugger, B. Kamber, B. Riniker, and P. Sieber, *Experientia*, 1976, **32**, 246.

Hypocalcaemic activity is drastically reduced by removal of the *C*-terminal-amide group, chain shortening, or opening of the disulphide ring, but unaffected or enhanced by modification of the *N*-terminal amino-group.

Other information on structure–activity relationship was obtained by chemical modification of the porcine and bovine calcitonins.

Acylation of porcine and bovine calcitonin at the amino-terminus gives increased hypocalcaemic activity,[17] as does *N*-terminal acetylation of hCT.[15] However, simultaneous acylation of the terminal NH_2 and the OH of Tyr[12] in both bovine

[14] H. Yamanchi, M. Shiraki, M. Otani, M. Matsuo, and H. Orimo, *Endocrinol. Japonica*, 1977, **24**, 281.
[15] W. Rittel, R. Maier, M. Brugger, B. Kamber, B. Riniker, and P. Sieber, *Experientia*, 1976, **32**, 246.
[16] R. Maier, M. Brugger, H. Bruckner, B. Kamber, B. Riniker, and W. Rittel, *Acta Endocrinol.*, 1977, **85**, 102.
[17] M. Merle, G. Lefevre, J. F. Staub, D. Raulais, and G. Milhaud, *Biochem. Biophys. Res. Commun.*, 1977, **79**, 1071.

and porcine calcitonins leads to a loss of potency, indicating the importance of the tyrosine residue for biological activity.

Biosynthesis. A biosynthetic calcitonin precursor with mol. wt. 15 000 dalton has been reported using wheat germ and reticulocyte lysate cell-free systems for the translation of messenger RNA from human medullary thyroid carcinoma [18] and from rat medullary thyroid carcinoma and cod fish ultimobranchial glands.[19] However, a much larger precursor for calcitonin of mol. wt. 65 000 dalton is also observed [20, 21] when messenger RNA from human and rat medullary thyroid carcinoma and other human tumours is translated in the frog oocyte.

Biological Effects. In addition to the well documented action of calcitonin as a potent inhibitor of bone resorption, it has been shown to have other biological effects. Most of these have been reviewed.[22, 23] More recently, interest has focused on the effects of calcitonin on the nervous system. There is evidence to suggest that calcitonin may be a neurotransmitter. Intracerebroventricularly (i.c.v.) administered calcitonin has been shown to have a clear-cut analgaesic effect in rabbits [24] and in mice.[25] Although the mechanism of this analgaesic effect is not known, it is not inhibited by narcotic antagonists and is therefore unlikely to be mediated through opiate receptors.[25, 26] However, analgesia is significantly inhibited by calcium injected simultaneously *via* the same route [27] and this inhibition is similar to that seen when calcium is administered simultaneously with morphine. Intravenous infusion of calcitonin in rats produces an increase in the brain levels of 5-hydroxy-tryptamine and acetylcholine esterase activity.[28] However, it is not clear whether these effects are due to the lowering of plasma calcium by calcitonin or whether they are directly attributable to the hormone. Intraventricular injection of calcitonin into anaesthetized male rats results in a significant dose-related increase in plasma prolactin. This effect is not observed following intravenous injection of calcitonin. However, calcitonin mildly stimulates prolactin release from anterior pituitary cells in culture.[29] Calcitonin is also reported to inhibit feeding in rats when given subcutaneously or intraventricularly.[30]

Extra-thyroidal Calcitonin. Although the presence of calcitonin in the nervous system of mammals is not confirmed, yet an immunoassayable human calcitonin-like molecule has been found in the nervous system of several primitive chordates

[18] R. H. Goodman, J. W. Jacobs, and J. F. Habener, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 932.
[19] J. W. Jacobs, J. T. Potts, jun., N. H. Bell, and J. Habener, *J. Biol. Chem.*, 1979, **254**, 10 600.
[20] C. J. M. Lips, J. Van Der Sluys Veer, J. A. Van Der Donk, R. H. Van Dam, and W. H. L. Hackeng, *Lancet*, 1978, *i*, 16.
[21] J. A. Van Der Donk, C. J. M. Lips, W. H. L. Hackeng, R. H. Van Dam, and J. GoudsWaard, *J. Mol. Medicine*, 1978, **3**, 95.
[22] T. J. Martin, ref. 3, p. 34.
[23] L. J. Deftos, *Adv. Intern. Med.*, 1978, **23**, 159.
[24] A. Pecile, S. Ferri, P. C. Braga, and V. R. Olgiati, *Experientia*, 1975, **31**, 332.
[25] M. Yamamoto, F. Kumagai, S. Tachikawa, and H. Maeno, *Eur. J. Pharmacol.*, 1979, **55**, 211.
[26] P. Braga, S. Ferri, A. Santagostino, V. R. Olgiati, and A. Pecile, *Life Sci.*, 1978, **22**, 971.
[27] M. Satoh, H. Amano, T. Nakazawa, and H. Takagi, *Res. Commun. Chem. Path. Pharmacol.*, 1979, **26**, 213.
[28] A. M. Nakhla and A. P. N. Majumdar, *Biochem. J.*, 1978, **170**, 445.
[29] Y. Iwasaki, K. Chihara, J. Iwasaki, H. Abe, and T. Fujita, *Life Sci.*, 1979, **25**, 1243.
[30] W. J. Freed, M. J. Perlow, and R. J. Wyatt, *Science*, 1979, **206**, 850.

and in the brain of hagfish.[31] Calcitonin has also been localized by immunochemical studies in the neural ganglion of *Ciona intestinalis*.[32]

Based on immunochemical localization of calcitonin in the intermediate and anterior lobes of rat pituitary, Deftos and his co-workers [33, 34] have postulated that calcitonin may be part of the 31 K-precursor to corticotropin and β-lipotropin. However, the localization of calcitonin in the pituitary has not been confirmed in other laboratories.[31, 35, 36] Furthermore, analysis of a tryptic digest of the 31 K-precursor, or sequence analysis of cloned cDNA coding for the protein has failed to demonstrate the presence of calcitonin within this molecule.[35, 37]

Parathyroid Hormone (PTH).—A recent review has dealt with the chemistry, biosynthesis, secretion, metabolism, and measurement of parathyroid hormone.[38]

Chemical Structure. Investigation of the disputed residues at positions 22, 28, and 30 in the amino-terminal region of human PTH (hPTH) proposed by Brewer *et al.*[39] (B) and Niall *et al.*[40] (N) has attracted several investigators.

Indirect evidence, based on the biological and immunological comparison of synthetic hPTH-(1—34) corresponding to both proposed sequences with native PTH, suggests that Niall's sequence is more likely to be correct. Thus, hPTH-(1—34) N is immunochemically identical to the native hormone in a number of radioimmunoassay systems using seven different antisera, whereas striking differences are found between the native hormone and hPTH-(1—34) B.[41] Furthermore, the native hormone reacts similarly to hPTH-(1—34) N and not to hPTH-(1—34) B, with an antiserum specific for the 29—33 region suggesting that position 30 in the native hormone is occupied by Asp[42] as proposed by Niall *et al.*[40] In a bioassay, hPTH-(1—34) N is more potent than hPTH-(1—34) B. The dose–response pattern of hPTH-(1—34) N is closer to that of the native hormone than is the pattern shown by Brewer's peptide in activating renal adenyl cyclase.[43] In other human and chick renal cortical adenyl cyclase systems hPTH-(1—34) N is 140 times more potent than hPTH-(1—34) B [44] and is equivalent to bPTH. Bovine PTH-(1—34) differs in only three amino-acids from hPTH-(1—34) N, but in six amino-acids from hPTH-(1—34) B.

[31] I. MacIntyre, T. R. Arnett, D. J. Brown, F. Galan Galan, S. I. Girgis, R. M. Rogers, E. Spanos, J. C. Stevenson, and Q. Bone, ref. 2, p. 193.
[32] H. A. R. Fritsch, S. Van Noorden, and A. G. E. Pearse, *Cell and Tissue Research*, 1979, **202**, 263.
[33] L. J. Deftos, D. Burton, B. D. Catherwood, H. G. Bone, J. G. Parthemore, R. Guillemin, W. B. Watkins, and R. Y. Moore, *J. Clin. Endocrinol. Metabol.*, 1978, **47**, 457.
[34] L. J. Deftos, D. Burton, H. G. Bone, B. D. Catherwood, J. G. Parthemore, R. Y. Moore, S. Minick, and R. Guillemin, *Life Sci.*, 1978, **23**, 743.
[35] E. Weber, K. H. Voigt, R. E. Mains, and B. A. Eipper, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 360.
[36] J. Mendelsohn, R. D'Agostino, C. Eggleston, and S. B. Baylin, *J. Clin. Invest.*, 1979, **63**, 1297.
[37] S. Nakanishi, A. Inoue, T. Kita, M. Nakamura, A. C. Y. Chang, S. N. Cohen, and S. Numa, *Nature (London)*, 1979, **278**, 423.
[38] G. N. Hendy, S. E. Papapoulos, I. G. Lewin, and J. L. H. O'Riordan, ref. 4, p. 1.
[39] H. B. Brewer, jun., T. Fairwell, R. Ronan, G. W. Sizemore, and C. D. Arnaud, *Proc. Natl. Acad. Sci. USA*, 1972, **69**, 3585.
[40] H. D. Niall, R. T. Sauer, J. W. Jacobs, H. T. Keutmann, G. Segre, J. L. H. O'Riordan, G. D. Aurbach, and J. T. Potts, jun., *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 384.
[41] G. V. Segre and J. T. Potts, jun., *Endocrinology*, 1976, **98**, 1294.
[42] T. J. Visser, C. J. Buurman, and J. C. Birkenhager, *Acta Endocrinol.*, 1979, **90**, 90.
[43] J. D. Monet, C. A. Bader, E. d'Herbigny, and J. L. Funck-Bretano, *FEBS Lett.*, 1978, **96**, 79.
[44] N. H. Hunt, S. MacNeil, and T. J. Martin, *Biochem. Biophys. Res. Commun.*, 1978, **81**, 581.

Niall's sequence of the *N*-terminal region has been confirmed and the remainder of the sequence determined by Keutmann and his co-workers [45, 46] using a new batch of hPTH isolated from parathyroid adenomas.

Human PTH differs at 11 positions from the bovine and porcine hormones (Figure 2). The most marked differences occur at the central positions of the

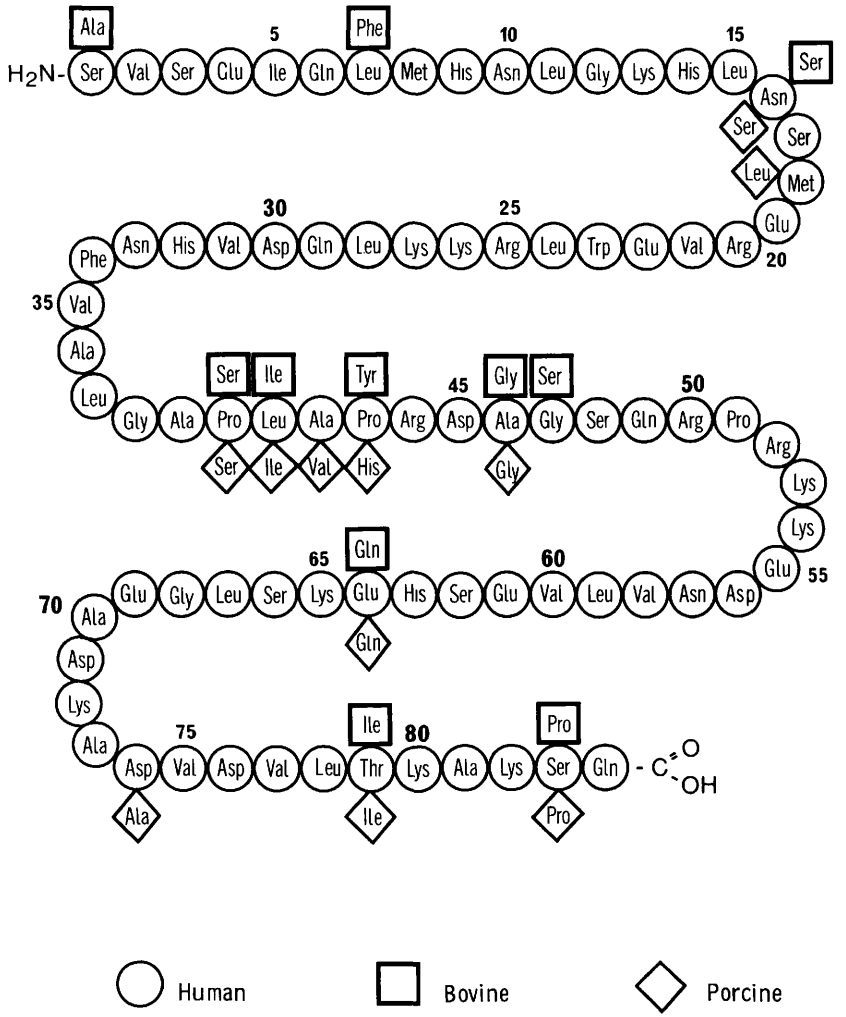


Figure 2 *Amino-acid sequences of human, bovine, and porcine* PTH
(From H. T. Keutmann, G. N. Hendy, M. Boehnert, J. L. H. O'Riordan, and J. T. Potts, jun., *Biochemistry*, 1978, **17**, 5723)

[45] H. T. Keutmann, G. N. Hendy, M. Boehnert, J. L. H. O'Riordan, and J. T. Potts, jun., *J. Endocrinol.*, 1978, **78**, 49.

[46] H. T. Keutmann, G. N. Hendy, M. Boehnert, J. L. H. O'Riordan, and J. T. Potts, jun., *Biochemistry*, 1978, **17**, 5723.

molecule where six of the eight residues in the segment 40—47 show species variation. Proline residues are especially abundant in this region and replacement of these is likely to cause conformational changes in the molecule. This could affect immunological cross-reactivity between the human and other species of the hormone.

The carboxy-terminal region is well conserved. However, the changes at positions 79 and 83 may be sufficient to impair immunological cross-reactivity, as observed with anti-bPTH sera directed towards this region of the molecule.[47, 48]

Structure-Function Studies with Synthetic Peptides. The amino-terminal region (1—34) of parathyroid hormone contains all the structural requirements necessary for full biological activity both *in vitro* and *in vivo*.[49, 50] Modifications of this region in bovine PTH have been studied extensively leading to the synthesis of analogues with enhanced biological activity or increased stability.

Only two modifications of bPTH-(1—34) are known to cause enhancement of biological activity *in vitro* in the rat renal adenyl cyclase assay. Both are at the carboxy terminus: substitution of tyrosine for phenylalanine at position 34 [51] and the substitution of carboxamide ($CONH_2$) for the terminal carboxylic acid group.[52] An analogue which combines these two structural features, [Tyr^{34}]bPTH-(1—34) amide, has been synthesized [53] and shows an additive enhancement of biological activity *in vitro*, being three times more active than unmodified bPTH-(1—34).

Replacement of the Met^{8} and Met^{18} residues with norleucine confers resistance to oxidation.[51] This sulphur-free analogue retains 40% of the biological activity of unsubstituted bPTH-(1—34) *in vitro*, which demonstrates that the methionine residues are not essential for biological activity.

The biological potency of this stable analogue is enhanced by modification of the *C*-terminus.[51, 53] [Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(1—34)-amide has twice the potency of the unmodified hormone and can be labelled to a high degree of specific activity with radioiodine without loss of biological activity. Such iodinated analogues have been used in the characterization of PTH receptors.[54]

A list of synthetic analogues of bPTH-(1—34) with enhanced activity or stability is shown in Table 2. Modification of the Trp^{23} residue by attachment of an *o*-nitrophenyl sulphenyl group to the indole nitrogen yielding [Nle^{8}, Nle^{18}, NPS-Trp^{23}, Tyr^{34}]bPTH-(1—34)-amide did not result in the formation of an inhibitor.[53]

[47] G. N. Hendy, P. M. Barling, and J. L. H. O'Riordan, *Clin. Sci. Mol. Med.*, 1974, **47**, 567.
[48] T. M. Murray, S. A. Muzaffar, J. A. Parsons, and H. T. Keutmann, *Biochemistry*, 1975, **14**, 2705.
[49] J. T. Potts, jun., G. W. Tregear, H. T. Keutmann, H. D. Niall, R. Sauer, L. J. Deftos, B. F. Dawson, M. L. Hogan, and G. D. Aurbach, *Proc. Natl. Acad. Sci. USA*, 1971, **68**, 63.
[50] G. W. Tregear, J. Van Reitschoten, E. Greene, H. T. Keutmann, H. D. Niall, B. Reit, J. A. Parsons, and J. T. Potts, jun., *Endocrinology*, 1973, **93**, 1349.
[51] M. Rosenblatt, D. Goltzman, H. T. Keutmann, G. W. Tregear, and J. T. Potts, jun., *J. Biol. Chem.*, 1976, **251**, 159.
[52] J. A. Parsons, B. Rafferty, D. Gray, B. Reit, H. T. Keutmann, G. W. Tregear, and J. T. Potts, jun., in 'Calcium Regulating Hormones', ed. R. V. Talmage, M. Owen, and J. A. Parsons, Excerpta Medica, Amsterdam, 1975, p. 33.
[53] M. Rosenblatt and J. T. Potts, jun., *Endocrinol. Res. Commun.*, 1977, **4**, 115.
[54] G. V. Segre, M. Rosenblatt, B. L. Reiner, J. E. Mahaffey, and J. T. Potts, jun., *J. Biol. Chem.*, 1979, **254**, 6980.

Table 2 *Analogues of bPTH (1—34)*

Synthetic peptide	*Potency[a]/ MRC units/mg*$^{-1}$	*Relative potency*	*Ref.*
bPTH-(1—34)	5400	100	*b*
[Tyr^{34}]bPTH-(1—34)	7500	140	*b*
bPTH-(1—34)-amide	14800	270	*c*
[Tyr^{34}]bPTH-(1—34)-amide	16800	300	*d*
[Nle^{8}, Nle^{18}]bPTH-(1—34)	2300	40	*b*
[Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(1—34)	4100	76	*b*
[Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(1—34) -amide	10100	190	*d*
[Nle^{8}, Nle^{18}, NPS-Trp^{23}, Tyr^{34}] bPTH-(1—34)-amide	10600	200	*d*

[a] *In vitro* rat renal cortical adenyl cyclase assay. [b] M. Rosenblatt, D. Goltzman, H. T. Kerstman, G. W. Tregear and J. T. Potts, jun., *J. Biol Chem.*, 1976, **251**, 159. [c] J. A. Parsons, B. Rafferty, D. Gray, B. Reit, and H. T. Keutmann, in 'Calcium Regulating Hormones', ed. R. V. Talmage, M. Owen, and J. A. Parsons, Excerpta Medica, Amsterdam, 1975, p. 33. [d] M. Rosenblatt and J. T. Potts, jun., *End. Res. Commun.*, 1977, **4**, 115.

This indicates that an unmodified Trp^{23} is not essential for hormone binding, which is in contrast with the effect of a similar modification in ACTH.[55]

Modification of arginine at positions 20 and 25 in hPTH-(1—34) with cyclohexane-1,2-dione to form [DHCH-Arg^{20}, DHCH-Arg^{25}]hPTH-(1—34) results in a drastic reduction of renal cortical adenyl cyclase activation.[56]

Parathyroid Hormone Inhibitors. It has been shown previously that synthetic fragments, based on the (3—34) sequence of bPTH, lack the structural determinants necessary for the activation of adenylate cyclase in rat renal membranes but still bind to the renal receptors, thus causing competitive antagonism of bPTH-(1—34) or native bPTH-(1—34) in stimulating adenyl cyclase *in vitro*.[57]

The antagonistic potency of bPTH-(3—34) is increased three-fold by introduction of the structural features associated with enhancement and stabilization of biological activity of the full agonist bPTH-(1—34). [Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(1—34)-amide, synthesized[58] by the solid-phase method, lacks agonist activity at doses 500 times those required to elicit maximal adenyl cyclase activation by native bPTH *in vitro* in canine renal cortical membranes. Co-incubation of the renal membranes with antagonist and native bPTH or [Nle^{8}, Nle^{18}, Tyr^{34}]-bPTH-(1—34)-amide at equimolar concentrations causes *ca.* 50% inhibition of bPTH-stimulated adenyl cyclase activity. At higher concentrations the antagonist causes total suppression of bPTH-stimulated adenyl cyclase activity. Moreover, attachment of the bulky *o*-NPS group to the indole nitrogen of Trp^{23} in the antagonist does not affect significantly the inhibitory potency indicating that an unmodified tryptophan at position 23 is not essential for receptor binding.

[55] W. R. Moyle, Y. C. Kong, and J. Ramachandran, *J. Biol. Chem.*, 1973, **248**, 2409.
[56] M. Rosenblatt, G. L. Shepard, G. A. Taylor, and J. T. Potts, jun., *Biochemistry*, 1978, **17**, 3188.
[57] D. Goltzman, A. Peytremann, E. Callahan, G. W. Tregear, and J. T. Potts, jun., *J. Biol. Chem.*, 1975, **250**, 3199.
[58] M. Rosenblatt, E. N. Callahan, J. E. Mahaffey, A. Pont, and J. T. Potts, jun., *J. Biol. Chem.*, 1977, **252**, 5847.

Adenyl cyclase activity in intact monolayer cultures of human giant cell tumours of the bone and of neonatal foreskin can also be used as an *in vitro* test system for PTH.[59] In these systems, the inhibitory potency of [Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(3—34)-amide is identical to that previously reported for canine renal cortical adenyl cyclase.[58] However, it has yet to be shown that antagonism *in vitro* is accompanied by inhibition *in vivo*.

In order to determine the minimum sequence required to inhibit adenyl cyclase stimulation by PTH, six shorter fragments of the analogue [Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(3—34)-amide have been synthesized[60] by the solid-phase method. These fragments contain sequences 5—34, 7—34, 10—34, 15—34, 20—34, and 25—34. None of the peptide fragments show agonist activity in the canine renal adenyl cyclase system at concentrations as high as 10^{-4} M. However, all these peptides, with the exception of fragment 25—34, inhibit totally a maximally active dose of PTH (see Table 3). Step-wise shortening of the *N*-terminus

Table 3 *PTH antagonists*

Analogue	*Molar ratio of inhibitor : PTH required for* 50% *inhibition of adenyl cyclase*[a, b]
[Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(3—34)-amide	1 : 1
[Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(5—34)-amide	12 : 1
[Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(7—34)-amide	30 : 1
[Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(10—34)-amide	70 : 1
[Nle^{8}, Nle^{18}, Tyr^{34}]bPTH-(15—34)-amide	370 : 1
[Tyr^{34}]bPTH-(20—34)-amide	700 : 1
[Tyr^{34}]bPTH-(25—34)-amide	Fails to inhibit at 4000 : 1

[a] In canine renal cortical membranes. [b] J. E. Muhaffey, M. Rosenblatt, G. L. Shepard, and J. T. Potts, jun., *J. Biol. Chem.*, 1979, **254**, 6496.

requires progressively higher doses of the shortened peptide to inhibit adenyl cyclase activation. Fragment 20—34 is capable of full inhibition of PTH-stimulated adenylate cyclase activation, although at a 700-fold higher dose than the 3—34 fragment. This and the complete lack of inhibition by fragment 25—34 at a molar ratio of 4000 : 1 indicates that some or all of the residues within the 20—24 sequence may be important for binding of the peptide to renal receptors. This conclusion is corroborated by the drastic loss of activity following the modification of Arg^{20} and Arg^{25} in hPTH.[56]

Other Synthetic Work. Fragments hPTH-(44—68) and hPTH-(53—84) have been synthesized [16, 62] with the aim of using them as immunogens to develop antisera directed specifically at these two sequences and to determine whether regions of the

[59] S. R. Goldring, J. E. Mahaffy, M. Rosenblatt, J. M. Dayer, J. T. Potts, jun., and S. M. Krane, *J. Clin. Endocrinol. Metabol.*, 1979, **48**, 655.
[60] J. E. Mahaffy, M. Rosenblatt, G. L. Shepard, and J. T. Potts, jun., *J. Biol. Chem.*, 1979, **254**, 6496.
[61] M. Rosenblatt, H. T. Keutmann, G. W. Tregear, and J. T. Potts, jun., *J. Medicin. Chem.*, 1977, **20**, 1452.
[62] M. Rosenblatt, G. V. Segre, G. W. Tregear, G. L. Shepard, G. A. Tyler, and J. T. Potts, jun., *Endocrinology*, 1978, **103**, 978.

molecule other than the amino-terminal sequence participate in biological activity. Both fragments lack agonist activity *in vitro* and *in vivo* and fail to inhibit the action of native PTH *in vitro*, indicating a lack of receptor binding affinity.

The 30 amino-acid *N*-terminal sequence of bovine parathyroid hormone precursor (pre-pro-parathyroid hormone)[63] has been synthesized[64] using the solid-phase method. Difficulties were encountered during synthesis and purification due to the marked hydrophobicity of the so called signal sequence. The availability of such synthetic signal sequences will enable the investigation of proteolytic processing that follows pre-hormone biosynthesis and also the production of specific antisera for immunolocalization studies of the precursor.

Labelled Compounds. A technique for labelling bPTH to high specific activity with tritium by [^{3}H]methyl exchange has been described.[65] [^{3}H]bPTH has full *in vitro* biological activity and full immunological reactivity. The method is of potentially wide application for the preparation of biologically active labelled peptides. It consists of methylating methionine residues with [^{3}H]methyl iodide to yield [^{3}H]methyl sulphonium salts, followed by demethylation with 2-mercaptoethanol to regenerate bPTH containing some [^{3}H]Met.

Biological Effects. Apart from the known effects of PTH on bone and kidney, other biological actions have also been described. Apparently, PTH has an intrinsic vasodilatory action.[66] In anaesthetized rats and dogs i.v. injections of either native bPTH or synthetic bPTH-(1—34) produce a dose related hypotension. This hypotensive action is not inhibited by α- or β-adrenergic, cholinergic, or histaminergic blocking agents. A coronary vasodilatory effect of bPTH-(1—34) has also been reported.[67]

Exogenously administered or endogenously stimulated PTH produces a significant increase in plasma-renin activity (PRA) and sodium excretion in dogs by an unknown mechanism.[68, 69]

Ectopic Production. Biosynthesis of PTH-like peptides by a human squamous cell carcinoma in culture [70] and production of immunoreactive PTH by lymphoblastic leukaemia cells in culture has been described.[71]

[63] B. Kemper, J. F. Habener, R. C. Mulligan, J. T. Potts, jun., and A. Rich, *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 3731.
[64] M. Rosenblatt, J. F. Habener, G. A. Tyler, G. L. Shepard, and J. T. Potts, jun., *J. Biol. Chem.*, 1979, **254**, 1414.
[65] D. L. Christie, W. S. Hancock, and P. M. Barling, *Endocrinology*, 1979, **105**, 1020.
[66] P. K. T. Pang, T. E. Tenner, jun., J. A. Yee, M. Yang, and H. Janssen, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 675.
[67] M. F. Crass, tert., and P. K. T. Pang, *Science*, 1980, **207**, 1087.
[68] J. M. Smith, D. R. Mouw, and A. J. Vander, *Am. J. Physiol.*, 1979, **236**, 311.
[69] H. R. Powell, D. A. McCredie, and E. Rotenberg, *Endocrinology*, 1978, **103**, 985.
[70] J. W. Hamilton, C. R. Hartmann, D. H. MacGregor, and D. R. Cohn, *J. Clin. Endocrinol. Metabol.*, 1977, **45**, 1023.
[71] N. K. C. Ramsay, D. M. Brown, M. E. Nesbit, P. F. Coccia, and W. Krivit, *J. Paediat.*, 1979, **94**, 623.

7 Vasoactive Peptides

Contributed by P. D. Roy

Bradykinin.—Reviews have covered the chemistry and biological activity of bradykinin analogues,[1] bradykinin receptors,[2] and immunological aspects of bradykinin relating to antibody production[3] and radioimmunoassay methodology.[4]

Table 1 lists new bradykinin analogues with available biological data reported in 1979. It includes a large series of octapeptide analogues of des-[Arg^9]-bradykinin, synthesized for studies on the structural features associated with activation of, and binding to, the recently characterized B_1 kinin receptor in the rabbit aorta. In this assay system des-[Arg^9]-bradykinin is six-fold more active than bradykinin and consequently was selected as the standard agonist. The results obtained with a series of alanine analogues, (1), (5)—(8), (18)—(20), derived by systematic replacement of each residue of des-[Arg^9]-bradykinin, indicate that activation of the receptor is a function of the *C*-terminus (see α^E values, Table 1). The myotropic activities of the latter analogues are seen to be reduced to varying degrees relative to des-[Arg^9]-bradykinin, except in the case of the [Ala^6]-octapeptide analogue (18). This maintains equivalent activity since presumably replacement of Ser^6 by alanine is an approximately isosteric interchange. The effect of replacing the phenylalanine residues in position 5 and/or 8 by aliphatic residues (leucine and cyclohexylalanine) or aromatic residues (*O*-methyl tyrosine and D-phenylalanine) was also examined. The data here indicate that Phe^5 is involved in the binding of des-[Arg^9]-bradykinin to the rabbit aorta receptor, whilst Phe^8 is associated with activation of the receptor. Antagonists were obtained on replacement of Phe^8 by the aliphatic substituents cyclohexylalanine (21) or leucine (23), or on substitution of both phenylalanines with cyclohexylalanine (14). These three analogues were devoid of intrinsic activity but retained receptor-binding capacity and thereby exhibited inhibitory properties. An interesting anomaly in this series appears to be the [D-Phe^8]-octapeptide analogue (22) which in contrast to the other 8-substituted analogues [(20), (21), (23), (24)], displayed high agonist activity, four-fold greater than des-[Arg^9]-bradykinin. The synthesis of des-[Arg^9]-bradykinin and analogues was by the solid-phase method utilizing the Merrifield resin and an automated peptide synthesizer. The latter was appropriately modified with a series of cooled ($-15\,^\circ C$) amino-acid reservoirs to reduce decomposition of Boc-amino-acid symmetrical anhydrides used for all coupling steps.[5, 6]

In contrast the introduction of isoleucine into positions 5 and/or 8 of bradykinin gave analogues (13), (17), and (25), with weak agonist activity but disappointingly no antagonist activity in rat uterus or rat blood pressure assay systems. This was due to the poor receptor affinity of these analogues and it is argued that future

[1] J. M. Stewart in 'Handbook of Experimental Pharmacology', Vol. 25: Bradykinin, Kallidin, and Kallikrein, Supplement, ed. E. G. Erdös, Springer-Verlag, Berlin, F.R.G., 1979, p. 357.
[2] C. E. Odya and T. L. Goodfriend, ref. 1, p. 287.
[3] M. Stocker, U. Hilgenfeldt, and F. Gross, *Experientia*, 1979, **35**, 1113.
[4] T. L. Goodfriend and C. E. Odya in 'Methods of Hormone Radioimmunoassay' (2nd edn.), ed. B. M. Jaffe and H. R. Behrman, Academic Press, New York, 1979, 909.
[5] S. St.-Pierre, P. Gaudreau, J. N. Drouin, D. Regoli, and S. Lemaire, *Can. J. Biochem.*, 1979, **57**, 1084.
[6] J. N. Drouin, P. Gaudreau, S. A. St.-Pierre, and D. Regoli, *Can. J. Physiol. Pharmacol.*, 1979, **57**, 562.

Table 1 *Analogues of bradykinin*

1 5 9

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg

Bradykinin

Compound number	*Structure*[a]	*Biological activity*[b]	*Test*[c]	*Ref.*
(1)	[Ala^{1}]-(1—8)	6.2%	B	5
		$\alpha^{E} = 1.0$	B	5
(2)	[β-Ala^{1,4},Gly^{2,3,5,6}]-(1—6)			10
(3)	*cyclo*[-Lys^{1},Gly^{6},Arg^{9}-]	*cf.* bradykinin	A	11
(4)	[β-Ala^{1,9},γ-Abu^{6}]			10
(5)	[Ala^{2}]-(1—8)	3.6%	B	5
		$\alpha^{E} = 1.0$	B	5
(6)	[Ala^{3}]-(1—8)	<1.0%	B	5
(7)	[Ala^{4}]-(1—8)	<1.0%	B	5
(8)	[Ala^{5}]-(1—8)	5.5%	B	5
		$\alpha^{E} = 1.0$	B	5
(9)	[Cha^{5}]-(1—8)	84.0%	B	5
		$\alpha^{E} = 1.0$	B	5
(10)	[D-Phe^{5}]-(1—8)	<1.0%	B	5
(11)	[Leu^{5}]-(1—8)	0.2%	B	5
		$\alpha^{E} = 1.0$	B	5
(12)	[Tyr(Me)^{5}]-(1—8)	<1.0%	B	5
		$\alpha^{E} = 1.0$	B	5
(13)	[Ile^{5}]	+	A	7
		+	C	7
(14)	[Cha^{5,8}]-(1—8)	*	B	5
		$\alpha^{E} = 0$	B	5
(15)	[D-Phe^{5,8}]-(1—8)	<1.0%	B	5
(16)	[Tyr(Me)^{5,8}]-(1—8)	<1.0%	B	5
(17)	[Ile^{5,8}]	+	A	7
		+	C	7
(18)	[Ala^{6}]-(1—8)	100%	B	5
		$\alpha^{E} = 1.0$	B	5
(19)	[Ala^{7}]-(1—8)	2.2%	B	5
		$\alpha^{E} = 0.6$	B	5
(20)	[Ala^{8}]-(1—8)	<1.0%	B	5
		$\alpha^{E} = 0.8$	B	5
(21)	[Cha^{8}]-(1—8)	*	B	5
		$\alpha^{E} = 0$	B	5
(22)	[D-Phe^{8}]-(1—8)	400%	B	5
		$\alpha^{E} = 1.0$	B	5
(23)	[Leu^{8}]-(1—8)	**	B	5
(24)	[Tyr(Me)^{8}]-(1—8)	<1.0%	B	5
(25)	[Ile^{8}]	+	A	7
		+	C	7
(26)	β-Ala-bradykinin	+	A	9
		+/++	C	9
(27)	NH ‖ Me-C-bradykinin	+	D	9
		+	A	9
		+	C	9
		+	D	9

Table 1 (*cont.*)

Compound number	*Structure*[a]	*Biological activity*[b]	*Test*[c]	*Ref.*
(28)	H_2N-CO-bradykinin	+	A	9
		+ + +	C	9
		+ + + +	D	9
(29)	Gly-Gly-Gly-Arg-bradykinin			15

[a] Abu, γ-aminobutyric acid; Cha, cyclohexylalanine.

[b] Percentage values shown are relative to des-[Arg^9]bradykinin (100%). Symbols for potency: 0—0.99% +, 1—9.99% + +, 10—99.9% + + +, 100% or greater + + + + (relative to bradykinin, + + + +). α^E, intrinsic activity expressed as a fraction of [des-Arg^9]bradykinin (α^E = 1.0). * and ** indicate inhibition (of increasing potency).

[c] *In vivo*: A, rat blood pressure. *In vitro*: B, rabbit aorta; C, rat uterus; D, guinea-pig ileum.

attempts to develop antagonists should centre on replacement of phenylalanine residues by rigid cyclic amino-acids with the aim of retaining binding capacity.[7]

An extensive study has been carried out on the activities and antibody-binding characteristics of previously reported 9-substituted analogues and fragments of bradykinin. The results obtained have further demonstrated the importance of the *C*-terminal arginine residue for biological activity (assay in guinea-pig ileum), and in addition have shown that the same residue is also essential for binding to antibody raised against bradykinin when linked to ovalbumin *via* toluene di-isocyanate. Interestingly the fragments (2—9)- and (3—9)-bradykinin, with intact *C*-termini, showed 2.5 and 8.75 times greater antibody-binding activity, respectively, than the parent nonapeptide.[8]

The structure–activity relationships for the *N*-terminus of bradykinin have been further examined. *N*-Terminal extension with β-alanyl or acetimidoyl gave analogues (26) and (27), respectively, with greatly diminished bradykinin activity, whilst carbamoylation at this position provided a fully active analogue (28). It is concluded that loss in activity is not solely a function of steric hindrance at the *N*-terminus, as previously suspected following the relative inactivity of Boc-bradykinin. None of the three analogues showed inhibitory activity.[9] The solid-phase synthesis of other β-alanine analogues, (2) and (4), has been reported.[10]

The cyclic bradykinin analogue (3) in which the *C*-terminal carboxyl group is coupled to the ε-amino-group of lysine in position 1, shows equivalent activity to bradykinin in the rat blood pressure lowering assay. It appears that this particular mode of cyclization also leads to prolongation of *in vivo* activity.[11]

Conformational changes of bradykinin with increasing temperature have been monitored using c.d. and ^{13}C n.m.r. spectroscopy. The results obtained suggest that there is a *ca.* 20% probability of the molecule existing in a γ-turn conformation.[12] Variation in the ^1H n.m.r. spectrum as a function of pH has been used to study the side-chain conformation of bradykinin residues.[13] Conformational differences

[7] A. T. Chiu, A. R. Day, J. A. Radding, and R. J. Freer, *J. Med. Chem.*, 1979, **22**, 334.
[8] L. W. Redman, D. Regoli, and E. R. Tustanoff, *Can. J. Biochem.*, 1979, **57**, 529.
[9] V. S. Chauhau, G. T. Young, and N. G. Bowery, *Bio-org. Chem.*, 1979, **8**, 333.
[10] V. Skaric, M. Topic-Bulic, and D. Skaric, *Croat. Chem. Acta*, 1978, **51**, 347.
[11] G. Cipens, F. Mutulis, and I. Misina, *Ger. Offen.*, 2 853 840, 28th June, 1979.
[12] J. R. Cann, R. E. London, J. M. Stewart, and N. A. Matwiyoff, *Int. J. Pept. Protein Res.*, 1979, **14**, 388.
[13] K. Lintner, S. Fermandjian, S. St.-Pierre, and D. Regoli, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 803.

between bradykinin, and a number of its fragments and analogues, have been discussed in relation to biological activity.[14]

Angiotensin.—The renin–angiotensin system has been reviewed in relation to hypertension,[16] activation,[17] inhibition,[18] radioimmunoassay,[19] and angiotensin receptors.[20]

Table 2 lists new angiotensin* analogues with biological activities reported mainly during 1979. The goal of research efforts remains the development of

Table 2 *Analogues of angiotensin*

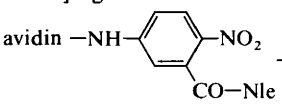
1 5 8
Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
Angiotensin II

Compound number	*Structure*[a]	*Biological activity (Myotropic/ Pressor)*	*Other*	*Test*[b]	*Ref.*
(30)	[Asp(NMe$_2$)^1]	70%		C	28
		6.5%[c]		A	28
		α^E = 1.0[c]		A	28
(31)	[des-Asp^1, HOCH$_2$CO-Arg^2, Leu^8]		*		23
(32)	[des-Asp^1, H$_2$NOCH$_2$CO-Arg^2, Leu^8]		*		24
(33)	[des-Asp^1, D-Ala^2, Leu^8]	1.4%[d]		C	26
(34)	[Sar^1, β-HTyr^4]	2.0%		C	29
(35)	[Asn^1, Phe(4-NH$_2$)^4]	24.0%		C	27
(36)	[Asn^1, Phe(4-NO$_2$)^4]	0.1%		C	27
(37)	[Asn^1, Tyr(3,5-Me$_2$)^4]	2.2%		C	27
(38)	[Asn^1, D-Tyr(3,5-Me$_2$)^4]	1.4%		C	27
(39)	[Sar^1, Phe(4-NO$_2$)^4, Val^5]	43.0%[f]		A	31
(40)	[Sar^1, Phe(4-N$_3$)^4, Val^5]	54.0%[f]		A	31
(41)	[Sar^1, Phe(4-N$_2^+$), Val^5]	98.0%[f]		A	31
(42)	[Sar^1, Phe(4-N$_2^+$)^{4,8}, Val^5]	96.0%[f]		A	31
(43)	[Sar^1, Phe(4-NO$_2$)^{4,8}, Val^5]	83.0%[f]		A	31
(44)	[Sar^1, Phe(4-N$_3$)^{4,8}, Val^5]	56.0%[f]		A	31
(45)	[Asn^1, Tyr(3-Bzl)^4, Phe(4-NO$_2$)^6]	<0.1%[d]		C	25
(46)	[Asn^1, Tyr(3-Bzl)^4, Phe(4-NO$_2$)^8]	0.2%[d]		C	26
(47)	[Sar^1, β-HIle^5]	2.8%		C	29
(48)	[4-formylbenzoyl-Asn^1, Val^5]	8.0%[e]		A	30
(49)	[fluoresceinthiocarbamyl-Asn^1, Val^5]	1.0%[e]		A	30
(50)	[Sar^1, Val^5, Phe(4-NO$_2$)^8]	89.0%[f]		A	31
(51)	[Sar^1, Val^5, Phe(4-N$_3$)^8]	46.0%[f]		A	31

[14] K. Lintner, S. Fermandjian, and D. Regoli, *Biochimie*, 1979, **61**, 87.
[15] A. C. M. Camargo, H. Caldo, and M. L. Reis, *J. Biol. Chem.*, 1979, **254**, 5304.
[16] J. D. Swales, *Pharmacol. Ther.*, 1979, **7**, 173.
[17] F. H. M. Derkx, B. N. Bouma, M. P. A. Schalekamp, and M. A. D. H. Schalekamp, *Nature (London)*, 1979, **280**, 315.
[18] M. A. Ondetti and D. W. Cushman, *Annu. Rep. Med. Chem.*, 1978, **13**, 82.
[19] A. E. Freedlander and T. L. Goodfriend, ref. 4, p. 887.
[20] M. A. Devynck and P. Meyer, *Adv. Pharmacol. Ther.*, 7th Proc. Int. Congr. Pharmacol., ed. J. Jacobs, Pergamon Press, Oxford, 1978, **1**, 279.

* The term angiotensin is used to refer specifically to [Asp^1,Ile^5]angiotensin II; other derivatives are named with reference to this.

Table 2 (*cont.*)

Compound number	Structure[a]	Biological activity (Myotropic/ Pressor)	Other	Test[b]	Ref.
(52)	[Sar[1], Val[5], Phe(4-N_2^+)[8]]	72.0%[f]		A	31
(53)	[Asn[1], Pya[6]]	3.5%[d]		C	25
(54)	[Asn[1], D-Pya[6]]	0.1%[d]		C	25
(55)	[Asn[1], Ima[6]]	0.4%[d]		C	25
(56)	[Asn[1], D-Ima[6]]	<0.1%[d]		C	25
(57)	[Asn[1], Phe(4-NH_2)[6]]	<0.1%[d]		C	25
(58)	[Asn[1], Phe(4-NO_2)[6]]	<0.1%[d]		C	25
(59)	[Asn[1], Dab[6]]	<0.1%[d]		C	25
(60)	[Sar[1,7]]	22.0%		B	21
		17.0%		C	21
(61)	[Sar[1,7], Leu[8]]	<0.1%	*	B	21
		0.7%		C	21
(62)	[Sar[1,7], Leu[8]]	<0.1%	*	B	21
		0.2%		C	21
(63)	[Sar[1,7], Ala[8]]	<0.1%	*	B	21
		<0.1%		C	21
(64)	[Sar[1,7], Met[8]]	<0.1%	*	B	21
		0.8%		C	21
(65)	[Sar[1,7], Thr(Me)[8]]	<0.1%	*	B	21
		0.1%		C	21
(66)	[Sar[1,7],DL-*a*Ile[8]]	0.7%	*	B	21
		12.5%		C	21
(67)	[Sar[1], Ala(Me)[7], Ile[8]]		*	B	22
(68)	[Sar[1], DL-Nip[7], Ile[8]]		*	B	22
(69)	[Sar[1], Ile[8]]	<0.1%	**	B	21
		1.1%		C	21
(70)	[Asn[1], Ahp[8]]	11.5%		C	26
(71)	[Asn[1], Nle[8]]	7.3%		C	26
(72)	[Asn[1], Len[8]]	1.2%		C	26
(73)	[Asn[1], Ind[8]]	0.1%	*	C	26
(74)	[Asn[1], Phe(4-NH_2)[8]]	52.5%		C	26
(75)	[Asn[1], Phe(4-NO_2)[8]]	15.0%		C	26
(76)	[β-HIle[5]]	29.2%		C	29
(77)	[rhodaminethiocarbamyl]-angiotensin	4.0%[e]		A	30
(78)	[2-methoxy-2,4-diphenyl-3(2*H*)-furanone]-angiotensin	3.0%[e]		A	30
(79)	[(2-nitro-5-azidobenzoyl)-Nle]angiotensin	0.5%[e]		A	30
(80)		0.1%[e]		A	30

[a] β-HTyr = β-homotyrosine; β-HIle = β-homoisoleucine; Pya = β-(2-pyridyl)alanine; Ima = β-(2-imidazoyl)alanine; Dab = 2,4-diaminobutyric acid; *a*Ile = *allo*isoleucine; Nip = nipecotic acid (3-piperidine carboxylic acid); Ahp = 2-aminoheptanoic acid; Ind = 2-aminoidane-2-carboxylic acid; avidin = egg white protein.

[b] A, rabbit aorta; B, rat uterus; C, rat pressor activity.

[c] Affinity relative to [Asn[1]]angiotensin; α^E = intrinsic activity.

[d] Relative to [Asn[1], Val[5]]angiotensin (100%).

[e] Affinity relative to angiotensin.

[f] Mean residual activity after treatment with photolabile analogue.

* and ** indicate inhibition (of increasing potency).

antagonists with low pressor activities and increased resistance to enzymatic degradation for use in the long-term management of human hypertension. This in turn has led to further work on the evaluation of angiotensin receptors utilizing in some cases specifically designed analogues.

The solid-phase synthesis of angiotensin antagonists containing sarcosine in position 7 has been reported.[21] Replacement of proline in this position of [Sar^1]angiotensin provides an analogue (60) which retains significant agonist activity (22.0%) in rat uterus, which suggests receptor interaction is not severely affected by this modification. The same interchange of proline for sarcosine has been applied to a series of known angiotensin inhibitors to produce the inhibitory analogues (61)—(66). However, these analogues still retain low, but significant, pressor activity. The [Sar^{1,7},DL-*a*Ile^8]angiotensin analogue (66) is seen to have unusually high agonist activity in both *in vitro* and *in vivo* rat assay systems. Moreover, none of the above analogues is a more potent antagonist than [Sar^1,Ile^8]angiotensin (69) in rat uterus.

The replacement of proline in position 7 of [Sar^1,Ile^8] by *N*-methylalanine or DL-nipecotic acid gives analogues (67) and (68), respectively, which also inhibit angiotensin activity in rat.[22] Other angiotensin antagonist analogues (31) and (32) have been described in patent reports.[23, 24]

The role in pressor activity of the imidazole nitrogens in His^6 of [Asn^1]-angiotensin has been examined through synthesis and evaluation of analogues (45), (53)—(59). In this series, substitution of histidine in general produced analogues with weak pressor activities in rat. Since the isohistidine analogue [Asn^1,Ima^6]angiotensin (55) (Ima = β-(2-imidazolyl)alanine) is essentially inactive it appears that the alignment of the heterocyclic nitrogens is a factor more important than retention of the imidazole structure. The synthesis of [Asn^1,Pya^6]angiotensin (53) (Pya = β-(2-Pyridyl)alanine) involved problems of extensive racemization (78%) of Boc-L-Pya on activation, despite the use of 1-hydroxybenzotriazole as an additive.[25]

The [Asn^1]angiotensin analogues (70)—(75) in which Phe^8 has been replaced by aliphatic or aromatic amino-acid residues have been used to study the role of position 8 in pressor activity. High pressor activity (52.5%) in rat was obtained with the [Asn^1,Phe(4-NH$_2$)^8]angiotensin analogue (74), but substitution of the *para*-position of the aromatic ring of Phe^8 by the bulkier nitro-substituent gave an analogue (75) with lower activity (15.0%). Pressor activity was almost abolished on substitution of position 8 with the 2-aminoindane-2-carboxylic acid (Ind) residue where rotational freedom of the phenyl ring is lacking. This analogue (73) was found to be a more potent antagonist than [Leu^8]angiotensin, but not [Sar^1,Leu^8]-angiotensin. Among the [Asn^1]angiotensin analogues substituted in position 8 with aliphatic residues 2-aminoheptanoic acid (70), norleucine (71), and leucine (72), a linear relationship was observed between pressor activity and increase in hydropho-

[21] G. J. Moore, E. M. Oudeman, D. Ko, and D. M. Nystrom, *J. Med. Chem.*, 1979, **22**, 1147.
[22] G. J. Moore and E. M. Ko, *Can. J. Physiol. Pharmacol.*, 1979, **57**, 763.
[23] L. Kisfaludy, O. Nyeki, M. Szirmai, E. Karpati, K. Gidai and L. Szporny, *Ger. Offen.*, 2831271, 1st March, 1979 (*Chem. Abs.*, 1979, **91**, 39880r).
[24] L. Kisfaludy, O. Nyeki, M. Szirmai, E. Karpati, K. Gidai, and L. Szporny, *Ger. Offen.*, 2831534, 1st Feb., 1979 (*Chem. Abs.*, 1979, **91**, 39877v).
[25] K. Hsieh and E. C. Jorgensen, *J. Med. Chem.*, 1979, **22**, 1199.

bicity. The lower activity of the leucine analogue (72) compared to the corresponding norleucine analogue (71) indicates that branching of the side-chain decreases agonist activity. These results overall provide useful structure–activity information for an approach to the design of inhibitory analogues with low pressor activity.[26]

The contribution of tyrosine in position 4 of [Asn[1]]angiotensin in pressor activity has been studied through analogues in which the hydroxy-group is replaced by the amino (35) or nitro (36) group, or flanked by two methyl groups in the *ortho*-position [analogues (37) and (38)]. Whilst [Asn[1],Phe(4-NO_2)[4]]angiotensin (36) was relatively inactive (0.1%) in the rat pressor activity, the corresponding *p*-aminophenyl analogue (35) retained significant activity (24.0%) suggesting a requirement for proton donation in hydrogen-bond formation at the receptor level. Reduced potencies of the dimethyltyrosine analogues (37) and (38) may be due to reduced hydrogen-bond formation as a result of steric hindrance by the *ortho*-methyl groups.[27]

The replacement of asparagine in [Asn[1]]angiotensin by the more hydrophobic N^4-dimethylasparagine residue yields an analogue (30) with *ca.* 70% pressor activity in rat. This modification did not alter intrinsic activity though a marked reduction in affinity relative to [Asn[1]]angiotensin was observed in rabbit aorta, indicating hydrogen-bond or steric requirements in position 1 of [Asn[1]]angiotensin for full expression of activity. Specificity of action of analogue (30) was demonstrated by competitive inhibition experiments.[28]

Angiotensin analogues with increased resistance to degradation by chymotrypsin were obtained on incorporating β-homotyrosine in position 4 of [Sar[1]]angiotensin (34) or β-homoisoleucine in position 5 of [Sar[1]]angiotensin (47) or angiotensin (76). However, there was no corresponding increase in *in vivo* duration of action suggesting degradation occurs by cleavage at other positions or involves other peptidases.[29]

Three fluorescent *N*-terminal analogues of [Asn[1],Val[5]]angiotensin and angiotensin (49), (77), and (78), and two corresponding reactive analogues (48), and (79) have been synthesized for use in light microscopic visualization and covalent labelling, respectively, of vascular receptor sites. The affinity of these analogues for rabbit aorta was in the range 0.1—8.0%. Difficulties were experienced in the purification of the fluorescein (49) and rhodamine (77) analogues.[30]

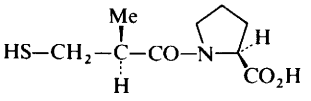
Several photolabile [Sar[1],Val[5]]angiotensin analogues (39)—(44) and (50—(52) have been used for evaluation of smooth-muscle receptors. From the series reported the best labelling peptides were those containing 4-nitrophenylalanine in position 4 (39) and 4-azidophenylalanine in position 8 (51).[31]

^{1}H N.m.r. titration studies on the conformation of angiotensin indicate the molecule is extended in acidic solution and folded at neutral pH due to interaction of charged terminal groups.[32] The structural requirements for biological activity in

[26] K. Hsieh, E. C. Jorgensen, and T. C. Lee, *J. Med. Chem.*, 1979, **22**, 1038.
[27] K. Hsieh, I. C. Kiraly-Olah, E. C. Jorgensen, and T. C. Lee, *J. Med. Chem.*, 1979, 1044.
[28] P. Cordopatis, J. Brison, J. Dirkx, and D. Theodoropoulos, *Experientia*, 1979, **35**, 849.
[29] K. Stachowiak, M. C. Khosla, K. Plucinska, P. A. Khairallah, and F. M. Bumpus, *J. Med. Chem.*, 1979, **22**, 1128.
[30] R. E. Galardy, S. S. Stafford, M. L. Schaefer, H. Ho, K. A. LaVorgna, and J. D. Jamieson, *J. Med. Chem.*, 1978, **21**, 1279.
[31] E. H. F. Escher and G. Guillemette, *J. Med. Chem.*, 1979, **22**, 1047.
[32] S. Premilat and B. Maigret, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 534.

the His-Pro region in angiotensin analogues has been studied by ^{1}H and ^{13}C n.m.r. spectroscopy. The results obtained suggest that the *cis*-isomer of histidine imidazole hydrogens (C-2 and C-4) is important for receptor binding.[33] The results of a conformational comparison of [Val^{5},Trp^{8}]angiotensin and [des-Asp^{1},Val^{4},Trp^{8}]-angiotensin (angiotensin III analogue) by fluorescence measurements over a range of pH values indicate the existence of folded structures in the *C*-terminal segment of both peptides. Small differences in conformational parameters are evident though it is not certain whether this forms the basis for receptor specificity.[34]

Potentiating Peptides and Enzyme Inhibitors.—The antihypertensive effect of the orally active angiotensin-converting enzyme inhibitor captopril [2-D-methyl-3-mercaptopropanoyl-proline, SQ 14225; compound (81)] has been the subject of



(81)

intensive research, and numerous publications have appeared relating to clinical trials in man[35-40] as well as investigations in various animal models.[41,42] The results obtained from clinical studies in man indicate that this agent offers a new, efficient, and well-tolerated approach to the management of hypertension. It is now anticipated that captopril will soon be successfully marketed.

The development of potent and specific inhibitors of angiotensin-converting enzyme as antihypertensive drugs has been reviewed.[43] An essentially similar approach has now led to the mercapto-compounds, 2-benzyl-3-mercaptopropanoic acid (82) and 2-mercaptomethyl-5-guanidinopentanoic acid (83) which are potent

$$HS \cdot CH_2 \cdot CH(CH_2Ph) \cdot CO_2H$$

(82)

$$HS \cdot CH_2 \cdot CH(CH_2 \cdot CH_2 \cdot CH_2 \cdot NH \cdot C(:NH) \cdot NH_2) \cdot CO_2H$$

(83)

and specific inhibitors of carboxypeptidase A and B, respectively.[44] The latter success has in turn fully validated the original approach to the design of enzyme inhibitors based on models of enzyme active sites.

[33] M. Liakopoulou-Kyriakides and R. E. Galardy, *Biochemistry*, 1979, **18**, 1952.
[34] P. W. Schiller, *Can. J. Biochem.*, 1979, **57**, 402.
[35] G. A. Turini, H. R. Brunner, M. Gribic, B. Waeber, and H. Gavras, *Lancet*, 1979, *i*, 1213.
[36] H. R. Brunner, B. Waeber, and H. Gavras, *Lancet*, 1979, *i*, 832.
[37] R. Fagard, A. Amery, P. Lijnen, and T. Reybrouck, *Circulation*, 1979, **59** and **60** (suppl. II), 227.
[38] H. R. Brunner, H. Gavras, B. Waeber, G. R. Kershaw, G. A. Turini, R. A. Vukovick, D. N. McKinstry, and I. Gavras, *Ann. Internal Medicine*, 1979, **90**, 19.
[39] K. M. Baker, D. W. Johns, C. R. Ayers, S. C. Williams, M. R. Yancey, M. J. Peach, and R. M. Carey, *Circulation*, 1979, **59** and **60** (suppl. II), 144.
[40] L. Favre, M. Fraser, D. N. McKinstry, and J. W. Hollifield, *Kidney Int.*, 1979, **15**, 585.
[41] J. F. E. Mann, W. Rascher, R. Dietz, A. Schoemig, and D. Ganten, *Clin. Sci.*, 1979, **56**, 585.
[42] T. R. Vrobel, J. N. Cohn, and R. J. Bache, *Circulation*, 1979, **59** and **60** (suppl. II), 227.
[43] D. W. Cushman, H. S. Cheung, E. F. Sabo, and B. Rubin, *Fed. Proc.*, 1979, **38**, 2778.
[44] M. A. Ondetti, M. E. Condon, J. Reid, E. F. Sabo, H. S. Cheung, and D. W. Cushman, *Biochemistry*, 1979, **18**, 1427.

The synthesis of numerous analogues of captopril (81) has been described mainly in patent reports. These include analogues with variations to the side-chain of captopril to give the corresponding halogeno-,[45] phosphonoacyl-,[46] and phosphinyl-[47] compounds (84)—(86), or those in which replacement of the proline residue by carbocyclic analogues,[48] tryptophan,[49] or tyrosine [50] has been carried out.

$$HS \cdot CH_2 \cdot CH(CF_3) \cdot CO\text{-Pro}$$

(84)

$$(HO)_2P(O) \cdot CH_2 \cdot CO\text{-Pro}$$

(85)

$$Ph(CH_2)_3 \cdot P(O)(OCH_2Ph) \cdot CH_2 \cdot CO\text{-Pro}$$

(86)

The fate of a labelled analogue of bradykinin-potentiating peptide 9a (BPP9a), [^{3}H]-Glp-^{1}Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro, has been followed after intravenous injection in rabbit. The nonapeptide (8%) and three constituent fragments, Glp-Trp (60%), Glp-Trp-Pro-Arg-Pro-Gln (20%) and Glp-Trp-Pro-Arg-Pro-Gln-Ile (12%), were rapidly removed from blood and excreted in urine. This pattern of degradation is not a function of blood or urine enzymes but apparently occurs in the kidney. The results obtained explain the relatively short-lasting depressor effect of BPP(9a) in the light of its resistance to attack by proteinase and peptidase enzymes, including angiotensin-converting enzyme itself.[51]

Tachykinins.—Methods for radioimmunoassay of substance P,[52] bombesin, and bombesin-related peptides [53] have been reviewed.

Attempts to develop specific and competitive inhibitors of substance P (87) are continuing. From a series of analogues synthesized on the solid phase, [D-Phe^9]-substance P showed very weak agonist activity, but no significant antagonist

$$\overset{1}{} \qquad \overset{5}{} \qquad \overset{10}{}$$

$$Arg\text{-}Pro\text{-}Lys\text{-}Pro(Gln\text{-}Gln\text{-}Phe\text{-}Phe\text{-}Gly\text{-}Leu\text{-}Met\text{-}NH_2$$

(87)

activity when tested in guinea-pig ileum. The [Ile^8]-substance P analogue was found to be twice as active as the parent compound.[54] Weak antagonist activity is reported for the *C*-terminal heptapeptide analogue, [Glu(*N*-Me$_2$)^5]-(5—11)-

[45] E. R. Squibb and Sons, Inc., *U.S. Patent*, 4 154 934, 15 May, 1979 (*Chem. Abs.*, 1979, **91**, 74 899a).
[46] E. R. Squibb and Sons, Inc., *U.S. Patent*, 4 151 172, 24 Apr., 1979 (*Chem. Abs.*, 1979, **91**, 108 230t).
[47] E. R. Squibb and Sons, Inc., *U.S. Patent*, 4 168 267, 18 Sept., 1979 (Chem. Abs., 1980, **92**, 76 922x).
[48] A. Sugie and J. Katsube, *Chem. Pharm. Bull.*, 1979, **27**, 1708.
[49] Santen Pharmaceutical Co. Ltd., *Jpn. Kokai Tokkyo Koho*, **79**, 52 073, 24 Apr., 1979 (*Chem. Abs.*, 1979, **91**, 108 233w).
[50] Santen Pharmaceutical Co. Ltd., *Jpn. Kokai Tokkyo Koho*, **79**, 92 937, 23 Jul., 1979 (*Chem. Abs.*, 1980, **92**, 76 920v).
[51] L. C. Martin, J. W. Ryan, G. H. Fisher, A. Chung, M. Epstein, and J. M. Stewart, *Biochem. J.*, 1979, **184**, 713.
[52] E. A. Mroz and S. E. Leeman, ref. 4, p. 121.
[53] J. H. Walsh and H. C. Wong, ref. 4, p. 581.
[54] G. Rackur, I. Yamaguchi, J. J. Leban, U. Björkroth, S. Rosell, and K. Folkers, *Acta Chem. Scand. Ser. B*, 1979, **33**, 375.

substance P. This particular modification is seen to increase both hydrophobicity and steric hindrance at the *N*-terminus.[55]

Degradation studies in rat brain preparations on partial sequences of substance P indicate that Boc-blocking at the *N*-terminus of the corresponding free peptides can increase resistance to proteolysis. Such stabilized analogues, in particular the blocked hepta- and octa-peptide *C*-terminal fragments of substance P, appear to be potentially useful tools for probing the receptor of substance P.[56] In this connection the preparation of a labelled hexapeptide substance P fragment, [acetyl-Lys^{6},Phe(4-^{3}H)^{7},Nle^{11}]-(6—11)-substance P, with full activity on smooth muscle has been reported. The analogue was obtained by the catalytic tritiation of [acetyl-Lys^{6},Phe(4-Cl)^{7},Nle^{11}]-(6—11)-substance P.[57]

The solid-phase synthesis of substance P has been used in part to evaluate a new and readily prepared polar support. The undecapeptide amide product was obtained in an overall yield of 47%.[58]

Substance P from bovine hypothalamus has been isolated and purified. The amino-acid sequence was rigorously determined and shown to be identical with that previously reported. There was no evidence of the existence of a different form of substance P.[59]

The skin of frog continues to be the source of new and interesting tachykinins with counterparts in the mammalian system. The undecapeptide, Ala-Pro-Gly-[Ile^{3}]-angiotensin II, has been isolated from the skin of the Australian frog *Crinia georgiana* and shown to have angiotensin-like activity in smooth muscle preparations.[60] The mucosa of the mammalian and avian gut has now been shown to contain one or more peptides with activities similar to bombesin and litorin.[61]

8 Enkephalins, Endorphins, and Related Peptides

Contributed by P. W. Schiller

Introduction.—More than 600 papers on all aspects of enkephalins and endorphins published during 1979 bear testimony to the undiminished vigorous research efforts in the field of endogenous opiates. The present article will be limited mainly to the presentation of new structure–activity relationships established with enkephalin- and endorphin-analogues and to the elucidation of the conformational element in opioid peptide action. We will also focus upon studies of opiate receptors at the molecular level, since they have greatly benefitted from the application of peptide synthetic methods. 'Current Contents, Life Sciences' (Institute for Scientific Information, Philadelphia, U.S.A.) and the 'Medline' file (National Library of Medicine, Bethesda, U.S.A.) have been the initial sources of references.

Most aspects of opioid peptides have been covered by numerous review articles of recent appearance. General reviews on enkephalins and endorphins include those

[55] N. Pinas, C. P. Poulas, and D. Theodoropoulos, *FEBS Lett.*, 1979, **108**, 45.
[56] S. Blumberg and V. I. Teichberg, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 347.
[57] M. Bienert, H. Niedrich, I. Teplan, and K. Medzihradszky, *J. Labelled Compd. Radiopharm.*, 1978, **14**, 577.
[58] R. Arshady, E. Atherton, M. J. Gait, K. Lee, and R. C. Sheppard, *J. Chem. Soc., Chem. Commun.*, 1979. 423.
[59] R. Carraway and S. E. Leeman, *J. Biol. Chem.*, 1979, **254**, 2944.
[60] V. Erspamer, P. Melchiorri, T. Nakajima, T. Yasuhara, and R. Endean, *Experientia*, 1979, **35**, 1132.
[61] V. Erspamer, G. F. Erspamer, P. Melchiorri, and L. Negri, *Gut*, 1979, **20**, 1047.

by Miller and Cuatrecasas [1, 2] and by Kuschinsky.[3] Articles dealing predominantly with the biology of opioid peptides have been presented by Beaumont and Hughes,[4] Rossier and Bloom,[5] and Holaday and Loh.[6] New developments in the characterization of opiate receptors have been dealt with in detail by Simon [7] and by Snyder *et al.*[8] Structure–activity relationships have been reviewed by Morley [9] (enkephalins) and by Li [10] (endorphins). Reviews dealing with the common precursor of ACTH and endorphin were written by Eipper and Mains [11] and by Chrétien *et al.*[12] The role of enkephalins and endorphins in the gastrointestinal tract was summarized by Ambinder and Schuster.[13] A mini-review by Meites *et al.*[14] covers neuroendocrine functions of opioid peptides and North [15] published a comprehensive article on the action of exogenous and endogenous opiates on single neurones. The importance of endorphins in psychiatry was assessed in a review by Ananth and Callanan [16] and Snyder [17] commented on the clinical relevance of opiate receptor and opioid peptide research.

New Opioid Peptides, Biosynthesis and Degradation.—Lewis *et al.*[18] were able to separate all the known enkephalins and endorphins by high performance liquid chromatography (h.p.l.c.) in a single run. H.p.l.c. also proved to be an invaluable tool for the detection and isolation of new opioid peptides.

Takagi *et al.*[19, 20] reported the isolation of a dipeptide, Tyr-Arg, from bovine brain, which displayed a relatively long-lasting analgesic effect in the mouse-tail pinch test. However, *in vitro* the dipeptide showed low activity in the guinea-pig ileum assay and in the opiate receptor binding assay. The authors concluded that Tyr-Arg produces analgesia by *releasing* [Met^5]enkephalin and by weakly inhibiting enkephalin degradation enzymes. N^{α}-acetylated forms of β-endorphin and β-LPH-(61—87) were isolated from porcine pituitaries by Smyth *et al.*[21] Inactivation of β-endorphin and its fragments by acetylation might be of importance for storage purposes or for the maintenance of normal levels of opiate activity in the central nervous system. Interestingly, β-endorphin isolated from salmon pituitaries [22]

[1] R. J. Miller and P. Cuatrecasas, *Vitam. Horm.* (New York), 1978, **36**, 297.
[2] R. J. Miller and P. Cuatrecasas in 'Neurochemical Mechanisms of Opiates and Endorphins' (Adv. Biochem. Psychopharmacol. Vol. 20), ed. H. H. Loh and D. H. Ross, Raven Press, New York, 1979, p. 187.
[3] K. Kuschinsky, *Klin. Wochenschr.*, 1979, **57**, 701.
[4] A. Beaumont and J. Hughes, *Annu. Rev. Pharmacol. Toxicol.*, 1979, **19**, 245.
[5] J. Rossier and F. Bloom, ref. 2, p. 165.
[6] J. W. Holaday and H. H. Loh, ref. 2, p. 227.
[7] E. J. Simon, ref. 2, p. 31.
[8] S. H. Snyder, S. R. Childers, and I. Creese, ref. 2, p. 543.
[9] J. S. Morley, *Annu. Rev. Pharmacol. Toxicol.*, 1980, **20**, 81.
[10] C. H. Li, ref. 2, p. 145.
[11] B. A. Eipper and R. E. Mains, *Endocrinol. Rev.*, 1980, **1**, 1.
[12] M. Chrétien, S. Benjannet, F. Gossard, C. Gianoulakis, P. Crine, M. Lis, and N. G. Seidah, *Can. J. Biochem.*, 1979, **57**, 1111.
[13] K. F. Ambinder and M. M. Schuster, *Gastroenterology*, 1979, **77**, 1132.
[14] J. Meites, J. F. Bruni, D. A. Van Vugt, and A. F. Smith, *Life Sci.*, 1979, **24**, 1325.
[15] R. A. North, *Life Sci.*, 1979, **24**, 1527.
[16] J. Anant and T. S. Callanan, *Comprehensive Psychiatry*, 1979, **20**, 246.
[17] S. H. Snyder, *Nature* (*London*), 1979, **279**, 13.
[18] R. V. Lewis, S. Stein, and S. Udenfriend, *Int. J. Pept. Protein Res.*, 1979, **13**, 493.
[19] H. Takagi, H. Shiomi, H. Ueda, and H. Amano, *Eur. J. Pharmacol.*, 1979, **55**, 109.
[20] H. Takagi, H. Shiomi, H. Ueda, and H. Amano, *Nature* (*London*), 1979, **282**, 410.
[21] D. G. Smyth, D. E. Massey, S. Zakarian, and M. D. A. Finnie, *Nature* (*London*), 1979, **279**, 252.
[22] H. Kawauchi and M. Tubokawa, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 1249.

seems to occur only in the N^{α}-acetylated form. A novel opioid peptide, α-neoendorphin, was isolated from pig hypothalami by Kangawa and Matsuo [23] and its sequence was partially determined as: Tyr-Gly-Gly-Phe-Leu-Arg-(Pro, Gly, Tyr_2, Lys_2, Arg). α-Neoendorphin was shown to be five times more potent than β-endorphin in the guinea-pig ileum assay. Whether or not this peptide is related to a precursor of [Leu^5]enkephalin remains to be demonstrated. Goldstein's group [24, 25] isolated an extremely potent opioid peptide, dynorphin-(1—13), from porcine pituitaries. The sequence of this peptide, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys, contains [Leu^5]enkephalin in the *N*-terminal part. Since dynorphin-(1—13) is 700 times more potent than [Leu^5]enkephalin in the guinea-pig ileum assay, its possible role as a precursor of the latter peptide seems unlikely. Stern *et al.*[26] isolated a new opioid peptide with the sequence Tyr-Gly-Gly-Phe-Met-Arg-Phe from bovine adrenal chromaffin granules and striatal extracts, which is probably derived from a larger protein, unrelated to β-endorphin.

While significant progress on the formation of β-endorphin from the pro-ACTH/endorphin precursor has been made,[11, 12] information about the biosynthesis of the enkephalins is still scarce. Tyr-Gly-Gly-Phe-Met(O)-Arg, a presumable precursor of [Met^5]enkephalin, was isolated from porcine hypothalami by Huang *et al.*[27] Two putative enkephalin precursor peptides from bovine adrenal medulla have recently been purified and partially characterized by Udenfriend's group.[28] One is a [Met^5]enkephalin precursor containing two copies of the [Met^5]enkephalin sequence and the other one contains both the [Met^5]enkephalin and the [Leu^5]enkephalin sequence. While these peptides look promising as candidates for enkephalin precursors, there is now evidence to indicate that β-endorphin is not involved in the biosynthesis of enkephalins.

Enkephalin degradation has been the subject of several recent investigations. The carboxypeptidase A inhibitors, D-phenylalanine and β-phenylpropionic acid, were shown to reduce enkephalin degradation in the bath fluid surrounding electrically stimulated guinea-pig ileum.[29] A convenient fluorometric assay based on resonance energy transfer occurring in the analogue Tyr-Gly-Gly-Phe-Met-NH-$(CH_2)_2$-Dns was developed by Guyon *et al.*[30] for monitoring enkephalin degradation by aminopeptidases. Bajusz [31] provided evidence that introduction of D-amino-acids in position-2 of enkephalin not only increases resistance against aminopeptidases but also enhances affinity for opiate receptors. Several contributions dealt with the cleavage of the Gly-Phe bond in enkephalins by carboxydipeptidase ('enkephalinase'). A h.p.l.c.-study of enkephalin degradation by mouse striatum extracts

[23] K. Kangawa and H. Matsuo, *Biochem. Biophys. Res. Commun.*, 1979, **86**, 153.
[24] L. I. Lowney, S. B. Gentleman, and A. Goldstein, *Life Sci.*, 1979, **24**, 2377.
[25] A. Goldstein, S. Tachibana, L. I. Lowney, M. Hunkapiller, and L. Hood, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6666.
[26] A. S. Stern, R. V. Lewis, S. Kimura, J. Rossier, L. D. Gerber, L. Brink, S. Stein, and S. Udenfriend, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6680.
[27] W.-Y. Huang, R. C. C. Chang, A. J. Kastin, D. H. Coy, and A. V. Schally, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 6177.
[28] S. Kimura, R. V. Lewis, A. S. Stern, J. Rossier, S. Stein, and S. Udenfriend, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 1681.
[29] D. Della Bella, A. Carenzi, V. Frigeni, and V. Santini, *Neuropharmacol.*, 1979, **18**, 719.
[30] A. Guyon, J. Barbet, B. P. Roques, J.-P. Swerts, B. Malfroy, and J.-C. Schwartz, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 919.
[31] S. Bajusz, *Pharmazie*, 1979, **34**, 352.

revealed both aminopeptidase and carboxydipeptidase activity.[32] Since enkephalin degradation by carboxydipeptidase was not efficiently inhibited by captopril,[33, 34] it can be concluded that 'enkephalinase' and 'kinase-II' are not identical enzymes even though they show some similarities. Fournié-Zaluski *et al.*[35] showed 'enkephalinase' to be an enzyme of low substrate specificity and also pointed out a correlation between the enhanced biological activity of several enkephalin analogues and their poor recognition by 'enkephalinase'. A conclusive study[36] revealed that α- and γ-endorphin and des-Tyr^1-α- and des-Tyr^1-γ-endorphin are endogenous peptides in rat pituitary and brain and not mere isolation artefacts.

Structure–Activity Relationships in Various Assay Systems.—Numerous new activities of enkephalins and endorphins have been described in recent articles and reviewing all of them would be beyond the scope of this article. However, most new enkephalin and endorphin analogues were tested in well established assays. Among the *in vitro* bioassays the most frequently used were those based on inhibition of electrically stimulated contractions of the guinea-pig ileum (GPI) or the mouse vas deferens (MVD). The availability of an ever increasing number of radiolabelled opiate agonists and antagonists with high receptor affinity has led to the frequent use of binding assays in recent years. The large number of different procedures used in the past for measuring *in vivo* analgesic activities has been drastically reduced and the rat or mouse tail flick test is now accepted as the method of choice by most authors in the field. Determination of adenylate cyclase activity and of growth hormone and prolactin secretion has also resulted in interesting structure–activity data. Many investigators performed several different assays for testing new analogues. Multiple assays have become a necessity in view of the often observed discrepanices between structure–activity data obtained in different assay systems.

New methodology and biological activities of analogues in each assay system will be presented in separate sections in the following. Aside from new analogues, old ones are sometimes listed again in order to allow for easy comparison. Molar potencies relative to standard compounds have been used as a measure of activity.

Isolated Tissue Assays. The MVD- and the GPI-assay remained the standard methods for testing the activity of new analogues. According to the receptor classification of Lord *et al.*,[37] GPI contains mainly μ-receptors, which are more sensitive to alkaloid opiates than to enkephalins, while δ-receptors, found in MVD, display higher affinity for enkephalins than for opiates. Shaw[38] recently introduced a field stimulated rat rectum preparation as a new assay for opiate activity. Structure–activity studies with opioid peptides and with alkaloid opiate agonists and antagonists revealed that the opiate receptors in the rat rectum are most likely

[32] A. Guyon, B. P. Roques, F. Guyon, A. Foucault, R. Perdrisot, J.-P. Swerts, and J.-C. Schwartz, *Life Sci.*, 1979, **25**, 1605.
[33] J. P. Swerts, R. Perdrisot, B. Malfroy, and J. C. Schwartz, *Eur. J. Pharmacol.*, 1979, **53**, 209.
[34] W. R. Buckett, *Eur. J. Pharmacol.*, 1979, **57**, 267.
[35] M.-C. Fournié-Zaluski, R. Perdrisot, G. Gacel, J.-P. Swerts, B. P. Roques, and J.-C. Schwartz, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 130.
[36] J. Verhoef, J. G. Loeber, J. P. H. Burback, W. H. Gispen, A. Witter, and D. deWied, *Life Sci.*, 1980, **26**, 851.
[37] J. A. H. Lord, A. A. Waterfield, J. Hughes, and H. W. Kosterlitz, *Nature (London)*, 1977, **267**, 495.
[38] J. S. Shaw, *Br. J. Pharmacol.*, 1979, **67**, 428P.

of the δ-type. The potencies of new enkephalin- and endorphin-analogues in the MVD- and GPI-assays are listed in Table 1.

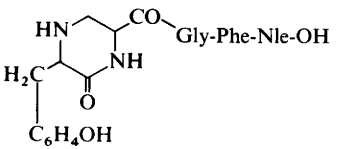
The tyrosyl residue in position-1 of enkephalin was the target of several interesting chemical modifications. Substitution of *p*-aminophenylalanine for tyrosine in [Leu^5]enkephalin resulted in an analogue (8) with low activity in the MVD-assay.[42] This result re-emphasizes the importance of an intact tyrosyl

Table 1 *Activity of analogues in isolated tissue assays*

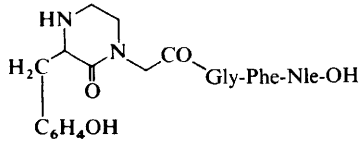
(a) *Enkephalins*

Compound number	*Structure**	*Test* MVD potency	*Ref.*	GPI potency	*Ref.*
1	D-Ala^2, Phe(*p*-NO$_2$)^4, Leu^5-NH$_2$			2270[a]	39
2	D-Ala^2, Leu^5, Lys(N^{ε}-Ac)^6-NH$_2$			360[a]	39
3	D-Ala^2, Δ^zPhe^4, Met^5-NH$_2$			5[c]	40
4	D-Ala^2, gPhe^4, *RS*-mLeu^5-NH$_2$			206[a]	41
5	D-Ala^2, gPhe^4, *RS*-mMet^5-NH$_2$			175[a]	41
6	D-Ala^2, gPhe^4, D-HCO-Leu^5			1464[a]	41
7	D-Ala^2, gPhe^4, D-HCO-Met^5			1499[a]	41
8	Phe(*p*-NH$_2$)^1, Leu^5	0.28[a]	42		
9	Glycol$^{2-3}$, Leu^5	0.21[a]	42		
10	Leu^5-NH-Ad	3.5[b]	43	1.6[b]	43
11	D-Ala^2, Leu^5-NH-Ad	4[b]	43	1.7[b]	43
12	3-Hydroxybenzyl-2,5-dioxo-piperazine-1-yl-acetyl, Des-Gly^2	<1[b]	44	<1[b]	44
13	3-Benzyl-2,5-dioxopipera-zine-1-yl-acetyl, Des-Gly^2	<1[b]	44	<1[b]	44
14	D-Met^2, Des-Met^5-NH$_2$	51[d]	45	216[d]	45
15	D-Nle^2, Pro^5-NH$_2$	146[d]	45	225[d]	45
16	D-Nle^2, Des-Met^5-NH$_2$	106[d]	45	274[d]	45
17	D-Met^2, Nle^5	3850[d]	45	57[d]	45
18	D-Met^2, Pro^5	201[d]	45	41[d]	45
19	D-Met^2, Pro^5-NH$_2$	790[e]	46	790[e]	46
20	H$_2$N-C(=NH)-Tyr^1, D-Met^2, Pro^5-NH$_2$	2500[e]	46	82300[e]	46
14	D-Met^2, Des-Met^5-NH$_2$	410[e]	46	1700[e]	46
21	H$_2$N-C(=NH)-Tyr^1, D-Met^2, Des-Met^5-NH$_2$	2100[e]	46	21100[e]	46
16	D-Nle^2, Des-Met^5-NH$_2$	840[e]	46	2170[e]	46
22	H$_2$N-C(=NH)-Tyr^1, D-Nle^2, Des-Met^5-NH$_2$	1800[e]	46	12600[e]	46
15	D-Nle^2, Pro^5-NH$_2$	1160[e]	46	1770[e]	46
23	H$_2$N-C(=NH)-Tyr^1, D-Nle^2, Pro^5-NH$_2$	1700[e]	46	7900[e]	46

[39] J. V. Castell, A. N. Eberle, V. M. Kriwaczek, A. Tun-Kyi, P. W. Schiller, K. Q. Do, P. Thanei, and R. Schwyzer, *Helv. Chim. Acta*, 1979, **62**, 525.
[40] R. E. Chipkin, J. M. Stewart, and C. H. Stammer, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 890.
[41] M. Chorev, R. Shavitz, M. Goodman, S. Minick, and R. Guillemin, *Science*, 1979, **204**, 1210.
[42] M. J. Quinn, F. J. Laska, and M. R. Fennessy, *Clin. Exptl. Pharmacol. Physiol.*, 1979, **6**, 535.
[43] R. Tomatis, S. Salvadori, E. Menegatti, and M. Guarneri, *Il. Farmaco*, 1979, **34**, 496.
[44] R. Tomatis, S. Salvadori, and M. Guarneri, *Il Farmaco*, 1979, **34**, 698.
[45] A. Z. Rónai, J. I. Székely, I. Berzétei, E. Miglécz, and S. Bajusz, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 1239.
[46] S. Bajusz, A. Z. Rónai, J. I. Székely, E. Miglécz, and I. Berzétei, *FEBS Lett.*, 1980, **110**, 85.

Compound number	*Structure**	*Test MVD potency*	*Ref.*	*GPI potency*	*Ref.*
19	D-Met^2, Pro^5-NH$_2$	1986[f]	47	517[f]	47
24	D-Met^2, *N*-Val^5-NH$_2$	2453[f]	47	727[f]	47
25	D-Ala^2, Pro^5-NH$_2$	366[f]	47	160[f]	47
26	D-Ala^2, *N*-Val^5-NH$_2$	5957[f]	47	320[f]	47
27	D-Ala^2, Car^4, Leu^5			121[a]	48
28	D-Ala^2, Car^4, Met^5-NH$_2$			411[a]	48
29	D-Ala^2, Ada^4, Leu^5			5[a]	48
30	D-Ala^2, Ada^4, Leu^5-NH$_2$			8[a]	48
31	Phe(NMe)^4, Met^5			18[a]	49
32	Trp(NMe)^4, Met^5			39[a]	49
33	Leu(NMe)^5			70[a]	49
34	Trp^4, Leu(NMe)^5			4[a]	49
35	Pro^5			1[a]	49
36	Trp^4, Pro^5			1[a]	49
37	Tyr(OMe)^1, Met^5			2[a]	49
38	Tyr(OMe)^1, Trp^4, Met^5			0.5[a]	49
39	$H_2N \cdot CH(C_6H_4OH \cdot CH_2) \cdot CH_2 \cdot CH_2 \cdot CO$-Gly-Phe-Met-$NH_2$			3[a]	50
40	$H_2N \cdot CH(C_6H_4OH \cdot CH_2) \cdot NH \cdot CH_2 \cdot CO$-Gly-Phe-Met-$NH_2$			56[a]	50
41	$H_2N \cdot CH(C_6H_4OH \cdot CH_2) \cdot NH \cdot CH_2 \cdot CO$-Gly-Phe-Met-ol			83[a]	50
42	H-Tyr-NH·$CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CO$-Phe-Met-$NH_2$			<0.3[a]	50
43	H-Tyr-NH·$CH_2 \cdot CH_2 \cdot NH \cdot CH_2CO$-Phe-Met-OH			<1[a]	50
44	H-Tyr-NH·$CH_2 \cdot CH_2 \cdot NH \cdot CH_2CO$-Phe-Met-ol			0.3[a]	50
45	H-Tyr-NH·$CH_2 \cdot CH_2 \cdot NH \cdot CH_2CO$-Phe-Met-$NH_2$			0.3[a]	50
46				inactive[a]	51

[47] Y. Audigier, H. Mazarguil, and J. Gros, *FEBS Lett.*, 1980, **110**, 88.
[48] R. Schwyzer in 'Peptides: Structure and Biological Function', ed. E. Gross and J. Meienhofer, Pierce Chemical Co., Rockford, Ill., 1979, p. 993.
[49] P. W. Schiller and J. St.-Hilaire, *J. Med. Chem.*, 1980, **23**, 290.
[50] D. Hudson, R. Sharpe, and M. Szelke, *Int. J. Pept. Protein Res.*, 1980, **15**, 122.
[51] J. DiMaio, P. W. Schiller, and B. Belleau, ref. 48, p. 889.

Compound number	*Structure**	*Test MVD potency*	*Ref.*	*GPI potency*	*Ref.*
47				inactive[a]	51
48	Tyr($^{+}$NMe$_3$)^1, Leu^5-OMe, I$^-$			0.2[b]	51
49	Tyr^0	28[a]	52	15[a]	52
50	Desamino-Tyr^0	0[a]	52	0[a]	52
51	Phe^0	50[a]	52	18[a]	52
52	Tyr^0, Phe^1	0[a]	52	0[a]	52
53	Arg^0	22[a]	52	17[a]	52
54	Lys^0	25[a]	52	20[a]	52
55	Desamino-Lys^0	0[a]	52	0[a]	52
56	Gly^0	18[a]	52	30[a]	52
57	β-Ala^0	0[a]	52	0[a]	52
58	$H_2N(CH_2)_3$-CO-Tyr^1	0[a]	52	0[a]	52
(b)	*β-Endorphins and α-neoendorphins*				
I	Met(O)^5-β_h-EP	30[g]	53	12[g]	53
II	Leu^5-β_h-EP	113[g]	53	13[g]	53
III	Gly^{31}-β_h-EP			168[g]	54
IV	Gly^{31}-β_h-EP-NH$_2$			200[g]	54
V	Gly^{31}-β_h-EP-Gly-OH			132[g]	54
VI	Phe^{27}, Gly^{31}-β_h-EP			128[g]	55
VII	Cys^{17}-Cys^{26}, Phe^{27}, Gly^{31}-β_h-EP			128[g]	55
VIII	Cys^{11}-Cys^{26}, Phe^{27}, Gly^{31}-β_h-EP			186[g]	55
IX	Cys^7-Cys^{26}, Phe^{27}, Gly^{31}-β_h-EP			7[g]	55
X	Des-Gly^2-β_c-EP			1[h]	56
XI	Des-Met^5-β_c-EP			7[h]	56
XII	Des-Thr^6-β_c-EP			97[h]	56
XIII	Des-Ser^{10}-β_c-EP			117[h]	56
XIV	Des-Gln^{11}-β_c-EP			100[h]	56
XV	Des-Thr^{12}-β_c-EP			117[h]	56
XVI	Des-Pro^{13}-β_c-EP			110[h]	56
XVII	Des-Leu^{14}-β_c-EP			140[g]	56
XVIII	Des-Val^{15}-β_c-EP			123[g]	56
XIX	Des-Asn^{20}-β_c-EP			111[g]	56
XX	Des-Ile^{22}-β_c-EP			197[g]	56
XXI	α-NEP-(1—6)			8[a]	57

[52] G. Gacel, M.-C. Fournié-Zaluski, E. Fellion, B. P. Roques, B. Senault, J.-M. Lecomte, B. Malfroy, J.-P. Swerts, and J.-C. Schwartz, *Life Sci.*, 1979, **24**, 725.

[53] B. M. Cox, M. Ross, A. Goldstein, and R. M. Palmour, *Brain Res.*, 1979, **165**, 311.

[54] C. H. Li, D. Yamashiro, L.-F. Tseng, W.-C. Chang, and P. Ferrara, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3276.

[55] J. Blake, W.-C. Chang, and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, **14**, 275.

[56] C. H. Li, ref. 48, p. 823.

[57] H. Matsuo, K. Kangawa, Y. Nakagawa, N. Chino, and M. Igarashi, ref. 48, p. 873.

Compound number	*Structure**	*Test* *MVD potency*	*Ref.*	*GPI potency*	*Ref.*
XXII	α-NEP-(1—9)			330[a]	57
XXIII	α-NEP-(1—9)-NH_2			800[a]	57

Potency: [a]% Potency relative to [Met[5]]enkephalin. [b]% Potency relative to [Leu[5]]enkephalin. [c]% Potency relative to [D-Ala[2], Met[5]]enkephalinamide. [d]% Potency relative to [D-Met[2], Pro[5]]enkephalinamide. [e]% Potency relative to normorphine. [f]% Potency relative to morphine. [g]% Potency relative to β_h-endorphin. [h]% Potency relative to β_c-endorphin.

* Entire structural formulae are given for compounds (39—47). Otherwise, the numbers indicating the position of amino-acids refer to the sequence of [Met[5]]enkephalin (H-Tyr[1]-Gly[2]-Gly[3]-Phe[4]-Met[5]-OH), unless a β-endorphin (β-EP) or α-neoendorphin (α-NEP) sequence is specified. The subscripts h and c depict human and camel β-endorphin, respectively.

Abbreviations: Δ^zPhe, α,β-dehydrophenylalanine; gPhe, *gem*-diaminomethane substituted with a side-chain corresponding to that of phenylalanine; mLeu, malonic acid residue substituted with a side-chain corresponding to that of leucine; mMet, malonic acid residue substituted with a side-chain corresponding to that of methionine; Ad, adamantyl; Car, *o*-carboranylalanine; Ada, (*S*)-2-amino-3-(1-adamantyl)-propionic acid (adamantylalanine); Cbm, cyclobutylmethyl; Hyph, 2-hydroxy-3-(*p*-hydroxyphenyl)-propionic acid; the symbol -ol designates the amino alcohol obtained by reduction of the α-carboxyl group of an amino-acid.

hydroxy-group for opiate activity. Guanidation of the *N*-terminal amino-group produced analogues (20—23) which were more active in the GPI- and MVD-assays than their amino-terminated parent compounds.[46] The addition of a guanidino-group shifts the positive charge by 1.2 Å and provides the capacity for hydrogen-bond formation. A diketopiperazine analogue of [Leu[5]]enkephalin (12) [44] showed low activity. This was to be expected, since acylation of the amino-group in the tyramine moiety of enkephalin is known to destroy activity (see ref. 61). Conformationally restricted analogues, containing a piperazinone structure in the *N*-terminal part of the sequence, (46, 47) were found to be inactive in the GPI-assay.[51] Since in these compounds the tyramine moiety is perfectly superimposable on the corresponding entity in the rigid morphine molecule, this unexpected result suggests that the conformation of the tyramine fragment in the receptor-bound peptide may be different from that present in the alkaloid. Gacel *et al.*[52] prepared various analogues (49—58) with additional amino-acids attached to the *N*-terminal and on the basis of the observed opiate activities the authors concluded that an ammonium group linked to the α-carbon of the *N*-terminal amino-acid is required for activity, whereas its position relative to the backbone can be somewhat modified.

Substitution of phenylalanine in position-4 of enkephalin with various unnatural amino-acids resulted in a number of interesting analogues. Introduction of a nitro-group in the *para*-position of Phe[4] in [D-Ala[2],Leu[5]]enkephalinamide produced a compound (1) with very high potency in the GPI assay.[39] Substitution of the α-β-dehydro derivative of phenylalanine for Phe[4] in [D-Ala[2],Met[5]]enkephalinamide (3) enhanced potency by a factor of five relative to the parent compound.[40] Schwyzer's group [48] prepared several enkephalin analogues containing the 'fat' amino-acids carboranylalanine (Car) or (*S*)-2-amino-3-(adamantyl)-propionic acid (Ada) in place of Phe[4] (27—30). The low activity of the [Ada[4]]enkephalins compared to the corresponding [Car[4]]enkephalins can be explained with the pseudoaromatic character of Car which is lacking in the purely aliphatic Ada. For the purpose of conformational studies by fluorescence techniques (see below) Schiller and St.

Hilaire[49] prepared several analogues with tryptophan or N^{α}-methyltryptophan substituted in position-4 (32, 34, 36, 38). Small but significant differences in potency between the [Trp^4]analogues (32) and (34) and their corresponding [Phe^4]analogues (31 and 33) were observed in the GPI-assay.

The role of the residue in position-5 for opiate activity was investigated by several groups. Audigier *et al.*[47] introduced norvaline in this position and the resulting analogues (24, 26) proved to be more active than the corresponding [Pro^5]peptides (19, 25) both in the GPI- and the MVD-assay. In order to enhance the lipophilic character of enkephalins, Tomatis *et al.*[43] prepared analogues carrying an adamantyl group at the *C*-terminal (10, 11). These peptides were more active in the *in vitro* assays than the corresponding parent compounds with an unsubstituted carboxamide-group at the *C*-terminal. The importance of the *C*-terminal residue in determining the character of opiate activity was investigated by Rónai *et al.*[45] (14—18). It was found that an aliphatic residue in position-5 and an unmodified carboxy-group at the *C*-terminal are the structural requirement for efficient binding to δ-receptors. On the other hand, the interaction with μ-receptors is not appreciably affected by structural modification at the *C*-terminal of enkephalin and omission of the residue in position-5 produced analogues with even enhanced activity in the GPI-assay. Extension of [D-Ala^2,Leu^5]enkephalin at the *C*-terminal with N^{ε}-acetyllysine resulted in an analogue (2) with good activity in the GPI-assay.[39] This result indicates that attachment of enkephalins to carrier molecules or Sepharose derivatives *via* the ε-amino-group of the added lysine is feasible.

Several interesting modifications of the peptide backbone of enkephalin were performed. In order to enhance stability against enzymatic degradation Chorev *et al.*[41] reversed one or more of the peptide bonds by synthesizing so-called retro-inverso-analogues of enkephalinamides. This was achieved by substitution of *gem*-diaminoalkyl (g) residues and malonyl (m) residues (4—7). The topochemistry of the side-chain residues in these compounds is analogous to that of related peptides with non-modified backbones. As could be expected, the potencies of the retro-inverso analogues in the GPI-assay were comparable to those of the corresponding parent peptides with non-modified backbones. Replacement of a peptide bond by a glycol bond as in the case of glycol$^{2-3}$-[Leu^5]enkephalin (9) caused a drastic drop in potency in the MVD-assay which has been interpreted in terms of reduced backbone flexibility.[42] Several analogues (39—45) containing CH_2—CH_2 or CH_2—NH bonds in place of peptide bonds were prepared by Hudson *et al.*[50] Such replacements of the Tyr^1-Gly^2 peptide bond produced analogues with retained activity in the MVD-assay, while modification of the Gly^2-Gly^3 peptide linkage reduced potency by more than two orders of magnitude. The results also indicate that the tyrosine carbonyl group *per se* as well as the proposed hydrogen bond between the amino-group of Phe^4 and the carbonyl-group of Tyr^1 are not important structural elements in the biologically active conformation. A cyclic enkephalin analogue, *cyclo*-[(NHCH$_2$CH$_2$)Tyr-Gly-Gly-Phe-Met] was synthesized by the same group and found to be totally inactive in both the GPI- and MVD-assay.

In human β-endorphin, oxidation of Met^5(I) or its replacement with Leu (II) reduces potency by approximately one order of magnitude in the GPI-assay, in contrast to the MVD-assay, where (II) was slightly more active than β-endorphin

and (I) still retained one-third of the potency of the latter peptide.[53] Various performed modifications at the *C*-terminal of human β-endorphin resulted in three analogues (III—V) which were all more potent than the native peptide in the GPI-assay.[54] The same group [55] synthesized three interesting analogues of human β-endorphin containing disulphide bridges (VII—IX). In the GPI-assay, analogues with cystine bridges between positions 17 and 26 or 11 and 26 were as active as the corresponding open-chain analogue (VI), whereas a disulphide bridge between positions-7 and -26 led to an 18-fold decrease in potency. Li [56] also reported the properties of camel β-endorphin analogues with deletion of a single amino-acid (X—XX). In the GPI-assay, omission of Gly^2 or Met^5 produced a substantial decrease in potency, while deletion of single amino-acids between positions-6 and -22 did not greatly affect activity.

Matsuo *et al.*[57] synthesized several fragments of α-neoendorphin (α-NEP) (XXI—XXIII). The carboxamidated 9-peptide (XXIII) is more active than native α-neoendorphin and seven times more potent than β-endorphin in the GPI-assay.

Opiate Receptor Affinities. Binding assays can be performed under a variety of different conditions. Variables include the selection and preparation of tissue, the choice of the radiolabelled ligand, the composition and temperature of the incubation medium and the incubation time. [^{3}H]Naloxone and [^{3}H]etorphine were the most frequently used radioligands for the collection of structure–activity data with new peptide analogues. However, [^{125}I]- or [^{3}H]-labelled derivatives of enkephalin and endorphin and their analogues have also been introduced and approximately ten different radiolabelled opioid peptide analogues have been described in the recent literature. Detailed studies of the binding characteristics of various radiolabelled opiates and opioid peptides will be presented in the section on 'Characterization of Opiate Receptors' (see below). Relative affinities of new enkephalin- and endorphin analogues are presented in Table 2.

Table 2 *Opiate receptor affinities*

(a) *Enkephalins*

Compound number	*Structure*	*Affinity*	*Ref.*
1	D-Ala^2, $Phe(p\text{-}NO_2)^4$, Leu^5-NH_2	3090[a]	39
2	D-Ala^2, Leu^5 $Lys(N^{\varepsilon}\text{-}Ac)^6$-$NH_2$	390[a]	39
19	D-Met^2, Pro^5-NH_2	207[b]	47
		571[c]	47
24	D-Met^2, *N*-Val^5-NH_2	283[b]	47
		667[c]	47
25	D-Ala^2, Pro^5-NH_2	50[b]	47
		133[c]	47
26	D-Ala^2, *N*-Val^5-NH_2	293[b]	47
		1333[c]	47
31	$Phe(NMe)^4$, Met^5	18[d]	49
32	$Trp(NMe)^4$, Met^5	46[d]	49
33	$Leu(NMe)^5$	44[d]	49
34	Trp^4, $Leu(NMe)^5$	31[d]	49
35	Pro^5	6[d]	49
36	Trp^4, Pro^5	5[d]	49
37	$Tyr(OMe)^1$, Met^5	2[d]	49

Table 2 (*cont.*)

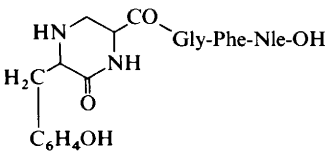
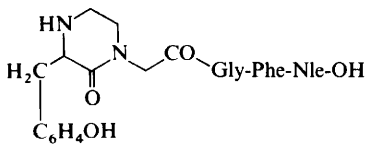
Compound number	*Structure*	*Activity*	*Ref.*
38	Tyr(OMe)[1], Trp[4], Met[5]	<1[a]	49
39	$H_2N \cdot CH(CH_2 \cdot C_6H_4 \cdot OH)CH_2\ CH_2 \cdot CH_2 \cdot CO$-Gly-Phe-Met-$NH_2$	9[e]	50
40	$H_2N \cdot CH(CH_2 \cdot C_6H_4 \cdot OH) \cdot CH_2 \cdot NH \cdot CH_2 \cdot CO$-Gly-Phe-Met-$NH_2$	50[e]	50
41	$H_2N \cdot CH(CH_2 \cdot C_6H_4 \cdot OH) \cdot CH_2 \cdot NH \cdot CH_2 \cdot CO$-Gly-Phe-Met-ol	200[e]	50
42	H-Tyr-NH $\cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CO$-Phe-Met-$NH_2$	1[e]	50
43	H-Tyr-NH $\cdot CH_2 \cdot CH_2 \cdot NH \cdot CH_2 \cdot CO$-Phe-Met-OH	5[e]	50
44	H-Tyr-NH $\cdot CH_2 \cdot CH_2 \cdot NH \cdot CH_2 \cdot CO$-Phe-Met-ol	3[e]	50
45	H-Tyr-NH $\cdot CH_2 \cdot CH_2 \cdot NH \cdot CH_2 \cdot CO$-Phe-Met-$NH_2$	3[e]	50
46		inactive[a]	51
47		inactive[a]	51
48	Tyr($^+NMe_3$)[1], Leu[5]-OMe, I^-	0.6[a]	51
49	Tyr[0]	30[f]	52
50	Desamino-Tyr[0]	>1000[f]	52
51	Phe[0]	30[f]	52
52	Tyr[0], Phe[1]	200[f]	52
53	Arg[0]	40[f]	52
54	Lys[0]	40[f]	52
55	Desamino-Lys[0]	>1000[f]	52
56	Gly[0]	20[f]	52
57	β-Ala[0]	280[f]	52
58	$H_2N(CH_2)_3$-CO-Tyr[1]	900[f]	52
59	Arg[0], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	141[g]	58
60	Glu[0], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	9[g]	58
61	Phe[0], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	109[g]	58
62	D-Phe[0], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	0.1[g]	58
63	Tyr(NAc)[1], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	0.6[g]	58
64	Tyr(NMe)[1], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	120[g]	58
65	Tyr(NCbm)[1], D-Ala[2], Phe(NMe)[4], Met(O)[5]ol	10[g]	58
66	Tyr(NMe_2)[1], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	60[g]	58
67	Tyr(OMe)[1], D-Ala[2], Phe (NMe)[4], Met(O)[5]-ol	3[g]	58
68	D-Tyr[1], D-Ala[2], Phe(NMe)[4], Met(O)[5]-ol	0.3[g]	58
69	D,L-Hyhp[1], D(Ala[2], Phe(NMe)[4], Met(O)[5]-ol	< 0.1[g]	58
70	D-Ala(NMe)[2], Phe(NMe)[4], Met(O)[5]-ol	46[g]	58
71	D-Ser[2], Phe(NMe)[4], Met(O)[5]-ol	8[g]	58
72	D-Met[2], Phe(NMe)[4], Met(O)[5]-ol	36[g]	58
73	D-Met(O)[2], Phe(NMe)[4], Met(O)[5]-ol	60[g]	58
74	D-Ala[2], Phe(*p*-Cl)[4], Met(O)[5]-ol	29[g]	58
75	D-Ala[2], Phe(*p*-NO_2)[4], Met(O)[5]-ol	133[g]	58
76	D-Ala[2], Phe(NMe, *p*-NO_2)[4], Met(O)[5]-ol	222[g]	58

Table 2 (*cont.*)

Compound number	*Structure*	*Activity*	*Ref.*
77	D-Ala^2, Phe(NMe)^4, Met^5-ol	31[g]	58
78	D-Ala^2, Phe(NMe)^4, Met(O$_2$)^5-ol	109[g]	58
79	D-Ala^2, Phe(NMe)^4, D-Met(O)^5-ol	11[g]	58
80	D-Ala^2, Phe(NMe)^4, *S*-Me-Cys(O)^5-ol	10[g]	58
81	D-Ala^2, Phe(NMe)^4, Leu^5-ol	14[g]	58
82	D-Ala^2, Phe(NMe)^4, Pro^5-ol	9[g]	58
(b) *Endorphins*			
I	Met(O)^5-β_h-EP	57[h]	53
II	Leu^5-β_h-EP	72[h]	53
		79[h]	53
III	Gly^{31}-β_h-EP	127[i]	54
IV	Gly^{31}-β_h-EP-NH$_2$	250[i]	54
V	Gly^{31}-β_h-EP-Gly-OH	153[i]	54
XXIV	Arg^0-β_h-EP	2[j]	59
		0.2[k]	59

[a] % Affinity relative to [Leu^5]enkephalin. Whole rat brain pellet, [^{3}H]naloxone, 0 °C. [b] % Affinity relative to morphine. Whole mouse brain pellet, [^{3}H]etorphine. [c] % Affinity relative to morphine. Whole mouse brain pellet, [^{3}H]-[D-Ala^2, Leu^5]enkephalinamide. [d] % Affinity relative to [Met^5]enkephalin. Whole rat brain pellet, [^{3}H]naloxone, 0 °C. [e] % Affinity relative to [Met^5]enkephalin. Whole rate brain pellet, [^{3}H]naloxone, 25 °C. [f] K_i[nM]. Mouse striatum. [^{3}H]-[Leu^5]enkephalin, 30 °C. [g] % Affinity relative to H-Tyr-D-Ala-Gly-MePhe-Met(O)-ol. Whole rat brain pellet, [^{3}H]naloxone, 25 °C. [h] % Affinity relative to β_h-endorphin. Whole guinea-pig brain pellet, [^{3}H]etorphine, 23 °C. [i] % Affinity relative to β_h-endorphin. Whole rat brain pellet, [^{3}H]-[Tyr^{27}]β_h-endorphin. [j] % Affinity relative to β_h-endorphin.[^{3}H]naloxone. Other conditions not specified. [k] % Affinity relative to β_h-endorphin. [^{3}H]naloxone; Na^+ = 100 μM. Other conditions not specified.

The relative affinities of compounds (1), (2), (19), (24), (25), and (26) correlate well with their activities determined with isolated tissue preparations (Table 1). With analogues (33) and (34) discrepancies between affinity and opiate activity in the GPI-assay were observed [49] and taken as evidence for the existence of several subclasses of opiate receptors. Good correlation between receptor affinity and activity (GPI-assay) was demonstrated for the isosterically modified analogues (37—45). The lack of affinity of the analogues containing a piperazinone structure (46, 47) and the weak binding of the compound with a quaternized *N*-terminal amino-group (48) are also in agreement with the results of the GPI-assay.[51]

The Sandoz group [58] synthesized a series of interesting analogues (59—82), using the highly potent and enzyme-resistant enkephalin analogue H·Tyr-D-Ala-Gly-Phe(NMe)-Met(O)ol (FK-33'824) as a starting point. Replacement of the *N*-terminal amino-group by the isosteric hydroxy-group (69) abolished affinity. Substitution of a single methyl-group on the terminal amino-group resulted in an analogue (64) with slightly enhanced affinity as compared to FK-33'824, while *N*-dimethylation (66) weakened binding significantly, in agreement with the result obtained by DiMaio *et al.*[51] Substitution of the bulky cyclobutylmethyl group on the α-amino-group of Tyr^1 (65) also reduced afffinity by an order of magnitude and acetylation of the same group (63) had an even more drastic effect on receptor

[58] J. Pless, W. Bauer, F. Cardinaux, A. Closse, D. Hauser, R. Huguenin, D. Roemer, H.-H. Buescher, and R. C. Hill, *Helv. Chim. Acta*, 1979, **62**, 398.
[59] J. Chang, B. T. W. Fong, E. Tau, W. J. Peterson, and M. Shimizu, ref. 48, p. 887.
[60] D. Roemer and J. Pless, *Life Sci.*, 1979, **24**, 621.

affinity, as previously shown by Eberle *et al.*[61] On the other hand, acylation of the amino-group with various amino-acids (59—61) produced analogues with more or less retained affinity, while *N*-terminal extension with D-phenylalanine (62) lowered affinity by three orders of magnitude. As pointed out by the authors, the affinity of analogues (59—61) may be due to enzymatic cleavage of the additional amino-acid, since the binding assay was performed at 25 °C in the absence of aminopeptidase inhibitors. In confirmation of the findings by Schiller *et al.*,[62] methylation of the tyrosyl hydroxy-group (67) also led to a substantial drop in affinity. Substitution of other D-amino-acids in position-2(70—73) reduced affinity as compared to the [D-Ala^2]-parent compound. Nitration in the *para*-position of the phenyl ring of Phe^4 or Phe(NMe)^4 resulted in analogues (75, 76) with enhanced affinity. However, the effect was not as dramatic as that observed upon performing the same modification with (D-Ala^2,Leu^5]enkephalinamide (1).[39] The *p*-chlorophenylalanine derivative (74) had one-third the affinity of FK-33'824. With the exception of the methionine-sulphone derivative (78), several performed substitutions in position-5 produced analogues with reduced opiate receptor affinity.

Endorphin analogues (I) and (II) displayed receptor affinities of 57% and 72%, respectively, relative to human β-endorphin.[53] These affinities show better correlation with the potencies observed in the MVD-assay than with those obtained with the GPI-assay. The enhanced affinities of analogues (III—V) relative to the native peptide are in agreement with the increase in opiate activity observed in the GPI-assay.[54] In the absence and presence of sodium chloride the *N*-terminal extension analogue of human β-endorphin, Arg^0-β_h-endorphin (XXIV), prepared by Chang *et al.*,[59] showed low affinities of 2 and 0.2%, respectively, relative to β_h-endorphin.

In vivo *Analgesic Activities.* A new intracisternal injection technique allowing administration of peptides directly to the brainstem, was introduced by Ueda *et al.*[63] The administration of [Met^5]enkephalin by this method produced relatively long-lasting analgesia, since enzymatic degradation was greatly diminished in comparison to intracerebroventricular injection.

Most of the new analogues were tested using the mouse or rat tail flick test with intracerebroventricular (i.c.v.), intracerebral (i.c.), intravenous (i.v.), subcutaneous (s.c.), or oral (p.o.) administration of the peptides. In most cases activities were expressed as molar potencies relative to morphine, enzyme-resistant enkephalin analogues or β-endorphin. Relative molar potencies of enkephalin and endorphin analogues are listed in Table 3.

In agreement with its *in vitro* activity, analogue (3) was five times more potent than [D-Ala^2,Met^5]enkephalinamide in the mouse tail flick test.[40] The authors suggested that, aside from enzyme resistance, a more favourable conformation of (3) could be responsible for the increase in activity. Comparison of the analgesic activities of analogues (16—19) with their potencies in isolated tissue assays revealed a GPI/MVD potency ratio near 1 as a criterion for high analgesic potency.[45] Guanidation of the α-amino-group (compounds 20—23) led to an increase in analgesic potency in the case of the 4-peptides, while the activity of the

[61] A. Eberle, O. Leukart, P. W. Schiller, J. L. Fauchère, and R. Schwyzer, *FEBS Lett.*, 1977, **82**, 825.
[62] P. W. Schiller, C. F. Yam, and M. Lis, *Biochemistry*, 1977, **16**, 1831.
[63] H. Ueda, H. Amano, H. Shiomi, and H. Takagi, *Eur. J. Pharmacol.*, 1979, **56**, 265.

Table 3 In vivo *data*

(a) *Enkephalins*

Compound number	*Structure*	*Activity*	*Ref.*
3	D-Ala^2, Δ^zPhe^4, Met^5-NH_2	331[a]	40
14	D-Met^2, Des-Met^5-NH_2	36[b]	45
		16[c]	45
15	D-Nle^2, Pro^5-NH_2	100[b]	45
		42[c]	45
16	D-Nle^2, Des-Met^5-NH_2	57[b]	45
		37[c]	45
17	D-Met^2, Nle^5	0.03[b]	45
18	D-Met^2, Pro^5	2.1[b]	45
		2.1[c]	45
19	D-Met^2, Pro^5-NH_2	6460[d]	46
		420[e]	46
20	H_2N-C(=NH)-Tyr^1, D-Met^2, Pro^5-NH_2	1050[d]	46
		65[e]	46
14	D-Met^2, Des-Met^5-NH_2	1210[d]	46
		220[e]	46
21	H_2N-C(=NH)-Tyr^1, D-Met^2, Des-Met^5-NH_2	21000[d]	46
		440[e]	46
16	D-Nle^2, Des-Met^5-NH_2	1640[d]	46
		120[e]	46
22	H_2N-C(=NH)-Tyr^1, D-Nle^2, Des-Met^5-NH_2	2000[d]	46
		795[e]	46
15	D-Nle^2, Pro^5-NH_2	2880[d]	46
		160[e]	46
23	H_2N-C(=NH)-Tyr^1, D-Nle^2-Pro^5-NH_2	110[d]	46
		60[e]	46
83	D-Ala^2, Met^5	3[e]	60
84	D-Ala^2, Met^5-NH_2	10[e]	60
85	D-Ala^2, Met(O)^5-NH_2	10[e]	60
86	D-Ala^2, Met^5-ol	14[e]	60
		5[f]	60
87	D-Ala^2, Met(O)^5-ol	33[e]	60
		10[f]	60
		7[g]	60
19	D-Met^2, Pro^5-NH_2	25[e]	60
		25[f]	60
		inactive[g]	60
88	D-Met^2, Pro^5-ol	10[e]	60
		6[f]	60
		inactive[g]	60
89	D-Met(O)^2, Pro^5-ol	100[e]	60
		100[f]	60
		inactive[g]	60
90	D-Ala^2, Phe(NMe)^4, Met(O)^5-ol	400[e]	60
		200[f]	60
		20[g]	60
91	D-Met^2, Phe(NMe)^4, Met^5-ol	13[e]	60
		13[f]	60
		inactive[g]	60
72	D-Met^2, Phe(NMe)^4, Met(O)^5-ol	100[e]	60
		100[f]	60
		7[g]	60

Compound number	*Structure*	*Affinity*	*Ref.*
92	D-Met(O)^2, Phe(NMe)^4, Met^5-ol	33[e]	60
		100[f]	60
		<7[g]	60
73	D-Met(O)^2, Phe(NMe)^4, Met(O)^5-ol	100[e]	60
		100[f]	60
		10[g]	60
71	D-Ser^2, Phe(NMe)^4, Met(O)^5-ol	10[e]	60
		10[f]	60
		inactive[g]	60
70	D-Ala(NMe)^2, Phe(NMe)^4, Met(O)^5-ol	100[e]	60
		100[f]	60
		<7[g]	60
64	Tyr(NMe)^1, D-Ala^2, Phe(NMe)^4, Met(O)^5-ol	300[e]	60
		1000[f]	60
		100[g]	60
93	Tyr(NMe)^1, D-Met(O)^2, Phe(NMe)^4, Met(O)^5-ol	100[e]	60
		100[f]	60
		13[g]	60
68	D-Tyr^1, D-Ala^2, Phe(NMe)^4, Met(O)^5-ol	<1[e]	60
94	D-Ala^2, D-Phe(NMe)^4, Met(O)-ol	1[e]	60
		3[f]	60
(b) *Endorphins*			
III	Gly^{31}-β_h-EP	83[h]	54
IV	Gly^{31}-β_h-EP-NH$_2$	225[h]	54
V	Gly^{31}-β_h-EP-Gly-OH	217[h]	54
X	Des-Gly^2-β_c-EP	inactive[i]	56
XI	Des-Met^5-β_c-EP	20[i]	56
XII	Des-Thr^6-β_c-EP	73[i]	56
XIII	Des-Ser^{10}-β_c-EP	92[i]	56
XIV	Des-Gln^{11}-β_c-EP	79[i]	56
XV	Des-Thr^{12}-β_c-EP	96[i]	56
XVI	Des-Pro^{13}-β_c-EP	23[i]	56
XVII	Des-Leu^{14}-β_c-EP	75[i]	56
XVIII	Des-Val^{15}-β_c-EP	24[i]	56
XIX	Des-Asn^{20}-β_c-EP	46[i]	56
XX	Des-Ile^{22}-β_c-EP	57[i]	56
XXIV	Arg^0-β_h-EP	10[j]	56

[a] Mouse tail flick i.c., % potency relative to [D-Ala^2, Met^5]-enkephalinamide. [b] Rat tail flick i.c.v., % potency relative to [D-Met^2, Pro^5]-enkephalinamide. [c] Rat tail flick, i.v., % potency relative to [D-Met^2, Pro^5]-enkephalinamide. [d] Rat tail flick, i.c.v., % potency relative to morphine. [e] Rat tail flick, i.v., % potency relative to morphine. [f] Rat tail flick, s.c., % potency relative to morphine. [g] Rat tail flick, p.o., % potency relative to morphine. [h] Mouse tail flick, i.c.v., % potency relative to β_h-endorphin. [i] Mouse tail flick, i.c.v., % potency relative to β_c-endorphin. [j] Potency relative to β_h-endorphin. Test conditions not specified.

5-peptide analogues was reduced.[46] In contrast to this result, the *in vitro* opiate activities of both 4- and 5-peptides were enhanced by guanidation. Furthermore, the effect of guanidation was influenced by the residue in position-2 and this influence seemed to depend on the route of application. As the authors point out, these findings are indicative of the complexity of events in the CNS leading to analgesia. The development of the long-lasting orally active Sandoz-analogue,

FK-33'824, (90) is illustrated with the series of compounds (83—90).[60] A number of analogues (64, 68, 70—73, 91—94) based on FK-33'824 showed analgesic potencies which were in good agreement with their opiate receptor affinities. In comparison to morphine, the most active analogue of this series (64) is ten times more potent (s.c.) and equipotent (p.o.).

Klis *et al.*[64] observed analgesic potencies of [Tyr(OMe)^1,Leu^5]enkephalin and [Tyr(3-NH_2)^1,Leu^5]enkephalin comparable to that of [Leu^5]enkephalin in the rat hot plate test (i.c.v.). In comparison to [Leu^5]enkephalin the analgesic effect of both analogues was of longer duration. The analgesic activity of [Tyr(OMe)^1,Leu^5]enkephalin is in disagreement with the very low *in vitro* opiate activities of this analogue [58, 62] and may be due to *O*-demethylation *in vivo*. I.c.v. injection of the retro-inverso-analogues (6) and (7) caused catatonia and analgesia in rats lasting up to three hours,[41] which demonstrates the extraordinary enzyme-resistance of these compounds. None of the piperazinone analogues (46, 47) was active in the rat tail flick test (i.c.v.).[51] The same compounds were also inactive in the mouse hot plate test with the exception of analogue (47) with D-configuration at the chiral centre of the piperazinone ring, which was three times more potent than [Met^5]enkephalin. However, the effect of the latter analogue could only be reversed by very high concentrations of naloxone and, therefore, it may not be mediated by μ-receptors [37] for which morphine and naloxone display high affinity.

In agreement with their enhanced *in vitro* opiate activities, endorphin analogues (IV) and (V) displayed higher analgesic potency than β_h-endorphin.[54] Endorphin analogues with deletion of a single amino-acid (X—XX) all showed lower analgesic activity in the mouse tail flick test (i.c.v.) than β_e-endorphin[56] The correlation between potencies observed in the *in vivo* analgesic tests and in the *in vitro* assay (GPI) was not very good. In contrast to its very low receptor affinity, [Arg^0]-β_h-endorphin (XXIV) was 10% as active as β_h-endorphin in an *in vivo* analgesic test.[59]

Other Biological Activities. A semi-quantitative assay, based on recovery of co-ordinated movement, was used for testing several analogues containing α-aminoisobutyric acid (Aib) substituted for Gly^2 or Gly^3.[65] Substitution of Aib for Gly introduces conformational restrictions at positions -2 and -3 of the peptide backbone. Since all analogues showed either retained or enhanced activity relative to [Leu^5]enkephalin, the biologically active conformation seems to be accessible to these conformationally restricted analogues. Active [Aib^2]-analogues containing Tyr(3-NO_2) in position -1 or Met-NH-$(CH_2)_2$-dansyl in position -5 were also synthesized for the purpose of n.m.r. and fluorescence studies, respectively. In a study on behavioural effects, Tseng *et al.*[66] compared the activities of β-endorphin, [D-Ala^2,D-Leu^5]enkephalin and morphine on inducing loss of righting reflex, catalepsy, and hypothermia, whereby significant differences between the three compounds were observed. Dupont *et al.*[67] determined the activity of several

[64] W. A. Kliś, E. Nawrocka, I. Z. Siemion, A. Plech, Z. Stachura, and Z. S. Herman, *Bioorg. Chem.*, 1979, **8**, 255.

[65] R. Nagaraj, T. S. Sudha, S. Shivaji, and P. Balaram, *FEBS Lett.*, 1979, **106**, 271.

[66] L.-F. Tseng, T. J. Ostwald, H. H. Loh, and C. H. Li, *Psychopharmacol.*, 1979, **64**, 215.

[67] A. Dupont, L. Cusan, L. Ferland, D. H. Coy, and F. Labrie, in 'Clinical Neuroendrocrinology: A Pathophysiological Approach', ed. G. Tolis, J. B. Martin, F. Labrie, and F. Naftolin, Raven Press, New York, 1979, p. 161.

enkephalin and endorphin analogues on prolactin and growth hormone release in rats. Interestingly, the pentafluorophenylalanine analogue [D-Ala^2,Phe(F-)$_5$^{4}, Met^5]enkephalinamide was five times more active than (D-Ala^2, Met^5]enkephalinamide as stimulator of growth hormone and prolactin release, while the reversed potency relationship is observed in the *in vitro* GPI-assay (see 1978 review). It is also noteworthy that the duration of action of [D-Ala^2]-β-endorphin was increased relative to that of β-endorphin in these assays. The effect of [Met^5]enkephalinamide and [D-Met^2,Pro^5]enkephalinamide on adenylate cyclase in various rat brain regions was investigated by Wolleman *et al.*,[68] whereby interesting differences between the activity patterns of the two peptides were observed. Immunochemical studies with a number of β-endorphin analogues revealed that the immunoreactivity is located in the *C*-terminal segment (residues 22—31) of β-endorphin.[69,70] Furthermore, the results of these investigations showed a lack of correlation between opiate and immunological activity of β-endorphin analogues.

Conformation of Enkephalins and Endorphins.—Further information about the conformation of enkephalin in unsolvated form, in the crystalline state, and in solution has been obtained. Facets of the receptor-bound conformation have been revealed by pharmacological evaluation of conformationally restricted analogues [51,65] (see previous sections).

Manavalan and Momany [71] performed empirical energy calculations for nine active enkephalin analogues and concluded that equivalent configurations can be assumed by all of them. Theoretical analysis of enkephalin conformations by the Monte-Carlo method [72] indicated the predominance of extended conformations of enkephalin at low pH, while at pH = 7 the existence of folded conformations is predicted and explained by an electrostatic $^+NH_3$—COO^- head-to-tail interaction. These predictions are in disagreement with the experimental finding of Schiller [73] who found no difference in the average intramolecular Tyr^1-Trp^4 distance between the cationic and zwitterionic forms of the active analogue [Trp^4,Met^5]enkephalin in dilute aqueous solution.

The *X*-ray structure of [Leu^5]enkephalin [74] is in the process of being revised.[75] Prangé and Pascard [76] determined the crystal structures of two enkephalin fragments, H·Tyr-Gly-Gly-Phe·OH and H·Gly-Gly-Phe-Leu·OH. Analysis of the former peptide resulted in a 4 → 1 hydrogen-bonded β-turn with a phenol–phenyl separation of 10.9 Å, which is comparable to the average Tyr^1-Trp^4 intramolecular distance (9.3 Å) determined for H·Tyr-Gly-Gly-Trp-·OH in aqueous solution by a fluorescence energy-transfer experiment.[77] The observed

[68] M. Wolleman, A. Szebeni, S. Bajusz, and L. Graf, *Neurochem. Res.*, 1979, **4**, 627.
[69] W.-C. Chang, H.-W. Yeung, and C. H. Li, *Int. J. Pept. Protein Res.*, 1979, **13**, 278.
[70] C. H. Li, W.-C. Chang, D. Yamashiro, and L.-F. Tseng, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 693.
[71] P. Manavalan and F. A. Momany, ref. 48, p. 893.
[72] S. Premilat and B. Maigret, *Biochem. Biophys. Res. Commun.*, 1979, **91**, 534.
[73] P. W. Schiller, *Biochem. Biophys. Res. Commun.*, 1977, **79**, 493.
[74] G. D. Smith and J. F. Griffin, *Science*, 1978, **199**, 1214.
[75] T. L. Blundell, L. Hearn, I. J. Tickle, R. A. Palmer, B. A. Morgan, G. D. Smith, and J. F. Griffin, *Nature (London)*, 1979, **205**, 220.
[76] T. Prangé and C. Pascard, *Acta Crystallogr.*, 1979, **B35**, 1812.
[77] P. W. Schiller, C. F. Yam, and J. Prosmanne, *J. Med. Chem.*, 1978, **21**, 1110.

distance of 6.3 Å between the hydroxy-group and the amino-group of Tyr^1 is close to the distance between the corresponding hydroxy-group and tertiary nitrogen in the crystal structure of morphine. The *C*-terminal 4-peptide of enkephalin assumes a folded conformation of the 'hairpin-type' and is not stabilized by an intramolecular hydrogen bond.

Numerous studies on the solution conformation of enkephalin by n.m.r. techniques and optical spectroscopy have been performed. Evidence for self-aggregation in the concentration range from 10^{-1} to 10^{-4} M was first reported by Khaled *et al.*[78] N.m.r. studies by Higashijima *et al.*[79] confirmed the latter finding, while Stimson *et al.*[80] found no concentration dependence of n.m.r. parameters in the range 0.001—0.025 M. The differences between these findings are striking and require further investigations. Further evidence in favour of a time-averaged, preferred backbone conformation in aqueous solution [81, 82] and in Me_2SO,[80] is in disagreement with the results obtained in a study performed in H_2O–Me_2SO mixtures,[79] which were interpreted in terms of an equilibrium between folded and extended conformers. Stimson *et al.*[80] interpreted recent n.m.r. data in favour of a Type I β-bend centred on Gly^3-Phe^4 and the results of a n.m.r. study by Gacel *et al.*[52] suggested that the 5 → 2 hydrogen-bonded β-bend is retained in enkephalin analogues containing an additional amino-acid at the *N*-terminal. On the basis of results obtained by paramagnetic probe techniques,[81] similarity between the crystal structure of [Met^5]enkephalin [74] and its solution conformation was proposed. In contrast to these results, a recent n.m.r. study [82] indicated that none of the amide protons is engaged in a hydrogen bond and a study [79] of the nuclear Overhauser effect on the zwitterionic form of [Met^5]enkephalin is inconsistent with a hydrogen bond between the amino-group of Met^5 and the carbonyl-group of Gly^2. Measurement of the intramolecular Tyr^1-Trp^4 distance by fluorescence energy transfer in a series of [Trp^4]enkephalin analogues, designed to prevent formation of various proposed hydrogen bonds, revealed that folded conformations in dilute aqueous solution need not be stabilized by intramolecular 4 → 1 or 5 → 2 hydrogen bonds between amino- and carbonyl-groups of the backbone or between the tyrosyl hydroxyl and a backbone carbonyl.[49] A c.d. study by Hollósi *et al.*[83] showed no conformational difference between [Pro^5]analogues of enkephalin with D-amino-acids in position 2 and [Met^5]enkephalin in aqueous solution between pH = 3 and 5. Several studies dealt with the conformation and relative flexibility of side-chains in positions 1, 4, and 5. A n.m.r. rotating-frame relaxation study[84] indicated that the tyrosyl residue in [Met^5]enkephalin is rigidly fixed. On the basis of the results of a n.m.r. study with ^{13}C enriched [Leu^5]enkephalin in Me_2SO, it was claimed [80] that the tyrosine side-chain conformation in the peptide is similar to the

[78] M. A. Khaled, M. M. Long, W. D. Thomson, R. J. Bradley, G. B. Brown, and D. W. Urry, *Biochem. Biophys. Res. Commun.*, 1977, **76**, 224.
[79] T. Higashijima, J. Kobayashi, U. Nagai, and T. Miyazawa, *Eur. J. Biochem.*, 1979, **97**, 43.
[80] E. R. Stimson, Y. C. Meinwald, and H. A. Scheraga, *Biochemistry*, 1979, **18**, 1661.
[81] B. A. Levine, D. L. Rabenstein, D. Smyth, and R. J. P. Williams, *Biochem. Biophys. Acta*, 1979, **579**, 279.
[82] L. Zetta, F. Cabassi, R. Tomatis, and M. Guarneri, *Eur. J. Biochem.*, 1979, **95**, 367.
[83] M. Hollósi, Z. Dobolyi, and S. Bajusz, *FEBS Lett.*, 1980, **110**, 136.
[84] H. E. Bleich, A. R. Day, R. J. Freer, and J. A. Glasel, *Biochem. Biophys. Res. Commun.*, 1979, **87**, 1146.

conformation of the tyramine part in morphine. Filippi *et al.*[85] interpreted tyrosine fluorescence data, obtained in aqueous solution, in favour of the existence of a hydrogen bond between the tyrosyl hydroxyl and a carbonyl of the enkephalin backbone. However, this interpretation is not warranted by the results (*cf.* ref. 49) and an earlier fluorescence study [73] had clearly indicated that conformers with this type of hydrogen bond are not predominant in aqueous solution. Using n.m.r. spectroscopy, Kobayashi *et al.*[86] found several different conformations (rotamers) for the side-chain of Phe[4]. Other n.m.r. data [82] also indicated high flexibility of the Phe[4] side-chain as well as three different rotamer configurations for the side-chain of Met[5]. In contrast to these results, other recent n.m.r. data [80] seemed to suggest that the side-chains of Phe[4] and Met[5] exist predominantly in one configuration (t_g^-). In summary, no consensus at all has been reached on self-aggregation, conformational heterogeneity, backbone structure, and side-chain conformations of enkephalin in solution.

Information about the conformation of endorphins is still scarce. The results of a recent n.m.r. study [81] suggested that β-endorphin has some conformational constraints and it was proposed that the region encompassing residues 14—18 is relatively ordered compared to the more extended *C*-terminal segment. Houghten *et al.*[87] investigated β-endorphin fragments and deletion peptides by n.m.r. spectroscopy and found no significant conformational differences between the shortened chains and the native peptide in aqueous solution. However, differences were observed in 50% MeOH or 1% SDS. A c.d. study performed by Wu *et al.*[88] revealed the existence of a partially α-helical conformation of β-endorphin in various lipid solutions.

Characterization of Opiate Receptors.—In 1979 a considerable amount of new information on binding characteristics and subclasses of opiate receptors in various tissues has accumulated and further attempts at receptor labelling, solubilization, and characterization have been made.

Ferrara *et al.*[89] studied the binding of [^{3}H-Tyr^{27}]-β_h-endorphin to rat brain membrane preparations. In the absence and presence (100 mM) of sodium apparent dissociation constants of 0.3 and 2.5 nM, respectively, were observed. A somewhat higher dissociation constant (2.5 nM) in the absence of sodium was observed with a radiolabelled derivative of [D-Ala^2]-β_h-endorphin in the rat brain system.[90] The same authors also found that the effects of cations on binding of endorphins are different from those found for enkephalin binding. This observation led to the conclusion that β-endorphin and enkephalin either have different modes of binding to the same receptor or that they bind to different receptors. The affinity of the radioiodinated Sandoz-analogue FK-33'824 for rat brain opiate receptors (K_d = 0.42 nM) [91] is comparable to that of β-endorphin.[89] The latter study also

[85] B. Filippi, P. Giusti, L. Cima, G. Borin, F. Ricchelli, and F. Marchiori, *Int. J. Pept. Protein Res.*, 1979, **14**, 34.
[86] J. Kobayashi, U. Nagai, T. Higashijima, and T. Miyazawa, *Biochim. Biophys. Acta*, 1979, **577**, 195.
[87] R. A. Houghten, D. Yamashiro, and C. H. Li, ref. 48, p. 869.
[88] C.-S. C. Wu, N. M. Lee, H. H. Loh, J. T. Yang, and C. H. Li, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 3656.
[89] P. Ferrara, R. Houghten, and C. H. Li, *Biochem. Biophys. Res. Commun.*, 1979, **89**, 786.
[90] E. Hazum, K.-J. Chang, and P. Cuatrecasas, *J. Biol. Chem.*, 1979, **254**, 1765.
[91] R. M. Kream and R. S. Zukin, *Biochem. Biophys. Res. Commun.*, 1979, **90**, 99.

demonstrated that FK-33'824 binds equally well to μ- and δ-receptors and that its dissocation from opiate receptors is much slower than that of other enkephalins. Chang and Cuatrecasas[92] studied binding of [^{125}I]-[D-Ala^2,D-Leu^5]enkephalin, [^{3}H]naloxone, and [^{3}H]dihydromorphine to rat brain membrane preparations and distinguished between 'enkephalin'-receptors and 'morphine'-receptors. In the case of [D-Ala^2,D-Leu^5]enkephalin a Scatchard-plot of the binding data revealed the existence of two binding sites with dissociation constants of 0.8 and 6 nM. The results of displacement studies with enkephalin fragments and various opiates seemed to indicate that the Phe^4-residue constitutes the major structural difference between enkephalin and morphine for receptor recognition. Since [D-Ala^2]enkephalin amide and endorphin interact with a larger number of binding sites than several enkephalin analogues and opiates, they may bind to both μ- and δ-receptors with high affinity.[93] In contrast to the findings by Kream and Zukin,[91] Chang *et al.*[94] observed higher affinity of FK-33'824 for morphine receptor sites than for enkephalin receptor sites in rat brain. Furthermore, differences in the distribution of binding in rat brain were observed for [^{125}I]FK-33'824,[^{3}H]naloxone, and [^{125}I]-D-Ala^2,Leu^5]enkephalin. Waterfield *et al.*[95] compared the binding of [^{3}H]-[Met^5]enkephalin and [^{3}H]-[Leu^5]enkephalin to guinea pig brain membranes and found high- and low-affinity binding sites for both ligands. The dissociation constants for both sites were found to be lower for [Met^5]enkephalin than for [Leu^5]enkephalin and the number of high-affinity binding sites was higher for [Met^5]enkephalin than for [Leu^5]enkephalin. The results of this study led to the conclusion that the existence of more than one type of enkephalin (δ) binding site is unlikely. In a recent study, Pert *et al.*[96] distinguished between GPT-sensitive (type 1) and GTP-insensitive (type 2) receptors. Type 1 receptors were shown to have high affinity for alkaloid opiates, while the type 2 receptors bound enkephalins preferentially. A study of the distribution of the two receptor types in rat brain indicated that type 1 receptors are enriched in areas which mediate analgesia.

In a comparative binding study, Leslie and Kosterlitz[97] demonstrated that the ratio of [Met^5]enkephalin to dihydromorphine binding is in favour of the peptide in rat brain and in favour of the alkaloid in the myenteric plexus of the guinea-pig, the mouse vas deferens is intermediate. Chavkin *et al.*[98] detected saturable high-affinity opiate binding sites in the adrenal medulla, but not in the adrenal cortex. The existence of specific non-opiate receptors for β-endorphin in cultured human lymphocytes was demonstrated by Hazum *et al.*[99] These receptors do not bind enkephalins or alkaloid opiates and their physiological role is unknown.

More direct evidence for the existence of two classes of opiate receptors was obtained by elegant cross-protection studies. Robson and Kosterlitz[100] showed

[92] K.-J. Chang and P. Cuatrecasas, *J. Biol. Chem.*, 1979, **254**, 2610.
[93] M. G. C. Gillan, H. W. Kosterlitz, and S. J. Paterson, *Br. J. Pharmacol.*, 1979, **66**, 86P.
[94] K.-J. Chang, B. R. Cooper, E. Hazum, and P. Cuatrecasas, *Mol. Pharmacol.*, 1979, **16**, 91.
[95] A. A. Waterfield, F. M. Leslie, J. A. H. Lord, N. Ling, and H. W. Kosterlitz, *Eur. J. Pharmacol.*, 1979, **58**, 11.
[96] C. B. Pert, D. P. Taylor, A. Pert, M. A. Herkenham, and J. L. Kent, ref. 48, p. 845.
[97] F. M. Leslie and H. Kosterlitz, *Eur. J. Pharmacol.*, 1979, **56**, 379.
[98] C. Chavkin, B. M. Cox, and A. Goldstein, *Mol. Pharmacol.*, 1979, **15**, 751.
[99] E. Hazum, K.-J. Chang, and P. Cuatrecasas, *Science*, 1979, **205**, 1033.
[100] L. E. Robson and H. W. Kosterlitz, *Proc. R. Soc. London, Ser. B*, 1979, **205**, 425.

that the inactivation of μ-receptors in guinea-pig brain homogenates by phenoxybenzamine can be selectively protected by previous incubation with a ligand of high affinity for μ-receptors (dihydromorphine), whereas ligands with high affinity for δ-receptors ([D-Ala^2,D-leu^5]enkephalin) are more efficient in protecting δ-receptors. In a similar experiment Smith and Simon [101] used the sulphydryl-group alkylating agent *N*-ethylmaleimide for inactivation of opiate receptors in rat brain. Naltrexone and morphine were found to protect μ-receptors more efficiently than δ-receptors, whereas enkephalin analogues provided better protection of δ-receptors than alkaloid opiate agonists and antagonists. In conclusion, there is now a large body of evidence in favour of the existence of two classes of opiate receptors in rat or guinea-pig brain (μ- and δ-receptors; 'enkephalin'- and 'morphine'-receptors; type 1- and type 2-receptors). Unfortunately, a common designation of these two receptor types has not yet been adopted in the literature.

Hazum *et al.*[102] introduced image-intensified fluorescence microscopy for the visualization of opiate receptors in neuroblastoma cells. Using the biologically active fluorescent derivative Tyr-D-Ala-Gly-Phe-Leu-Lys-rhodamine,[103] these authors were able to demonstrate clustering of receptors on the cell surface. No subsequent internalization was observed and cluster formation was drastically reduced in the presence of sodium. Since sulphydryl reagents were shown [104] to prevent cluster formation, a sulphydryl–disulphide exchange reaction may be involved in this phenomenon. Lee *et al.*[105] prepared [D-Ala^2,Met^5]enkephalin-Tyr-*N*-(2-nitro-4-azidophenyl)ethylenediamine as a photoaffinity label for opiate receptors. The analogue displayed high affinity for opiate receptors in NG-108 cell membranes in the dark and its presence during photolysis of the membranes caused irreversible inactivation of the opiate receptors.

Using essentially the solubilization techniques of Simon *et al.*,[106] Zukin and Kream [107] obtained a soluble complex of radioiodinated FK-33'824 and rat brain opiate receptor. Chemical cross-linking of the enkephalin ligand to the receptor macromolecule was successfully performed with dimethyl suberimidate. In agreement with earlier results,[106] the macromolecular complex was shown to have a Stokes radius of 48 Å which corresponds to a molecular weight of 380 000 dalton for a spherical molecule. Sodium dodecyl sulphate electrophoresis revealed the existence of a subunit with a molecular weight of 35 000 dalton.

Clinical Tests with Enkephalins and Endorphins.—Based on the hypothesis that altered endorphin homeostasis may play a role in schizophrenia, several clinical investigations focused on the effects of endorphins and enkephalin analogues on schizophrenic and depressed patients. In particular, neuroleptic properties of [des-Tyr^1]-γ-endorphin were postulated and i.v. application of this peptide to a number of patients suffering from schizophrenic psychosis led to a marked reduction of

[101] J. R. Smith and E. J. Simon, *Proc. Natl. Acad. Sci. USA*, 1980, **77**, 281.
[102] E. Hazum, K.-J. Chang, and P. Cuatrecasas, *Science*, 1979, **206**, 1077.
[103] E. Hazum, K.-J. Chang, Y. Shechter, S. Wilkinson, and P. Cuatrecasas, *Biochem. Biophys. Res. Commun.*, 1979, **88**, 841.
[104] E. Hazum, K.-J. Chang, and P. Cuatrecasas, *Nature (London)*, 1979, **282**, 626.
[105] T. T. Lee, R. E. Williams, and C. F. Cox, *J. Biol. Chem.*, 1979, **254**, 11787.
[106] E. J. Simon, J. M. Hiller, and I. Edelman, *Science*, 1975, **190**, 389.
[107] R. S. Zukin and R. M. Kream, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 1593.

psychotic symptoms.[108] However, the results of behavioural tests with rats indicated that [des-Tyr^1]-γ-endorphin is not a haloperidol-like neuroleptic.[109] In agreement with this finding, the inability of [des-Tyr^1]-γ-endorphin to inhibit specific [^{3}H]spiroperidol binding from various regions of rat brain also suggests that this peptide is not acting at the classical neuroleptic receptor.[110] Short-term improvement in the symptoms of schizophrenic patients were also observed after intramuscular (i.m.) administration of the Sandoz-analogue FK-33'824.[111-113] Interestingly, the observed side-effects were less pronounced in the mental patients than in normal subjects. Foley *et al.*[114] investigated analgesic and hormonal effects of β-endorphin in humans. Since i.v. injection did not produce analgesia, it appears that β-endorphin is unable to cross the blood–brain barrier. I.v. administration caused a rapid rise in plasma prolactin, but had no effect on plasma growth hormone levels. I.c.v. applications at low doses elevated prolactin levels and reduced growth hormone in plasma rapidly. A relatively high dose (7.5 mg) was required to produce an analgesic effect which lasted for 19 h.

Further evidence for the development of tolerance to, and dependence on, opioid peptides was obtained. In a new approach, Crain *et al.*[115] demonstrated development of tolerance after chronic exposure of an explant of mouse spinal cord with attached dorsal root ganglia to the Sandoz analogue FK-33'824. By comparison of morphine, β-endorphin, and [D-Ala^2,Pro^5]enkephalinamide, Miglécz *et al.*[116] found a correlation between antinociceptive and tolerance-development/dependence capacities of these opiates in the rat. However, there are some indications that FK-33'824 and the Eli Lilly analogue (D-Ala^2,Met(NMe)^5]enkephalinamide (LY-127623) may be less 'additive' than conventional opiates (*cf.* ref. 17). In a promising new approach, Walter *et al.*[117] demonstrated that MIF and *cyclo*(-Leu-Gly-) block development of physical dependence on morphine in mice.

[108] W. M. A. Verhoeven, H. M. vanPraag, J. M. vanRee, and D. deWied, *Arch. Gen. Psychiatry*, 1979, **36**, 294.
[109] S. B. Weinberger, A. Arnsten, and D. S. Segal, *Life Sci.*, 1979, **24**, 1637.
[110] N. W. Pedigo, N. C. Ling, T. D. Reisine, and H. I. Yamamura, *Life Sci.*, 1979, **24**, 1645.
[111] A. Jørgensen, R. Fog, and B. Veilis, *Lancet*, 1979, 935.
[112] E. Krebs and J. Roubicek, *Pharmakopsychiat.*, 1979, **12**, 86.
[113] N. Nedopil and E. Rüther, *Pharmakopsychiat.*, 1979, **12**, 277.
[114] K. M. Foley, I. A. Kourides, C. E. Inturrisi, R. F. Kaiko, C. G. Zaroulis, J. B. Posner, R. W. Houde, and C. H. Li, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 5377.
[115] S. M. Crain, B. Crain, T. Finnigan, and E. J. Simon, *Life Sci.*, 1979, **25**, 1797.
[116] E. Miglécz, J. I. Székely, and Z. Dunai-Kovács, *Psychopharmacol.*, 1979, **62**, 29.
[117] R. Walter, R. F. Ritzmann, H. N. Bhargava, and L. B. Flexner, *Proc. Natl. Acad. Sci. USA*, 1979, **76**, 518.

Author Index

Aadland, E., 519
Aaviksaar, A., 95
Abadie, B., 121
Abano, D. A., 84
Abdallah, M. A., 236, 354
Abdella, P. M., 197
Abdel-Moez, M. S., 301
Abdolrasulnia, R., 185
Abdulaev, N. G., 144
Abe, H., 542
Abe, I., 392
Abe, K., 119, 149, 451, 535
Abe, N., 81
Abels, J., 41
Abiko, T., 150, 400, 511
Abner, K. E., 481
Abraham, E. P., 449
Abramov, Z. T., 87, 126
Abramova, V. V., 405
Abu-Al-Futuh, I. M., 33
Abuchowski, A., 157, 189
Abu Khaled, M., 277, 280
Abu Zarga, M. H., 21
Achari, A., 250
Acher, R., 128
Achtman, M., 252
Ackers, G. K., 336, 337
Adachi, H., 373, 382, 409, 528
Adachi, K., 340, 341
Adachi, R. I., 538
Adamczyk, D. L., 49
Adamich, M., 283
Adamietz, P., 95
Adams, B., 265
Adams, C. W., 127
Adams, D. O., 2
Adams, E., 304
Adams, J. B., 41
Adams, M. J., 236
Adams, M. L., 332
Adams, M. W. W., 57
Adams, R. F., 31
Addis, P., 49
Adiga, P. R., 39
Adolph, K. W., 248
Adrian, T. E., 468, 515, 527, 536
Aebi, U., 249
Afanasenko, G. A., 126
Agarwal, K. L., 518
Agarwal, N. S., 277, 496
Aggarawal, B. B., 175, 482, 483
Aggarwal, S., 143
Agnati, L. F., 515
Agosta, W. C., 277, 388
Agostini, G., 19
Agro, A. F., 271
Agrup, G., 34
Aguiar, A., 283
Aguilera, G., 499
Ahlman, H., 529, 520
Ahmad, F., 165, 265, 267
Ahmad, P. M., 165
Ahmadi, B., 64
Ahrens, H., 49
Aiba, M., 185
Aida, T., 444
Aimetti, J. A., 447
Airhart, J., 111
Aisen, P., 286
Aitken, A., 145
Ajisaka, K., 19
Ajo, D., 18
Akaboshi, M., 8, 31
Akaiwa, S., 63
Akaji, K., 373, 375, 382
Akasaka, K., 264, 279, 288
Akazaka, Y., 536
Akazawa, T., 53
Akazawa, Y., 373, 382
Åkerman, M., 75
Akhtar, M., 25, 159
Aki, K., 175
Akira, O., 511
Akita, T., 29, 373, 382, 403
Akiyama, M., 410, 456
Akiyama, S. K., 339
Akiyoshi, H., 532
Aksenov, S. I., 274, 290
Akwari, O. E., 519
Alakov, Y. B., 265
Alam, T., 93
Al-Ayash, A. I., 163
Albano, O., 519
Alben, J. O., 297
Albericio, F., 22
Albers, R. W., 326
Albers-Shonberg, G., 444, 457
Alberti, K. G. M. M., 534
Alberts, A. H., 18
Alberts, B. M., 75
Albertsson, P.-A., 329
Albuquerque, R. H., 515
Alden, R. A., 241
Aldridge, W. N., 169, 320
Alegakis, G., 519
Aleksiev, B., 306
Aleshina, N. V., 298
Alev-Behmoaras, T., 351
Alexander, C. B., 103, 143
Alexander, N. M., 34
Alexander Ross, J. B., 511
Alfsen, A., 149
Alger, J. R., 283
Al-Habib, A., 189
Alhanaty, E., 39
Al-Hassan, J., 433
Allan, R. D., 6
Allbrand, J. P., 274, 281
Allcock, H. R., 322
Allen, A. K., 91
Allen, F. H., 223
Allen, G., 185
Allen, J. K., 418
Allen, L. C., 256
Allen, M. C., 395
Allen, O., 287
Allen, P. C., 79
Allen, R., 476
Allen, R. C., 460
Allen, R. H., 60
Allen, R. L., 169
Allen, S. H. G., 39
Allerhand, A., 175, 284, 288
Allfrey, V. G., 388
Allgyer, T. T., 57
Allison, S. A., 349
Allmann, H., 135
Alma-Zeestraten, N. C. M., 291
Al-Nuri, M., 444
Aloj, S. M., 189
Alper, C. A., 81, 139
Alpert, A. J., 61
Alpin, R. T., 449
Alston, K., 179
Alumets, J., 510, 515, 519, 529, 530
Alvarado-Urbina, G., 512
Alvarez, E., 466
Åman, P., 99
Amano, H., 542, 559, 570
Ambartsumyan, N. S., 271
Ambinder, K. F., 559
Ambler, R. P., 133
Ambrong, V. W., 201
Ambrose, K. R., 15
Amelina, V. N., 404
Amery, A., 556
Amesz, H., 77
Amini, S., 315
Amiranoff, B., 528, 531
Amma, E. L., 226, 227

Amneus, H., 100
Amons, R., 131
Amos, L. A., 252
Amouric, M., 87
Amouyal, E., 30, 352
Amrhein, N., 3, 15
Amzel, L. M., 223
Anagnostaras, P., 456
Anant, J., 559
Ananthanarayanan, V. S., 254, 304
Anantharmaiach, G. M., 379, 383
Ancans, J. J., 403
Anderegg, R. J., 119, 120, 130, 144, 423
Andersen, D. K., 535
Andersen, R. N , 472
Andersen, T. T., 181, 481
Anderson, C. M., 236
Anderson, F. A., 17
Anderson, G., 380
Anderson, G. M., 34
Anderson, J. A., 420
Anderson, J. M., 62, 347
Anderson, J. N., 101
Anderson, N. L., 100
Anderson, P. M., 55
Anderson, R. G. W., 499
Anderson, W. L., 273
Andersson, K., 515
Andersson, L., 196
Andersson, T., 286
Anderton, B. H., 342
Ando, M., 95, 163, 167
Ando, S., 428
Ando, T., 73, 423
Andre, J. C., 347, 348
Andreasson, L. E., 318
Andreeva, A. P., 169
Andreeva, N. S., 234, 258
Andres, C. M., 49
Andres, R., 535
Andrev, D., 383
Andnànov, V. I., 437
Androszkiewicz, R., 404
Anfinsen, C. B., 77
Angelides, K. J., 339, 359
Angermann, K., 62
Anliker, M., 533
Annan, W. D., 34, 118
Anner, J., 271
Anselme, J.-P., 26
Antennis, M. J. O., 19, 275, 280, 417, 440
Antholine, W. E., 274
Anton, C. J., 309
Antonini, E., 41, 287, 335, 336
Anundi, H., 149
Anwar, R. A., 51
Anzai, J., 304, 409
Anzenbacher, P., 508
Anzinger, H., 370
Aoki, K., 103
Aonuma, S., 538
Aoshima, H., 61
Aoyagi, H., 6, 421, 428, 431
Aoyagi, T., 402, 451
Apold, J., 270, 273
Appella, E., 69, 123, 140, 142, 143
Applebaum, S. W., 183
Appleby, C. A., 287, 298
Applequist, J., 300
Appleton, D. W., 276
Appu Rao, A. G., 169
Araco, A., 161
Arad, T., 251
Aragoa, J. B., 308
Arai, M., 439
Arata, Y., 290
Araya, A., 132
Arcamone, F., 443, 445
Archibald, I. G., 236
Archimandritis, A. J., 519
Arendt, A., 381, 411, 456
Arentzen, R., 481
Arfmann, H.-A., 391
Argos, P., 111, 225, 245, 263
Arima, K., 311
Arimura, A., 465, 469, 512
Arisaka, F., 336
Arison, B. H., 444, 468
Arlaud, G. J., 81
Armitage, I. M., 283, 291
Armstrong, R., 123
Armstrong, R. N., 282
Arnaud, C. D., 543
Arnberg, L., 219
Arnett, T. R., 543
Arnheim, N., 271
Arnold, R., 513, 518
Arnon, R., 273
Arnone, A., 226
Arnsten, A., 579
Aronsson, A.-C., 95
Arotins, G., 437
Arrio-Dupont, M., 354
Arseniev, A. S., 288
Arshady, R., 385, 386, 558
Artigas, F., 31
Artymiuk, P. J., 229, 261, 263
Arutyunyan, A. M., 317, 318
Asakura, S., 252
Asakura, T., 340, 341
Asami, S., 53
Asano, A., 191
Asaoka, M., 12
Asaturyan, R. A., 17
Asboe-Hansen, G., 36
Aschauer, H., 132
Ashani, Y., 151
Ashida, T., 254
Ashihara, Y., 191
Aslanyan, V. M., 309
Asnes, C. F., 63
Asplund, K., 177
Aspray, T. E., 175
Assairi, L. M., 73
Assal, J. P., 502
Aster, R. H., 69
Astier, J. P., 133, 228
Astorri, C., 99
Asukura, T., 20
Ataka, M., 297
Atanasov, B., 305, 306
Atassi, M. Z., 153, 270, 271, 273, 403
Atger, M., 150
Atha, D. H., 329, 337
Atherton, E., 368, 385, 386, 436, 558
Atherton, F. R., 455
Atkinson, J. G., 14
Attanasio, A., 314
Attia, R. R., 531
Attie, A. D., 194
Au, F. Y., 194
Aubry, A., 18, 275, 293, 456
Auchet, J. C., 30, 350, 360
Auclair, C., 465
Audebert, R., 34, 35
Audemard, E., 179
Audhya, T. K., 118
Audigier, Y., 563
Auger, G., 400
Aull, J. L., 169
Auna, Z., 407
Aurbach, G. D., 514, 543, 545
Auricchio, F., 69
Aussel, C., 55
Austen, B. M., 248
Austen, K. F., 87, 89
Autric, F., 97
Au-Young, S., 363
Aviram, I., 163, 263
Axelrod, B., 185
Ayers, J. S., 40
Ayers, M., 342
Azanza, J.-L., 87
Azegami, M., 183
Azerad, R., 12
Azuma, I., 463
Azzi, A., 163, 202

Baba, S., 526
Baba, Y., 406
Babcock, G. T., 317
Bacci, M., 322
Bache, R. J., 556
Báchhawat, B. K., 187, 505
Bachmann, E. W., 34
Bachmann, P., 274
Bada, J. L., 23
Badalyants, I. P., 292
Baddoo, P., 61
Bade, D., 321
Bader, C. A., 543
Baenziger, J. U., 58
Bagger, S., 301
Bai, Y., 161
Baici, A., 276, 409
Baile, C. A., 523
Bailey, T. J., 108, 126, 137
Baines, B. S., 87, 193

Bajusz, S., 560, 562, 574, 575
Baker, K. M., 556
Baker, T. S., 252
Baksi, K., 73, 185
Balagurov, A. M., 221
Balaji, V. N., 246, 257
Balaram, P., 253, 267, 276, 297, 364, 411, 453, 456, 573
Balas, D., 524
Balaspiri, L., 487, 518
Balasubramanian, A. S., 93
Balasubramanian, T. M., 256
Bald, R., 187
Baldwin, D., 503
Baldwin, G. S., 122
Baldwin, J., 226
Baldwin, J. E., 447, 449
Baldwin, J. P., 250
Baldwin, R. L., 265, 266, 267, 268
Baldwin, T. O., 124
Balikov, S. R., 102
Balis, M. E., 95
Baliut, M., 201
Balko, B., 321
Ball, J. C., 44
Ballini, A., 271
Balny, C., 42
Balogun, M., 169
Balsamo, A., 448
Balszuweit, A., 370
Baltes, W., 25
Baltimore, D., 75
Baltzinger, M., 181
Bamburg, J. R., 107
Bandarin, V. A., 257
Bandekar, J., 293
Bandyopadhyay, P. K., 355
Banergee, S. K., 179
Bangerter, F., 276, 409
Bankowski, K., 487
Banks, G. R., 73
Banks, R. D., 125, 237, 264
Banks, W. A., 515
Bannister, J. V., 281
Bannister, W. H., 281
Bansal, M., 254, 257
Bapat, A. R., 169
Bara, Y. A., 431
Barakat, F., 77
Barany, G., 33, 388
Barata, L., 457
Baratova, L. A., 87, 126
Barbeau, D. L., 157, 327, 361
Barber, D., 317
Barber, J., 358
Barber, M., 24, 381, 407
Barber, R., 191
Barber, T. A., 4, 460
Barbet, J., 560
Barbezat, G. O., 519
Barbotin, J. N., 167
Barboy, N., 354
Bard, A. J., 7
Barden, N., 512
Barden, R. E., 165
Barisas, B. G., 337
Barker, R., 281
Barkley, M. D., 349
Barkovsky, E. V., 257
Barling, P. M., 545, 548
Barnabe, N., 45
Barnard, E. A., 151
Barnes, A. J., 468
Barnes, K., 146
Barnes, P., 244, 256
Barnick, J. W. F. K., 450
Barnickel, G., 462
Baron, M. H., 75, 157, 292
Baros-Stuliglowa, E., 408
Barra, D., 121
Barrach, H.-J., 62
Barrett, A. G. M., 447
Barrett, A. N., 183, 333
Barrett, G. C., 455
Barrett, J. C., 538
Barrieux, A., 109
Barrio, J. R., 364
Barrow, K. D., 418
Barry, C. D., 256
Barry, S., 39
Bartels, K., 241
Bartenev, V. N., 249
Barth, G., 317
Barth, J., 7
Barth, T., 378, 407, 409, 492
Barth, W. E., 446
Bartha, F., 338
Barthe, P. L., 471
Barthels, R., 370
Bartsch, R. G., 133
Barzu, O., 17
Basch, J. J., 101
Baserga, R., 315
Basham, T. Y., 141
Bashan, N., 39
Basheeruddin, K., 6
Baskin, R. J., 159
Bassagnani, L., 5
Bassedas, M., 22
Basso, N., 515
Basu, S.K., 65
Bataille, D., 526, 531, 533, 534, 538
Batcho, A. D., 14
Bates, O. J., 467
Bates, S. E., 66
Bathena, S. J., 469
Batke, J., 338
Bats, J. W., 275, 430
Battersby, M. K., 169
Battini, L., 501
Battistini, A., 161
Batzri, S., 523
Bauce, L. G., 408
Bauer, A., 403
Bauer, C.-A., 233
Bauer, H., 32, 87, 111, 122, 523
Bauer, K., 189
Bauer, R. S., 161
Bauer, W., 396, 569
Baugher, J. F., 30, 352
Baughman, M., 364
Baumann, C., 135
Baumann, H., 101
Baumann, J. B., 527
Bauminger, E. R., 323, 324
Baumont, M., 399
Baute, M. A., 418
Baute, R., 418, 419
Bautisha, J. G., 173
Baxter, A. J. G., 445
Baxter, J. D., 481
Baxter, R. L., 450
Bayard, B., 102
Bayer, E., 32, 314, 370, 389, 390, 392, 402
Bayley, P. M., 144, 313
Baylin, S. B., 530, 543
Baynes, J. W., 155, 194
Bazin, M., 352
Bazzone, T. J., 87, 159
Beabealashvilli, R. Sh., 185
Bear, R. S., 254
Beardsley, W., 523
Beaudet, A., 529
Beaudreau, G. S., 108, 137
Beaumont, A., 559
Beaumont, M., 468
Beaune, P., 67
Beaven, G. H., 510
Bech, A., 531
Becher, B., 357
Bechet, J. J., 197
Bechtel, F., 19
Bechtel, K. C., 34, 118
Bechtolsheimer, H.-H., 369
Beck, E. A., 103
Beck, M., 8
Beckage, M. J., 282
Becker, E. L., 276, 404
Becker, H. D., 514
Becker, J. H., 256, 410
Becker, J. M., 267, 275, 303
Becker, J.-U., 57
Becker, J. W., 91, 134
Becker, R. R., 108, 126, 137
Becker, W., 345
Beckers, J. F., 484
Beddard, G. S., 345
Bednarek, M., 421
Beebee, T. J. C., 71
Beegen, H., 334
Beeler, D. L., 270
Begg, G. S., 140
Begley, D., 181, 270
Behar, J., 533
Behe, M. J., 341
Behnke, W. D., 305, 313
Behrens, C., 470, 472
Behrman, E. J., 29
Beijer, H. J. M., 527
Beintema, J. J., 126, 283
Beissner, R. S., 44, 241

Belaich, J. P., 30, 290
Belchich, L. A., 25
Bell, A. W., 161
Bell, E. A., 1, 2, 3
Bell, J. E., 123, 282
Bell, N. H., 542
Bell, P. B., 109
Bell, R. H., jun., 514
Bellard, S., 223
Belleau, B., 563
Bellisario, R. L., 126, 127
Bello, J., 194
Belonogova, O. V., 325
Belostotskij, V. M., 169
Beltz, W. R., 437
Belyanova, L. P., 87, 126
Benassi, C. A., 405
Benbasat, J. A., 330
Bender, M. L., 189
Bendzko, P., 260
Benedetti, E., 19, 253, 292
Benes, M., 39
Benezra, R., 55
Benfield, P. A., 123
Bengtsson, G., 31
Benjamin, D. C., 273
Benjannet, S., 559
Benko, B., 286
Ben-Naim, A., 255
Benne, R., 77
Ben Neriah, Y., 179
Bennesch, R. E., 341
Bennett, A., 150
Bennett, C. D., 115, 128, 468, 513
Bennett, E. L., 151
Bennett, H. P. J., 61, 470
Bennett, J. C., 137
Bennick, A., 60, 148
Benoit, J., 399, 468
Benoit, R., 512
Benoiton, N. L., 1, 26, 27, 276, 369, 379, 380, 413
Benovic, J. L., 287
Benson, E. S., 317
Bentley, P. H., 443
Bentz, H., 135
Ben-Yosef, Y., 58, 93
Beppu, M., 161
Beppo, T., 311
Ber, E., 306
Berelowitz, M., 519
Berestein, S. F., 271
Berezin, B. B., 17, 25
Berezin, I. V., 25, 161, 171
Berg, A., 67
Berg, A. I., 325
Berg, J. M., 253
Berg, N. O., 515
Berg, R. A., 110
Berger, I., 157
Berger, M., 502
Berger, R. L., 321
Bergman, L. W., 269
Bergman, M. D., 406, 473
Bergquist, C. H., 465
Bergson, G., 7
Bergstrand, S. J., 400, 468, 512
Bergstrom, J. D., 97
Berhanu, P., 538
Berk, P. D., 70
Berkowitz, J. M., 524
Berlin, K. D., 437
Berliner, L. J., 161
Berman, A. H., 358
Berman, A. L., 295
Berman, E., 8
Berman, H. A., 151
Berman, H. M., 250
Bernabeu, C., 187
Bernas, A., 30, 352
Berndsen, R. G., 98
Berndt, H., 399, 406
Bernstein, J., 364
Bernstein, M. A., 288
Bersohn, R., 286
Berson, S. A., 475
Berstad, A., 519
Bertani-Dziedzic, L., 34
Berthet-Colominas, C., 254
Berthold, W., 77
Berthou, J., 231
Berti, A., 153
Bertini, I., 285
Bertino, J. R., 97
Bertles, J. F., 341
Bertolini, A. D., 153
Bertrand, P., 323
Berz, F. W., 196
Berzétei, I., 562
Berzinis, A. P., 286
Beschastnaya, N. V., 403
Besser, G. M., 465, 475
Besson, F., 21, 425
Besson, J., 526, 529, 531, 533
Besterman, H. S., 515, 527, 534, 536
Beswick, F. B., 527
Betcher-Lange, S. L., 191
Bethell, G. S., 40
Bethge, P. H., 222, 228
Bettay, A. L., 465
Bevins, C. L., 173
Bewley, T. A., 181, 308, 394, 478, 479, 481, 482
Bey, P., 11
Beychok, S., 340
Beyerman, H. C., 29, 155, 400
Bhandary, K. K., 250
Bhargava, H. N., 498, 579
Bhargava, P. M., 89
Bhat, R., 329
Bhat, T. N., 245, 258
Bhatnagar, P. K., 422
Bhatnagar, R. S., 304
Bhattacharyya, S. C., 18
Bhavanandan, V. P., 58
Bholah, M. A., 2
Bianchetta, J. D., 124
Bianchi, G. E., 5
Bianchi, R., 222
Biancini, B., 5
Biber, J., 177
Bickel, M., 525
Bickelhaupt, F., 2, 450
Bicksler, J., 469
Bidaud, J., 124
Bideau, J. P., 19
Bidney, D. L., 71
Bieber, A. L., 97
Bieber, L. C., 36
Bieker, L., 175
Bielka, H., 187
Biellman, J.-F., 44, 165, 236, 239, 354
Biemann, K., 119, 120, 130, 144, 423
Bienert, M., 558
Bieth, J. G., 42, 163, 165, 284
Bigam, G., 274
Bigelis, R., 47
Bigelow, C. C., 265
Bigi, A., 254
Bijtholt, M. M. C., 333
Billington, D. C., 16
Binder, B. R., 87
Bink, G., 36
Birch, G. R., 523
Birdsall, B., 274
Birdsall, W. J., 289
Birk, Y., 183, 308, 472, 474, 480
Birkenhager, J. C., 543
Birnbaum, E. R., 289, 363
Birnbaumer, L., 511
Birnbaumer, M. E., 181
Birr, C., 378, 388, 398, 400, 428, 435, 498, 500
Bischoff, J. L., 23
Biscoglio de Jimenez Bonino, M. J., 169, 394
Biserte, G., 102, 129
Bishop, C. A., 34, 392
Bishop, J. M., 105
Bisset, G. W., 407
Bisson, R., 202
Bitar, K. N., 519, 524, 526
Bittner, M., 75
Biz-Zozero, J. P., 121
Bjarnason, T., 314
Bjerrum, O. J., 103
Björk, I., 137, 157
Björk, P., 55
Bjoerklund, A., 529
Bjoerkman, S., 406
Björkroth, U., 408, 557
Black, B., 142
Black, J. A., 132
Blackburn, A. M., 515
Blackburn, P., 73
Blakemore, J. I., 175
Blackman, B. D., 65
Blaha, K., 275, 295, 299, 303, 304, 378, 422, 430, 492
Blair, E. L., 519
Blair, N. E., 23

Blake, C. C. F., 125, 229, 237, 261, 263, 264
Blake, J., 373, 394, 395, 397, 471, 478, 479, 564
Blake, T. J., 438
Blackmore, J. I., 272
Blakley, R. L., 193, 281
Blanchard, R. A., 270
Blanchette, L., 476
Blaner, W. S., 187
Blanot, D., 400
Blasio, B. D., 292
Blass, E. M., 523
Blatterspiel, R., 124
Blauer, G., 312
Bleich, H. E., 276, 278, 404, 575
Blicharska, B., 290
Blinks, J. R., 344
Blobel, G., 106, 126, 136, 140
Blödorn, J., 239, 507
Bloemhoff, W., 408
Blombäck, B., 138, 165
Blomberg, F., 102, 456
Blondin, G. A., 177
Bloom, F., 359
Bloom, J. W., 309
Bloom, K. S., 101
Bloom, S. R., 127, 468, 513, 514, 515, 517, 527, 528, 529, 530, 531, 533, 534, 536
Bloom, W., 333
Bloomfield, G. A., 482, 483
Bloomfield, V. A., 330, 331
Blount, J. F., 14, 421
Blout, E. R., 280, 289, 430, 433, 452
Bloxham, D. P., 173, 197
Blumberg, S., 558
Blumenkrantz, N., 36
Blumenstein, M., 278, 279, 282, 408
Blundell, T. L., 234, 258, 499, 510, 574
Blunt, D. J., 23
Blyumenfeld, L. A., 317, 318
Bobrzecka, K., 173
Bocchi, V., 371
Bock, E., 103
Bock, K. W., 51
Bock, S. C., 102
Bodanszky, A., 409, 528, 529
Bodanszky, M., 368, 374, 407, 409, 524, 528
Bode, W., 264
Bode, H.-J., 101
Bode, J., 171, 193
Boden, G., 526
Boden, N., 281
Boders, C. L., 159
Bodley, J. W., 145, 165
Böger, P., 165
Boehnert, M., 115, 544
Boerger, H. W., 514
Boesch, C., 288
Boezi, J. A., 73
Bof, M., 157
Bogardt, R. A., 133
Bogdanov, A. A., 364
Boggaram, V., 42
Boggs, N. T., tert, 9, 413
Boghosian, R. A., 49
Bognar, R., 4
Bogomolni, R. A., 356
Bogorad, L., 109
Boguta, G., 30
Boheim, G., 453
Bohlen, P., 35
Bohm, H., 187
Bohme, F., 173
Bohn, B., 337
Bohn, E. W., 73
Bohnet, H. G., 465
Boisson, D., 31
Boissonnas, R. A., 487
Boivin, P., 49
Bojan, O., 17
Bojanovska, V., 407
Bojarski, C., 349
Bojesen, G., 4, 461
Boles Bryan, D., 448
Boletto, N., 515
Bolger, M. B., 264
Bolhuis, P. A., 81
Bolivar, F., 499
Boller, T., 2
Bollum, F. J., 104
Bolman, R. M., tert, 518
Bologa, M., 17
Bolognesi, M. C., 232, 242
Bolotin, C., 483
Bolotina, I. A., 307
Bolscher, B. G. J. M., 283
Bolts, J., 179
Bommer, U.-A., 187
Bonatti, S., 136
Bonaventura, C., 329
Bonaventura, J., 329, 333
Bondareva, V. M., 510
Bondy, S. C., 18
Bone, H. G., 543
Bone, Q., 543
Bonewald, L. F., 480
Bonfils, S., 513, 518
Boni, R., 430
Bonicel, J. J., 124
Bonilavri, E., 13
Bonnafons, J.-C., 41
Bonner, W. A., 23
Bonner, W. M., 171
Bonnett, R., 26, 358
Bonora C. M., 19, 254, 290, 292, 303, 308, 389, 400, 519
Bonting S. L., 521, 527
Bookchin, R. M., 340
Bookjans, G., 165
Boon, P.-J., 266, 393
Boorman, G. A., 539
Booth, N. A., 81, 139
Boothe, J. H., 449
Borca, E., 221
Bordas, J., 254
Borders, C. J. L., 194
Borders, C. L., jun., 165
Bordier, C., 109, 339
Bordiu-Obanza, E., 465
Borel, J. P., 33, 35
Borghi, C., 531
Borghi, E., 285
Borgstroem, A., 127
Borgstrom, B., 285
Borin, G., 306, 308, 375, 396, 400, 576
Borisov, I. L., 25
Borisy, G. G., 341
Bornstein, J., 405
Bornstein, P., 136
Borowski, E., 404
Borsos, T., 79
Bory, S., 12
Bosch, L., 136
Boschetti, E., 61
Base, G. H., 297
Bosisio, E., 16
Bosnich, B., 6
Boss, A., 53
Bossa, F., 121
Bossinger, J., 339
Bostwick, J. R., 98
Botelho, L. H., 134
Botella-Llusia, J., 465
Botes, D. P., 148
Bothner-By, A. A., 280
Bothwell, M. A., 326
Botos, C. R., 409
Bottari, S., 153
Bouchilloux, S., 93
Bouffard, F. A., 445
Boulter, D., 134, 146
Bouma, B. N., 552
Bourque, D. P., 101
Bousard, N., 93
Boussard, G., 275, 293, 456
Bouveret, P., 102, 110
Bovermann, G., 453
Bowden, P. E., 474
Bower, J. D., 464
Bowers, C.-Y., 406, 466
Bowers, H., 474
Bowery, N. G., 403, 551
Bowey, F. S. B., 519
Bowien, B. U., 127
Bowman, B. H., 150
Bowman, R. L., 299
Box, S. J., 444
Boxer, S. G., 286
Boyd, D. B., 449
Boyd, P. A., 63
Boydens, R., 447
Boyle, M. D. P., 79
Boyle, S. M., 187
Boyles, W. J., 24
Boyse, E. A., 400
Bracha, M., 111
Bracht, A., 157
Bradaczek, H., 462

Bradbury, E. M., 250, 276, 290
Bradley, C. H., 277
Bradley, R. J., 575
Bradley, W. A., 149
Bradshaw, R. A., 111, 467
Brady, R. O., 103
Brady, S. F., 410, 434, 468, 512
Bränden, C. I., 44, 238, 239
Braga, P. C., 542
Braganza, J. M., 527
Bragg, P. D., 189, 192
Brahmachari, S. K., 254, 257, 304
Brahms, J., 298
Brahms, J. G., 299, 303, 304, 433
Brahms, S., 299, 303, 304, 433
Bramhall, J. S., 161, 191
Bramson, H. N., 282
Brand, J. S., 111, 194
Brand, L., 359
Brandenburg, D., 394, 399, 498, 500, 503
Brandt, A., 5
Brandt, W. F., 129
Branfman, A. R., 32
Brantl, V., 145
Branton, D., 110
Branyas, N., 372, 466
Bratby, D. M., 401
Bratcher, S. C., 185, 193
Bratt, P., 177
Braude, I. A., 61, 71, 77
Brauer, A. W., 34, 118
Brauer, D., 131
Braughler, J. M., 57
Braun, D. G., 140
Braun, F., 448
Braunitzer, G., 132, 133, 147
Braunstein, A. E., 121
Brawer, J. R., 503
Bray, R. P., 290
Brayden, J. E., 111
Brayer, G. D., 233, 234, 248, 260
Brazeau, P., 512
Bredberg, L., 357
Breddam, K., 87, 159
Breederveld, K., 81
Breese, H., 326
Breese, K., 98
Bregman, M. D., 511
Brems, D. N., 181
Brenna, L. A., jun., 515
Brenner, D. S., 289
Brenner-Holzach, O., 123
Brent, T. P., 73
Bresciani, F., 39
Breslow, E., 179, 278, 356
Brett, M., 185
Brevet, A., 71
Brew, K., 111, 146
Brewer, C. F., 58, 290
Brewer, H. B., jun., 116, 543
Brewer, J. M., 165
Brewster, D., 464
Bricas, E., 400
Brice, M. D., 223
Bridgen, P. J., 77
Bridges, J. W., 167, 169, 320
Briehl, R. W., 341
Briggs, J., 524
Briggs, M. T., 15, 403, 517
Briggs, R. W., 438
Briley, P. A., 122
Brimacombe, R., 187, 199
Brimhall, B., 108, 132, 137
Brink, L., 61, 510
Brison, J., 277, 403, 555
Brisson, N., 187
Brittain, T., 316
Brnjas-Kraljevic, J., 287
Brobjer, T., 42
Brockbank, W. J., 87
Brocklehurst, K., 87, 153, 193
Brodbeck, U., 151
Brodie, A. F., 210
Brodrick, J. W., 404
Brody, R. S., 97
Broer, Y., 528
Bronson, D., 43
Brooks, G., 443
Brooks, J. S., 15
Broome, S., 499
Broor, S. L., 519
Broughton, C. G., 233
Brouilette, C. B., 189
Brousseau, R., 500
Brouwer, F. A. S., 527
Brouwer, J., 99
Brouwer, M., 134
Brouwers, L., 447
Brown, A. G., 444
Brown, A. J. L., 42
Brown, A. S., 134, 137
Brown, B. A., 145
Brown, B. E., 407
Brown, C. E., 271, 279
Brown, D., 444
Brown, D. H., 301
Brown, D. J., 543
Brown, D. L., 342
Brown, D. M., 548
Brown, E., 83
Brown, E. M., 282, 514
Brown, F. C., 533
Brown, F. F., 277
Brown, G. B., 575
Brown, J. C., 514, 533, 535
Brown, J. L., 41
Brown, J. N., 256, 366, 432
Brown, J. R., 436
Brown, L. R., 288
Brown, M., 104, 512
Brown, M. F., 284
Brown, M. R., 468
Brown, M. S., 65, 499
Brown, P. J., 69
Brown, P. R., 61
Brown, R. D., 58, 175, 290
Brown, R. J., 40
Brown, S. A., 39
Brown, W. D., 133
Brown, W. E., 195
Brownlee, M., 504
Brownstein, M. J., 105
Broyart, J., 528
Brtnik, F., 409
Bruckner, H., 541
Bruckner, P., 135
Bruderlein, H., 364
Brudzynski, T. M., 89, 189
Brugger, M., 541
Brumbaugh, E. E., 328
Brundish, D. E., 395, 472
Brunfeldt, K., 388
Bruni, J. F., 559
Brunner, H., 6
Brunner, H. R., 556
Brunner, R., 420
Brunori, M., 336
Brunzell, J. D., 535
Bruschi, M., 133, 134, 228
Bruton, C. J., 77
Bryant, M. G., 107, 514, 515, 529
Buc, H., 364
Bucci, E., 264, 327, 361
Buchan, A. M. J., 518, 535
Buchanan, K. D., 510, 526
Bucher, D., 127
Bucher, D. J., 33
Buchholz, M., 369, 370
Buck, C. A., 102
Buck, R., 2
Buckett, W. R., 561
Buckley, T. F., 448
Buczylko, J., 198
Budd, D. L., 285
Budesinsky, M., 19, 275
Budorf, M., 476
Budzikiewicz, H., 21
Buechter, K., 22
Bückmann, A. F., 37
Büldt, G., 252
Büllesbach, E., 394, 498, 504
Buescher, H.-H., 396, 569
Buettner, G. R., 460
Buffa, R., 530
Buhl, P. H., 1
Buillemin, K., 512
Buishvili, Y. G., 291
Bukolova-Orlova, T., 306
Bulgakova, O. S., 525
Bull, H. B., 98
Bull, H. R., 326
Bull, T., 280
Bullard, B., 258, 329
Bullock, C., 342
Bullock, D. G., 346
Bullock, D. W., 148
Bumpus, F. M., 402, 555
Bunce, R. A., 346
Bundi, A., 274, 366
Bunera, V. N., 163
Bunn, H. F., 169

Bunnenberg, E., 317
Burback, J. P. H., 561
Burch, J. B. E., 171
Burchall, J. J., 123
Burgen, A. S. V., 274, 281, 284
Burger, D. R., 61
Burger, M., 531
Burgess, R. J., 265
Burgess, R. R., 75
Burhol, R. G., 515, 526, 527
Burke, M. F., 392
Burke, R. L., 75
Burke, W. H., 482
Burleigh, B. D., 122, 191, 483
Burley, R. W., 116, 144
Burman, K. D., 36
Burnett, G., 16
Burns, P. C., 101, 338
Burns, P. D., 286
Burroughs, S. F., 200
Bursaux, E., 337
Burshtein, E. A., 343, 353, 353
Burstein, S., 479
Burstein, Y., 142, 147
Burtnick, L. D., 346, 363
Burton, D., 543
Burton, D. R., 274
Burton, M. P., 480
Bury, C., 368
Busch, H., 147
Buse, G., 119, 122, 123, 132
Busel, E. P., 353
Bush, C. A., 304, 433
Bushuev, V. N., 290, 306
Bushueva, T. L., 353
Butina, D., 11
Butkowski, R., 147
Butler, P. J. G., 248
Butler, T. A., 16
Butschak, G., 286
Butterworth, P. J., 155
Buttgereit, K., 39
Buurman, C. J., 543
Bycroft, B. W., 417, 458
Bye, J., 136
Byfield, P. G. H., 44, 539
Byrd, B. L., 16
Byrnes, D. J., 526
Bystrov, V. F., 288
Byyny, R. L., 475

Cabassi, F., 278, 575
Caciagli, V., 5
Cadman, E., 98
Cahnmann, H. J., 41
Cailla, H. L., 524
Calabrese, L., 281
Calam, J., 518
Calas, B., 411
Calderon, P., 514, 524
Caldés, T., 53
Caldo, H., 552
Callahan, A. P., 15, 16
Callahan, E. N., 546
Callanan, T. S., 559
Calleman, C. J., 28
Callens, R. E. A., 280, 440
Callens, S., 280
Calvet, P., 470
Calvo-Fernandez, P., 195
Cama, L. D., 445, 446
Camargo, A. C. M., 552
Camici, G., 153
Camier, M., 106
Campbell, D. G., 144
Campbell, I. D., 273, 285
Campbell, P., 202
Campbell, P. S., 101, 338
Campbell, R. D., 81, 139
Campling, M. R., 282
Campos-Carieres, M., 159
Camus, J. C., 531
Cancedda, F., 130
Candelas, G., 110
Canioni, P., 285
Cann, J. R., 263, 279, 336, 551
Cannon, D., 521
Cannon, M., 60
Cano, A., 73
Cano-Iglesias, R., 465
Canopmo, P., 285
Canova-Davis, E., 472
Canti, G., 285
Cantor, C. R., 361
Capaldi, R. A., 163, 197, 252
Capasso, R., 185
Capasso, S., 121, 276
Capel M., 101
Caplaneris, T., 407
Caplow, M., 343
Capp, M., 61
Cappugi, G., 153
Capra, J. D., 109, 140, 143, 269
Caprioli, R. M., 119
Caputo, T. M., 200
Carafoli, E., 70
Caranikas, S., 413
Cardemil, E., 185
Cardenas, J. M., 126
Cardillo, R., 17, 417
Cardin, A. D., 305, 313
Cardinaux, F., 396, 569
Carenzi, A., 560
Carey, R. M., 556
Carlberg, I., 169
Carlberg-Bacq, C. M., 59
Carlei, F., 515
Carlier, M. F., 161
Carlquist, M., 128, 467, 526, 528
Carlsen, J., 407
Carlson, H. E., 524
Carlson, J. D., 183
Carlsoo, B., 523
Carlstedt-Duke, J., 69
Carmichael, D. F., 183
Carne, A. F., 144, 251
Carne, T., 169
Carnegie, P. R., 65
Caron, F., 73
Caron, M. G., 38
Carpenter, F. H., 399
Carpenter, G., 536
Carpenter, G. B., 222
Carpino, L. A., 368
Carpy, A., 418
Carr, D., 10
Carrano, C. J., 420, 424
Carraway, R., 128, 558
Carrea, G., 41, 335, 538
Carrell, H. L., 250
Carrey. E. A., 265
Carrington, P. H., 22
Carroll, M. C., 109
Carroll, R. J., 510, 512
Carruthers, J. R., 221, 222
Carson, J. F., 26
Carta, S., 143
Carter, C. W., 220
Carter, C. W., jun., 220
Carter, D. C., 518
Carter, T. J. N., 346
Carter, W. G., 69
Cartwright, B. A., 223
Carvalho, E. F., 406
Cary, P. D., 280
Cascio, S., 91
Cascone, O., 394
Case, D. A., 225, 256, 261, 322
Casella, L., 9
Casellato, M. M., 538
Casey, M., 448
Casey, R., 60
Casey, R. P., 163
Casimir, J., 3
Casnati, G., 371
Casone, O., 169
Caspar, D. L. D., 248
Caspers, J., 438
Cass, A. E. G., 281
Cassigneul, J., 521
Cassim, J. Y., 315
Castel, A., 116, 136
Castelino, J. B., 482
Castell, D. O., 533
Castell, J. V., 194, 374, 396, 562
Castellano, S., 19
Castellino, F. J., 173, 282
Castensson, S., 406
Castineira, M., 21, 293
Castle, A. G., 53
Castro, D., 340
Cataland, S., 533, 534
Catherwood, B. D., 543
Cathiard, A. M., 532
Catt, K. J., 499
Caughey, W. S., 296
Cavalieri, P., 436
Cavatorta, P., 30, 351
Cave, A., 289
Caviezel, M., 7
Cawley, D. B., 153
Cawston, T. E., 87
Cayley, G., 281
Cayley, P. J., 281

Cebula, T. A., 51
Ceccnetti, P., 501
Cechova, D., 183
Cecka, J. M., 108
Cellucci, M. D., 177
Cerami, A., 504
Cerdonio, M., 322
Cerletti, N., 202
Cerrini, S., 411
Cervera, M., 98, 194
Chaabouni, A., 63
Chabre, M., 295
Chacko, E., 447
Chacko, K. K., 18
Chadha, K. C., 77
Chae, Q., 357
Chaiken, I. M., 106, 265, 273
Chakravarty, P. K., 438
Challberg, M. D., 75
Challis, J. R. G., 474
Chalvignac, M.-A., 122
Chamberlain, J. P., 103
Chambers, B. B., 44
Chambers, J. L., 222
Chambers, S. M., 43
Champagne, D., 306
Champagne, J., 514
Champagne, M., 298
Chan, S. H. P., 100, 338
Chan, S. J., 512
Chan, W., 531
Chan, W. W.-C., 157
Chan, W. Y., 277
Chan, Y.-L., 409
Chance, B., 346, 357
Chance, R. E., 498
Chandra, H. S., 99
Chandramouli, N., 407
Chandrasekaran, R., 246, 290, 411
Chandross, R. J., 254
Chaney, M. O., 447
Chang, A. C. Y., 476, 543
Chang, C. C., 16, 288
Chang, C.-D., 387
Chang, C.-J., 442
Chang, C. T., 299
Chang, C. T. C., 189
Chang, D., 130, 383
Chang, E. L., 331
Chang, H., 287, 503
Chang, J., 569
Chang, J. C., 322
Chang, J. J., 307
Chang, J. Y., 117
Chang, K.-J., 272, 404, 576, 577, 578
Chang, P. K., 15
Chang, R. C. C., 560
Chang, S. T., 181
Chang, T.-M., 527, 530
Chang, W.-C., 397, 404, 564, 574
Chang, Y.-S., 502
Changchien, L. M., 187
Changeux, J.-P., 144, 259
Chanley, J. D., 31
Channabasavaiah, K., 466
Chany, C., 71, 77
Chao, Y. H., 286
Chapman, D., 289
Chapman, J. A., 183
Chapoy, L. L., 350
Chappelle, E. W., 347
Charbon, G. A., 524, 527
Charbonneau, H., 97
Chard, T., 474
Chari, R. V. J., 13
Charles, D., 43
Charlier, J., 157
Charlton, S. C., 281
Charnas, R. L., 443
Charro-Salgado, A., 465
Chatelain, P., 531
Chaturvedi, N., 465
Chaudhuri, M. K., 387
Chaudhuri, S., 95
Chauhan, V. S., 19, 383, 403, 551
Chauvet, J., 128
Chauvet, M. T., 128
Chauvet-Monges, A. M., 30, 290
Chauvin, M. M., 51
Chauvin, Y., 13
Chavez, L. G., 266
Chavkin, C., 577
Chawla, R. K., 127 473
Chayviálle, J. A., 467, 515, 526
Cheetham, P. S. J., 98
Cheetham, R. C., 340
Chelbi-Alix, M. K., 71, 77
Chen, B., 332
Chen, C.-A., 33
Chen, C. S., 456
Chen, F. M. F., 26, 27, 276, 369, 379, 380, 413
Chen, H. C., 308
Chen, J. F., 159
Chen, J. T., 287
Chen, M. S., 331
Chen, R., 147, 161, 284
Chen, R. F., 35
Chen, S., 179
Chen, S. S., 307
Chen, T. C., 403
Chen, Y.-C., 73
Cheng, C. M., 69
Cheng, K. W., 484
Cheng, L. J., 420
Cheng, L. Y., 169
Cheng, Y.-C., 53
Chen-Kiang, S., 103, 108, 113
Chen-Marotel, J., 153
Cherenkevitch, S. N., 351
Chernyak, D. V., 99
Chernyak, V. Y., 99
Cheron, C., 35
Cheronis, J., 17
Cherry, P. C., 443, 447
Chersi, A., 143
Chertov, O. Yu., 185
Chestukhin, A. V., 404
Cheung, G., 91
Cheung, H. C., 335, 363
Cheung, H. S., 556
Cheung, P. H.-C., 360
Cheung, S., 167
Cheung, S. T., 380
Cheung, W. Y., 53
Chey, W. Y., 526, 527, 530
Chiancone, E., 41, 287, 335, 336
Chiang, R., 321
Chiappelli, F., 98
Chiba, S., 167
Chibata, I., 10, 17
Chick, W., 499
Chieregatti, G., 167
Chiga, M., 69
Chignell, C. F., 436
Chihara, K., 542
Childers, S. R., 559
Childers, W. M., 23
Chillemi, F., 405
Chillemi, R., 2
Chilson, O. P., 273
Chinkjian, J. G., 73
Chino, N., 564
Chioccara, F., 3
Chipens, G. I., 256, 367, 377, 403, 472, 496
Chipkin, R. E., 562
Chipman, D. M., 364
Chirikjian, J. G., 73, 165
Chiron, C. A., 352, 355, 356
Chiu, A. T., 403, 551
Chiu, A. Y., 128
Chiu, C. C., 227, 340
Chiu, Y.-Y. H., 143
Chlebowski, J. F., 283
Cho, J., 360
Choay, J., 407, 462
Chock, P. B., 285, 355
Chock, S. P., 355
Chopra, C., 440
Chorbanov, B., 306
Chorev, M., 453, 455, 465, 562
Chothia, C., 226, 257, 259, 262
Chou, M. W., 57
Chou, P. Y., 257, 258
Chou, T. S., 447
Choudhury, A. M., 401, 517
Choughuley, A. S. U., 27
Chow, T. H., 167
Chowdhury, A., 436
Chowdhury, L. A., 455
Chowdrey, H. S., 407
Chowdry, V., 200
Choy, R., 512
Choy, Y.-M., 60
Chrambach, A., 99, 100, 332
Chrétien, M., 476, 482, 559
Christensen, B. G., 444, 445, 446, 447
Christensen, M., 407

Christensen, T., 388
Christensen, U., 181
Christiansen, J., 518, 531
Christie, D. L., 548
Christie, E. A., 159
Christie, M. A., 447
Christner, J. E., 93
Christofides, N. D., 515, 536
Christopanides, N. D., 468
Christophe, J., 514, 524, 531
Christou, G., 323
Chu, A. H., 327
Chu, G. Y., 361
Chu, K. Y., 401, 517
Chu, S.-C., 502
Chua, M. M., 179
Chua, N.-H., 100, 102, 126
Chujo, R., 290
Chukhrova, A. I., 83
Chun, P. W., 328
Chung, A., 557
Chung, C. S., 287
Chung, D., 470, 472, 475
Chung, D. W., 148
Chung, J., 84,
Churáček, J., 107
Churchich, J., 187
Churchich, J. E., 183
Ciajolo, A., 19, 292
Ciardelli, T. L., 438
Ciaschi, R., 409
Cidlowski, J. A., 177
Cima, L., 306, 396, 576
Cinader, B., 272
Cintrón, N. M., 61
Cipens, G., 407, 551
Cipollo, K. L., 163, 189
Ciriello, S., 34
Citri, N., 173
Citti, L., 501
Cjypers, P., 275
Claeys, M., 531
Claflin, J. L., 140
Claiborne, A., 57
Claridge, F. C., 169
Clark, A. G., 83
Clark, A. M., 450
Clark, D., 474
Clark, D. L., 91
Clark, D. S., 275
Clark, M. W., 253
Clark, R., 318
Clarke, A. E., 58, 59
Clarke, D. E., 163
Clarke, F. M., 251
Clarke, R. G., 98, 326
Clarke, S. E., 171, 227
Clarkson, R. W., 15
Claude, H., 276
Claude, R., 279
Clauss, R., O., 519
Clay, K. L., 17, 31
Cleator, I. G. M., 534
Clemens, J. A., 533
Clement, J., 399, 468
Clement, L. T., 141
Clementi, E., 244, 256
Clements, P. R., 165
Cleveland, D. W., 109, 342
Clin, B., 288
Clizbe, L. A., 8
Closier, M. D., 447
Closse, A., 396, 569
Closset, J., 484
Cobb, M. H., 500
Coccia, P. F., 548
Cocco, L., 281
Coderre, J., 389
Codogno, P., 128
Cody, V., 18
Coffer, D. L., 421
Cohen, C., 250
Cohen, D. J., 34
Cohen, F. E., 247, 260
Cohen, G. N., 122
Cohen, I. A., 224
Cohen, J. S., 274, 283, 284, 286, 408
Cohen, L. W., 165
Cohen, M. B., 16
Cohen, N. D., 183, 333, 334
Cohen, P., 106, 124
Cohen, S., 524, 536
Cohen, S. G., 323, 324
Cohen, S. N., 103, 476, 543
Cohn, D. R., 548
Cohn, J. N., 556
Cohn, M., 282
Coito, C., 440
Cole, H. H., 482
Cole, J. R., 423
Cole, R. D., 171
Coleman, J. E., 283
Coleman, M. S., 73
Coleman, P., 181
Coles, C. J., 187, 281
Coletta, F., 19
Coligan, J. E., 140
Colle, A., 142
Colletti, C., 187
Colley, P. W., 418
Collier, G. E., 187
Collier, R. J., 130, 161
Collins, J. H., 43, 110, 361
Collins, T. C., 256
Collman, J. P., 320
Colman, R. F., 167, 173, 175
Colman, R. W., 83
Colomb, M. G., 60, 81
Colombo, R., 405
Colton, D. G., 519
Columelli, S., 67
Comings, D. E., 54, 165
Commerene, D., 13
Commoy-Chevalier, M. J., 71, 77
Compans, R. W., 137
Conboy, J. G., 104
Conde, F. P., 187
Condon, M. E., 556
Congote, L. F., 61
Conlon, J. M., 510
Conn, K. M., 291
Connell, G. E., 144
Connelly, J. A., 47
Connolly, B. A., 167, 171
Connor, W. M., 322
Conover, W. W., 290
Consiglio, E., 189
Conti, F., 424
Conway, R., 406
Conway, T. T., 448
Cook, G. C., 515
Cook, G. M. W., 65
Cook, J. C., jun., 452
Cook, K. H., 268
Cook, R. A., 47
Cooke, R., 53
Cookingham, R. E., 296
Coon, M. J., 49
Cooper, B. R., 577
Cooper, C. W., 518
Cooper, M., 17
Cooper, M. F., 474
Cooper, R. D. G., 445
Cooperman, B. S., 185
Copeland, T. D., 137
Copland, G. T., 99
Copp, D. H., 539
Coppes, R. I., 51
Corazziari, E., 525
Corbett, D. F., 444
Cordopatis, P., 403, 407, 456, 555
Corfield, A. P., 41
Corgier, M., 61
Corlström, K., 55
Cormier, M. J., 97, 358
Cornelis, A., 280
Cornell, D. G., 305
Corongin, G., 244, 256
Correa, F. M. A., 521
Cort, J. H., 492
Cosani, A., 276
Coscia, C. J., 49
Cosmatos, A., 405, 502, 503
Cossart, P., 122
Cossement, E., 447
Cosson, J. J., 157, 175
Costantino, P., 391
Costenbader, C. L., 514, 523
Coté, J., 512
Cote, T. E., 167
Cotrait, M., 19, 421
Cottam, L. G., 363
Cottin, P., 87
Cotton, R. J., 22
Cottreau, D., 49
Cottrell, B. A., 137, 138
Coughlan, M. P., 191
Courseille, C., 18
Cowburn, D., 277, 388
Cowley, A. W., jun., 487
Cox, B. M., 564, 577
Cox, L. A.-M., 77

Cox, M. G., 436
Cox, T. T., 171
Coy, D. H., 372, 465, 466, 469, 512, 533, 560, 573
Coyle, S., 369, 401
Cozzone, A. J., 102
Cozzone, P. J., 285
Crain, B., 579
Crain, P. F., 4
Crain, S. M., 579
Cram, D. J., 18
Cramer, L. E., 345
Crane-Robinson, C., 280
Crang, A. J., 357
Crapster, B. B., 200
Crass, M. F., tert., 548
Crastes de Paulet, A., 165
Craven, G. R., 187
Crawford, I. P., 127
Crawford, J. L., 249
Crawford, J. S., 16
Crawford, L. V., 189
Crawford, N., 53
Crawhall, J. C., 31
Crea, R., 481, 499
Credo, R. B., 83
Creese, I., 559
Creighton, T. E., 101, 266, 267, 268, 273
Cremonesi, P., 41, 335
Crepeau, R. H., 227, 252, 341
Crespi, H. L., 251
Cresswell, P., 66
Crettol-Järvinen, A., 109, 339
Creutzfeldt, C., 518
Creutzfeldt, W., 518, 524, 525, 533, 534, 538
Crevat, A., 30, 290
Crewe, A. U., 332
Crews, D., 482
Crine, P., 476, 559
Crippen, G. M., 257, 258, 260
Crisba, J., 276
Croitoru, I., 527
Cronan, J. E., 51, 66
Cronin, J. R., 35, 111
Crooke, S. T., 147
Crookston, J. H., 340
Cros, F., 110
Cross, C. E., 136
Cross, T. A., 291
Crossley, L. G., 173
Crothers, D. M., 289
Crotti, P., 448
Crowther, R. A., 249
Crumpton, M. J., 270
Cruse, W. B. T., 253
Csopak, H., 283
Cuatrecasas, P., 272, 404, 505, 559, 576, 577, 578
Cuendet, P. A., 296
Cueni, L. B., 87
Cummings, R. D., 51
Cummins, P., 250
Cung, M. T., 18
Cunningham, B. A., 91, 134, 140
Cunningham, L. W., 159
Cunningham, R. P., 73
Curman, B., 141
Curphey, T. J., 369
Curran, W. V., 449
Curran-Patel, S., 309
Curti, B., 165
Curtius, H. C., 31
Cusan, L., 465, 573
Cushley, R. J., 282
Cushman, D. W., 552, 556
Cutfield, J. F., 240, 500
Cutfield, S. M., 240, 394, 500, 501
Cutz, E., 531
Cuypers, P., 19
Cybulsky, D. L., 159
Cysyk, K. L., 32
Czarniecki, S., 299
Czech, M. P., 197
Czerwinski, E. W., 228

Dabrowiak, J. C., 460
Daddona, P. E., 153
Daehne, S., 345
Däumigen, M., 260, 266, 407
D'Agnolo, G., 161
Dagorn, J. C., 523, 527
D'Agostino, R., 543
Dahl, K. H., 155
Dahl, O., 38
Dahlquist, F. W., 197
Dahlquist-Edberg, U., 123
Dahlstroem, A., 529, 530
Dahmus, M. E., 64
Dailey, H. A., 163
Dajani, E. Z., 519
D'Alagui, M., 409
D'Albis, A., 197
Dale, B. A., 105
Dale, J., 430
Dale, R. E., 347, 349
D'Alessio, G., 126, 307
Dallas, J. L., 284, 460
D'Allesio, E., 185
Dalton, H., 49
Dalton, J., 358
Daly, M. V., 200
Dalziel, K., 53, 169
Damaille, J. G., 289
Damassa, D. A., 524
Dan, P., 119
Dancewicz, A. M., 30
Danenberg, K. D., 189
Danenberg, P. V., 97, 189
Dangworth, G., 23
Danho, W., 412, 498
Daniel, E., 333, 344
Daniel, M., 133
Danielsson, A., 523
Danishefsky, S., 8
Dao, M. L., 45
D'Arcy, J. B., 31
Dardenne, G., 3
Darling, D., 23
Darnall, D. W., 363
Darnell, J. H., 492
Daron, H. H., 169
Dars, F. F., 181
Das, B. C., 407, 425, 436, 440
Das, M., 197
Das, R. H., 73
Das, T. P., 322
das Gupta, A., 75
das Gupta, C., 73
das Gupta, H., 87
Dash, R. J., 519
Dauber, P., 255
Daufau, M. L., 499
Daures, M. F., 289
Daus, H., 39
Daussant, J., 102
Davankov, V. A., 17, 24, 25
Daves, G. D., 61
Davey, J., 271
Davidovich, M. A., 350
Davidovitch, Y. A., 378
David-Pfeuty, T., 342
Davidson, B. E., 122, 173
Davidson, J. M., 136
Davidson, J. N., 60
Davidson, W. S., 155
Davie, E. W., 81, 137, 138, 139, 148
Davie, J. M., 140
Davies, D., 98
Davies, J. S., 433
Davis, A., 263
Davis, A. E., tert., 81, 139
Davis, D. G., 280, 434
Davis, D. J., 181
Davis, F. F., 157, 189
Davis, L. C., 329, 331
Davis, M. A., 14
Davison, B. L., 75
Davril, M., 369
Davydov, R. M., 317, 318
Dawson, B. F., 545
Dawson, J. D., 318
Day, A. R., 276, 278, 369, 403, 404, 551, 575
Day, J. F., 155
Dayer, J. M., 547
Dazai, S., 400
De, A. U., 22
De Abreu, R. A., 183, 339
Deal, W. C., 98, 331
Dean, J., 272
Dean, K. J., 62
Dean, P. D. G., 43, 64
Dearborn, D. G., 193, 283
Dearborn, D. J., 274
Deatherage, J. F., 225, 226
Deb, K. K., 277, 314, 496
Debas, H. T., 514, 535
De Benedetti, E., 161
Deber, C. M., 404, 430

De Bianchi, A. G., 189
De Bony, J., 288
Debrunner, P. G., 286, 321, 323, 324
Debruyne, I., 95
De Castiglione, R., 519
Dechter, J. J., 275
De Clercq, E., 61, 71, 77, 419
Declerq, J. P., 219, 440
Deconinck, M., 394
De Corpo, J. J., 22
Dedkova, V., 29
Deeley, M., 127
Deetz, J. S., 29
Deffieux, G., 418
Deftos, L. J., 542, 543, 545
Degani, C., 163
Degani, Y., 163
de Gasparo, M., 397, 398, 501
Degelaen, J., 277, 284
De Graaf, F. K., 146
De Graaf, J. M., 116, 136
De Graaf-Hess, A. C., 361
De Haan, R. L., 500
De Haas, G. H., 125, 181, 283
De Haën, C., 511
Deibel, M. R., 73
de Jersey, J., 30, 337
De Jong, R. J., 242
De Kok, A., 183, 339, 361
De Kruijff, B., 288
Delacourte, A., 342
Delaney, R., 45
De Lange, R. J., 129, 130, 171
Delanoy, R. L., 498
Delannoy, J.-P., 55
De Laveleye, F., 447
Delbaere, L. T. J., 233, 234, 248, 260
Delcambe, L., 425
De Léan, A., 512
Deleers, M., 438
Delepelaire, P., 100
De Ley, J., 133
Dell, A., 122
Dell, H. D., 35
Della Bella, D., 560
Dellacherie, E., 63
Della-Fera, M. A., 523
Del Mar, E. G., 404
Delmotte, F., 356
de Loze, C., 292
Del Pra, A., 18, 254, 409
Del Salvatore, B., 519
De Luca, M., 49
De Magistris, L., 512
Demaille, J. G., 97, 125, 183
De Marco, A., 279, 424
Dembinski, A. B., 515
De Meyts, P., 500
Demling, L., 515
Demol, P., 527, 538
Dempsey, A., 24
De Neef, P., 531
Denisov, G. S., 21, 291
Denning, J. W., 347
Dennis, E. A., 283
De Pierre, J. W., 169
De Pont, J. J. H. H. M., 159, 521, 527
Depreter, J., 394
Deranleau, D. A., 511
Derechin, M., 364
de Recondo, A.-M., 73
Derkx, F. H. M., 552
Dermody, W. C., 406
De Ropp, J. S., 287
Derricott, C., 18
Derrien, M., 407, 462
Dershwitz, M., 287
Derugin, N., 527
Desai, N. N., 91
De Santis, P., 391, 436
Deschodt-Lanckman, M., 531
Deschrijver, P., 277
Descomps, B., 165
Descotes, G., 6
Desgres, J., 31
Deshmane, S. S., 368, 409, 529
Desjardins, C., 482
Deslauriers, R., 267, 275, 278, 293, 410, 435
De Smet, M.-J., 167, 280
Desnick, R. J., 55
Desruisseaux, H., 126
Dessen, P., 330
Destro, R., 222
Desvigne, A., 515
De Tar, D. F., 20, 275
Determann, H., 20
De Titta, G. T., 253
de Traglia, M. C., 111, 194
Detrich, H. W., 342
Dettmar, P. W., 464
Dettwiler, M., 57
Devadas, B., 378
Devaney, P. W., 321
Deveney, C. W., 521
Devillers-Thiery, A., 126, 144
Devitt, P., 533
De Voort, F. R., 327
de Vries, A. C. J., 98
De Vries, J., 183, 339
Devynck, M. A., 552
Dewes, H., 135
de Wied, D., 472, 498, 561, 579
De Wit, J. L., 291
de Wulf, H., 497
Deziel, M. R., 169
Dhaese, P., 136
Dhaliwal, H. S., 177
d'Herbigny, E., 543
Dhundale, A. R., 388, 437
Diaconescu, C., 498
Diarra, B., 5
Diaz, J., 399, 468
Di Blasi, R., 275
Di Blasio, B., 19, 253
Dickinson, K. H., 445
Dickoff, W. W., 473
Dickson, D. P. E., 323, 324
Dickson, M. R., 253, 334
Di Corleto, P. E., 57
Di Donato, A., 126
Diem, M., 21, 299, 300
Diem, P., 500
Dietz, R., 556
Dietze, F., 527
Dijkman, R., 181
Dill, K., 288
DiMagno, E. P., 515
Di Maio, J., 563
Dimaline, R., 520, 530, 532
Di Marchi, R. D., 179, 394
Dimeli, A., 407
Dimicoli, J. L., 163, 165, 284
Dimitriadis, G. J., 102
Dimitrijenic, L., 55
Dininger, N. B., 533
Di Ninno, F., 447
Diopoh, J., 198
Di Pascale, G., 289
Dipasquale, A., 473
Di Pietro, A., 157
Dirks, G., 20
Dirkx, J., 277, 403, 555
DiScipio, R. G., 81, 139
Dissing, J., 38
Distler, J. J., 93
Divor, A. R., 479
Dixit, S. N., 135
Dixon, G. H., 147
Dixon, J. E., 57, 183, 478
Djerassi, C., 317
Djondjurov, L., 101
Dlouha, M., 221
Do, K. Q., 7, 396, 562
Doan, N., 321
Dobashi, A., 35, 392
Dobbs, R. E., 510
Dobolyi, Z., 575
Dobryszyeka, W., 169, 315
Dobson, C. M., 16, 273, 289
Dockray, G. J., 401, 513, 517, 518, 520, 521, 522, 530, 532
Dockter, M. E., 177
Dodd, G., 191
Dodd, J., 524, 532
Dodgson, K. S., 171
Dodkin, S. M., 307
Dodson, E. J., 240, 428, 499, 500
Dodson, G. C., 394
Dodson, G. G., 240, 499, 500, 501
Dodvik, H., 362
Doebber, T. W., 479
Doerr-Schott, J., 519
Dohme, F., 187
Dohnal, J. C., 100
Doi, Y., 391
Dolhofer, R., 504
Dolinnyi, A. I., 305
Dolly, J. O., 151
Dolva, L. O., 519

Domazetis, G., 292
Dombradi, G. B., 169
Domeier, L. A., 18
Domiano, P., 448
Domschke, S., 515, 531
Domschke, W., 515, 531, 535
Doneen, B. A., 181, 394, 481
Donelson, J. E., 75
Dooijewaard, G., 57, 282
Dooley, D. M., 318
Doolittle, R. F., 137, 138
Doonan, S., 121, 157
Dopheide, T. A. A., 136, 137
Dorman, D. E., 447
Dornand, J., 41
Dorrington, K. J., 145
Dorsey, C. C., 346
Dose, K., 81, 201
Doskocilova, D., 276
Dossena, A., 371
Doubleday, A., 223
Doumeng, C., 87
Douzou, P., 42
Dowben, R. M., 345
Dowd. P., 10
Dowd, S. R., 401
Dowdall, M. J., 189
Dower, S. K., 290
Dowrer, N. W., 163
Doyen, N., 111, 192
Doyle, D., 101
Doyle, T. W., 448
Drabarek, S., 16
Drabble, W. T., 38
Drach, G., 153
Drahota, Z., 175
Drakenberg, T., 289
Draper, M. W., 471
Drapier, A. M., 34
Drauz, K., 5
Drazin, R. E., 130
Dreiling, D. A., 515
Drent, G., 241
Drenth, J., 242
Drescher, D. G., 35, 111
Drewes, S. E., 173, 405, 501
Dreyfus, M., 364
Driskili, D. R., 519
Drivas, G., 519
Drobnica, L., 28, 169
Dronova, L. A., 308
Drovin, J. N., 403, 549
Dryburgh, J. R., 533, 535
Dubick, M., 136
Dubochet, J., 249, 252
DuBois, G. C., 123
Dubrasquet, M., 518
Dubs, A., 262, 279
Dubz, A., 268
Duc, J. A., 334
Ducastaing, A., 87
Duckworth, H. W., 161
Dudich, E. I., 361
Dudkiewicz, J., 358
Dudkin, S. M., 126
Duffield, A. M., 21, 32
Dufourcq, J., 288, 356
Dugas, H., 187
Dugat, D., 444
Dulaney, J. T., 58
Dunai-Kovács, Z., 579
Dunford, H. B., 171, 311
Dunkley, P. R., 62
Dunlap, R. B., 44, 163, 189
Dunn, A. J., 498
Dunn, B. M., 42
Dunn, J. T., 480
Dunnick, W., 61
Duplaa, A.-M., 81
Dupont, A., 512, 573
Dupont, C., 528, 531, 538
Dupre, D. B., 350
Duquesne, M., 352
Durchschlag, H., 98, 326
Durden, D. A., 33
Durette, P. L., 407, 462
Durham, N. N., 437
Durieu, O., 153
Durocher, G., 351
Durrant, J. L., 49
Durst, T.. 444
Dutta, A. S., 406
Duve, H., 505
du Vigneaud, V., 485
Dwek, R. A., 179, 274, 290
Dwivedi, A., 321, 324
Dwulet, F. E., 133
Dyckes, D. F., 191, 407
Dyer, P. Y., 334
Dykes, G. W., 227, 341
Dzau, V. J., 89
Dzieduszycka, M., 381

Eaker, D., 130, 189
Earnshaw, W. C., 249
Eastabrook, G., 134
Easter, B. R. D., 173, 405, 501
Easterby, J. S., 169
Easwaran, K. R. K., 22, 274, 434
Eaton, W. A., 340
Ebata, M., 457
Ebeid, A. M., 531
Ebel, R. E., 296
Eberle, A. N., 13, 396, 397, 473, 562, 570
Eberspaecher, J., 2
Ebert, R., 533
Ebisawa, H., 8
Ebner, K. E., 147, 181
Ebrey, T. G., 357
Eckel, R. H., 535
Eckelman, W. C., 29
Eckert, H., 369
Eckhardt, G., 441
Eckstein, F., 201
Edalji, R., 341
Eddeland, A., 527
Edelhoch, H., 510
Edelman, G. M., 134, 140
Edelman, I., 578
Edelstein, M. S., 358
Edelstein, S. J., 227, 252, 341
Ederle, A., 522
Edgren, R. E., 406
Edholm, L. E., 34
Edin, R., 529, 530
Edmonds, J. W., 253
Edmondson, D. E., 187, 281
Edmundson, A. B., 269
Edsall, J. T., 98
Edvinsson, L., 533
Edward, J. T., 22
Edwards, A. V., 531, 533
Edwards, R. A., 44
Edy, V. G., 61
Effenberger, F., 5
Efimov, A. V., 246, 258, 284
Efros, M. B., 404
Egami, F., 8
Egan, W., 287
Egberts, E. H., 522
Eggleston, C., 543
Eggleston, J. C., 530
Eglington, A. J., 444
Egmond, M. R., 288
Egorov, A. M., 167
Egorov, I. A., 24
Ehnholm, C., 97
Ehrenberg, B., 357
Ehrenberg, L., 28
Ehrhardt, M. E., 134
Eibl, H., 251
Eichberg, J., 98
Einarson, B., 105
Einarsson, R., 280
Eipper, B. A., 128, 475, 476, 543, 559
Eisenach, J. C., 151
Eisenberg, E., 355
Eisenberg, S., 75
Eisenfeld, J., 347
Eisenman, R. N., 110
Eisler, K., 397, 398, 501
Ek, K., 99
Ekisola, O. B., 169
Ekizyan, N. G., 306
Eklund, H., 44, 238, 239
Eklund, S., 533
Ekman, P., 123
El-Abadelah, M. M., 21
Elahi, D., 535
ElAmin, B., 383
Elashoff, J., 518
El-Bayoumi, M. A., 337, 362
Elder, K., 136
Eley, M. H., 101, 338
Elfinger, M. J., 155
Elgar, G. J., 271, 279
Elgavish, G. A., 171, 290
Elgin, S. C. R., 129
Elicio, N., 515
Ellestad, G. A., 461
Ellfolk, N., 310
Elliot, B., 169

Elliott, B. M., 320
Elliott, J. I., 165
Ellis, D., 447
Ellis, E., 479
Elmaleh, D. R., 17
Eloranta, T., 97
Elsaas, L. J., sec., 500
Elsayed, S., 270, 273
Elson, S. W., 444
Elsworth, J. F., 439
Elwell, M., 49
Elzinga, M., 130, 179
Ema, M., 10
Emdin, S. F., 240, 500
Emeis, J. J., 83
Emmerson, P. T., 149
Emmett, J. C., 374
Emptage, M. H., 323
Emson, P. C., 529, 532
Emson, P. M., 515
Emura, J., 393, 500
Endean, R., 127, 558
Endermann, R., 177
Endo, M., 45
Endo, T., 391, 458, 526
Endo, Y., 167
Endoh, M., 250
Eneroth, P., 515
Enfield, D. L., 87, 137
Enfield, D. N., 122
Enfinger, R. R., 157
Engel, A., 252
Engel, J., 334
Engel, P. C., 193
Engel, R. G., 362
Engel, S. L., 520
Engelhard, M., 387
Engelman, D. M., 236, 264
Engeser, H., 123
England, R. D., 133
Englander, S. W., 341
Englert, A., 348
English, A. R., 446
English, M. L., 455
Englund, P. T., 75
Engstrom, G. W., 440
Engström, L., 123
Engström, Y., 75
Enns, C. A., 157
Ensinck, J. W., 514
Enzmann, F., 499
Epand, R. M., 167
Epelbaum, J., 529, 533
Epp, O., 241
Epstein, D. J., 151
Epstein, M., 557
Epton, R., 386
Erickson, B. W., 388
Erickson, J. S., 77
Ericsson, L. H., 124, 137
Erikson, R. C., 448
Eriksson, B., 220
Eriksson, S., 75
Eriquez, L. A., 449
Erlich, H. A., 103
Ermak, N. M., 399
Ernest, I., 446
Ernst, R. R., 273, 274
Ernster, L., 169
Erspamer, G. F., 558
Erspamer, V., 127, 558
Ersson, B., 220
Escher, E., 403
Escher, E. H. F., 555
Eshdat, Y., 192
Espelie, K., 136
Esser, A. F., 139
Estess, P., 140
Estival, A., 523
Eto, S., 518
Eto, T., 175
Eugene, E. H. J. M., 283
Eulitz, M., 140
Evangelopolous, A. E., 272
Evans, C. S., 2
Evans, D. L., 458
Evans, D. R., 157
Evans, G., 35, 54
Evans, J., 69
Evans, J. C., 507
Evans, J. R., 444
Evans, P. R., 125, 237, 264
Evans, R. F., 30, 352
Evans, W. H., 62
Evdakov, V. P., 288
Everitt, M. T., 87, 124
Evshigneeva, R. P., 404, 405
Ewenstein, B. M., 140
Eyzaguirre, J., 185

Face, M., 183
Fagard, R., 556
Fahien, L. A., 63, 167
Fahnestock, S. R., 187
Fahrenkrug, J., 526, 527, 528, 529, 530, 531, 532, 533
Fahy, C., 106
Failli, A., 404, 434
Faini, G. J., 347
Fairwell, T., 116, 543
Faith, M. R., 482
Faivre, M., 515
Falasca, A., 91
Falasco, J. D., 523
Falk, K., 285
Falk, K. E., 286
Falk, P., 247, 260
Falkenberg, P., 131
Falkmer, S., 500
Familletti, P. C., 61
Fan, C. C., 362
Fan, D. P., 87
Fang, W.-F., 523
Fanning, T. G., 187
Fara, J. W., 524
Farley, J. R., 159
Farmer, S. W., 474, 482
Farooqui, A. A., 59, 91
Farorova, O. O., 191
Farrell, H. M., 101
Farrell, P. G., 22
Farver, O., 311
Fasco, M. J., 272
Fasiolo, F., 181
Fasman, G. D., 257, 258, 292, 315
Faszholz, S., 22
Fatánia, H. R., 53
Fauchard, C., 399, 468
Fauchère, J. L., 13, 397, 471, 570
Faucon, J. F., 356
Faulstich, H., 426, 427
Fauque, G., 133
Favilla, R., 30, 351
Favora, O. O., 271
Favre, L., 556
Fearon, D. T., 70
Fedeli, W., 411
Fedorov, A. A., 234
Fedoryak, D. M., 405
Fedotov, V. P., 399, 405
Feeney, J., 274, 281, 284, 438
Feeney, R. E., 4, 26, 179, 195
Feher, G., 177
Fehler, J. R., 461
Fehlner, J. R., 4, 460
Fehske, K. J., 155
Feigina, M. Y., 144
Feitelson, J., 354
Felber, J. P., 538
Feldman, I., 354
Feldmann, K., 282, 285
Feller, W. F., 73
Fellion, E., 564
Fellows, L., 3
Felt, V., 31
Fendler, J. H., 18, 349
Fenichel, R. L., 469
Fenn, M. D., 366
Fenna, R. E., 242
Fennessy, M. R., 562
Fenwick, G. R., 3
Fenyvesi, E., 36
Ferber, E., 65
Ferderigos, A., 405, 502
Ferderigos, N., 405, 502, 503
Ferger, M. F., 485
Ferguson, W. S., 171
Ferguson-Miller, S., 163
Ferland, L., 515, 573
Fermandjian, S., 275, 279, 300, 314, 421, 430, 456, 470, 551, 552
Fernandez-Cruz, A., 465
Ferrara, L., 290
Ferrara, P., 404, 564, 576
Ferrari, C., 27
Ferraz, C., 183
Ferreira, C., 189
Ferreira, I. C., 350
Ferreira de Castro, E., 518
Ferri, S., 542
Ferris, J. P., 8
Ferrone, S., 142

Fessas, P., 177
Fetterroll, B. M., 33
Feurle, G. E., 513
Fex, G., 329
Fichot, O., 73
Ficht, T. A., 75
Fiddler, M. B., 58
Fiedler, F., 87, 461
Fiedler, J., 35
Field, F. H., 21
Field, S., 533
Fiete, D., 58
Fietzek, P. P., 135
Figarella, C., 87, 527
Fiksinski, K., 358
Filatov, A. V., 274, 290
Filatova, M. P., 403
Filenkova, N. V., 404
Filimonov, V. V., 265
Filippi, B., 306, 308, 396, 400, 525, 576
Filippovich, E. I., 405
Filleau, M. J., 418, 419
Findeisen, A., 349
Findlay, J. B. C., 177, 185, 187
Fine, J. M., 99
Fini, C., 34
Fink, G. R., 47
Finke, U., 533
Finkelstein, A. V., 247, 260
Finlay, J. W., 28
Finlayson, A. J., 28
Finn, F. M., 472
Finney, J. L., 244, 256
Finnie, M. D. A., 559
Finnigan, T., 579
Firmin, J. L., 3
Fischer, A. G., 183
Fischer, E. H., 183
Fischer, E. R., 125
Fischer, G. H., 410
Fischer, J. E., 530, 531
Fischer, R. S., 437
Fischetti, V. A., 148
Fischman, A. J., 277, 496
Fish, W. W., 137
Fishback, J. L., 71
Fisher, G. H., 557
Fisher, J., 443
Fisher, L. M., 73
Fisher, R. R., 183
Fisher, S. E., 49
Fisher, W. K., 119, 133
Fitt, P. S., 61
Fitton, J. E., 122
Fitzgerald, P. M. D., 226
Fitzpatrick, M. L., 536
Fjellstroem, K. E., 519
Flack, H. D., 219
Flanagen, M. T., 347
Flanigan, K., 264, 361
Flastner, M. S., 159
Flegel, M., 409
Fleischhauer, J., 258, 300
Fleischer, S., 281
Fleming, G. R., 345, 350, 362
Fleming, P. J., 355
Flengsrud, R., 81
Fless, G. M., 84
Fletcher, A. R., 28, 373
Fletcher, D. J., 513
Fletterick, R. J., 235, 245, 258
Fletton, R. A., 444
Flexner, L. B., 498, 579
Flink, I. L., 179
Flinois, J. P., 67
Flippen-Anderson, J. L., 433
Fliss, H., 192
Floridi, A., 34
Floss, H. G., 420
Flouret, G., 407
Flynn, T. G., 155
Foda van der Hoorn, M. M. E. L., 181
Fodor, E., 522
Föhles, J., 498
Foekens, J. A., 66
Foelsch, U. R., 524
Förg-Brey, B., 87
Förster, L. S., 351, 353
Fog, R., 579
Foglio, M., 445
Fohlman, J., 112, 130, 141, 189
Fok, K.-F., 465
Foley, K. M., 579
Folkers, K., 383, 400, 406, 408, 466, 557
Folkersen, J., 102
Folkhard, W., 252
Foltmann, B., 112, 122
Fonda, M. L., 167
Fondy, T. P., 44
Fong, B. T. W., 569
Fonina, L. A., 437
Fontana, A., 185, 265, 273, 307
Foote, C. S., 29
Forand, R., 106
Forcina, B. G., 123
Ford, C. C., 347
Forde, M. D., 33
Fordyce, A. M., 125
Forell, M. M., 523, 524
Forgac, M. D., 279
Forlani, L., 336
Formisano, S., 189, 510
Forrest, H. S., 253
Forrest, J. A. H., 519
Forsen, S., 274, 285, 286–288
Forsgren, B., 55
Forsham, A., 181, 270
Forssmann, W. G., 535
Forsyth, J. M., 220
Fossel, E. T., 280
Foster, J. A., 4
Fothergill, J. E., 81, 139
Fothergill, L. A., 185
Fonad, H., 19
Foucault, A., 561
Fougereau, M., 121, 142
Fournier, C., 289
Fournié-Zaloski, M.-C., 561, 564
Fowden, L., 1
Fowler, A. G., 248
Fox, B. L., 392
Fox, C. F., 197, 201, 578
Fox, I. H., 49
Fox, J., 314
Fox, J. L., 381
Fox, J. W., 130, 179
Fox, T. O., 66
Frackowiak, D., 358
Francavilla, A., 519
Francerchi, C., 91
Franceschi, G., 443, 445, 447
Francke, W., 32
Francois, C., 59
Franek, F., 173, 361
Frangione, B., 137
Frank, G., 111, 116, 245, 263
Frank, H., 32, 392
Frankel, R. B., 323
Frankel, R. D., 220
Franklin, E. C., 137
Franks, N. P., 251
Franz, H., 272
Frappier, F., 439, 442
Fraser, C. M., 65
Fraser, M., 556
Fraser, R. D. B., 253, 254
Frauenfelder, G. A., 264
Frauenfelder, H., 225, 261
Frazier, W. A., 91, 155, 177
Frederich, R. C., 66
Freed, J. H., 41
Freed, W. J., 542
Freedlander, A. E., 552
Freedman, J. A., 338
Freedman, R., 268
Freeman, A., 40
Freeman, I. L., 33, 111
Freer, R. J., 276, 278, 369, 403, 404, 503, 551, 575
Freer, S. T., 241
Freese, E., 55, 57
Frei, J., 34
Freidinger, R. M., 410, 434, 455
Freimuth, U., 173
Freist, W., 173, 183
Freitheim, K., 179
French, J. K., 169
French, J. S., 49
Frère, J. M., 284, 449
Frerichs, H., 534
Fresco, J. R., 109
Fresen, H. G., 69
Frey, M., 133, 228
Frey, M. H., 291
Frey, P., 106
Frey, P. A., 191
Freytag, J. W., 100
Fric, I., 299, 303, 422, 492
Fridkin, M., 295, 367, 382
Fridlandsky, F., 150
Fridovich, I., 57, 187
Friedel, K., 403
Friedman, A. M., 17
Friedman, C. I., 529

Friedman, F. K., 340
Friedman, M., 33
Friedman, M. E., 169
Friedrich, A., 275, 430, 431
Friedrich, P., 169
Friesen, H. J., 498
Frigeni, V., 560
Frigeri, L. G., 480
Fritsch, H. A. R., 543
Fritsch, W. P., 514, 525
Fritz, H., 87
Froehlich, J. A., 84
Froehlich, P. M., 351
Froehner, S. C., 67
Frolov, E. H., 325
Fromageot, P., 275, 430, 518
Fronticelli, C., 264, 361
Froyssinet, J. M., 360
Fruton, J. S., 219
Fryzuk, M. D., 6
Fuchs, S., 314
Fuchsman, W. H., 298
Fuenles, J. J., 14
Fuess, H., 275, 430
Fuganti, C., 17
Fuganti, G., 417
Fugate, R. D., 344
Fuhrman, J., 142
Fujii, A., 458, 459, 460
Fujii, N., 29, 373, 375, 382
Fujii, S., 279
Fujii-Kuriyama, Y., 67
Fujimoto, S., 29
Fujimoto, W. Y., 510, 514, 535
Fujino, M., 470, 511, 535
Fujioka, M., 187
Fujisawa, H., 53
Fujisawa, Y., 449
Fujishiro, K., 89
Fujita, T., 453, 523, 542
Fugita, Y., 47, 55, 57
Fujiwara, K., 284
Fujiyama, T., 450
Fukada, Y., 191
Fukae, K., 231
Fukagawa, Y., 440
Fukano, K., 61
Fukatsu, S., 6
Fukazawa, H., 175
Fukuchi, M., 526
Fukuda, H., 378
Fukuda, T., 93
Fukuhara, K., 450
Fukui, S., 119, 149, 167, 191, 451
Fukunaga, M., 179
Fukushima, T., 518
Fukutome, A., 303
Fukuyama, K., 229
Fukuyama, M., 95
Fuller, B. B., 406, 473
Fuller, S. D., 252
Fuller, W., 249
Fullmer, C. S., 108, 289
Fulton, J. A., 61
Funakoshi, S., 29, 373, 375, 382
Funatsu, G., 187
Funck-Bretano, J. L., 543
Fung, C. H., 288
Furey, W., jun., 222, 223
Furie, B., 270
Furie, B. C., 270
Furlan, M., 103
Furnelle, J., 514, 524
Furr, B. J. A., 406
Furst, P., 32
Furthmayr, H., 161
Furusaki, A., 19
Fushiya, S., 3
Futterman, S., 357
Fuwa, K., 284
Fuxe, K., 515

Gaastra, W., 146
Gabbay, K. H., 169
Gabel, D., 100
Gabellec, M.-M., 55
Gacel, G., 561, 564
Gadasi, H., 43, 110, 272
Gadenne, M. C., 298
Gadret, M., 418
Gaeta, F. C. A., 18
Gafni, A., 171, 290, 355, 357, 361, 363
Gagelmann, M., 42
Gaginella, T. S., 533
Gagnon, J., 136
Gainey, P. C., 77
Gait, M. J., 385, 500, 558
Gaitonde, M. K., 35
Galaev, I. J., 25
Galaktionov, S. G., 256, 496
Galand, C., 49
Galan Galan, F., 543
Galardy, R. E., 278, 555, 556
Galbo, H., 530
Galitskii, N. M., 253, 437
Galley, W. C., 344, 356
Galliani, C., 447
Gallo, A. B., 482
Gallop, P. M., 35, 169
Gally, H. U., 252
Galpin, I. J., 371, 376, 401
Galtier, D., 399
Gambaro, A., 19
Ganapathy, S., 20
Ganda, O. P., 534
Gandy, W. E., 35, 111
Gangarosa, M. A., 61
Gani, V., 183
Ganoza, M. C., 77
Ganten, D., 515, 556
Gantt, E., 358
Garaud, J. C., 519
Garbers, D. L., 53
Garcia-Pardo, A., 137, 143
Gard, D. L., 109
Gard, F. R. N., 394
Gardiner, K. J., 336
Gardner, D. G., 514
Gardner, J. D., 357, 513, 514, 523, 528, 531
Gardner, J. E., 322
Gardner, W. S., 34
Garel, M. C., 340
Garibov, R. E., 320
Garish, M., 179
Garland, F., 335, 363
Garland, P. B., 196
Garlick, R. L., 132
Garmin, J. L., 54
Garnak, M., 47
Garner, C. D., 323
Garner, J., 181
Garner, W. H., 179, 394
Garrell, R. L., 341
Garrick, L., 169
Garrigou-Lagrange, C., 21
Garsky, V. M., 469
Garver, F. A., 61
Garvin, J. E., 100
Garzillo, A. M., 121
Gaspar, V., 8
Gasparič, J., 107
Gasparov, V. S., 287
Gasson, J., 478
Gates, F. T., tert., 140
Gates, R. E., 245, 258
Gatley, S. J., 16, 36
Gattegno, D., 424
Gatti, G., 17, 417
Gattier, D., 468
Gattner, H.-G., 498
Gaud, H. T., 337
Gaudin, C., 30, 290
Gaudreau, P., 403, 549
Gaudry, M., 12
Gauldie, J., 519
Gautheron, D. C., 65, 157, 357
Gavras, H., 556
Gavras, I., 556
Gawley, R. E., 9, 413
Gayda, J. P., 323
Gaymard, F., 11
Geahlen, R. L., 191, 201, 343
Gebauer, G., 91
Gee, A. P., 79
Gee, R., 47
Gefter, M. L., 73
Gehrke, C. W., 31
Geiduschek, E. P., 339
Geiger, R., 87, 138, 471, 473, 499, 525
Geisler, N., 290
Geissler, A. W., 131
Gelder, F. B., 83
Gelin, B. R., 245, 255
Gelin, M. L., 515
Geller, L. I., 525
Gellert, M., 73
Gelman, R. A., 330
Gelpi, E., 31
Gengenbach, B. G., 47
Gennis, R. B., 362
Gentinetta, R., 151
Gentleman, S. B., 560
Geoghegan, K. F., 26, 195
Geokas, M. C., 404

George, A. L., jun., 165
George, S., 179
Gerard, D., 348, 355
Gerard, K. W., 179
Gerard, S., 62
Gerber, G. E., 120, 144
Gerber, L. D., 61, 560
Gerday, C., 289
Gergely, J., 201
Gergely, P., 53
Gerhardt, J., 3
Gerhardt, K. O., 31
Gerich, J., 512
Gerig, J. T., 284
Gerken, T. A., 193, 283
Gerlo, E., 157
Germain, G., 219, 440
Germeiner, P., 169
Gerner, T., 524
Gershkovich, A. A., 376
Gershon, H., 403
Gertler, A., 183
Gervais, C., 65
Gespach, C., 526, 534
Gething, M. J., 136
Getoff, N., 352
Gevorkyan, N. M., 309
Geynet, P., 38
Ghai, G., 65
Ghatei, M. A., 127, 515, 517, 536
Gheorghiu, Z., 221
Ghiringhelli, D., 17
Ghiron, C. A., 30, 191
Ghisla, S., 357
Ghose, A. K., 22
Ghosh, A. C., 119, 130, 423
Ghosh, C., 191
Ghosh, N., 187
Ghuysen, J.-M., 284, 449
Giachetti, A., 530, 531
Gianazza, E., 99
Gianoulakis C., 559
Gibbons, I., 123
Gibbons, J. J., 22
Gibbons, W. A., 264, 278, 279, 280
Gibbs, J., 523
Gibson, D., 132
Gibson, D. R., 101
Gibson, D. T., 41
Gibson, Q. H., 337
Gibson, R. G., 519
Gidai, K., 554
Gielen, H.,-G., 399
Giesbrecht, P., 462
Giesen, M., 131
Gil-Av, E., 17, 392
Gilbert, H. J., 38
Gilbert, J.-P., 411
Gilbert, W., 499
Gilbert, W. A., 42
Gilchrist, T. L., 6
Gilden, R. V., 137
Gildersleeve, D., 105
Giles, M. B., 406
Giles, N. H., 53
Gill, S. J., 336, 337
Gillan, M. G. C., 577
Gillard, R. D., 301
Gillbro, T., 31
Gillessen, D., 404, 521
Gilman, J. G., 286
Gilmore, D. J., 251
Gilon, C., 35, 411, 436
Gilpin, M. L., 443
Gilroy, J., 146
Ginsberg, B. H., 500
Ginsberg, M. H., 83
Ginzburg, O. F., 436
Gioannini, T. L., 202
Giori, P., 405
Giralt, E., 22, 29, 383
Girard, A. E., 446
Girard, J., 527
Girard, Y., 14
Girgis, S. I., 539, 543
Girling, R. L., 226, 227
Girin, S. K., 374
Giro, M. G., 254
Girodet, J., 513
Girotti, A. W., 169
Girshovich, A. S., 187
Gisin, B. F., 280, 388, 434, 437
Gispen, W. H., 561
Gitlow, S. E., 34
Giudice, L. C., 106, 175, 314, 484
Giusti, P., 306, 396, 576
Given, B., 503
Givol, D., 179
Gjessing, L. R., 36
Glanville, R. W., 135
Glasel, J. A., 276, 278, 404, 575
Glaser, C. B., 308
Glaser, L., 58
Glass, J. D., 195, 369, 389
Glatz, P., 357
Glazer, R. I., 32
Gleason, J. G., 448
Gleason, R. E., 534
Gleaves, J. T., 134
Gleeson, P. A., 58, 59
Glibin, E. N., 436
Glick, B. R., 77
Glick, Z., 523
Glickson, J. D., 277, 284, 356, 460, 496
Gliemann, J., 173, 501
Glinskaya, O. V., 502
Glinski, R. L., 77
Glitzer, M. S., 468, 512
Glössl, J., 91
Glotov, B. O., 289
Glover, C. V. C., 129
Glover, G. I., 329
Glusker, J. P., 250
Gnemmi, E., 167
Go, A., 432
Go, N., 260, 433
Go, V. L. W., 515, 536
Goddard, P., 386
Godinot, C., 65, 157, 357
Godunova, L. F., 6
Goeddel, D. V., 481, 499
Goehring, W., 518
Goel, N. S., 260
Goeres, E., 521
Götz, H., 143
Götz, M., 404
Goff, D., 441
Gogiya, Z. V., 290, 306
Goingo, E. M., 167
Gold, L. I., 137, 165
Gol'danskii, V. I., 325
Goldberg, E. B., 249
Goldberg, M., 311
Goldberg, M. E., 191, 265
Goldfine, I. D., 521, 524
Goldin, S. M., 66
Golding, B. T., 16
Goldmacher, V. S., 66
Goldman, R. D., 330
Goldring, S. R., 547
Goldsmith, B., 9, 413
Goldsmith, M. R., 102
Goldstein, A., 560, 564, 577
Goldstein, A. L., 149
Goldstein, G., 400
Goldstein, J. L., 65, 499
Goldstein, L., 40
Golovanov, I. B., 256
Goltermann, N., 512, 515, 519, 522
Goltzman, D., 545, 546
Gomez, J. C., 289
Gomez-Pan, A., 465
Gomez-Sal, P., 293
Goni, G. M., 289
Gonser, U., 319
Gonzales-Fernandez, F., 289
Gonzales-Ros, J. M., 195
Gonzalez, M., 157
Good, H. P., 345
Goodenow, R. S., 75
Gooderham, K., 147
Goodfriend, T. L., 549, 552
Goodman, D. S., 149
Goodman, H. M., 481
Goodman, M., 131, 132, 276, 294, 303, 453, 455, 465, 562
Goodman, R. H., 542
Goodnough, J. E., 529
Goodwin, G. H., 145
Goodwin, P., 504
Goody, R., 264, 288
Gopinathan, K. P., 78
Gorbman, A., 525
Gorbunoff, M. J., 341
Gorden, P., 499
Gordon, A. H., 98
Gordon, E. M., 25
Gordon, J., 77, 103
Gore, M. G., 25
Goren, H. J., 295, 382, 408
Gorenstein, D. G., 161, 284
Gorin, F. A., 256
Gorinsky, B., 244
Gornall, A. G., 159

Gorodetsky, D. I., 87, 126
Gorovsky, M. A., 129
Gorski, J. P., *139*
Goshev, I., 98
Goss, N. H., 334
Gossard, F., 476, 559
Gosselin, L., 59
Gosteli, J., 446
Goth, A., 531
Gotkin, P. J., 300
Goto, H., 75
Goto, S., 364
Goto, Y., 57, 159
Gotoh, H., 62
Gotschlich, E. C., 145
Gottlieb, J., 333
Gotto, A. M., jun., 251, 403
Gotz, M., 434
GoudsWaard, J., 542
Goulas, P. R., 165
Gould, K. G., 193
Goulder, T. J., 534
Goumans, H., 77
Gourdji, D., 533
Goux, W. J., 175, 284
Govindjee, R., 357
Gowland, M. S., 458
Gozin, D. C., 263
Gozzer, C., 165
Grabowski, J., 358
Grace, D. E. P., 221, 229, 261, 263
Gracesa, P., 171
Gracheva, O. A., 438
Gracy, R. W., 101
Gräsbeck, R., 69
Graf, L., 574
Graham, J., 248
Graham, J. W., 529
Graham, N., 346
Grahn, D. T., 145, 161
Grainger, D. A., 181
Gramlich, V., 253
Gran, U. M., 263
Grand, D., 30, 352
Grande, H. J., 361
Grandi, C., 185, 307
Grandjean, J., 289
Granot, J., 282
Granozzi, G., 18
Grant, G. A., 111
Grant, J. A., 422
Grant, P. G., 185
Grasselli, P., 17
Gratton, E., 346
Gratzer, W. B., 510
Grau, U. M., 111, 245
Graves, D. J., 272
Gray, C. P., 120, 144
Gray, D., 545
Gray, D. W., 54
Gray, H. B., 318
Grebanier, A. E., 306
Green, B. J., 36
Green, D. W., 173
Green, E. A., 221
Green, G. D. J., 408
Green, N. M., 159, 347
Green, R., 194
Green, R. M., 77
Greenall, R. J., 249
Greenaway, F. T., 460
Greenberg, G. R., 515, 527
Greenberg, J. R., 187
Greene, E., 545
Greene, K., 512
Greengrass, C. W., 446
Greenwood, C., 316, 317
Greer, L., 329
Gregor, M., 515
Gregory, A. R., 420
Gregory, G. I., 443
Gregory, H., 537
Gregory, R., 201
Gregory, R. A., 401, 405, 515, 517, 520, 521
Gregson, R. P., 401
Greigger, P. P., 322
Grenader, A. K., 253
Grenier, J. F., 528
Gres, L., 518
Greuer, B., 131
Gribic, M., 556
Gribov, L. A., 293
Grieg, J. T., 153
Griffin, J. F., 574
Griffith, M. J., 189
Griffith, O. W., 51
Griffith, W. P., 24
Griffiths, G. R., 171
Grigg, R., 25
Grillo, M., 271
Grimaud, E., 61
Grimell, F., 161
Grisham, C. M., 157, 284
Grishkova, V. P., 298
Groff, J. P., 281
Gros, J., 563
Gross, F., 549
Gross, H. J., 4
Gross, J., 101, 338
Gross, T., 26
Grossman, M. I., 514, 518, 525, 528
Groswasser, J., 93
Grossweiner, L. I., 30, 352
Grouselle, D., 533
Grove, J., 36
Grove, J. F., 439
Grove, M. G., 159
Grube, D., 510,
Gruber, K. A., 61, 115, 329
Gruber, P., 10
Grütter, M. G., 231
Gruezo, F., 91
Grujic-Injac, B., 132
Grulich, R., 460
Grumbach, M. M., 479
Grunberg-Manago, M., 187
Grundy, S. M. W., 183
Grushka, E., 35
Gruszecka, E., 15
Gruzdev, A. D., 349
Grynpas, M., 60
Grzybowska, J., 404
Gschwind, H.-P., 43
Gschwind, U., 43
Gualerzi, C., 146, 185
Gualtieri, R., 407
Guarino, D. U., 126, 127
Guarner, V., 515
Guarneri, M., 278, 279, 374, 404, 405, 562, 575
Guebitz, G., 35, 392
Guedj, R., 11
Guegon, R., 399, 468
Guengerich, F. P., 99, 272
Guerrier, D., 97
Guerrier, P., 271
Guesnon, S., 337
Guestzow, K., 147
Guggi, A., 374
Guiard, B., 126, 227
Guibe-Jampel, E., 25
Guidi-Morosini, C., 221
Guidoni, A. A., 124
Guillemette, G., 555
Guillemin, R., 455, 465, 478, 543, 562
Guillemin, R. C. L., 514
Guillory, R. J., 157, 175, 179
Guillot-Edelheit, G., 25
Guinand, M., 425
Guinn, W. M., 487
Guise, A., 134
Guiso, N., 271
Gullick, W. J., 311
Gullotti, M., 9
Gumbiner, B., 305
Gunar, V. I., 17
Gund, P., 421
Gundberg, C. M., 35
Gunnar, K., 274
Gunsalus, I. C., 286
Gunter, M. J., 405
Gupta, M. K., 295
Gupta, P. K., 27
Gupta, R. K., 282, 286, 287, 288, 318
Gupta, R. R., 322
Gupta, V. D., 295
Gupte, S. S., 157
Guranowski, A., 55
Gurd, F. R. N., 133, 179, 256, 286
Gurevich, A. Z., 288
Guschlbauer, W., 57
Gust, D., 20
Gustafson, M. E., 194
Gustafsson, J.-Å., 31, 55, 67, 69 515.
Gustavsson, H., 287
Gustchina, A. E., 234, 258
Guthenberg, C., 95
Gutowsky, H. S., 286
Gutte, B., 260, 266, 407
Gutweniger, H., 202
Guyon, A., 560, 561

Guyon, F., 561
Guzman, N. A., 110
Guzman, S., 515
Gyorgydeak, Z., 27
Gyr, K., 527

Haagsman, H. P., 148, 202
Haakanson, R., 514, 515, 519, 520, 529, 530
Haar, H.-P., 346
Haas, E., 348
Haase, B. A., 98, 325
Habeeb, A. F. S. A., 153, 271
Habener, J. F., 542, 548
Haber, E., 89, 171
Habermehl, K.-O., 159
Hack, E., 51
Hackeng, W. H. L., 542
Hackenthal, E., 83
Hadjiioannou, T. P., 382
Hadley, M. E., 277, 406, 473
Haecki, W. H., 519
Håkanson, R., 510
Haellgren, R., 519
Haertlé, T., 57
Haffner, P. H., 283
Hagaman, E. W., 442
Hagaman, J., 539
Hage, G., 538
Hageman, J. H., 183
Hageman, T. C., 138
Hagen, A., 521
Hagen, D. S., 291
Hagen, I., 103
Hagenmaier, H., 286
Hager, D. A., 75
Hager, L. P., 321
Hagerman, P. J., 266
Haggerty, D. F., 98
Hagler, A. T., 255, 256
Hagler, L., 51
Haglund, U., 531
Haiech, J., 289
Haimo, L. T., 342
Haimoto, Y., 19
Hajdu, J., 151, 155, 169
Hajnal, F., 522
Hakimelahi, G. H., 445
Hakomori, S-i., 69
Hakvoort, T. B. M., 81
Halama, J. R., 16
Halban, P. A., 502
Haldar, J., 407, 485, 487
Hale, G., 125, 183
Haley, B. E., 167, 191, 201, 343
Hall, D. O., 57, 317
Hall, L. D., 288
Hall, M. J., 455
Hall, N., 49
Hall, N. T., 31
Hall, R., 465
Hall, W. G., 523
Hall, Z. W., 109
Halle, J. C., 22
Hallenga, K., 277
Halley, B. A., 284
Halmer, K., 264
Halpern, B., 32
Halpert, J., 130
Halter, F., 519
Halvorson, H. R., 330
Ham, N. S., 22
Hama, T., 34
Hamada, A., 146
Hamaguchi, K., 159, 231, 307
Hamaguchi, Y., 167
Hama-Inaba, H., 75
Hamanaka, E. S., 447
Hamaoka, T., 179
Hamashima, Y., 448
Hamazaki, H., 93
Hamilton, J. A., 434
Hamilton, J. W., 147, 149, 548
Hamilton, T. A., 105
Hammarström S., 59
Hammes, G. G., 161, 339, 359
Hammons, M., 253
Hamperly, J. L., 134
Hampl, H., 71
Hamprecht, B., 514
Han, G. Y., 368
Han, K. K., 309
Hanabusa, K., 409
Hanania, G. I. H., 256
Hanaoka, F., 64
Hancock, F. E., 401
Hancock, W. S., 34, 40, 113, 392, 548
Handa, B. K., 401
Handschumacher, R. E., 15
Haneishi, T., 439
Haniu, M., 123
Hanker, J. P., 465
Hanly, S. P., 175
Hanna, S., 159
Hannah, J., 444
Hanoune, J., 38
Hansburg, D., 140
Hansen, A. E., 300
Hansen, B., 501
Hansen, S. I., 42
Hansky, J., 514, 526
Hanson, J. C., 220
Hanssen, K. F., 519
Hansson, B., 329
Hansson, C., 34
Hanstein, W. G., 201
Hantgan, R. R., 330
Hanus, V., 29
Hapner, K. D., 91
Hara, A., 49
Hara, K., 482
Hara, M., 512
Hara, S., 35
Harada, K., 1, 6, 8, 9, 11
Harami, T., 319
Harasawa, S., 536
Haratz, A., 91
Harding, D. R. K., 392
Hardman, M. J., 354
Hardman, R., 33
Hardy, G. W., 125, 237, 264, 401
Hardy, P. M., 4, 14, 192, 365, 371
Hare, F., 347
Hare, P. E., 1, 17, 392
Hargreaves, R. T., 461
Harkey, M. R., 287
Harkins, R. N., 185
Harlos, K., 251
Harmison, G. G., 175
Harmon, S. J., 314
Haron, Y., 3
Harper, A. A., 538
Harrington, M. E., 18
Harris, B. G., 49
Harris, C. M., 4, 5, 460, 461
Harris, G., 159, 361
Harris, J., 18
Harris, J. I., 125, 405, 517, 521
Harris, J. M., 346
Harris, N. D., 44
Harris, R. L. N., 15
Harris, T. M., 4, 5, 460, 461
Harrison, J. E., 145
Harrison, J. M., 61
Harrison, P. G., 324
Harrison, R. A., 60
Harrison, S. C., 220
Hart, G. W., 111
Hartley, B. S., 63, 122
Hartman, F. C., 285
Hartmann, C. R., 548
Hartmann, M. K., 35
Hartt, J. E., 250
Hartzell, C. R., 279
Harvey, R. F., 520, 522
Harwood, J. P., 499
Hasan, S. K., 27
Hase, T., 134
Hasegawa, H., 62
Hasegawa, M., 53, 410, 456
Hasegawa, T., 183
Haser, R., 125, 133, 228, 237, 264
Hash, J. H., 307
Hashiba, N., 19
Hashim, G. A., 406
Hashimoto, C., 311
Hashimoto, M., 445
Hashimoto, T., 61, 448
Hashizume, H., 284
Haslinger, E., 442
Hass, G. M., 87, 145, 161
Hass, P. E., 101
Hassall, C. H., 410, 441, 455
Hasselmann, D., 9
Hassner, A., 411, 436
Hashings, J. W., 359
Hata, T., 179
Hata, Y., 228
Hatanaka, S., 2
Hatanaka, S. I., 449
Hatano, H., 30, 61, 279

Hatano, M., 311, 316
Hatfield, G. W., 127
Hattingh, J., 29
Haubrich-Morrec, T., 77
Haudegoud, J. P., 13
Haugen, G. R., 345
Hauptman, H., 219
Hausamen, T. U., 514
Hauschka, P. V., 120
Hause, D. W., 35
Hauser, D., 396, 569
Hauser, H., 177
Hauser, M., 346
Hausler, J., 1
Haussler, M. R., 69
Hauzer, K., 58
Havranova, M., 183
Hawiger, J., 69
Hawkes, G. E., 424
Hawkes, R. B., 231
Hawley, D. E., 253, 334
Hayakawa, M., 421
Hayakawa, T., 302, 304
Hayashi, H., 129, 450
Hayashi, M., 523
Hayashi, R., 161
Hayashi, T., 25, 100, 254, 294, 309, 409
Hayashida, T., 478, 479
Hayaishi, O., 73
Hayase, N., 26
Hayek, B., 301
Hayes, J. D., 110
Hayes, M. B., 286
Hayes, R. L., 16
Hayon, E., 344
Hayzer, D. J., 13
Haze, G., 150
Hazel, J. P., 18
Hazelwood, R. L., 407
Hazen, E. E., jr., 358
Hazum, E., 404, 505, 576–578
Heading, R. C., 519
Heald, S. M., 224
Hearn, L., 574
Hearn, M. T. W., 34, 40, 63, 113, 392
Hebdon, G. M., 159
Hecht, S. M., 8, 78, 458
Hedin, A., 59
Hedlung, B. E., 317
Heersche, J. N. M., 539
Hegeman, G. D., 175
Hehir, S., 279
Heidemann, E. R., 410
Heiderer, R., 8
Heiland, I., 131
Heimark, R. L., 81, 138
Heimer, E. P., 387
Heinrich, P. C., 54
Heitz, F., 280
Heitz, P., 519
Heitz, P. U., 514
Hejtmancik, K., 514
Heldin, C.-H., 44
Helene, C., 289, 344, 351, 356
Helgeson, R. C., 18
Helle, K. B., 103
Helle, O., 136
Hellemans, J., 536
Heller, A., 17
Heller, E., 128
Helliwell, J. R., 236
Helmreich, E. J. M., 282, 285
Helmstaedter, V., 535
Helmuth, R., 252
Hemmasi, B., 390
Hemmes, P., 22
Hemmi, H., 450
Hemmings, M. A., 291
Hemmink, J., 83
Hemon, Ph., 65
Hemperley, J. J., 91
Henderson, G. B., 177
Henderson, L., 526
Henderson, L. A., 194
Henderson, L. E., 101, 137, 338
Henderson, R., 252
Hendricks, J. G., 526
Hendrickson, W. A., 221, 222, 233
Hendriks, F. F. A., 181
Hendy, G. N., 543, 544, 545
Hengartner, H., 125
Hengartner, U., 14
Heng-Khoo, C. S., 136
Hengstenberg, W., 282
Henkel, W., 135
Hennecke, H., 272
Hennen, G., 484
Henning, U., 147, 177
Henriksen, O., 69, 140
Henrikson, K. P., 39
Henry, J. P., 344
Henry, R. W., 526
Henschen, A., 112, 138, 145
Hensens, O. D., 444, 457
Hensley, P., 332
Henson, R., 281
Hepburn, P., 173
Heppenheimer, K., 410
Herbert, E., 475, 476
Herkenham, M. A., 577
Herlihy, W. C., 120, 144
Herman, K. M., 271
Herman, M. M., 101
Herman, R. H., 51
Herman, Z. H., 404
Herman, Z. S., 573
Hermans, J., 330
Hermans, L., 49
Hermodson, M., 185
Hermodson, M. A., 112, 146, 194
Hermoso, J., 133
Herner, R. C., 2
Heromi, K., 187
Heron, C., 338
Herper, P., 17
Herranz, J., 21, 293
Herrlich, P., 77
Herrmann, S. H., 60
Hersh, L. B., 49
Hershberger, M., 344
Herscheid, J. D. M., 419, 420
Hershcopf, R. J., 535
Hershey, J. W. B., 360
Hershman, J. M., 524
Hertes, M. P., 189
Hertz, L., 33
Herz, J. M., 159
Herzfeld, J., 341
Hess, B., 310
Hess, G. P., 364
Hess, U., 26
Hessel, B., 112, 138
Hesterberg, L. K., 331
Hever, J., 20
Heumann, W., 187
Heusel, G., 19, 388
Heward, C. B., 406, 473
Hewat, A. W., 221
Heyneker, H. L., 481, 499
Heywood, S. M., 77
Hiatefi, Y., 201
Hibbard, L., 81
Hichwa, R. D., 16
Hickman, B. J., 100
Hicks, B., 352
Hidaka, J., 301
Hideaki, J., 3
Hider, R. C., 324
Higashijima, T., 278, 280, 575, 576
Higgins, R. C., 64
Higgs, H., 223
Highsmith, S., 264, 288
Higuchi, H., 400
Hikichi, K., 276
Hikino, H., 3
Hildebrand, J. H., 245, 256
Hilderman, R. H., 77
Hilgenfeldt, U., 83, 549
Hilger, F., 55
Hill, D. W., 34
Hill, E. A., 79
Hill, H., 287
Hill, H. A. O., 281, 287
Hill, M., 197
Hill, R. C., 396, 569
Hill, R. L., 51, 93, 173, 282
Hill, T., 17
Hiller, J. M., 578
Hillier, I. H., 256
Hillman, J. D., 47
Hilschmann, N., 143
Hilsted, J., 530
Hilton, B. D., 264, 279
Hilz, H., 39, 95
Himeno, M., 87
Himmel, M. E., 326
Hincke, M. T., 159, 189, 313
Hindersson, P., 102
Hinman, M., 476
Hino, T., 30

Hinoo, H., 451
Hinshaw, J. V., jun., 392
Hinz, H.-J., 341
Hirai, K., 446, 448
Hirama, M., 8
Hirani, S., 93
Hiraoki, T., 276, 447
Hirata, H., 159
Hirata, Y., 537
Hirayama, T., 397
Hiroaki, O., 411
Hiromi, K., 362
Hirono, S., 232
Hirose, T., 499
Hirose, Y., 1
Hiroshi, N., 288
Hirota, R., 524
Hirs, C. H. W., 255, 284
Hirsch, J., 522
Hirschauer, C., 87
Hirschfeld, T., 347
Hirschfield, J., 444
Hirschmann, R., 464, 468, 512
Hirschowitz, B. I., 519
Hirst, B. H., 519
Hirt, J., 400
Hirth, L., 355
Hiskey, R. G., 9, 33, 276, 277, 413
Hitchman, A. J. W., 145
Hixon, C. S., 183
Hixson, S. M., 200
Hiyama, N., 45
Hizunio, N., 153
Hjelmeland, L. M., 99
Hlasta, D. J., 445, 448
Hlavack, J., 378, 492
Hnatowich, D. J., 17
Ho, C., 287, 290
Ho, H., 555
Ho, N. T., 287
Ho, P., 526
Hoard, L. G., 222
Hobus, P. A., 71
Hock, R. A., 69, 538
Hochstrasser, K., 146
Hodes, Z. I., 22, 256
Hodge, J. L., 61
Hodges, R. S., 390
Hodgins, L. T., 89
Hodgkin, D. C., 219, 240, 499
Hodgkin, J. C., 34
Hodgson, K. O., 253, 452
Hoebeke, J., 93
Högberg, B., 55
Högberg-Raibaud, A., 191
Hoekfelt, T., 515, 529, 530
Hoel, D., 31
Hoeprich, P., 465
Hoerl, M., 282
Hoerlein, D., 135
Hoes, C., 408
Hoffman, P., 17
Hoffmann, F. M., 66
Hoffmann, G. E., 53
Hoffmann, J. J., 423
Hoffmann, M., 411, 456
Hofmann, K., 401, 472
Hofmann, T., 144, 145, 148
Hofrichter, J., 340, 341
Hofstee, B. H. J., 79
Hogan, M. L., 545
Hogberg-Raiband, A., 265
Hogge, L. R., 422
Hol, W. G. J., 241, 242
Holaday, J. W., 559
Holbrook, J. J., 354, 360
Holden, J. A., 51
Holden, K. G., 448
Holdsworth, E. S., 98
Holick, W., 446
Holladay, L. A., 75, 307, 327
Hollaender, H., 15
Holland, D., 474
Holland, L. D., 345
Holldorf, A. W., 45
Hollenberg, M. D., 69, 538
Hollifield, J. W., 556
Holliger, C., 533
Hollingshead, C. J., 248
Hollins, B. M., 127, 473
Hollins, P. J., 500
Hollósi, M., 575
Holloway, F. H., 374
Holly, F. W., 400, 410, 434, 468, 512
Holm, I. M., 525
Holm, J., 42, 146
Holm, R. H., 281, 323
Holmes, E. W., 91
Homes, J. E., 537
Holmes, K., 288
Holmes, M. C., 281
Holmes, R., 523
Holmgren, A., 265
Holmquist, A. L., 522
Holmquist, B., 87, 159
Holowka, D. A., 161
Holroyde, M. J., 406
Holst, J. J., 531, 532
Holtzer, H., 101
Holzer, H., 89
Holzwarth, A. R., 345
Homyk, M., 189, 192
Hong, K. M., 348
Hongo, C., 17
Hongo, M., 526
Honig, G. R., 340
Honma, K., 146
Honma, M., 155
Honzatko, R. B., 243
Hood, A. J. C., 538
Hood, J. D., 444
Hood, J. M., 142
Hood, K., 313
Hood, L., 108, 140, 142, 144, 401, 517, 560
Hood, L. E., 118, 128, 129, 149
Hoogerhout, P., 408
Hoogsteen, K., 281, 444
Hook, D. J., 440
Hook, J. H., 308
Hooper, E. A., 183
Hoopes, E., 23
Hope, H., 19
Hope, J., 474
Hopfer, U., 177
Hopkins, C. R., 518, 537
Hopp, T. P., 91, 115, 134, 140, 146
Hoppe, B., 525
Hoppe, J., 173, 183
Hora, S., 392
Hořejší, V., 58, 91
Hori, C., 97
Hori, K., 75
Hori, M., 450
Hori, Y., 439
Horigarne, T., 179
Horiike, K., 313
Horikawa, H., 5, 11
Horikawa, S., 66
Horikoshi, H., 406
Horio, T., 62
Horitsu, K., 295
Horiuchi, S., 169
Horiuti, Y., 97
Hornnes, P. J., 534
Horrobin, D. F., 524
Horrocks, W. D., jun., 362, 363
Horsburgh, C., 244
Horton, H. R., 40, 181, 353
Horuk, R., 504
Horvath, L. B., 65
Horwitz, J., 329
Hosaka, K., 51
Hosein, B., 148
Hoshi, M., 57
Hoshimaru, M., 51
Hoskins, R., 83
Hosoda, H., 153
Hosomi, S., 165
Hospital, M., 18
Hossain, M. B., 433
Hossdorf, T., 531
Hotta, K., 93
Hou, C. T., 45
Houde, R. W., 579
Houghten, R. A., 112, 195, 442, 479, 481, 576
Houghton, R. L., 185
Houpt, K. A., 523
Houpt, T. R., 523
Houston, L. L., 153
Houston, T. E., 226, 227
Hovmöller, S., 219
Howard, J., 105, 271
Howard, J. B., 125, 147, 175
Howarth, O. W., 266, 275, 283
Howarth, T. T., 444
Howat, H. T., 527
Howlett, G. J., 98, 326
Hozumi, T., 179, 481
Hrkal, Z., 83
Hrncir, S., 29

Hruby, V. J., 16, 277, 278, 314, 392, 406, 407, 473, 496, 510, 525
Hsiao, H.-Y., 41
Hsiao, S., 523
Hsiao-Ping Moore, H., 151
Hsieh, K., 402, 403, 554, 555
Hsiung, H. M., 500
Hshung, J. C., 286
Hu, V. W., 177
Huang, C. H., 315
Huang, D. H., 277, 496
Huang, J. S., 87
Huang, P. C., 171
Huang, S., 47
Huang, S. S., 87
Huang, T., 287
Huang, T.-F., 165
Huang, W.-Y., 560
Huang, Y., 285
Hubbard, C. R., 18
Hubbuch, A., 412
Huber, R., 241, 244, 263, 264
Hubert, E., 167
Hubert, J.-C., 271
Hudeček, J., 508
Hudson, A. L., 407
Hudson, B. G., 100, 147, 149
Hudson, D., 396, 563
Hudson, P. J., 125, 237
Hübner, K., 123
Huebscher, W., 396
Huehns, E. R., 340
Huennteus, F. M., 177
Hufford, C. D., 450
Hughes, G. J., 4, 14, 113, 117, 118, 192
Hughes, J., 559, 561
Hughes, J. J., 249
Hugli, T. E., 139
Huguenin, R., 396, 569
Huguenin, R. L., 487
Hui Bon Hoa, D., 528, 531
Huisman, T. H. J., 61, 226, 227
Hull, S. E., 428, 440
Hul, W. E., 276, 285, 288, 424, 457, 459
Hulmes, D. J. S., 254
Hulstaert, P. F., 527
Hultin, T., 187
Humble, E., 123
Hummelink, T., 223
Hummelink-Peters, B. G., 223
Humphrey, C., 530, 531
Humphrey, R. R., 406
Humphries, J., 406, 466
Hungerford, M., 93
Hunkapiller, M., 401, 517, 560
Hunkapiller, M. W., 118, 128, 144, 279
Hunston, D. L., 335
Hunt, E., 443, 444
Hunt, N. H., 543
Hunt, S., 253
Hunter, E. P., 21
Hunter, R. L., 21
Hunton, J. F., 438
Hunziker, P., 118
Hurley, L. H., 250
Hurnaus, R., 10
Hurrell, J. G. R., 138, 406
Husek, P., 31
Huser, H., 140
Hutchinson, A. J., 418
Hutchinson, D. W., 155
Hutchinson, S. A., 450
Hutchison, J. B., 520, 521
Hutt, M. P., 406
Huynh, B. H., 256, 322, 323
Hyams, R. L., 15
Hyde, J. S., 274
Hyde, P. V. B., 514
Hynes, J., 97
Hynes, R. O., 165

Ibanez, I. B., 183, 284
Ibrahimi, I. M., 104, 124
Ichihara, A., 19
Ichihara, S., 100, 177
Ichikawa, T., 380
Ichimura, S., 302
Ide, K., 448
Ideda, S., 292
Idler, D. R., 480
Igarashi, H., 89
Igarashi, M., 564
Igi, K., 301
Ihara, H., 25
Iio, T., 189, 361
Iijima, H., 194
Iimori, T., 12
Iino, K., 447
Iitaka, Y., 232, 253, 459
Ikeda, S., 303, 367, 409
Ikeda, Y., 73
Ikegami, A., 350
Ikehara, M., 75
Ikenaka, K., 407, 462, 463
Ikenaka, T., 140
Ikkai, T., 360
Ikota, N., 6
Illarionova, N. G., 502
Illmer, K., 534
Illyustrov, N. V., 325
Imae, T., 303
Imahori, K., 38, 125, 181
Imai, K., 337
Imai, T., 134
Imai, Y., 311
Imakubo, K., 351, 356
Imakule, K., 30
Imamura, S., 97
Imamura, T., 99
Imanishi, Y., 303
Imbault, P., 78
Immer, H., 404, 434
Imura, H., 536
Inada, Y., 191
Inagaki, F., 283, 457
Inagami, T., 81
Inage, M., 463
Inazu, T., 367
Infante, A. J., 143
Ingemann, H. S., 146
Ingham, K. C., 483
Ingelman-Sundberg, M., 31, 67
Inglis, A. S., 116, 119, 138, 144
Inman, J. K., 505
Innis, R. B., 521
Ino, T., 219
Inokuchi, H., 22
Inomata, K., 377, 378
Inoue, A., 179, 475, 476, 543
Inoue, K., 45, 53, 187, 409, 528
Incue, M., 169
Incue, Y., 290
Inouye, K., 61, 393, 439, 498, 500
Inouye, M., 334
Inouye, S., 334, 439
Inturrisi, C. E., 579
Inubushi, T., 280
Inukai, M., 439
Iodal, M., 531
Ireland, C. R., 98
Irie, H., 28, 375, 382, 451
Irie, M., 185, 189
Irmscher, G., 453
Irons, L. I., 91
Irving, C. S., 288
Irwin, M. J., 440
Isaac-Sodeye, W. A., 340
Isaacson, M. S., 332
Isab, A. A., 279
Isackson, P. J., 71
Iseli, M., 303, 433
Isemura, S., 148
Isemura, T., 101, 338
Isenman, D. E., 171, 268, 290
Ishibashi, S., 515
Ishida, N., 447
Ishihama, A., 75, 185
Ishii, K., 153
Ishii, S., 183, 189
Ishii, Y., 148
Ishikawa, C., 148
Ishikawa, E., 167, 198
Ishikawa, H., 291, 362, 371
Ishikura, K., 448
Ishikura, T., 440
Ishimitsu, S., 29
Ishimori, A., 526
Ishiwari, K., 276
Ishiye, M., 75
Ishizaki, H., 312
Iskric, S., 406
Island, D. P., 475
Isogai, A., 418, 439
Isowa, Y., 380
Israelstam, D., 17
Issleib, K., 370
Itakura, K., 481, 499
Ito, H., 376
Ito, K., 382
Ito, S., 73

Ito, T., 6
Itoh, S., 524
Itoh, Z., 536
Ito-Mega, S., 93
Ivanov, V. T., 253, 288, 428, 437
Ivanova, A. I., 405
Ivanova, Ts. K., 191
Ivanova, V., 305
Ivari, R. D., 57, 481
Iversen, L. L., 531, 532
Iwai, J., 470, 473
Iwai, K., 129, 171
Iwai, S., 179
Iwaki, M., 125
Iwakura, M., 175
Iwamoto, K., 435
Iwanaga, S., 89, 138
Iwano, Y., 446, 448
Iwasaki, J., 542
Iwasaki, T., 5, 11, 14, 15, 148
Iwasaki, Y., 542
Iwase, H., 31
Iwatsuki, N., 524
Iyengar, R. B., 149
Izawa, T., 12
Izdebski, J., 381
Izmailova, V. N., 305
Izui, K., 181
Izumi, K., 402
Izumiya, N., 6, 12, 20, 381, 421, 428, 431, 441
Izvarina, N. L., 502

Jaben, S., 59
Jack, T. R., 29
Jackman, D. E., 446
Jackson, A. G., 401
Jackson, N. J., 515
Jackson, T. A., 18
Jackson, W. R. C., 179
Jacobi, P. A., 10
Jacobs, E., 183
Jacobs, J. W., 542, 543
Jacobs, P. M., 8, 14
Jacobs, R., 286
Jacobs, S., 272, 505
Jacobsen, C., 155, 338
Jacobsen, H., 510
Jacobsen, J., 155, 338
Jacobsen, E. D., 523
Jacobsen, G. R., 57
Jacobson, L., 274
Jacobson, M. K., 49
Jacq, C., 73
Jadot, J., 3
Jaeck, G., 196
Jäckle, H., 102
Jaeger, E., 28, 374, 520, 525
Jähnig, F., 251
Jaenicke, R, 264, 334
Järvinen, M., 83
Jaffe, B. M., 515, 528
Jaffe, C. L., 200
Jagenburg, R., 527
Jagendorf, A., 161
Jago, G. R., 173
Jain, S. C., 250
Jakes, R., 129
Jakob, F., 87, 122
Jakoby, W. B., 51
Jallon, J. M., 330
Jamaluddin, M., 99
James, B. D., 292
James, G. T., 157
James, L. B., 33
James, M. N. G., 230, 233, 234, 248, 260
James, T. L., 281, 283, 287
Jameson, D. M., 346
Jamieson, J. D., 555
Janado, M., 61, 101, 153
Janin, J., 245, 258
Janolino, V. G., 353
Jansen, E. H. J. M., 283
Janski, A. M., 272
Janssen, H., 548
Janssens, J., 536
Jansson, R., 527
Janzen, D. H., 3
Jao, T. C., 510
Jaramillo-Jaramillo, C., 465
Jardetzky, O., 246, 264, 288, 290
Jardetzky, T. S., 246, 290
Jarrar, A. A., 21
Jarreau, F.-X., 442
Jarrell, H. C., 278
Jarrousse, C., 534
Jarry, J. P., 346
Jarvis, D., 173, 375, 395
Jasensky, R. D., 422
Jaureguiberry, C. G., 425
Javaherian, K., 315
Jaworski, R. J., 285
Jaynes, E. N., 185
Jazwinski, S. M., 140
Jean-Baptiste, E., 471
Jeck, R., 155
Jecny, J., 421
Jeffcoate, W. J., 475
Jeffrey, P. D., 333
Jeffries, T. M., 34
Jekel, P. A., 126
Jelinski, L. W., 291
Jellenz, W., 35, 392
Jenkins, S. E., 236
Jenkins, W. T., 12
Jensen, H., 8
Jensen, H. P., 301
Jensen, L. H., 220, 229
Jensen, M. H., 155
Jensen, R. T., 514
Jensenuis, J., 102
Jensenuis, J. C., 102
Jentoft, J. E., 193, 283
Jentoft, N., 193, 274, 283
Jentsch, K. D., 73
Jerina, D. M., 123
Jermyn, M. A., 58
Jett, M. F., 42
Jibson, M. D., 258, 314, 480
Jimenez, J. S., 183
Jirgensons, B., 265, 305, 309
Jodal, M., 533
Jönsson, B., 244, 256
Jönsson, M., 139
Jørgensen, A., 579
Jörnvall, H., 121, 127, 128, 137, 155, 239, 467, 517, 528
Johannssen, W., 73
Johansen, N. L., 28, 372
Johannson, B. G., 315
Johansson, G., 37
John, D. I., 447
Johner, P. E., 280
Johns, A., 533
Johns, D. W., 556
Johns, E. W., 145, 147
Johnsen, R. H., 31
Johnson, A. G., 522
Johnson, B. C., 45
Johnson, C. E., 323
Johnson, D., 183
Johnson, D. A., 447
Johnson, D. F., 202
Johnson, D. G., 510
Johnson, E. F., 109
Johnson, E. R., 273
Johnson, J., 340
Johnson, J. D., 337, 361, 362
Johnson, K. A., 341
Johnson, L. N., 230, 236
Johnson, L. P., 62
Johnson, L. R., 515, 519
Johnson, M., 317
Johnson, M. G., 498
Johnson, M. H., 34, 118
Johnson, M. L., 337, 510
Johnson, N. D., 118
Johnson, P., 358
Johnson, P. M., 315
Johnson, R. E., 353, 510
Johnson, S. C., 126
Johnson, W. C., 298
Johnson, W. H., 410, 441
Johnston, D. B. R., 445
Johnston, I. R., 73
Jokinen, M. G., 8, 449
Jollès, J., 124, 134, 149
Jollès, P., 124, 134, 149, 231
Jolly, D. W., 33
Jonak, R., 254
Jonas, A., 157, 327, 361
Joncant, M., 171
Jones, A., 241
Jones, A. J., 418
Jones, B. N., 133
Jones, C. R., 280
Jones, G. H., 75
Jones, G. L., 47
Jones, J. H., 24, 26, 28, 373, 381, 407
Jones, N. D., 447
Jones, P. G., 460

Jones, R. B., 285
Jones, R. T., 132
Jones, S., 279
Jones, T. A., 244
Jones, W. M., 155
Jonissek, G., 403
Jonn, T. M., 73
Jonsson, B. H., 284
Joppich, M., 390
Joppich-Kuhn, R., 354
Jordan, M., 25
Jorde, R., 515
Jordis, U., 458
Jorge, M. I. B. M., 350
Jorgensen, E. C., 402, 403, 554, 555
Jori, G., 153, 344, 358
Jose, F., 445
Josefsson, L., 407
Josephs, R., 227, 340
Joshi, V. C., 57
Joshi, V. K., 40
Jošt, K., 378, 407, 487, 492
Josting, D., 51
Joubert, F. J., 130, 148
Jourdian, G. W., 93
Joyeau, R., 83
Judd, W., 63
Jue, D. L., 34, 118
Jukes, T. H., 499
Julliard, J. H., 123
Jung, G., 19, 302, 388, 389, 402, 453
Jung, J., 358
Jung, M., 449
Jung, W., 39
Juretschke, H. P., 283
Jurnak, F. A., 243
Jursik, F., 301
Just, G., 445
Justus, P. G., 527
Jyotishmati, U., 8

Kaback, H. R., 175, 177
Kabat, E. A., 91
Kachelhoffer, J., 528
Kaczka, E. A., 444
Kader, J. C., 65
Kadiri, A., 352
Kadis, B. M., 33
Kadoma, Y., 304
Kaegi, J. H. R., 282, 347, 355
Kaeppli, F., 191
Kafatos, F. C., 105
Kagamiyama, H., 121, 125
Kagawa, Y., 159
Kagayama, A., 22, 294
Kageyama, M., 65, 111
Kahan, F. M., 444
Kahan, J. S., 444
Kahn, A., 49
Kahn, C. R., 499, 500
Kahn, P. C., 298
Kai, Y., 356
Kaifu, R., 407
Kaiko, R. F., 579
Kainosho, M., 19, 119, 148, 440
Kaiser, E. T., 282
Kaito, A., 317
Kajiwara, S., 103
Kakegawa, T., 17
Kakimoto, T., 10
Kakitani, H., 256
Kakitani, T., 256
Kakolja, S., 447
Kakudo, M., 3, 228, 229
Kakumoto, Y., 518
Kaladas, P. M., 91
Kalb, A. J., 224, 268, 274, 279, 394
Kalef, E., 273
Kalei, U. O., 366
Kalk, K. H., 241, 242
Kalman, J. R., 4, 438, 460
Kalonsek, F., 104, 179, 183
Kalos, A., 519
Kalous, V., 508
Kalra, V. K., 201
Kamaluddin, 8
Kamber, B., 374, 397, 398, 469, 501, 512, 541
Kamen, M. D., 133, 134
Kamenskii, Y. A., 317
Kameshita, I., 181
Kamikubo, T., 75
Kaminir, L. B., 126
Kaminski, Z., 10
Kaminsky, L. S., 272
Kaminyo, T., 51
Kamio, M., 20
Kamiya, R., 252
Kamiya, T., 445
Kamiya, Y., 4, 119, 149, 451
Kamogawa, H., 410
Kamoto, K. O., 395
Kanaoka, M., 439
Kanarek, L., 153
Kanaya, K., 167
Kanaya, T., 393, 500
Kanchisa, M. I., 269
Kanda, Y., 149
Kandel, M., 159
Kandel, S. I., 159, 285
Kandler, O., 149, 461
Kanehisa, M. I., 260
Kaneko, S., 449
Kaneko, T., 532
Kanellis, P., 407
Kanellopoulos, J., 60
Kaneto, A., 532
Kanety, H., 362
Kang, A. H., 135
Kang, C., 361
Kang, S., 20
Kang, S. Y., 286
Kangawa, K., 560, 564
Kani, Y., 301
Kanmera, T., 6, 421
Kannapell, C. C., 101, 338
Kanno, T., 523
Kano, K., 97
Kantor, J. A., 73
Kapitonova, K. A., 249
Kaplan, E. R., 519
Kaplan, H., 173, 506
Kaplan, H. S., 75
Kaplan, N. O., 77, 97
Kaplan, S. L., 479
Kapmeyer, H., 77
Kapoor, M., 185
Kaptein, R., 280, 283
Karaboshyan, L. V., 306
Karasaki, Y., 404
Karic, L., 308
Karimov, Z., 438
Karle, I. L., 253, 428, 431, 433
Karle, J., 219
Karlsson, B., 286
Karlsson, R., 428
Karoly, E., 448
Karpati, E., 554
Karpeiskaya, E. I., 6
Karpeiskii, M. Y., 231
Karplus, M., 221, 225, 245, 255, 256, 260, 261, 269, 322
Karr, T. L., 341, 342
Kartha, G., 194
Karush, F., 179, 196
Kasai, H., 253
Kasche, V., 100
Kashtanova, N. L., 288
Kasper, C. B., 110
Kassab, R., 179
Kassner, R. J., 285, 337
Kastell, J. V., 98
Kastin, A. J., 465, 473, 533, 560
Kastner, M. E., 224
Kasvinsky, P. J., 332
Kaszuba, Z., 523
Katada, M., 318
Katakai, R., 292, 294, 391
Kataoka, S., 30
Katar, M., 91
Katayama, K., 137
Katchalski-Katzir, E., 295, 348, 382
Kather, H., 528
Katlic, A. W., 58
Katnik, I., 169, 315
Kato, F., 49
Kato, K., 87
Kato, M., 536
Kato, N., 45, 189
Kato, S., 30
Kato, T., 20, 407, 428, 431, 441, 451
Kato, Y., 19
Katrukha, G. S., 5, 367
Katsoyannis, P. G., 405, 502, 503
Katsube, J., 557
Katsube, Y., 229
Katsuda, N., 87
Katsuka, N., 17

Katsuki, H., 175, 181
Katsumi, M., 301
Katsunuma, T., 157
Katsuura, G., 524
Katunuma, N., 89
Katz, B. J., 24
Katz, D. H., 157, 179
Kauffman, D., 112, 122
Kaufman, C., 10
Kaur, J., 91
Kaura, A. C., 447
Kauska, M., 16
Kauzaki, T., 449
Kawabe, K., 34, 377
Kawai, K., 8, 31, 536
Kawai, M., 301, 428
Kawai, T., 294
Kawai, Y., 34
Kawakami, M., 145
Kawamoto, K., 8, 31
Kawamura, T., 526
Kawanaka, M., 526
Kawano, H., 34
Kawano, K., 67, 314
Kawano, T., 409, 528
Kawano, Y., 448
Kawasaki, C., 402, 406
Kawasaki, K., 401, 402, 406
Kawashima, S., 38
Kawatani, H., 470
Kawato, S., 350
Kawauchi, H., 127, 470, 473, 475, 480, 559
Kawski, A., 349
Kay, C. M., 159, 189, 308, 311, 313
Kay, E., 183, 305
Kayasseh, L., 527
Kayne, F. J., 282
Kayser, R. H., 173
Kazi, Z. A., 27
Kazim, A. L., 153, 270, 271, 403
Kaziro, Y., 165, 291
Kazlauskas, R., 417
Kedersha, N. L., 110
Keech, D. B., 334
Keefe, M., 183
Keegstra, K., 111
Keesey, J. K., 47
Keglevic, D., 406, 461
Kehry, M., 142
Keiderling, T. A., 317
Keira, T., 357
Keleti, T., 338
Kelleher, P. C., 79
Keller, O., 369
Keller, R. M., 286
Keller-Juslen, C., 425
Kellermann, O., 71
Keller-Schierlein, W., 424
Kelley, J., 111
Kelley, W. N., 51, 153
Kellogg, M. S., 447
Kelly, I. D., 281
Kelly, J. A., 230
Kelly, J. S., 524, 532
Kelly, K. A., 514, 536
Kelly, P. A., 465
Kelly, R. C., 9
Kemp, G. D., 165
Kemp, J. D., 51
Kemp, J. E. G., 447
Kemper, B., 548
Kempf, T., 150
Kempner, E. S., 99
Kende, H., 2
Kendrick-Jones, J., 129
Keniry, M. A., 309
Kennard, O., 223, 253, 460
Kennedy, D. S., 77
Kennedy, W. P., 161, 284
Kenner, G. W., 371, 376, 396, 401, 405, 517
Kenny, J. W., 187
Kent, J. L., 577
Kent, S. B. H., 383, 387
Kent, T. A., 320
Kenyan, B. L., 175
Kenyan, G. L., 201
Keppens, S., 497
Keradjopoulos, D., 45
Kerby, A. L., 125
Kerchentsev, M. A., 301
Kerckaert, J.-P., 102
Kerl, J. E., 61
Kerling, K. E. T., 408
Kernebeck, K., 374
Kerr, M. A., 139
Kerr, V. N., 16
Kershaw, G. R., 556
Kerstein, W., 175
Kersten, W., 201
Kerwin, J. F., 449
Keshavarz, E., 111
Kessel, D., 167
Kesseler, L. L., 271
Kessels, H. J., 23
Kessler, H., 275, 430, 431, 432
Kessner, A., 89
Ketterer, B., 169
Ketterer, H., 515
Kettner, C., 89, 181, 189, 191
Keul, V., 191
Keutmann, H. T., 127, 128, 484, 539, 543, 544, 545, 547
Kewenter, J., 529, 530
Khairallah, L. H., 332
Khairallah, P. A., 402, 555
Khaled, M. A., 428, 432, 575
Khalef, S., 183
Khalifah, R. G., 62, 155, 193
Khalilulina, K. Kh., 428
Khan, N. H., 6
Khan, S. M., 363
Khandanyau, A. Z., 307
Kharakoz, D. P., 22
Khatri, H., 25
Khaw, B. A., 171
Khazhakyan, L. V., 292
Khedkar, P. D., 13
Khodkova, E. M., 404
Khodyrera, S. N., 181
Khorana, H. G., 120, 144
Khorlin, A. Y., 32, 111
Khosla, M. C., 402, 555
Khouri, H., 21
Khrapov, V. V., 320
Khrapovitskii, V. P., 351
Kibby, J. J., 460
Kibirev, V. K., 405
Kibly, J. J., 4
Kida, M., 449
Kida, S., 17
Kidd, G. H., 109, 110
Kido, A., 526
Kiefer, H., 201
Kielland, S. L., 405
Kiener, P. A., 285
Kienle, M. G., 16
Kierkegaard, P., 220
Kierstead, R. W., 14
Kihara, K., 380
Kikkawa, I., 448
Kikuchi, K., 503
Kikuchi, T., 93
Kilara, A., 40
Kilber, R. D., 55
Kim, C. U., 448
Kim, J. J., 472
Kim, M. H., 529
Kim, M. S., 526, 527
Kim, S., 472
Kim, Y. H., 298
Kim, Y. S., 53
Kimball, M. R., 244
Kimura, H., 169
Kimura, N., 148
Kimura, S., 61, 303, 560
Kincaid, R. L., 64
Kindinger, J., 105, 271
Kindt, T. J., 140
King, G. I., 251
King, K., 23
King, T. E., 123, 286
King, T. J., 323
Kingdon, H. S., 189, 539
Kings, M., 405
Kingsbury, D. W., 179
Kingston, B. L., 145
Kingston, E. E., 21
Kingston, I. B., 145
Kinney, Y. E., 32
Kinnunen, P. K. J., 97
Kinoshita, H., 371, 377, 378
Kinoshita, S., 1
Kinoshita, T., 439
Kinosita, K., jun., 350
Kinsella, J. E., 59
Kinzel, V., 42
Kiraly-Olah, I. C., 402, 555
Kirby, E. P., 89, 189
Kirby, G. W., 420
Kirchnerova, J., 22
Kirkpatrick, J. R., 515, 536

Kirpichnikov, M. P., 289
Kirschenbaum, D. M., 98
Kirschner, K., 43, 57
Kirschner, M. W., 109, 342
Kirshna, R. V., 13
Kiruko, M., 3
Kiselev, A. V., 144
Kisfaludy, L., 26, 28, 372, 395, 402, 470, 517, 554
Kisfalvi, I., 527
Kishchenko, G. P., 349
Kishi, Y., 418
Kishner, S., 356
Kisliuk, R. L., 282
Kiso, Y., 373, 382, 403, 470, 472
Kisseler, L. L., 191
Kissinger, P. T., 34
Kistler, J., 249
Kita, T., 476, 543
Kitabashi, F., 412
Kitade, K., 29, 373
Kitagawa, K., 29, 373, 382, 403
Kitagawa, T., 424
Kitamura, K., 93, 146
Kitamura, T., 527
Kitayana-Yokokawa, C., 183
Kitazawa, S., 539
Kitmura, S., 163
Kito, Y., 29
Kitson, T. M., 155
Kivirikko, K. I., 97
Kiyohara, T., 148
Klabunovskii, E. I., 6
Klapdor, R., 513
Klapperstück, M., 83
Klapproth, K., 95
Klareskog, L., 69
Klarman, A., 333
Klausner, Y., 411, 436
Kledzik, G. S., 465
Kleemann, A., 28
Klein, D. C., 29, 499
Klein, F. K., 441
Klein, M. P., 424
Klein, R., 352
Kleine, R., 83
Kleinschmidt, T., 133
Klemes, Y., 173
Klemm, P., 145, 146
Klevan, L., 289
Kley, H.-P., 140
Klibanov, A. L., 66
Klibanov, A. M., 161
Klimov, P. K., 520
Klimovich, V. M., 296
Klis, W. A., 404, 573
Klotz, I. M., 198, 335, 341
Klusha, V. E., 472
Klutchko, C., 153
Kmiotek, E., 63, 167
Knapp, F. F., 15
Knappenberg, M., 277
Knecht, R., 140
Knight, A. E. W., 362
Knight, P., 198
Knisatschek, H., 189
Knoblauch, M., 533
Knof, S., 28, 374, 520, 525
Knott-Hunziker, V., 173, 446
Knowles, J. R., 443
Knowles, P. F., 281
Knox, J. R., 258
Knox, R. S., 350
Ko, D., 403, 554
Ko, E. M., 403, 554
Kobata, A., 93
Kobayashi, J., 278, 575, 576
Kobayashi, N., 523
Kobayashi, S., 12
Kobayashi, T., 377, 446, 447
Kobayashi, Y., 8, 314
Kobazashi, M., 503
Kobetsu, M., 81
Koblet, H., 101, 338
Kobzareva, V. P., 312
Koch, D. D., 34
Koch, M., 254
Kochman, H., 181
Kochman, K., 181
Kochman, M., 198
Kochwa, S., 333
Kociolek, K., 38
Kocourek, J., 58
Kodaka, M., 294
Kodama, M., 276
Koehler, C., 530
Koehler, G., 352
Koehler, K. A., 9, 33, 276, 277, 413
Koehler, M. M. D., 102
Koenig, H., 149
Koenig, S. H., 285
Koenig, W., 525, 531
König, W. A., 32
Koeppe, R. E., 253, 452
Koester, M. K., 313
Koester, V. J., 345
Koga, K., 10
Koga, Y., 523
Kohler, G., 269
Kohlmiller, N. A., 125
Kohn, L. D., 189
Kohno, M., 75
Kohno, T., 392
Kohut, J., 187
Koida, M., 181
Koide, T., 83
Koivusalo, M., 45
Koizumi, F., 526
Koizumi, M., 526
Koizumi, R., 2
Kojima, H., 121, 153
Kojima, K., 102
Kolaskar, A. S., 255
Kolasov, I. V., 312
Kolattukudy, P. E., 53
Kolb, E., 125
Kolb, H. J., 70
Kolb, L. M., 81
Kolb, M., 7, 11
Kolb, W. P., 81
Kolby, J., 49
Kollman, P. A., 257
Kollonitsch, J., 11
Kolodziejczyk, A., 19, 381, 411, 456
Kolpak, F. J., 249
Kolterman, O., 503
Kolts, B. E., 527
Kom, E. D., 272
Komiya, K., 61
Komiyama, T., 21
Komolov, I. S., 399
Komorita, T., 300
Komoriya, A., 258, 268
Komoto, T., 294
Komyama, T., 301
Konaka, R., 61
Konda, Y., 451
Kondo, H., 189, 282, 361, 362
Kondo, K., 133, 409
Kondo, M., 25, 428
Kondo, T., 518
Kondor, P., 430, 432
Konev, S. V., 351
Kong, Y. C., 546
Konieczny, L., 173
Konig, W., 374
Konigsberg, W., 101, 199, 338
Konishi, K., 99
Konishi, T., 159
Konno, S., 455
Konopinska, D., 408
Konrad, M., 264, 288
Konstantinov, A. A., 317
Kontani, T., 299
Konturek, S. J., 514
Konze, J. R., 2
Koornstra, W., 58
Kopecký, J., 175
Kopelovitch, V. M., 17
Kopeyan, C., 130
Kopoldova, J., 29
Kopp, F., 296
Koppel, D. E., 355
Koppel, G. A., 445, 447
Kopple, K. A., 304
Kopple, K. D., 256, 366, 432, 433
Kopriwa, B. M., 503
Kopylow, A., 523
Korc, M., 524
Korcek, L., 189
Kordon, C., 529, 533
Korf, J., 35
Korhonen, T. K., 338
Korkushko, O. V., 527
Korn, A. P., 29, 526
Korn, E. D., 43, 110
Kornberg, A., 75
Kornbluth, R., 44
Korner, M., 532
Korpela, T. K., 43
Korri, K. K., 51

Kortmann, H., 87
Kortt, A. A., 89
Korytuyk, W., 27
Korzhova, L. P., 291
Kosaka, K., 532
Kosower, E. M., 362
Kost, A. A., 364
Kosta, L. D., 514
Kostansek, E. C., 428, 432
Kosterlitz, H. W., 561, 577
Kostyk, T. G., 112
Kostyo, J. L., 479
Koszalka, G. W., 73
Kotake, H., 371, 377, 378
Kotra, R. K., 1
Kotsira-Engonopoulos, C., 407
Koul, A. K., 393
Kourides, I. A., 484, 579
Kousvelari, E. E., 106, 271
Kouvonen, I., 69
Kovyama, T., 345
Kovacheva, S., 191
Kovács, K., 155, 517
Kovacs, K. L., 21
Kowal, P., 103
Kowalski, K., 173
Kowalski, M. M., 69
Koyama, J., 81, 451
Koyama, K., 409, 451, 470, 473, 528
Kozhanova, Z. E., 99
Kozlov, I. A., 99
Kozma, S., 59
Kraal, B., 116, 136
Kraas, E., 518
Kraek, G., 430
Krämer, C., 147
Krajcarski, D. T., 351
Krajicek, A., 420
Krakow, J. S., 155
Kramer, P., 430
Kramp, D. C., 354
Krane, S. M., 547
Kranenburg, P., 400
Kraska, B., 276
Kraszewski, A., 499
Kratzin, H., 143
Krauhs, E., 150
Krauss, E. R., 316
Kraut, J., 241
Kraybill, W., 61
Krchňák, V., 409
Kream, R. M., 165, 576, 578
Krebs, E. G., 183, 201, 579
Krebs, H., 264
Krebs, T., 191
Kreielsheimer, G., 523
Krejci, I., 496
Krenitsky, T. A., 73
Kreppein, W., 535
Kress, L. F., 83, 183
Kresse, K., 91
Kriausakal, N., 23
Kricheldorf, H. R., 276, 391, 457
Krieger, D. E., 388
Krieger, D. T., 105, 475
Krigbaum, W. R., 258, 268
Krikis, A. Y., 377
Krimm, S., 293
Krishna, N. R., 277, 460, 496
Krishnamachari, N., 249
Krishnamoorthy, R., 101
Krishnan, N., 326
Kristian, P., 28
Kristiansen, T., 58
Krit, N. A., 403
Krivit, W., 548
Krivtsov, V. F., 405
Kriwaczek, V. M., 396, 473, 562
Krob, P. M., 77
Krøll, J., 102
Kronenberg, L. H., 104
Kronman, M. J., 185, 193
Krstulovic, A. M., 34, 61
Kruck, T. P. A., 276
Kruese, J., 357
Kruger, H., 148
Krull, I. S., 403
Krupa, T. S., 5, 410
Kruse, K., 32
Kruse-Larsen, C., 519
Krutzsch, H. C., 87, 113, 142
Kuan, K. N., 47
Kublis, G. G., 472
Kubo, K., 100, 101, 338
Kubota, M., 397
Kubota, S., 513
Kubota, T., 448
Kudo, A., 391
Kudo, T., 181
Kudryashova, N. V., 163
Kuebel, B., 10
Kübler, D., 42
Kuehl, W. M., 269
Kuehn, K., 135
Kühn, M., 39, 425
Kümel, G., 39
Kugo, K., 254, 294, 409
Kuhl, C., 534
Kuiper, H. A., 287, 336
Kuipers, G. F., 316
Kukla, D., 244
Kula, M.-R., 37
Kulbe, K. D., 49
Kulczycki, A., 69
Kulesha, I. D., 400
Kulikov, S. V., 436
Kulo, M., 297
Kulprathipanja, S., 17
Kumagai, F., 542
Kumagai, H., 155
Kumar, D., 503
Kumar, G., 201
Kumar, K. G., 127
Kumar, P., 377
Kumarasamy, R., 103
Kumazaki, T., 189
Kumikawa, M., 400, 511
Kunda, S. K., 39
Kunicki, T. J., 69
Kuntz, I. D., 257, 258
Kuntz, R. R., 30, 352
Kunz, H., 369
Kuo, K. C., 31
Kuo, M., 280
Kupfer, A., 183
Kupfer, J. M., 300
Kuramitsu, S., 231, 307
Kuramochi, H., 189
Kurbatov, V. A., 163
Kurecki, T., 83, 183
Kurganov, A. A., 17
Kuriyama, K., 451
Kurland, R. J., 281
Kurochkin, A. V., 289
Kuroda, K., 276, 379, 380
Kurosawa, Y., 410
Kurose, H., 424
Kurosky, A., 150
Kurstak, E., 103, 191
Kurtz, D. M., jun., 281
Kusaka, T., 47
Kusano, G., 3
Kusba, J., 349
Kuschinsky, K., 559
Kushkulei, L. M., 299
Kusumi, T., 157
Kusumoto, S., 2, 407, 462, 463
Kutzbach, C., 138
Kuusi, N., 102
Kuusi, T., 97
Kuwahara, A., 38
Kuwata, S., 10, 412, 451
Kuznetsova, I. A., 87, 126
Kuzuya, T., 505
Kveder, S., 406
Kvenvolden, K. A., 23
Kwok, F., 183
Kwong, S., 341
Kyogoku, Y., 314

Laangstroem, B., 7
Laatsch, H., 33
Labhardt, A., 229
Labhardt, A. M., 265, 266
Labie, D., 101, 340
Labischinski, H., 462
Labo, G., 519
Labrie, F., 465, 512, 573
Laburthe, M., 528, 529, 531, 532, 538
Lachman, L. B., 15
Lachmann, P. J., 60
Lacks, S. A., 64
Ladenstein, R., 241
Ladesic, B., 406, 461
Ladik, J. J. 22, 256
Ladygina, M. E., 298
Lafont, D., 6
Lafrancier, P., 407
Lagarias, J. C., 441, 442
Laggner, P., 251
Lagutkina, V. I., 405

Lai, C. Y., 130
Lai, M. Z., 83
Laidler, K. J., 30, 351
Laidler, P., 173
Lake, J. A., 253
Laloi-Diard, M., 25
Lalthantluange, R., 133
Lam, E., 177
Lam, K. S., 110
Lam, M. K. T., 187
La Mar, G. N., 285, 286, 287
Lamas, L., 189
Lambert, J. M., 187
Lambert, M., 514, 524, 531
Lambert, R. W., 455
Lambin, P., 99
Lambris, J., 177
Lambros, T. J., 387
Lamers, C., 524, 531
Lami, H., 352
Lam-Thanh, H., 300
Lamy, F., 4
Lamy, J., 134
Lancelot, G., 276, 289
Lancet, D., 112, 141, 268
Land, E. J., 25
Landelius, J., 519
Landini, D., 13
Landuyt-Caufriez, M., 438
Lane, J. D., 103
Lane, L. K., 157
Lane, M. D., 63
Lang, G., 319, 320
Lang, H., 135
Lange, R. H., 239, 507
Langer, J. A., 253
Langerman, N., 347
Langry, K. C., 285
Langs, D. A., 18, 253
Langstrom, B., 16
Lanka, E., 187
Lankisch, P. G., 525
Lanyi, J. K., 356
Lapanje, S., 255
Lapidot, A., 288
Lapiere, C. M., 135
LaPorte, D. C., 53
Lapoumeroulie, C., 101
Lappi, D. A., 77
Lapresle, C., 111, 192
Lapuk, V. A., 83
Largman, C., 404
Larkins, B. A., 146
Larner, J., 503
Larochelle, J. F., 4
Larscheid, M. E., 14
Larsen, B., 392
Larson, K., 42
Larsson, L.-I., 512, 515, 517, 518, 521, 522, 530
Larsson, L. O., 220
Lartey, P. A., 364
Lashkov, V. N., 367, 436
Laska, F. J., 562
Laskin, A. I., 45
Laskowski, E. J., 323
Laskowski, M., 83, 183
Laszlo, P., 280, 289
Lattman, E. E., 248, 250
Lau, A. L. Y., 355
Lau, C. K., 171
Lau, H. K., 270
Lau, K.-M., 60
Lauber, M., 106
Laughrea, M., 290
Laugier, R., 527, 538
Lauransan, J., 21
Lauritsen, K., 531
Lauritsen, K. B., 534
Laursen, R. A., 145
Laustriat, G., 348, 355
Lauterwein, J., 288
Lauton, R. G., 198
Laval, J., 524
Lavee, A. E., 538
Lavie, E., 312
Lavielle, S., 512
Lavis, V. R., 511
La Vorgna, K. A., 555
Lavrentev, G. A., 24
Lavrik, O. I., 191
Lawrence, F., 533
Laws, W. R., 354, 359
Lawther, R. P., 127
Laycock, M. V., 2
Layne, P. P., 279, 408
Lazarev, Yu. A., 295
Lazarides, E., 109
Lazarus, L., 526
Lazarus, N. R., 504, 505
Lazdunski, M., 200
Lea, P. J., 1
Leach, S. J., 21, 138, 293, 435
Leader, H., 151
Leander, S., 189
Leanza, N. J., 444
Leary, T. R., 145, 161
Lease, R. A., 461
Leavis, P. C., 344, 351, 356
Leban, J. J., 408, 557
Lebedev, A. N., 185
Leberman, R., 165
Lebl, M., 407, 487
Le Bras, G., 122
Lecavalier, D., 31
Lechasseur, P., 32
Leclerc, M., 348
Leclercq, J., 447
Lecomte, J.-M., 564
Lecoq, J. C., 61
Led, J. J., 19, 274
Ledden, D. J., 266, 393
Lederer, E., 407, 425, 462
Lederer, F., 126, 227
Lederman, I., 462
Ledneva, R. K., 364
le Doucen, C., 42
Ledvina, M., 4
Lee, B. K., 259
Lee, C. A., 57
Lee, C.-L., 270
Lee, C. M., 515
Lee, C.-Y., 42, 43, 47, 60, 95, 472
Lee, G., 189
Lee, G. E., 444
Lee, G.-Li, 273
Lee, H.-C., 57
Lee, H.-W., 33
Lee, J., 30, 358, 496
Lee, J. C., 331, 342
Lee, J. Y., 352
Lee, K., 385, 558
Lee, K. S., 35, 111
Lee, K. Y., 250, 526, 527
Lee, L., 289
Lee, L. L. Y., 342
Lee, M. C., 33
Lee, N. M., 576
Lee, P. C., 523
Lee, R. H., 256
Lee, R. T., 91
Lee, S., 6, 316, 421, 431, 441
Lee, S. L., 27
Lee, T., 153
Lee, T. C., 402, 403, 555
Lee, T. J., 153, 235
Lee, T. S., 3
Lee, T. T., 201, 578
Lee, T.-Y., 235, 344
Lee, V. J., 420
Lee, W., 287
Lee, W. K., 287
Lee, Y. C., 91
Lee, Y.-H., 73, 165, 526
Leeman, S. E., 128, 557, 558
Leese, R. A., 347
Lefebvre, B., 35
Lefebvre, P. J., 510
Lefevre, G., 258, 541
Lefevre, M., 136
Lefkowitz, R. J., 38
Lefrancier, P., 462
Le Gall, J., 133, 134, 228
Legault, R., 444
Leger, J., 102
Leger, J. M., 418
Legg, J. I., 159
Lehman, I. R., 73
Lehman, L. D., 133
Lehmann, B., 28
Lehmann, G., 24
Lehnert, P., 525
Lehrer, S. B., 83
Lehrer, S. S., 344, 351, 356
Lehy, T., 518
Leibman, D. Ya., 169
Leighton, T., 75
Leighton, W. P., 31
Leimgruber, W., 14
Leitch, C. R., 110
Lemaire, S., 403, 470, 473, 474, 549
Lemay, A., 63, 192
Lemley, A. T., 296

Lemmon, R. M., 23
Lemon, M., 87
Lenkinski, R. E., 284, 356
Lennarz, W. J., 111
Lennette, E. P., 185
Lenstra, J. A., 126, 283
Leonard, K. R., 251, 252
Leonard, N. J., 364
Leonis, J., 394
Leonova, V. I., 256, 496
Leplawy, M. T., 10
Lepo, J. E., 51
Lerman, S., 175
Lernmark, A., 512
Leroux, J. P., 67
Leroy, F., 18
Lesavre, P. H., 139
Leshchinskaya, I. B., 126, 307
Leshem, R., 35
Lesk, A. M., 262
Leslie, F. M., 577
Leslie, L. J. F., 280
Lesniak, M., 173, 501
Lesniewska, I., 290
Lessard, G., 351
Lesser, B. H., 54
Lestienne, P., 163, 165, 284
Leukart, O., 13, 397, 403, 570
Leute, R., 175, 272
Lev, A. A., 438
Level, M.,407
Levin, M. J., 49
Levin, S. R., 538
Levin, W., 123, 134
Levine, B. A., 278, 289, 534, 575
Levine, M., 238
Levine, R., 499
Levinson, A. D., 105
Levinson, J. R., 103
Levintow, L., 105
Levison, S. A., 360
Levitan, P., 14
Levitskii, D. I., 288
Levy, A., 323
Levy, G. C., 460
Levy, M., 171
Levy, R. H., 261
Lewin, C. M., 36
Lewin, I. G., 543
Lewis, A., 296
Lewis, B. A., 360
Lewis, D. A., 534
Lewis, D. S., 177
Lewis, L. J., 480
Lewis, P. N., 171
Lewis, R. V., 61, 392, 559, 560
Lewis, S. D., 40
Lewis, V. J., 480
Lewis, W. G., 129
Leysath, G., 87
Lezoche, E., 515
Lhoste, J. M., 163
Li, C. H., 165, 181, 195, 258, 314, 394, 395, 397, 404, 470, 471, 472, 473, 474, 475, 478, 479, 480, 481, 482, 484, 559, 564, 573, 574, 576, 579
Li, N. C., 287
Li, R.-W., 23
Li, S. S.-L., 47
Li, T.-F., 502
Li, T. M., 285
Li, T. Y., 159
Liakopoulos-Kyriakides, M., 278, 556
Lian, J. B., 35
Liao, S., 59
Liao, T., 71, 192
Liberek, B., 19, 421
Libert, W., 447
Libman, L., 179, 356
Libor, S., 183
Licht, A., 171, 290
Licht, P., 482
Lichtner, R., 100, 338
Lidgard, G., 519
Lieb, W. R., 251
Liebman, A. A., 16
Liedberg, G., 520
Liefkens, T. J., 311
Liefländer, M., 151
Lien, E. L., 469
Liepnieks, J. J., 87
Liepnieks, J. S., 410
Lifchitz, A., 231
Lifson, S., 245, 255, 257
Light, A., 87, 191, 267, 410
Lijnen, P., 556
Likhtenshtein, G. I., 325
Liles, S., 474
Lilienblum, W., 51
Lilja, H., 288
Liljeqvist, G., 132
Lim, G., 448
Lim, V. I., 290, 306
Limburg, K., 4
Lin, A., 41
Lin, D.-X., 23
Lin, J.-K., 33
Lin, M. C., 511
Lin, T. Y., 161
Lin, W. K., 483
Lindberg, U., 129
Linde, S., 501
Lindell, T. D., 75
Lindemans, J., 41
Linder, R. E., 317
Lindkaer Jensen, S., 532
Lindley, P. F., 244
Lindman, B., 280, 283, 289
Lindroth, P., 34
Lindskog, S., 284
Lindstrom, J., 105
Linek, E. V., 447
Ling, J., 8
Ling, N., 475, 478, 512, 577
Ling, N. C., 579
Ling, N. R., 177
Ling, S. Y., 105
Lingappa, V. R., 140
Lingens, F., 2
Lingham, D. A., 6
Lintner, K., 279, 300, 314, 456, 551, 552
Lion, R., 187
Liotta, A. S., 105, 475
Lipkin, V. M., 185
Lipkind, G. M., 256, 452
Lipkowski, A., 407
Lips, C. J. M., 542
Lipscomb, W. N., 157, 243, 428, 432
Lipton, A., 524
Liquirer, J., 298
Lis, H., 200
Lis, M., 476, 559, 570
Lisowski, J., 315
List, D., 32
L'Italien, J. J., 145
Little, M., 150
Littlechild, J., 290
Litzenberger, J., 518
Liu, C. Y., 83
Liu, F., 157, 179
Liu, T.-N., 41
Liu, T.-Y., 145
Liu, Y. M., 358
Liu, Y.-S. V., 142
Liu, Y.-Y., 16
Live, D. H., 277, 388, 496
Lively, M. O., 196
Lizada, M. C. C., 2
Lizardi, P. M., 110
Llanos, O. L., 518
Llinas, M., 424
Lo, Y. S., 447
Lobanov, N. A., 144
Lobenstine, E. W., 298
Lobley, R. W., 523
Lochmeuller, C. H., 392
Locke, M. J., 21
Lockshin, A., 97
Lockwood, A. H., 342
Loeber, J. G., 561
Loefler, G., 35
Loehr, D. T., 284
Loehr, J. S., 171, 227
Löw, M., 372, 395
Logan, R. F. A., 519
Loginova, N. F., 367
Loh, E., 142
Loh, H. H., 559, 573, 576
Loh, Y. P., 106, 476
Lohman, P., 472
Lolkema, J., 57
Lombardi, P., 443, 445, 447
Lompre, A.-M., 102
Londesborough, J., 153
London, R. E., 263, 275, 279, 281, 551
Long, G. L., 98, 326
Long, M. M., 315, 575
Long, R. G., 468
Longmore, R. B., 436

Longworth, J. W., 355
Lonovics, J., 522, 524, 533
Loor, R., 59
Loosli, H. R., 425
Looze, Y., 394
Lopes, D. A., 143
López-Barea, J., 95
Lopez de Castro, J. A., 112, 141, 143
Lopez del Campo, G., 465
Lopez Delgado, R., 346
Lord, J. A. H., 561, 577
Lord, S. T., 278
Loren, I., 519, 529
Lorenz, D. N., 523
Lorenzi, G. P., 253, 302, 391
Losse, G., 404, 405
Lotan, N., 295
Lott, R. S., 383
Lottspeich, F., 112, 138, 145
Loucheux-Lerfebvre, M. H., 309
Lourie, A. W., 200
Love, W. E., 226
Lovell, F. M., 461
Low, B. W., 244
Low, M., 28, 402, 470, 517
Low, R. B., 111
Low, T. L. K., 142, 149
Lowbridge, J., 407, 485
Lowe, C. R., 38
Lowe, G., 161, 284, 442
Lowe, P. A., 75
Lowney, L. I., 560
Lowry, P. J., 470, 474, 475
Lozykowski, H. J., 299
Lu, A. Y. H., 123
Lu, T., 153
Lu, T.-H., 235
Lubas, B., 290
Lubin, B. H., 169
Lucacchini, A., 153
Lucente, G., 411
Luchinat, C., 285
Luckas, R. J., 151
Ludevid, M. D., 22, 29
Ludwig, B., 163
Ludwig, E., 173
Ludwig, M., 220
Lüdemann, H.-D., 334
Luederwald, I., 518
Luefler, G., 392
Luer, C. A., 258
Luerssen, K., 2
Lue Zundel, J., 177
Lugaro, G., 41, 538
Lugauskas, V., 307
Luisi, P. L., 276, 354, 380, 390, 409
Luk, K. F. S., 284
Lukacs, G., 457
Lullman-Rauch, R., 189
Luly, P., 503
Lumdblad, R. L., 539
Lumry, R., 344
Lumry, R. W., 161
Luna, E. J., 110
Lunardi, J., 157
Lund, P. K., 519, 520
Lundberg, J. M., 529, 530
Lundblad, R. L., 189
Lundgren, O., 531, 533
Lundquist, G., 519, 520
Lundt, B. F., 28, 372
Lussan, C., 347, 356
Luthra, N. P., 20, 275
Lutton, J. K., 66
Lutz, H., 118
Lux, G., 531
Luyben, D., 527
Luyckx, A. S., 510
Lyddiatt, A., 151
Lygren, I., 515
Lyman, S., 169
Lynch, J. E., 446
Lynch, T. J., 53
Lynen, F., 123, 165, 339
Lyngbye, J., 42, 146
Lynn, K. R., 87, 351
Lynn, W. S., 153
Lytle, F. E., 345

Ma, C.-Y., 98, 327
Maag, H., 421
Maassen, J. A., 199
Maat, L., 29
Mabuchi, I., 105
McAdon, J. M., 247, 258
McBride, R. W., 33
McCammon, J. A., 221, 261
McCandless, R. J., 271
McCann, S. M., 524, 529, 532
McCarty, M., 148
McCarty, R. E., 198
McCauley, J., 136
Maccecchini, M.-L., 106
Macchia, B., 448
Macchia, F., 448
Maccioni, R. B., 167
McCloskey, J. A., 4
McClure, W. O., 95
McConkey, E. H., 104
McConnell, M. L., 340
McCord, R. W., 302
McCorkindale, N. J., 450
McCormick, D. B., 84, 183
McCredie, D. A., 548
McCubbin, W., 313
McCubbin, W. D., 159, 189
McCulloch, J., 533
McCumber, L. J., 143
McDermott, J. R., 371
McDevitt, H. O., 103, 108
McDonald, D., 41
McDonald, J. M., 503
McDonald, M. J., 169
MacDonald, N. S., 16
McDonald, R. C., 236
McDonald, T. J., 127, 517
Mace, D., 75
McFarland, J. T., 159
McGahren, W. J., 461
MacGillivary, R. T. A., 146, 148
MacGregor, A. W., 102
MacGregor, D. H., 548
McGregor, D. N., 448
MacGregor, I. L., 527
McGuinness, E. T., 49
McGuire, J., 473
Machida, K., 22, 294
Machin, P. A., 230
Machleidt, W., 122
Machová, A., 409, 496
Maciag, T., 106
McInnes, A. G., 2
McIntosh, R. P., 358
MacIntyre, I., 539, 543
McIntyre, J. O., 281
McIntyre, L. J., 103
McIver, R. T., 21
Mackall, J., 63
McKay, D. B., 264
McKay, D. J., 47
McKenna, M. J., 105
McKenzie, L. J., 192
Mackenzie, S. L., 28, 31
McKerrow, J. H., 1
McKinley-McKee, J. S., 155
McKinnon, A. E., 347
McKinstry, D. N., 556
Mackintosh, I. R., 265
McLachlan, A., 132
McLachlan, A. D., 232, 234, 258
McLaren, J. V., 386
McLaughlin, A. E., 194
McLaughlin, C L., 523
McLaughlin, J. M., 85
MacLennan, A. P., 91
McLeod, B., 63
McLoughlin, G. P., 519
McLoughlin, J. L., 539
McMahon, T. W., 119
McManus, J., 512
McManus, M., 169
McMartin, C., 470
McMaster, W. R., 60
McMillan, M., 108
MacNeil, S., 543
McNeill, J. R., 487
McPhaul, M. J., 65
McPherson, A., 243
McPhie, P., 267
MacQuarrie, R., 169
McQuie, J. R., 293, 435
McQuillan, M. T., 512
MacRae, T. P., 253, 254
McShane, L. J., 445, 447
Madapallimattam, G., 60
Madar, D., 33
Madec, C., 21
Madhavarao, M. S., 437
Madison, V., 256, 433
Madjar, J.-J., 102
Madri, J. A., 161

Madsen, M. T., 16
Madsen, N. B., 235, 332
Madyastha, K. M., 49
Maeda, H., 334
Maeda, M., 406, 523, 526
Maeda, S., 11, 518
Maeda, Y., 250, 319
Mäkinen, E., 43
Maeno, H., 542
Mäntsälä, P., 121
Märki, F., 397, 398, 469, 501, 512
Maestle, W., 275, 430
Magdoff-Fairchild, B., 227, 340
Magdowski, G., 239, 507
Mage, R. G., 62, 103, 143
Magee, R. J., 292
Maggio, E., 175, 272
Maghuin-Rogister, G., 277, 484
Magne, A., 355
Magonov, S. N., 317, 318
Magoshi, J., 297
Magoshi, Y., 297
Mahadevan, P. R., 13
Mahaffey, J. E., 545, 546, 547
Mahbovba, M., 507
Mahdavi, V., 110
Mahn, T. H., 280
Mahoney, W. C., 112, 131, 132, 194
Mahuron, D., 99
Mahy, N., 31
Maida, T., 121
Maier, R., 471, 541
Maier, V., 510
Maigret, B., 278, 555, 574
Main, P., 220, 428
Mainardi, C. L., 135
Mainka, L., 157
Mains, R. E., 128, 475, 476, 543, 559
Mairanovsky, V. G., 367
Maita, T., 131, 132
Majamaa, K., 97
Majumdar, A. P. N., 542
Mak, A. S., 129
Makarova, N. A., 403, 472
Makhlouf, G. M., 519, 524, 526
Maki, H., 8, 31
Makino, K., 30
Makino, M., 138
Makino, N., 335
Mako, M., 503
Makofske, R. C., 387
Maksimenko, A. V., 161
Malabanos, J. J., 161
Malanik, V., 4
Malesci, A., 522
Maletti, M., 533
Maley, F., 54, 126, 127
Maley, G. F., 126, 127
Malfroy, B., 560, 561, 564
Malik, N. A., 290
Malinowski, D. P., 57, 187, 282
Malkiewicz, I., 297
Malley, A., 181, 270
Malling, C., 102
Mallinson, C. N., 468, 527
Malmstadt, H. V., 382
Malone, J. F., 25
Maloy, W. L., 127
Malsey, S., 248, 252
Malsky, M. L., 148
Malthouse, J. P. G., 153
Mamrack, M. D., 147
Man, E. H., 24
Mana, G., 153
Manabe, M., 536
Manabe, T., 102, 523
Manavalan, P., 574
Mandaro, R., 169, 226
Mandel, F., 305
Mandel, P., 55
Mandelkern, L., 275
Mandori, B., 271
Mandy, W. J., 143
Manera, E., 538
Mangeat, P., 129, 532
Mangrio, N. G., 301
Mani, J.-C., 41
Manikowski, H., 358
Manjula, G., 431
Manmade, A., 119, 130, 423
Mann, D. L., 112
Mann, J. F. E., 556
Mann, K. G., 81, 309, 358, 364
Mannen, S., 165
Mannervik, B., 42, 95, 169
Manning, J. M., 155
Manning, M., 407, 485, 487
Mansouri, A., 169
Mansuy, D., 67
Mantsch, H. H., 30, 275
Mantulin, W. W., 346
Manuck, B. A., 290
Manuel, Y., 142
Manzocchi, A., 16
Mara, P., 522
Marburg, S., 11
Marchalonis, J. J., 140
Marchelli, R., 371
Marchesi, V. T., 310
Marchiori, F., 306, 308, 375, 396, 400, 576
Marchis-Mouren, G., 121, 129, 532
Marco, R., 98, 194
Marcolin, H. E., 320
Marcus, F., 167
Marczak, I., 45
Marescaux, J., 528
Maret, G., 248
Margalit, R., 163
Marganiello, V. C., 181
Margel, S., 196
Margoliash, E., 163
Margolies, M. N., 34, 117, 118, 143, 388
Margolis, F. L., 271, 279
Margraff, R., 13
Maria, P., 278
Maricic, S., 286, 287
Marie, A. L., 321
Marieva, T. D., 17
Marinkovic, D. V., 122
Marin, C., 533
Marinetti, T. D., 177
Marino, G., 121
Marjason, J., 526
Mark, D. F., 75
Markert, C. L., 101
Markese, J., 105, 503, 510
Markin, E., 179
Markley, J. L., 183, 284
Marks, R. H. L., 159
Marks, V., 167, 534
Markus, I., 26
Markussen, J., 28, 372, 498
Marletta, M. A., 201
Marlicz, K., 523
Marlier, M., 3
Maroney, J. V., 198
Maroux, S., 87
Marquet, A., 12
Marquez, G., 510
Marr, G., 386
Marra, E., 157
Marraud, M., 18, 275, 293, 456
Marschel, A. H., 165
Marsh, C. A., 253, 334
Marsh, H. C., 277
Marsh, R. E., 221, 421
Marshall, C. E., 522
Marshall, G. R., 256
Marshall, M., 282
Marston, A., 376
Martens, J., 28
Martial, J. A., 481
Martin, B., 126, 146
Martin, E. W., jun., 534
Martin, J.-C., 157
Martin, J. H., 461
Martin, J. L., 16
Martin, L. C., 557
Martin, P. G., 126
Martin, R. B., 20, 274
Martin, T. J., 542, 543
Martinage, A., 129
Martinaud, M., 352
Martinek, K., 161
Martinelli, R. A., 108, 138
Martinez, G., 130
Martinez, J., 368, 374, 400, 407, 409, 529
Martinez-Carrion, M., 157, 195
Martini, F., 121
Martinko, J. M., 140
Martinson, H. G., 129, 171
Martynov, V. F., 405, 517
Marubayashi, Y., 294
Maruta, H., 43, 110
Marvin, D. A., 248, 252
Marwedel, B. J., 281
Mas, M. T., 198
Masahiro, T., 317

Mascaretti, O. A., 442
Masevich, C. G., 520
Mason, J. C., 526
Mason, M., 59
Masoro, E. J., 177
Massey, D. E., 146, 478, 559
Massey, V., 357
Masseyeff, R., 55, 198
Mastalerz, P., 15
Masuda, S., 175
Masuda, T., 25
Masuda-Mikawa, R., 67
Masuyima, T., 302
Mataga, N., 349
Matern, H., 89
Mateu, L., 251
Mather, I. H., 98
Matheson, A. T., 131
Matheson, I. B. C., 30
Matheson, R. R., 266
Mathews, F. S., 222, 228
Mathiaparanam, P., 405, 422
Mathur, K. B., 378
Matsabara, F., 448
Matson, G. B., 287
Matsoukas, J., 456
Matsubara, H., 134, 229
Matsubara, I., 250
Matsuda, G., 121, 131, 132
Matsuda, H., 439
Matsuda, Y., 311
Matsueda, G. R., 117, 388
Matsukawa, S., 311, 337
Matsukura, S., 536
Matsumoto, I., 40
Matsumoto, K., 1, 5, 11, 15, 53, 73, 435
Matsumoto, T., 19, 134
Matsumura, H., 444, 448
Matsumura, M., 532
Matsumura, S., 49
Matsunari, Y., 523
Matsuno, R., 362
Matsuo, H., 163, 541, 560, 564
Matsuo, Y., 527
Matsuoka, T., 526
Matsushima, A., 191
Matsushita, K., 36
Matsutani, H., 2
Matsutomo, K., 340
Matsuura, S., 283, 408
Matsuura, Y., 3, 228, 229
Matsuyama, M., 8
Matsuzaki, M., 28, 411
Matthews, B. W., 231, 232, 242
Matthews, C. N., 8
Matthews, D. A., 241, 281
Matthews, E. W., 539
Matthews, J. B., 256
Matthews, K. S., 175
Matthews, R., 287
Mattice, W. L., 302
Mattingley, P. G., 449
Matucha, M., 31
Matwiyoff, N. A., 263, 279, 281, 551
Matzen, P., 527
Mauch, H., 39
Maury, P., 32
Mawatari, K., 311, 337
Mawhinney, T. P., 77
Maxfield, F. R., 21, 246, 257, 293, 435
Maxwell, J. C., 296
May, L., 325
Mayer, F., 73
Mayer, H., 73
Mayer, R., 275, 276
Mayer, R. J., 271
Mayer, R. T., 49
Mayly, W. L., 39
Mayre, W., 302, 389, 453
Mayumi, T., 34
Mazarguil, H., 563
Mazo, A. M., 271
Mazza, F., 411
Mazzaferri, E. L., 534
Mazzarella, L., 121, 276
Mazzei, Y., 121
Mazzini, A., 30, 351
Mazzotta, D., 405
Meadows, M. D., 289
Means, G. E., 155, 383
Mecham, R. P., 4
Meddis, D., 474
Medzihradszky, K., 558
Mee, J. D., 511
Megaw, J., 175
Meguro, T., 539
Mehrabian, M., 171
Mehta, S. K., 519
Mehta, V., 519
Mei-Chang, K., 279, 280
Meienhofer, J., 381, 387, 436
Meier, L. K., 2
Meineke, G., 521
Meinwald, Y. C., 278, 293, 435, 575
Meissner, G., 21
Meister, A., 51
Meites, J., 559
Meitzner, E. P., 407, 462
Meizel, S., 89
Mekhjian, H. S., 533
Melchiorri, P., 127, 558
Melissinos, K., 519
Mellet, M., 35
Melmed, S., 524
Menchali, M., 73
Mendelsohn, G., 530
Mendelsohn, J., 543
Mendelson, R., 360
Mendlowski, M., 410, 434
Menegatti, E., 279, 404, 562
Mentzer, W., 169
Menzel, E. R., 346
Meot-Ner, M., 21
Meraldi, J. P., 430
Merchant, F. W., 510
Mercier, J.-C., 145, 150
Mercola, D., 258, 329
Meredith, G. S., 51
Meredith, M., 63
Merle, M., 258, 541
Mernet, M., 264
Merret, M., 125, 237
Merrien, M. A., 418
Merrifield, R. B., 33, 373, 383, 384, 385, 387, 388
Merril, C. R., 103
Merrill, A. H., 84
Mertens, M. L., 282, 347, 355
Mery, J., 411
Mescher, M. F., 60
Mesecke, S., 98
Meshalkina, L. E., 189
Mess, B., 201
Messenger, L. J., 51
Messripour, M., 33
Mestechkina, N. M., 63
Mester, L., 276
Mester, M., 276
Mesyanzhinov, V. V., 249
Metcalf, B. W., 13
Metcalf, G., 464
Metsikkö, K., 60
Metz, P., 171
Metzger, H., 60
Meuth, J. L., 133
Mevarech, M., 518
Meyer, H., 181
Meyer, P., 552
Meyer, T. E., 133, 134
Meyers, C. A., 469, 512, 533
Meyers, M. L., 30
Mezey, P. G., 22
Mezo, I., 466
Mezzetti, G., 59
Mich, T. F., 406
Michalski, C. J., 187
Michel, A., 277
Michel, F., 528
Michel, G., 425
Michel, S., 102
Michelson, A. M., 344
Michniewicz, J., 500
Mickelson, K. E., 83
Midwinter, G. G., 125
Mielczarek, E. V., 321
Mighell, A. D., 18
Miglécz, E., 562, 579
Mignucci, G., 409
Miguel, A. G., 54
Mihashi, K., 363
Mikairi, S., 167
Mikawa, T., 153
Mikes, O., 392
Miki, M., 363
Mildvan, A. S., 282, 285
Miler, M. P., 264
Milgrom, E., 150, 317
Milgrom, L. R., 358
Milhaud, G., 258, 541
Militello, G., 27

Millan, J., 399, 468
Miller, A., 254
Miller, C. H., 482
Miller, D. M., tert., 157, 240
Miller, D. R., 347
Miller, F. C., 36
Miller, G. H., 220
Miller, J. R., 223
Miller, L. J., 515
Miller, M. J., 339, 449
Miller, P. R., 280
Miller, R. D., 159
Miller, R. J., 559
Miller, R. L., 49, 57, 175
Miller, R. S., 61
Miller, S. L., 247, 258
Miller, T. A., 523
Miller, T. W., 444
Miller, W. H., 34, 49
Mills, F. C., 336, 337
Mills, J. B., 479
Milstein, C., 269
Milton, J. G., 344
Minaire, Y., 526
Minami, N., 219
Minasian, E., 293, 435
Minato, S., 185
Mincey, T., 321
Minematsu, Y., 20, 381
Mineyev, A. P., 316
Minick, S., 455, 478, 543, 562
Minotani, N., 173
Minster, D. K., 458
Minter, D., 161
Minton, A. P., 189
Mintz, G., 58
Miozzari, G., 481
Mirai, A., 185
Mironov, A. F., 404
Mironova, G. M., 221
Miroshnikov, A. I., 428
Misharin, A. Y., 173
Mishima, S., 15
Mishina, M., 51
Misina, I., 551
Misin'sh, I. P., 472
Misinya, I. P., 403
Misra, R., 34
Misuraca, G., 3
Mita, K., 302
Mitamura, S., 9
Mitani, K., 373
Mitchell, A. R., 387
Mitchell, G., 346
Mitchell, S. J., 515, 530, 531
Mitchell, W. M., 62, 475
Mitchinson, C., 265
Mitra, S., 198
Mitsuda, Y., 10
Mitsui, T., 251
Mitsui, Y., 232
Mitaal, C. M., 57
Mitterer, R. M., 23
Mitzlaff, M., 8
Mitznegg, P., 515, 531, 535
Miura, C., 406
Miura, H., 36
Miura, R., 153
Miura, T., 424
Miwa, M., 21, 301, 536
Miwa, T., 536
Miyadera, T., 448
Miyagawa, H., 406
Miyairi, S., 153, 167
Miyakawa, M., 362
Miyake, Y., 95
Miyano, O., 377, 378
Miyata, K., 473
Miyazaki, M., 301
Miyazawa, T., 10, 19, 165, 253, 278, 280, 283, 291, 412, 451, 457, 575, 576
Miyokaura, T., 177
Miyoshi, A., 514
Miyashi, E., 8
Miyoshi, M., 5, 11, 14, 15, 428
Mizuide, M., 297
Mizuno, K., 20, 175
Mizuno, T., 65, 111
Mizuno, Y., 40
Mizunuma, H., 53
Mizushima, S., 100, 177
Mizutani, A., 61
Mizutani, J., 61
Mkrtchyan, A. P., 292
Mochtar, I. A., 81
Modlin, I. M., 515, 531, 536
Modyanov, N. N., 185
Moe, J. G., 43
Möhring, J., 106, 487
Moelter, W., 131
Moellmann, G., 473
Moersch, G. W., 406
Moeschler, H. J., 411
Moews, P. C., 258
Moffat, K., 225, 226
Moffler, M. M., 51
Moh, P. P., 297
Mohanakrishnan, P., 22, 274
Mohlenstedt, M., 465
Mohr, P., 39
Moinier, D., 121
Molineux, I., 243
Mollica, G., 519
Molloy, K., 324
Molnar, J., 83
Molter, M., 431
Momany, F. A., 574
Monaco, H. L., 243
Monahan, J. W., 522
Monastyrskaya, G. S., 404
Monet, J. D., 543
Monges, A., 515
Monnerie, L., 346
Monnot, M., 300
Monod, J., 259
Monoi, H., 280
Monsigny, M., 344, 356
Moňstěk, J., 175
Montagu, M. C., 136
Montanari, F., 13
Montanaro, L., 361
Montarani, G., 121
Montecucco, C., 202
Montenay-Garestier, T. H. C., 275, 344
Monti, J. P., 30, 290
Mooberry, E. S., 460
Moody, A. J., 510, 531
Moonen, P., 148, 202
Moo-Pen, W. F., 34, 118, 340
Moore, C. H., 125, 196
Moore, G. J., 403, 554
Moore, P. B., 187, 290
Moore, R. Y., 543
Moore, S., 410, 405, 517
Moore, T. A., 159
Moore, W. T., jun., 482
Mopper, K., 34
Moran, D. M., 405
Moran, J. M., 405
Moran, L. A., 75
Moran, R. G., 97
Moran, T. F., 196
Morehouse, K. M., 31
Moreira, M. A., 146
Morel, A. F., 441
Morera, A. M., 532
Moretti, S., 77
Moretto, V., 400
Morgan, B. A., 464, 574
Morgan, D. J., 194
Morgan, F. J., 140
Morgan, G. J., 386
Morgan, K. C., 2
Morgan, L. M., 533, 534
Morgan, M. R. A., 64
Morgan, P. H., 507
Morgan, R. S., 247, 258
Morgat, J. L., 518
Morgenstern, E., 521
Morgenstern, R., 169
Mori, H., 527, 532
Mori, K., 380, 536
Mori, N., 153
Mori, S., 448
Mori, Y., 81
Moriguchi, M., 2
Morihara, K., 393, 394, 498, 500
Moriike, K., 183
Morikawa, S., 314
Morikawa, T., 539
Morimoto, H., 151
Morin, P., 289
Morin, R. B., 444
Morino, Y., 121, 153, 169
Morishima, H., 402, 451
Morishima, I., 287
Morita, S., 523
Morita, T., 89
Morita, Y., 319
Morito, N., 376
Moritoki, H., 382
Morley, J. E., 524

Morley, J. S., 15, 403, 464, 517, 559
Mornet, D., 179
Moroder, L., 374, 514, 518, 520, 525
Moroder, Z., 375
Morokuma, K., 121
Morosetti, S., 436
Morozova, L. G., 399
Morrill, M. E., 53
Morris, C. F., 75
Morris, D., 167
Morris, G. S., 2
Morris, H., 503
Morris, H. R., 122
Morris, J. A., 481
Morris, J. I., 155
Morris, J. M., 350, 362
Morris, M., 61, 115, 329
Morris, S. C., 81, 139
Morrisett, J. D., 251, 403
Morrison, C. A., 290
Morrison, I. E. G., 324
Morrison, R. J. S., 362
Morrow, C., 431
Morrow, J. S., 280, 452
Morten, D. J., 251
Morton, G. O., 461
Mosbach, K., 39, 196
Moscarello, M. A., 289
Moscovitz, A., 317
Moseley, M. H., 479
Moser, P. W., 34, 118
Moses, S., 39
Mosoda, H., 167
Mosolov, V. V., 308
Moss, D. S., 244
Moss, J., 55
Moss, S., 523
Motherwell, W. D. S., 223
Moule, M. L., 394, 502
Moult, J., 224
Mount, C. D., 105
Moura, I., 134
Mouw, D. R., 548
Movesyan, S. G., 306
Mowery, R. L., 316
Moyazawa, T., 284
Moyer, R. W., 75
Moyle, W. R., 546
Mozzi, R., 34
Mrevlishvili, G. M., 291
Mroz, E. A., 557
Mryanishi, T., 179
Mucchielli, A., 55
Muccio, D. D., 315
Muchmore, D. C., 197
Muchlemann, M., 34
Muehlethaler, K., 296
Mühlmann, H. P., 331
Müller, F., 53, 357
Mueller, G. C., 64
Mueller, K., 523
Müller, M., 85
Müller, R. K. M., 404, 521
Mueller, U. W., 102
Müller-Eberhard, H. J., 81, 139
Müller-Eberhard, U., 109
Münck, E., 319, 323
Muggeo, M., 500
Muirhead, H., 238
Mukaiyama, T., 376
Mukerjee, A. K., 377, 442
Mukerji, S. K., 89
Mukherjee, S. P., 153
Mukkur, T. K. S., 332
Mukohata, Y., 163
Mulac, B., 406
Mulder, E., 66
Mullen, E., 159
Muller, M., 473
Muller, T., 26
Muller, W. E., 155
Mulligan, R. C., 548
Mun, S. K., 322
Munch, J. D. B., 155
Munday, J. S., 22
Muneaux, C., 399, 468
Muneaux, Y., 399, 468
Munekata, E., 427, 539
Munier, R. L., 34
Muniyappa, K., 39
Munro, H., 530
Munro, I., 221, 264
Munro, I. H., 344, 354
Munson, B. R., 187
Murachi, A., 53
Murachi, T., 339
Murad, F., 57
Murai, A., 31, 119, 148, 440
Murakami, K., 17, 42
Murakami, M., 448
Murakami, W. T., 248
Murakami, Y., 435
Muramor, A. V., 185
Muramoto, K., 127, 470, 473
Murao, S., 450
Muraoka, Y., 458, 459, 460
Murate, H., 169
Murphy, D. B., 108
Murphy, P. T., 417
Murphy, R. C., 17, 31
Murphy, R. F., 510, 526
Murphy, W. A., 469
Murray, P., 531
Murray, R. K., 289
Murray, T. M., 195, 545
Murray-Rust, P., 223
Musha, S., 392
Mushens, J., 538
Mushika, Y., 14
Musick, W. D. L., 124, 239
Muszynska, G., 306
Mutopo, D. S., 155
Mutt, V., 127, 128, 467, 513, 515, 517, 520, 526, 528, 538
Mutter, H., 314, 402
Mutter, M., 292, 303, 314, 370, 379, 389, 402
Mutulis, F., 551
Muzaffar, S. A., 545
Myagkova, M. A., 121
Myasnikov, A. N., 121
Myrmel, K. H., 81

Nabedryk-Viala, E., 470
Naber, S., 499
Nabeshima, Y., 140
Nachtmann, F., 392
Nadelman, A., 448
Nader, W., 57
Naderi, S., 33
Nadezhdin, A., 171
Nadler, H. L., 58, 93
Nadolska, B., 411
Naegeli, H. U., 424
Näslund, L., 100
Naether, M., 345
Nafie, L. A., 21, 299, 300
Nafrady, J., 517
Nagai, K., 84, 526
Nagai, M., 311, 337
Nagai, S., 410
Nagai, U., 21, 278, 301, 425, 428, 575, 576
Nagai, Y., 100
Naganawa, H., 402, 451, 458, 459
Nagano, M., 448
Nagaoka, S., 290
Nagaraj, R., 276, 297, 411, 453, 573
Nagasawa, H., 418
Nagasawa, T., 54
Nagasawa-Fujimori, H., 54
Nagase, O., 397
Nagata, C., 276
Nagata, K., 36, 125, 181
Nagata, W., 444, 448
Nagayama, K., 273, 274, 282, 355
Nagel, R. L., 340
Nagelschmidt, M., 161
Nagura, M., 291
Nagy, A., 159
Nagy, S., 31
Nahm, H. S., 482
Naide, A., 181
Naider, F., 256, 267, 275, 303, 410
Naik, S. R., 519
Nair, C. M., 456
Naithani, V. K., 394, 498, 504
Najjar, V. A., 279, 387, 408
Nakache, M., 163
Nakagawa, M., 30
Nakagawa, Y., 564
Nakaguchi, O., 445
Nakai, S., 98, 111, 327
Nakai, T., 26
Nakajima, A., 254, 276, 294, 409
Nakajima, B., 409
Nakajima, M., 26, 439
Nakajima, T., 127, 558

Nakame, S., 526
Nakamori, H., 61, 101, 153
Nakamoto, N., 403
Nakamura, A., 6
Nakamura, H., 33, 459
Nakamura, I., 9, 11
Nakamura, K., 38
Nakamura, K. T., 232
Nakamura, M., 395, 470, 473, 476, 543
Nakamura, S., 148, 165, 291, 297, 382
Nakamura, T., 153, 335
Nakane, A., 165
Nakanishi, M., 29
Nakanishi, S., 475, 476, 543
Nakano, A., 284, 291, 435
Nakano, K., 448
Nakashima, S., 157
Nakashima, T., 274
Nakashima, Y., 199
Nakata, H., 53, 189
Nakata, Y., 403
Nakatani, H., 362
Nakatani, T., 458, 459
Nakataya, S., 532
Nakayama, K., 1
Nakayama, R., 61
Nakayama, T., 49
Nakazawa, T., 542
Nakhla, A. M., 542
Nalewaik, R. P., 107
Namba, K., 252
Nambara, T., 3, 153, 167
Namiki, M., 25
Nanasawa, M., 410
Nandi Majumdar, A. P., 519
Nannini, G., 448
Narahashi, Y., 87
Narai, G., 524
Narang, S. A., 500
Narasaki, Y., 518
Narasinga Rao, M. S., 169
Narayanan, R., 364
Narisada, M., 444
Narita, K., 133, 163, 539
Narita, M., 390
Naruse, H., 189
Nash, C. P., 19
Nashef, A. S., 4, 179
Nassal, M., 378, 435
Natarajan, S., 528
Natarajan, V., 78
Nath, A., 318
Nathan, M. M., 500
Nathans, A., 512
Nathanson, N. M., 109
Nathenson, S. G., 41, 140
Natsume, S., 448
Nau, H., 119
Naude, R. J., 470
Naumann, K., 2
Naumski, R., 461
Navalesi, R., 501
Nave, C., 248
Navon, G., 357
Nawrocka, E., 404, 408, 573
Nayyir-Mazhir, R., 285
Nazaikinskii, V. E., 308
Nazar, R. N., 149
Nealon, D. A., 47
Near, J. A., 155
Neave, E. J., 60
Nebelin, E., 407
Nebert, D. W., 99
Nedkov, P., 98
Nedopil, N., 579
Negishi, M., 67
Negri, D. J., 195
Negri, L., 558
Neher, R., 539
Neidle, S., 250
Neilands, J. B., 324
Neireiter, G. W., 179, 394
Nekola, M. V., 466
Nelles, L. P., 107
Nelson, D. J., 289
Nelson, K., 351
Nelson, S. D., 518
Nelson, T. E., 33
Nemethy, G., 256, 267, 293, 435
Nemetschek, T., 254
Nemetschek-Gansler, H., 254
Neri, N., 278
Nernethy, G., 22
Nesbit, M. E., 548
Nesheim, M. E., 81, 364
Ness, G. C., 51
Nesterov, V. P., 169
Nestor, J. J., jun., 485
Netzel, E., 123
Neu, H. C., 446
Neuberger, M. S., 63
Neuhauser, M., 32
Neuhoff, C., 518
Neuhoff, V., 98, 103
Neupokoeva, E. S., 6
Neurath, H., 87, 122, 124, 137, 145, 161
Neurohr, K. J., 30, 275
Neville, R. W. J., 504
Nevinsky, G. A., 191
Newall, C. E., 443, 447
Newcomer, M. E., 157, 240
Newmark, P., 365, 499
Newton, M. G., 19
Nexø, E., 69, 127, 538
Neya, S., 287
Nezlin, R. S., 173, 361
Nguyen, N. Y., 100
Niac, G., 17
Niada, R., 41, 538
Niall, H. D., 365, 539, 543, 545
Nibley, D. A., 347
Niccolai, N., 20, 264
Nice, E. C., 61
Nichol, L. W., 328
Nicholas, J. D., 244, 256
Nichols, B. P., 75
Nicholson, G. J., 32
Nicholson, W. E., 105
Nickles, R. J., 16
Nicholson, G. J., 392
Niclause, M., 348
Nicolaides, E. D., 406
Nicolaidou, P., 26
Nicolas, J. C., 165
Nicoll, C. S., 473
Nicolson, W. E., 475
Nicosia, S., 531
Nicot, C., 149
Nieburgs, A. C., 289
Niedrich, H., 558
Nief, F., 462
Nielsen, A. H., 102
Nielsen, N. C., 146
Nielson, A. J., 24
Nierhaus, K. H., 71
Nieto, J. L., 293
Nieuwenhuizen, W., 83
Nieuwenhuysen, P., 98
Niewiarowski, S., 89, 189
Niggli, V., 70
Nihei, J., 531
Nihel, K., 144
Nihira, T., 191
Nii, Y., 12
Nikai, S., 362
Nikaido, H., 251
Nikiforov, S. A., 185
Nikiforovich, G. V., 256, 496
Nikkilä, E. A., 97
Nikodem, V., 109
Nillus, S. J., 465
Nilsson, G., 31, 127, 517, 529, 530
Nisard, R. J. F., 266
Nishi, N., 254, 401, 409
Nishibu, M., 311, 337
Nishida, T., 101, 153
Nishigori, H., 181
Nishikawa, H., 428
Nishikawa, K., 261
Nishikawa, S., 75
Nishikaze, O., 271
Nishimoto, M., 34
Nishimoto, Y., 53
Nishimukai, H., 45
Nishimura, H., 191
Nishimura, J. S., 187
Nishimura, O., 470
Nishimura, S., 4, 253, 380
Nishina, Y., 313
Nishino, N., 85
Nishio, S., 20
Nishioka, A., 20
Nishira, H., 103
Nishitami, T., 14
Nishitani, Y., 448
Nishiya, T., 310
Nishizuka, Y., 49
Nisonoff, A., 140
Nitta, K., 185, 193
Niu, C. H., 275, 283, 408, 431, 433

Nivard, R. J. F., 393, 419, 471, 521
Nix, P. T., 266, 393
Noda, L. H., 51
Noda, M., 31
Noda, T., 539
Noe, B. D., 513
Noelken, M. E., 100
Noguchi, C. T., 287
Noguchi, J., 254, 409
Noguchi, K., 526
Nohira, H., 17
Nokihara, K., 399
Noltes, J. G., 18
Noltmann, E. A., 313
Noma, A. T., 33
Nomiyama, S., 536
Nomoto, C. S., 455
Nomoto, K., 3
Nomoto, S., 403, 423
Nonaka, K., 428
Nonaka, Y., 380
Noolandi, J., 348
Noordam, A., 29, 155
Noravyan, A. S., 292
Norden, B., 300
Nordling, K., 157
Nordman, C. E., 222
Norne, J. E., 280, 283, 287
Noronha, J. M., 185
Norskov-Lauritzen, L., 19
North, R. A., 559
Northrop, F. D., 149
Nose, M., 524
Nosoh, Y., 167, 173
Notario, V., 93
Novak, F. C., 287
Novak, R. F., 287
Novellino, E., 3
Nowack, C., 465
Nowaczyk, K., 349
Noyes, B. E., 518
Nozaki, M., 125
Nozawa, T., 311, 316, 317
Numa, S., 51, 475, 476, 543
Numata, Y., 17
Nummy, L. J., 447
Nunami, K., 12
Nurse, D., 161, 284
Nute, P. E., 131, 132
Nutt, R. F., 400, 464, 468, 512
Nyeki, O., 402, 517, 554
Nygard, O., 187
Nystroem, L. E., 129
Nystrom, D. M., 403, 554

Oatley, S. J., 229, 261, 263
Obara, M., 404
Obendorf, S. K., 225
Oberley, L. W., 460
Obermeier, R., 499
Oberthuer, W., 132
Obie, J. F., 518
O'Brien, J. S., 91
O'Brien, M. J., 169
O'Brien, P., 301
O'Brien, T. A., 362
Ochoa, J. L., 58
O'Connor, D. V., 347
O'Connor, K., 504
Oda, T., 187, 532
Odagiri, E., 105
Odaguchi, K., 524
O'Dea, M. H., 73
Odham, G., 31
O'Donnell, D. J., 437
O'Donnell, S., 169, 226
O'Dorisio, T. M., 510, 529, 533, 534
Odorzynski, T. W., 191, 267
Odya, C. E., 549
Oehler, E., 1, 12
Oekonomopulos, R., 302, 389, 402, 453
Oelofsen, W., 470
Oertel, R. P., 291
Oesterhelt, D., 364
O'Farrell, P. Z., 189
Ofengand, J., 187
Ofer, S., 323, 324
Offner, G. D., 134
Offord, R. E., 266, 392, 393, 502
Ogata, K., 185
Ogaura, M., 187
Ogawa, H., 28
Ogawa, S., 287
Ogawa, T., 2
Ogawara, H., 66
Ogilvie, A., 175, 201
Ogino, J., 526
Ogita, K., 181
Ogita, Z.-I., 101
Ogura, H., 9, 377
Ogushi, S., 167
Ohara, A., 29
O'Hare, M. J., 61
Ohe, Y., 129, 514
Ohgi, K., 185
Ohk, E., 446
Ohkawa, T., 61
Ohki, A., 523
Ohki, T., 276
Ohlsen, S. R., 401
Ohmae, M., 34
Ohmori, M., 380
Ohms, J. P., 286
Ohneda, A., 531
Ohno, M., 12, 404
Ohta, N., 406
Ohta, T., 26
Ohtaki, S., 167
Ohtsuka, A., 421
Ohtsuka, E., 75
Ohtsuki, M., 332
Ohtsuru, M., 179
Oi, N., 411
Oida, S., 446
Oikawa, K., 308
Oishi, M., 373, 382
Oka, H., 532
Oka, I., 95, 163
Oka, T., 97, 394, 498, 500
Oka, Y., 2
Okabayashi, M., 301
Okabe, K., 457
Okada, H., 1
Okada, K., 21, 410, 444, 448
Okada, M., 38
Okada, Y., 67, 191, 402
Okahashi, K., 292, 409
Okajima, T., 515
Okamoto, K., 470
Okamoto, M., 187
Okamoto, Y., 391
Okamura, M. T., 177
Okamura, Y., 84
Okano, K., 523, 526
Okawa, H., 17
Okawara, M., 391
Okazaki, T., 57
O'Keefe, D. H., 296
O'Keefe, R. B., 59
Okimura, Y., 49
Okinaka, M., 402
Okonjo, K., 169
Oku, T., 445
Okuda, H., 84
Okumura, Y., 423, 440
Okuyama, T., 102
Olah, A. J., 519
Olavesen A. H., 171
Olbe, L., 531
Oldfield, E., 251, 280, 286, 289
O'Leary, M. H., 285
Olefsky, J. M., 503
ole-Moi Yoi, O., 87, 89
Olesker, A., 457
Olgiati, V. R., 405, 542
Olinger, E. J., 531
Oliveira, E. B., 145
Oliver, J. M., 522
Oliver, R. S., 444
Olma, A., 10
Olomucki, M., 198
Olsen, M. O. J., 147
Olsen, O., 2
Olsen, R., 155
Olsen, R. K., 438
Olson, D. C., 34
Olson, J. L., 530
Olson, M. L., 300
Olthof, G. J., 222
Oltmann, L. F., 57
O'Malley, B. W., 181
Omar, S., 284
Omoto, S., 439
Ona, H., 448
Onda, M., 451
Ondetti, M. A., 520, 552, 556
O'Neal, C. C., 353
Onodera, I., 150, 400
Onoue, H., 444
Ontjes, D. A., 539
Onuma, K., 6
Oobatake, M., 261

Ooi, T., 261
Oomura, Y., 515
Oosawa, F., 350, 363
Oosterbaan, M., 419
Opella, S. J., 291
Opmeer, F. A., 472
Oppemann, H., 105
Oppenheim, F. G., 106, 271
Oppenheimer, L., 51
Oppenheimer, N. J., 55
Oratore, A., 271
Orci, L., 510
Ores, C., 333
Orii, Y., 317
Orimo, H., 539, 541
O'Riordan, J. L. H., 543, 544, 545
Orlando, J., 59
Orlek, B. S., 173, 446
Orloff, M. J., 514
Orlowski, R. C., 275
Orme-Johnson, W. H., 281, 323
Oroszlan, S., 101, 137, 338
Orr, H. T., 112, 141
Orth, D. N., 105, 475, 537
Ory-Lavollee, L., 518
Osa, T., 304, 409
Osawa, T., 161, 407
Osborne, W. C., 116, 144
Oscarson, J., 520
Osguthorpe, D. J., 247, 260
Osheroff, N., 163
Oshiro, S., 167
Oste, C., 187
Osterman-Golkar, G., 28
Osterrieth, P. M., 59
Ostwald, T. J., 573
O'Sullivan, J., 449
O'Sullivan, M. J., 167
Otani, M., 539, 541
Otani, S., 313
Otsubo, Y., 25
Otsuka, H., 439, 457
Otsuki, M., 523, 526
Ott, D. G., 16
Otte, M., 523, 524
Otte, S., 533
Ottenheym, H. C. J., 253, 419, 420, 428
Ottensmeyer, F. P., 29
Ottesen, B., 533
Otvos, J. D., 283, 291
Otwell, H. B., 163
Ouchi, R., 3
Oudeman, E. M., 403, 554
Oudet, P., 171
Ouyang, C., 165
Ovádi, J., 338
Ovchinnikov, Yu. A., 144, 253, 288, 437
Owyang, C., 515
Oyama, H., 467
Oyama, K., 380
Ozaki, Y., 5, 11, 15
Ozawa, M., 191
Ozawa, N., 67
Ozols, J., 113, 148

Packer, L., 159
Packman, L. C., 122
Padan, E., 175, 177
Padieu, P., 31
Padron, J. L., 22
Padron, R., 251
Padyukova, N. S., 231
Page, D. L., 81
Pages, M., 289
Pahlman, S., 58
Pai, R. C., 270
Paik, C. H., 29
Pain, R. H., 265, 267, 273
Pais, M., 439, 442
Painter, R. G., 83
Pajula, R.-L., 97
Pal, P. K., 167
Palacek, E., 315
Palacian, E., 185
Palade, G. E., 103
Palank, N. C., 181
Palasciano, G., 527
Palczuk, N. K., 157
Paleus, S., 132
Paleveda, W. J., jun., 400, 512
Palibroda, N., 17
Pallai, P., 465
Palm, D., 285
Palmer, G., 317
Palmer, R. A., 574
Palmerini, C. A., 34
Palmiter, R. D., 136
Palmour, R. M., 564
Palumbo, M., 276, 292
Palva, E. T., 159, 177
Palzic, M., 311
Pambrun, C., 346
Pampeno, C., 155
Pancoska, P., 299, 303, 422
Pande, C. S., 195, 369, 389
Pandey, R. C., 34, 452
Pandey, R. K., 378
Panella, C., 519
Pang, C. C. Y., 487
Pang, P. K. T., 548
Panigel, R., 357
Pantaloni, D., 342
Pantel, P., 179
Paolillo, L., 290
Papaefthymiou, G. C., 323
Papamichail, M., 177
Papapoulos, S. E., 543
Papazian, D., 66
Papkoff, H., 175, 482, 483
Paquin, R., 32
Paradies, H. H., 339
Paradisi, M., 411
Paradiso, P. R., 199
Parak, F., 321, 324
Pardos, B. T., 91
Parello, J., 289, 411
Parfett, C. L., 185
Parham, P., 60, 141
Parkar, M., 244
Parker, C. W., 69
Parker, D. M., 354
Parker, K. L., 105
Parker, T. L., 41
Parkhurst, L. J., 335
Parlow, A. F., 480
Paroutaud, P., 144
Parr, D. M., 144
Parr, G. R., 263
Parsons, J. A., 545
Parsons, P., 53, 67
Parsons, R. G., 103
Parsons, S. M., 122
Parsons, T. F., 175, 189, 277, 483
Parthasarathi, V., 219
Parthasarathy, R., 27, 456
Parthasarathy, S., 219
Parthemore, J. G., 543
Pasaribut, S. J., 406
Pascal, M., 73
Pascard, C., 574
Pascher, I., 251
Pasero, L., 121
Pasini, A., 9
Passarella, S., 157
Pasta, P., 41, 335, 538
Patel, L., 175, 177
Patel, R. N., 45
Patel, V. B., 53
Pater, A., 54
Paterson, S. J., 123, 577
Patrick, G. L., 420
Patrick, P., 123
Patterson, D., 60
Patterson, R. A., 63
Patthy, L., 155
Patton, G., 512
Pattus, F., 181
Patzelt, C., 510
Paucker, K., 77
Paude, C. S., 410
Paul, B., 27
Paul, C. H., 43
Pauling, L., 245, 256
Paulson, J. C., 93
Pavlovskii, A. G., 231
Pavone, V., 19, 292
Pawelczak, K., 411
Pawlikowski, M., 299
Paxton, R. J., 358
Payan, F., 133, 228
Paynovich, R. C., 399
Peach, M. J., 556
Peacock, D., 134
Peacock, S. C., 18
Peak, M. J., 175
Peale, A. L., 32
Pearce, C. J., 458
Pearlstein, E., 137, 165
Pearse, A. G. E., 513, 518, 529, 543
Pearson, L. J., 194

Pearson, R. H., 251
Pease, L. G., 275, 431, 433
Pechere, J. F., 289
Pecht, I., 171, 221, 264, 268, 290, 311, 354
Pecile, A., 405, 542
Pedersen, C. A., 498
Pedersen, P. L., 61
Pederson, R. A., 514
Pederson, T., 324
Pedersen, T. G., 146
Pedersen, V. B., 112, 122
Pedigo, N. W., 579
Pedone, C., 19, 253, 292
Pedrocchi-Fantoni, G., 17
Pedroza, E., 465, 466
Peeters, T. L., 536
Peggion, E., 276
Pehlevanian, M. Z., 538
Peikin, S. R., 523
Peillon, F., 531
Pellogrino, M. A., 142
Pellet, H., 97
Pelletier, M. J., 346, 526
Pelzig, M., 195, 369
Penin, F., 65, 157
Penke, B., 517, 518
Pennathur-Das, R., 169
Penniston, J. T., 70
Penny, G. S., 191
Pepys, M. B., 61
Percy-Robb, I. W., 110
Perdrisot, R., 561
Pereira, L. C., 350
Pereira, M. E. A., 91
Perera, J., 315
Perez-Infante, V., 465
Perham, R. N., 123, 125, 159, 183
Perkins, J. P., 66
Perkins, L. M., 11
Perkins, S. J., 230, 279
Perkone, I. K., 367
Perlman, J., 264, 361
Perlow, M. J., 542
Permutt, A., 467
Permyakov, E. A., 352, 353
Perone, S. P., 119
Perrauchin, J.-P., 394
Perrella, M., 334
Perret, B. A., 103
Perret, G., 515
Perreult, R. A., 163
Perrin, J. H., 309
Pershadsingh, H. A., 503
Pert, A., 577
Pert, C. B., 531, 577
Perutz, M. F., 225, 336
Pestaña, A., 73
Pestka, S., 61
Peterfreund, R. A., 95
Peterman, B. F., 30, 351
Petermann, C., 471
Peteron, S. M., 480
Peters, D., 256
Peters, J., 256
Peters, J. R., 161
Peters, M. J., 66
Peters, N., 256
Petersen, H., 528
Petersen, H. U., 187
Petersen, O. H., 524
Petersen, R. L., 282, 286, 318
Petersen, S. B., 19, 274
Peterson, E. A., 62
Peterson, G. L., 98
Peterson, J. A., 296
Peterson, M. A., 19
Peterson, P. A., 69, 112, 141, 149
Peterson, W. J., 569
Peticolas, W. L., 261
Petit, A., 30
Petitou, M., 407
Petrillo, E. W., jun., 410
Petrusek, R., 250
Petsko, G. A., 225, 261, 264
Pettersson, G., 530
Pettit, G. R., 5, 20, 406, 410
Peypoux, F., 21, 425
Peytremann, A., 546
Pfaender, P., 20
Pfaendler, H. R., 446
Pfeiffer, B. H., 249
Pfeil, H., 51
Pham Van Chuang, P., 518
Phan, D., 104
Philip, M., 99
Philipp, D. P., 53
Philips, M., 171
Philips, T. J., 320
Phillip, K., 98
Phillips, A. W., 123, 125, 237, 264
Phillips, D. C., 221, 229, 230, 261, 263
Phillips, E. L., 519
Phillips, G. N., jun., 250
Phillips, M., 476
Phillips, W. C., 248
Phillis, J. W., 515, 536
Philpot, R. M., 272
Philpott, H. G., 524
Philson, S. B., 286
Phipps, S. A., 24
Pho, D. B., 171
Photaki, I., 407, 413
Photos, E., 21
Piatelli, M., 2
Picciolo, G. L., 347
Pick, U., 159
Pickering, B. T., 472
Pickover, C. A., 264
Picot, D., 286
Pierce, J. G., 175, 189, 277, 314, 482, 483, 484
Pierce, M., 51
Pieronczyk, W., 6
Pierrot, M., 133, 228
Pietrzik, E., 314
Piez, K. A., 330
Pifat, G., 287
Pigram, W. J., 249
Pike, J. W., 69
Pilati, T., 222
Pilbeam, D. J., 2
Pilch, P. F., 197
Pillai, R. P., 282
Pillai, S., 187, 505
Pillai, V. N. R., 389
Piloty, M., 25
Pinas, N., 558
Pincus, M. R., 231
Pinget, M., 521, 522
Pinkus, G. S., 87
Pino, P., 302, 391
Pinset-Härström, I., 333
Pintor-Toro, J. A., 185
Piotrowska, K., 15
Piper, E. A., 281
Pipkorn, R., 378, 398, 435, 498, 500
Pirie, N. W., 37
Piriou, F., 275, 300, 421, 430
Pirkle, W. H., 35
Pisano, J. J., 33, 87, 113
Pisano, M. A., 449
Piszkiewicz, D., 43, 122
Pitcher, R. G., 271, 279
Pitha, J., 38
Pittman, R. C., 194
Pivcova, H., 276
Piyachurawata, P., 466
Pizzarello, S., 35, 111
Planck, S. R., 73
Plapp, B. V., 69, 185, 354
Plech, A., 404, 573
Pless, J., 396, 569
Plessier, J., 520
Pletnev, V. Z., 253, 437
Ploegh, H. L., 141
Ploegman, J. H., 241
Plucinska, K., 402, 555
Pluijms, W., 131
Pluscec, J., 25
Pluzhnikova, G. N., 399
Podack, E. R., 81
Podgornova, N. N., 366
Podgorski, M., 15
Podhradsky, D., 28
Podjarny, A. D., 249
Podkoscielny, W., 15
Podolsky, D. K., 55
Podolsky, R. J., 250
Poduska, K., 492
Poe, M., 281
Poglazov, B. F., 288
Pohl, S. L., 511
Poilleu, W. N., 341
Poisel, H. 1
Pokrić, B., 98
Polak, J. M., 513, 518, 529, 530, 535
Polevaya, L. K., 407
Polganov, G. M., 404

Policastro, P., 476
Polimeno, L., 519
Poljak, R., 223
Poljak, R. J., 143
Pollack, R. M., 173
Pollack, S., 99
Pollara, B., 83
Pollet, R. J., 98, 325
Pollock, G. E., 8
Polonsky, J., 418
Polosatov, M. V., 520
Pommerening, K., 39
Pommier, J., 41
Pon, C. L., 146
Pongor, S., 33
Pongs, O., 187
Ponnamperuma, C. 1
Ponnell, R., 79
Ponnuswamy, P. K., 258
Ponstingl, H., 150
Pont, A., 546
Ponzi, D. R., 447
Poole, J. A., 349
Popov, E. M., 256, 293, 452
Popov, V. O., 167
Popovic, Z. D., 346
Poppke, D. C., 330
Porath, J., 37
Porcellati, G., 34
Poretz, R. D., 91
Porter, G., 345, 358, 362
Porter, J. C., 530
Portner, A., 179
Porubcan, M. A., 183, 284
Porunkevich, E. A., 472
Poser, J. W., 194
Posner, B. I., 503
Posner, G. H., 359
Posner, J. B., 579
Potter, J. D., 361
Potter, J. M., 102
Potter, M., 142, 144
Pottle, M. S., 293, 435
Potts, J. T., jun., 539, 542, 543, 544, 545, 546, 547, 548
Poucher, R., 503
Pougeois, R., 157
Poulas, C. P., 558
Poulin, G., 33
Poulsen, C., 126
Poulsen K., 102
Pousette, Å., 55
Powell, D., 521
Powell, H. R., 548
Powers, D. A., 124
Powers, J. C., 85, 196
Powers, S. P., 21
Powls, R., 169
Poy, P., 519
Poyarkova, S. A., 405
Poyart, C., 337
Pozdnev, V. F., 366
Pozdnjakov, V. A., 187
Poznansky, M. J., 191
Prabhakaran, M., 258
Pradayrol, 467, 521, 524
Pradel, L. A., 171
Prager, E. M., 104, 124
Prahl, J. W., 81, 139
Praissman, M., 524
Prange, A. J., jun., 498
Prangé, T., 574
Prasad, B. V. V., 411
Prasad, M. R., 57
Prasad, R., 147
Prashanth, D., 255
Pratt, R. S., 65
Préaux, G., 147
Preels, J. P., 173
Premilat, S., 278, 348, 555, 574
Prendergast, F. G., 358, 364
Pressman, D., 149, 173
Prestayko, A. W., 147
Prestidge, R. L., 63
Price, B., 341
Price, N. C., 163
Price, P. A., 194
Prick, P. A. J., 419
Prieels, J. P., 282
Prieto, J. C., 528, 531
Primrose, S., 191
Primalov, P. L., 268, 438
Privat, J. P., 30, 350
Próchniewica, E., 153
Prockop, D. J., 309
Pronzato, P., 515
Propst, F., 514
Prosmanne, J., 574
Prota, G., 3
Protas, J., 18
Provost, K. L., 270
Pruetz, W. A., 25
Prusakov, A. N., 405, 517
Prusakov, V. E., 325
Przybylska, M., 420
Przybylski, M., 518
Psaro, R., 9
Ptak, M., 256, 421
Ptitsyn, O. B., 247, 260
Pučar, Z., 98
Pucci, P., 121
Pueppke, S. G., 91
Puett, D., 308
Puijk, W. C., 125, 181
Purich, D. L., 341, 342
Puszkin, S., 333
Putnam, F. W., 142, 143, 145
Pyerin, W., 42
Pytasz, G., 297

Qasim, M. A., 179
Quagliariello, E., 157
Quarles, R. H., 103
Quigley, G. J., 249
Quik, M., 531, 532
Quinn, J. E., 244, 256
Quinn, M. J., 562
Quiocho, F. A., 44, 157, 240, 241
Quiviron, C., 35
Raabe, A. B., 4, 460
Raba, M., 4, 95
Raba, R. 95
Rabbani, L. D., 179, 356
Rabenstein, D. L., 274, 278, 279, 575
Rabinowitz, J. C., 75
Rachmilewitz, E. A., 324
Racker, E., 159
Rackur, G., 383, 408, 557
Racusen, D., 103
Radda, G. K., 169
Radding, C. M., 73
Radding, J. A., 403, 551
Radhakrishna, A. S., 437
Radzyner, M., 533
Rae, I. D., 277, 293, 435
Rafecas, L., 383
Rafferty, B., 545
Rafferty, M. A., 200
Raftell, M., 271
Rafter, G. W., 175
Rafter, J. J., 31
Raftery, M. A., 144, 151
Rafto, S., 67
Ragan, C. J., 338
Raggi, A., 155
Raghavan N. V., 222
Raghavendra, K., 257
Raghow, 179
Ragnarsson, U., 123, 378
Rahamimoff, H., 66
Rahman, R., 419
Raikov, Z. D., 191
Raina, A., 97
Rainer, F., 282
Raizes, G. S., 535
Rajagoplan, K. V., 191
Rajananda, V., 4, 460, 461
Rajaniemi, H., 60
Rajh, H. M., 15, 521
Rajoria, D. S., 318
Ralston, E., 275
Ramachandran, J., 470, 472, 474, 482, 546
Ramachandran, K. L., 401, 517
Ramachandran, N., 191, 356
Ramage, R., 371, 401, 405, 517
Ramage, W. I., 28, 373, 381
Ramakrishnan, C., 431
Ramaley, R., 57
Ramalingam, K., 437
Ramani, R., 421
Ramponi, G., 153
Ramsay, N. K. C., 548
Rance, M. J., 464
Randall, E. W., 424
Randle, P. J., 125
Rand-Meir, T., 122
Randoux, A., 33
Ranganayaki, S., 8
Ranieri, G. A., 281
Ranieri-Raggi, M., 155
Rao, A., 177
Rao, A. J., 395, 471, 473

Rao, B. D. N., 282
Rao, C. N. R., 297
Rao, C. P., 297
Rao, D. N., 142
Rao, G. S. J., 39
Rao, K. K., 317
Rao, N. A., 39
Rao, N. V., 304
Rao, S. T., 18
Rao, T. N., 24
Rao, V. S. R., 24, 256, 462
Rapaka, R. S., 304, 432
Raper, J. H., 123
Rapoport, H., 441, 442
Raptis, S., 510
Rascati, R. J., 67
Rascher, W., 556
Rashetova, O. S., 5
Rashin, A. A., 247, 258
Rask, L., 69, 112, 141, 149
Rasmussen, J. R., 449
Rastetter, W. H., 419
Ratcliffe, R. W., 444
Rathnam, P., 165, 175, 482
Rattner, E. C., 102
Raulais, D., 539, 541
Rauterberg, J., 135
Ravazzola, M., 510
Ravestein, P., 149
Rawitch, A. B., 181
Rawlings, J., 281, 317, 318, 323
Rayford, P. L., 514, 515, 518, 533
Raymond, J., 87
Raymond, K. N., 420, 424
Rayner, D. M., 350, 351
Razjwin, A. P., 364
Re, L., 5
Read, A. E., 522
Reading, C., 173
Reagan, C. R., 479
Rearick, J. I., 51, 93
Reba, R. C., 29
Reboud, J.-P., 102
Reboul, A., 60, 81
Rebstock, M. C., 406
Recant, L., 469
Recasens, M., 55
Recktenwald, D., 201
Records, R., 317
Redding, T. W., 469
Reddy, B. S., 249, 250
Reddy, E. S. P., 89
Reddy, S. R. R., 163
Redfern, P. H., 34
Redfield, A. G., 282
Redhardt, A., 157
Redman, B., 101
Redman, L. W., 403, 551
Reeck, G. R., 71
Reed, C. A., 320
Reed, J., 310
Reed, J. D., 519
Reed, T. A., 310
Reel, J. R., 406
Rees, D. A., 288
Rees, L. H., 475
Rees, L. V. C., 324
Reeve, A. E., 62
Reeves, H. C., 47
Regnier, F. E., 61
Regoli, D., 279, 314, 403, 549, 551, 552
Rehacek, Z., 420
Rehfeld, J. F., 512, 513, 514, 515, 517, 518, 519, 521, 522
Reiche, H., 7
Reichen, J., 70
Reichert, L. E., jun., 175, 466
Reichert, U., 51
Reid, J., 556
Reid, K. B. M., 125, 139, 144
Reimarsson, P., 289
Reimer, J. A., 284
Reinarsson, P., 283
Reinartz, M.-L., 20
Reinbolt, J., 131
Reiner, B. L., 545
Reingold, I. D., 191
Reinheimer, J. D., 153
Reinshagen, H., 449
Reis, F. A. M., 441
Reis, M. L., 552
Reiser, J., 103
Reisfeld, R. A., 142
Reisine, T. D., 579
Reisler, E., 307
Reissmann, S., 403
Reit, B., 545
Reithmeier, R. A. F., 177
Rejnková, J., 83
Rembaum, A., 196
Remy, P., 181
Renart, J., 103
Renaud, A., 163, 165, 284
Renner, L., 165, 339
Renold, A. E., 502
Renthal, R., 159, 361
Renugopalakrishnan, V., 432
Renwick, A. G. C., 43
Requena, J., 251
Reschke, R., 320
Restaino, A., 519
Retey, J., 191
Retsema, J. A., 446
Retzios, A. D., 121
Reybrouck, T., 556
Reynolds, C., 534
Reynolds, C. D., 240, 499, 500
Reynolds, C. H., 169
Reynolds, G. A., 466
Reynolds, G. D., 392
Reynolds, J. A., 339
Reynolds, R. M., 410
Rhee, S. G., 285
Rhodes, G., 73
Rhodes, R. A., 526
Rhodes, R. E., 444
Rhugenda-Banga, N., 3
Rhynard, D., 319
Ribeiro, A., 303
Ribet, A., 467, 521, 524
Riblet, R., 142
Ricchelli, F., 306, 396, 576
Rice, A. C., 75
Rice, D., 280, 286
Rice, D. M., 289
Rice, D. W., 125, 237, 264
Rice, R. H., 133
Rich, A., 243, 249, 548
Rich, D. H., 422
Richards, F. M., 247, 259, 260, 262
Richards, J. H., 279
Richardson, B. C., 145
Richardson, C. C., 75
Richardson, J. S., 244, 246, 260
Richardson, M., 148
Richarz, R., 262, 268, 279
Richmond, T. J., 247, 260
Richter, G., 65, 149
Richter, W. J., 136
Rick, W., 525
Rickli, E. E., 34, 118
Ricquier, D., 65
Riddles, P. W., 193
Ridge, B., 405
Riedl, H., 254
Rieke, E., 39
Riemen, M. W., 373
Riepl, R. G., 329
Riggs, A., 329
Riggs, A. D., 499
Riggs, A. F., 132, 337
Righetti, P. G., 99, 101
Rigo, A., 281
Rigoult, J., 221
Rijken, D. C., 87, 181
Rikitake, K., 95, 163, 167
Riley, C. M., 34
Riley, M., 138
Rilling, H. C., 181
Rimpler, M., 403
Rinehart, K. L., jun., 34, 452, 458
Ring, D., 171
Ringel, I., 343
Ringrose, R. S., 455
Riniker, B., 397, 398, 501, 539, 541
Rink, H., 469, 512
Ripamonti, A., 254
Riskulov, R. R., 234
Risteli, J., 97
Rittel, W., 374, 397, 398, 501, 539, 541
Ritzmann, R. F., 498, 579
Rivett, D. E., 119
Rivier, J., 468, 512
Rivier, J. E., 115, 128, 468, 513
Rizack, M. A., 471
Rizza, R., 512
Rizzo, R., 391
Rizzo, V., 276, 302, 409
Robb, F. T., 175

Robb, R. J., 112, 141, 171
Robbens, J. E., 420
Robberecht, P., 531
Robbins, A., 71
Robbins, J. E., 91
Robbins, R. J., 350, 362
Robert-Galliot, B., 71, 77
Roberts, G. C. K., 274, 277, 281, 284, 313, 438
Roberts, J. D., 275, 431
Roberts, J. L., 475, 476
Roberts, M. F., 283
Roberts, N. A., 410
Roberts, P. M., 445
Roberts, T. G., 6
Robertson, B. H., 137
Robertson, P., 276
Robillard, G. T., 57, 167, 280, 282
Robin, J.-M., 87
Robins, D. J., 420
Robinson, A. G., 128
Robinson, E. A., 69, 140, 142
Robinson, G. H., 153
Robinson, G. W., 350, 362
Robinson, H. M., 501
Robinson, J. A., 191
Robinson, J. D., 159
Robinson, J. P., 308
Robinson, J. S., 474
Robinson, N., 159, 361
Robison, P. D., 187
Robson, B., 247, 256, 260
Robson, L. E., 577
Robson, R. J., 283
Rocchi, R., 374
Rocchiccioli, F., 442
Rocha, V., 127
Rochat, H., 130
Rock, C. O., 51, 54
Roda, L. G., 161
Rodbard, D., 332
Rodbell, M., 511
Rode, W., 97
Roderich, W., 277
Rodger, K., 117
Rodgers, J. R., 223
Rodkey, J. A., 115, 128, 468, 513
Rodrigues, J. De A., 129
Rodriguez, A., 187
Rodriguez, L. A. M., 447
Rodwell, J. D., 179
Roe, D. C., 284
Roemer, D., 396, 569
Römer-Lüthi, C. R., 151
Röming, R., 131
Roenfanz, M., 521
Roesch, P., 282
Roesterjans, H., 456
Rogers, M. J., 69
Rogers, R. M., 543
Rogers, T. B., 200
Rogozhin, S. V., 63
Rogues, B. P., 425
Rohde, H., 136
Rohrmann, G. F., 108, 137
Rohrschneider, L. R., 110
Rokhlin, O. V., 271
Rokushika, S., 61
Roland, E. A., 280
Roll, F. J., 161
Rolla, F., 13
Rollin, C. F., 131, 149
Rolny, P., 527
Romakov, Yu. A., 291
Romano, S., 244, 256
Romanovskii, P. Y., 377
Romberg, R. W., 337
Romeo, A., 411
Romig, W. R., 161
Romovacek, H., 401
Rónai, A. Z., 562
Ronan, R., 543
Ronca, G., 153
Ronnberg, M., 310
Roomer, A., 309
Rooney, C. S., 14
Roos, M. H., 105
Roossien, F. F., 282
Root, M. A., 498
Roques, B. P., 18, 560, 561, 564
Rorsman, H., 34
Rosa, J., 340
Rosa, J. J., 199
Rosa, M. D., 199
Rosa, P. A., 476
Rosai, A., 448
Rosaspina, E., 405
Rosato, F. E., 515
Rose, G. D., 246, 257, 258, 269
Rose, Z. B., 287
Rosegay, A., 462
Rosell, S., 408, 557
Rosemeyer, M. A., 340
Rosén, A., 99
Rosen, F. S., 81, 139
Rosen, L. S., 244
Rosen, S. D., 91
Rosenbaum, J. L., 342
Rosenberg, A., 317
Rosenberg, J. S., 270
Rosenberg, L. E., 104, 179, 183
Rosenberg, R. A., 195
Rosenberg, R. D., 270
Rosenblatt, M., 545, 546, 547, 548
Rosenblatt, S., 31
Rosenfeld, M. G., 109
Rosengren, A. M., 34
Rosengren, E., 34
Rosenquist, G. L., 522
Rosenthal, A. L., 64
Rosenthal, A. S., 505
Rosenthal, K. S., 424
Ross, J., 165
Ross, J. B. A., 331
Ross, M., 564
Ross, M. J., 481
Rosselin, G., 526, 528, 529, 531, 532, 533, 534, 538
Rossi, C. A., 91, 153
Rossi, G., 60
Rossi-Bernardi, L., 334
Rossier, J., 559, 560
Rossi-Fanelli, M. R., 41, 335
Rossky, P. J., 261
Rossmann, M. G., 111, 124, 225, 239, 245, 263
Rossodivita, A., 5
Rossomando, E. F., 61
Rotenberg, E., 548
Roth, F. L., jun., 530
Roth, J., 173, 500, 501
Roth, S., 51
Roth, W., 410
Rothe, M., 275, 430
Rothgeb, T. M., 133, 286
Rothstein, S. G., 65
Rotilio, G., 281
Rotondi, A., 69
Rotsztejn, W. H., 529, 533
Rottem, S., 324
Rottenberg, M., 151
Rottman, A. J., 523, 528
Roubicek, J., 579
Roumeliotis, P., 61
Rousset, M., 532
Rousslang, K. W., 511
Rouvière-Yaniv, J., 73, 145
Roveri, N., 254
Rovery, M., 124
Rowan, R., tert., 277, 496
Rowe, D. W., 136
Roy, C., 112, 130, 149
Roy, H., 306
Roy, J., 263
Roy, P. D., 405
Roy, R. N., 22
Roy, S., 175
Roy, S. K., 39
Roy, T. P., 450
Roy, U., 263
Royer, G. P., 41, 197, 379, 383
Rozhkova, G. D., 171
Rozovskaya, T. A., 185
Rozynov, B. V., 5
Rubatelli, F. F., 153, 358
Rubenstein, A. H., 503, 512
Ruberg, M., 533
Rubin, B., 520, 556
Rubinstein, M., 60, 61, 108, 113, 475
Rubenstein, P. A., 57
Rubinstein, S., 61
Rubira, M. R., 138
Ruchel, R., 338
Rucker, R. B., 136
Rudikoff, S., 140, 142, 144
Rudin, Y., 106
Rudinger, J., 173, 375, 395
Rudloff, V., 132
Rudman, D., 127, 473
Rudolph, F. B., 44, 241

Rudolph, R., 264
Rüchel, R., 101
Rueda, D. R., 254
Rüdiger, H., 91, 135
Rüegg, U. T., 173, 375, 395
Rühimaki, G. M., 175
Ruess, K. P., 151
Rueterjans, H., 283
Rüther, E., 579
Rumsby, M. G., 357
Rumynskaya, I. G., 21, 291
Rune, S. J., 526, 527
Runge, M. S., 342
Runswick, M. J., 155, 405, 517, 521
Rupley, J. A., 256, 353, 510
Rupnow, J. H., 57
Rupprecht, K. M., 8, 78
Rusak, A. F., 288
Rusakav, Y. I., 501
Rusch, R. M., 291
Rush, J. D., 323
Rushbrook, J. I., 110
Rushizky, G. W., 73, 185
Russel, E. S., 99
Russel, R. P., 100
Russel, S. M., 271
Russell, C., 159
Russell, J., 173
Russell, M. P., 185
Russkikh, E. V., 320
Russo, S. F., 362
Russu, G., 527
Ruswinkle, L. J., 444
Rusznak, I., 26
Rutter, R., 321
Ruveda, E. A., 441, 442
Ruysschaert, J. M., 438
Ryan, D. E., 134
Ryan, J. W., 410, 557
Ryan, R. J., 127, 484
Ryan, R. L., 95
Ryan, W. B. F., 8
Ryang, H. S., 29
Rydon, H. N., 4, 14, 192, 405
Rydzy, M., 290
Rylatt, D. B., 124
Ryley, H. C., 83
Rzeszotarska, B., 411

Saal-De Jong, M., 181
Saari, J. C., 357
Saavedra, J. E., 26
Sabai, Y., 153
Sabesan, M. N., 434
Sabo, E. F., 556
Sabri, S. S., 21
Sacchi, G., 165
Saccone, C., 157
Sacher, R., 122
Sada, S., 2
Sadler, J. E., 59, 93, 98, 326
Sadler, P. J., 290
Sadovnikova, N. V., 399
Saeed, T., 32
Saenger, N., 256
Saenger, W., 246
Saez, J. M., 532
Safe, S., 419
Saffen, E. E., 328
Saffouri, B., 519
Saffran, W. A., 337
Safro, M. G., 234
Saha, N. N., 18
Saheki, T., 157
Sahm, H., 45
Saibil, H. R., 295
Said, S. I., 515, 528, 529, 530, 531, 532, 533
Saier, M. H., 57
Saigo, K., 17
Saini, M. E., 151
Saint-Blancard, J., 231
St.-Hilaire, J., 563
St. Pierre, S. A., 279, 403, 549, 551
Saint-Yves, A., 289
Sairam, M. R., 165, 167, 482, 483, 484
Saito, A., 523
Saito, H., 73, 276, 382
Saito, J., 73
Saito, S., 532
Saito, Y., 22, 294
Saitoh, E., 148
Saitoh, T., 185
Sajgó, M., 395
Sakabe, K., 498
Sakabe, N., 498
Sakaguchi, K., 177
Sakai, K., 15, 49
Sakai, T., 97
Sakai, T. T., 460
Sakai, Y., 157, 167
Sakakibara, S., 153, 314, 393, 498, 500, 539
Sakamoto, C., 523, 526
Sakamoto, M., 440
Sakamoto, W., 271
Sakamoto, Y., 34, 505
Sakamura, S., 19
Sakata, S., 270, 403
Sakazaki, R., 451
Sakita, R., 536
Sakiyama, F., 175
Sakore, T. D., 249, 250
Saksoe, P., 518
Sakuno, A., 21
Sakurai, A., 4, 119, 149, 423, 451
Sakurai, K., 19
Salahuddin, A., 179, 265
Salardi, S., 303
Saleha, S., 6
Salem, E. M. S., 374
Salemme, F. R., 246, 257
Salisbury, S. A., 253
Salm, K., 288
Salmeen, I., 317
Salvadori, S., 374, 404, 562
Samaja, M., 334
Samama, J.-P., 44, 239
Samartsev, M. A., 405, 517, 520
Samet, J., 179
Sammes, P. G., 173, 418, 446
Samouilidis, I., 413
Sams, J. R., 319
Samskag, P. O., 31
Samson, W. K., 510, 524, 529, 532
Samulski, E. T., 276
Sanada, K., 148
Sanada, Y., 89
Sanasaryan, A. A., 437
Sanda, A., 185
Sandberg, B. E. B., 401
Sander, C., 245, 257
Sander, J., 33
Sanders, D. J., 519, 520
Sandlin, D. E., 406
Sandoval, I. V., 343
Sandow, J., 471, 473
Sandra, P., 32
Saneii, A., 465
Sangster, A. W., 2
Sankozi, M., 470
Sankran, H., 521
Sannia, G., 121
Sano, A., 455
Sano, S., 155
San Pietro, A., 181
Sansom, M. S. P., 236
Sansonno, D., 519
Santagostino, A., 542
Santaniello, E., 16
Santi, W., 35, 392
Santini, V., 560
Santome, J. A., 169, 394
Santora, A. C., 500
Santora, G. T., 47
Santoro, J., 283
Santus, R., 352
Sanyal, G., 62, 193
Saperstein, R., 468, 511, 512
Saranson, P. E., 424
Sarantakis, D., 469
Saraste, M., 338
Saravis, C. A., 99
Sargent, D. F., 411
Sargeson, A. M., 32
Sarhan, S., 36
Sari, J. C., 30, 290
Sari, J. P., 290
Sarkar, B., 276
Sarkar, S. K., 304, 433
Sarkisyan, M. S., 309
Sárközi, M., 395
Sarles, H., 523, 524, 527, 538
Sarris, A. H., 103
Sarson, D. L., 515, 534
Sarvazyan, A. P., 22
Sasaki, H., 61
Sasaki, I., 62
Sasaki, K., 498
Sasaki, N. A., 406

Sasaki, T., 28
Sasaki, Y., 75, 526
Sasaoka, K., 2
Sasara, P., 13
Sase, S., 290
Sasisekharan, V., 245, 246, 254, 257, 258
Sastry, R. V. R., 99
Sasvari, M., 518
Satake, H., 311
Sato, A., 244
Sato, E., 447
Sato, H., 512, 535
Sato, K., 428, 523
Sato, R., 133, 311
Sato, S., 155
Sato, T., 526
Satoh, H., 380
Satoh, M., 542
Satoh, S., 183
Sâtor, V., 195
Satow, Y., 232
Satre, M., 157
Satyshur, K. A., 18
Sauer, R. T., 543, 545
Saunders, D. J., 498
Saunieres, J. F., 527
Sautierre, P., 129
Savino, M., 391, 436
Savion, N., 524
Savithri, H. S., 39
Savoca, K. V., 157
Savrda, J., 275, 421
Sawada, F., 43
Sawada, H., 49
Sawada, S., 19
Sawai, Y., 73
Sawan, S. P., 283
Sawyer, L., 173
Sawyer, T. H., 147
Sawyer, T. K., 406, 473
Sawyer, W. H., 407, 485, 487
Sax, M., 222, 223
Saxena, B. B., 165, 175, 482
Sayers, G., 471
Scanlon, K. J., 97
Scanlon, M. F., 465
Scanu, A. M., 84, 157, 327, 361
Scarafile, C., 445
Scechter, Y., 505
Schaafsma, T. J., 291
Schaal, R., 22
Schaar, P. L., 363
Schachman, H. K., 121, 326, 332
Schaechtele, K. H., 285
Schaefer, L., 23
Schaefer, M. L., 555
Schaefer, K., 535
Schär, H.-P., 97
Schafer, H. J., 201
Schaffalitzky de Muckadell, O. B., 526, 527, 529, 530, 531, 532
Schafmayer, A., 514, 518
Schalekamp, M. A. D. H., 552
Schalekamp, M. P. A., 552
Schallert, T., 523
Schally, A. V., 465, 466, 469, 512, 533, 560
Schamper, T. J., 432
Schapiro, H., 515
Schattenkerk, C., 401
Schatz, G., 106, 202
Schatz, H., 535
Schatz, P. N., 316
Schauer, R., 41
Schechter, A. N., 272, 287
Schechter, I., 142, 147
Schechter, Y., 272, 274
Scheek, R. M., 280
Scheele, R. B., 332
Scheid, M. P., 400
Scheidt, W. R., 224
Scheinman, A. O., 289
Schejter, A., 163, 263
Schenk, H., 222
Schenkel-Hulliger, L., 471
Scheraga, H. A., 21, 22, 108, 138, 231, 246, 256, 257, 261, 266, 267, 277, 278, 433, 435, 575
Schermer, D., 253, 428
Schetters, H., 63
Scheurich, P., 201
Schevitz, R. W., 249
Schiessl, J., 53
Schilirò, G., 340
Schiller, P. W., 396, 471, 483, 524, 556, 562, 563, 570, 574
Schilling, J., 129, 140, 142
Schiltz, E., 91, 124
Schimandle, C. M., 53
Schimpl, A., 91
Schindler, M., 173
Schipper, D., 2, 450
Schipper, P. E., 300
Schirmer, R. H., 51, 123, 255
Schlabach, T. D., 61
Schlageck, J. G., 364
Schlegel, W., 99, 510
Schleich, T., 284
Schleicher, R. B., 60
Schlesinger, D. H., 115, 118, 400
Schlesinger, M. J., 111
Schlessinger, T., 363
Schletter, I., 9
Schlinder, M., 282
Schlitter, J., 157
Schmid, F. X., 266, 267, 268
Schmid, G., 334
Schmid, M. F., 242
Schmidmayr, W., 147
Schmidt, G. J., 34
Schmidt, G. W., 126
Schmidt, H., 175
Schmidt, M. F. G., 111
Schmidt, P. G., 286
Schmidt, R., 51
Schmidt, U., 1, 12
Schmidt, W. C., jun., 227
Schmidt-Adergan, U., 282
Schmitt, S. M., 445
Schmitt, H. W., 144, 251
Schmiedt, W., 523
Schmutz, J. L., 322
Schnackerz, K. D., 282, 285
Schneider, A. B., 173
Schneider, B., 521
Schneider, B. S., 522
Schneider, D. L., 179
Schneider, E. L., 538
Schneider, H. P. G., 465
Schneider, W. J., 57, 65
Schnek, A.-G., 394
Schoemig, A., 556
Schön, I., 372, 517
Schoenborn, B. P., 251
Schönert, H., 331
Schoenmaker, G. S., 57
Schoentgen, E., 124, 149
Scholten, T., 514
Scholtz, M., 345
Scholz, R., 143
Scholze, H., 244
Schomaker, V., 221
Schomburg, D., 432
Schon, I., 26, 402
Schonberg, R., 95
Schook, W., 333
Schrader, W. P., 83
Schrader, W. T., 181
Schramm, M., 532
Schrank, B., 132, 147
Schreckenbach, T., 364
Schreffler, D. C., 105
Schröder, C., 81
Schroeder, R., 2
Schroer, J. A., 505
Schuer, R., 49
Schütte, H., 45, 73
Schüttler, A., 394, 503
Schulte, T. H., 310
Schultz, R. M., 161
Schultzberg, M., 530
Schultz, C. E., 321, 323
Schulz, G. E., 123, 255, 260
Schulz, H., 485
Schulz, H. J., 527
Schulz, R. M., 284
Schulze, H., 71
Schur, J. M., 349
Schuster, M. M., 559
Schuster, S., 474
Schuster, T. M., 169, 226, 332
Schutt, C. E., 220
Schutz, A., 27
Schuurkes, J. A. J., 524
Schwabe, C., 314
Schwartz, B. D., 141
Schwartz, I., 187
Schwartz, I. L., 263, 492
Schwartz, J.-C., 560, 561, 564
Schwartz, K., 102, 110

Schwartz, T. W., 512
Schweikert, K., 151
Schwenk, D. A., 410, 434
Schwertner, E., 394, 501
Schwyzer, R., 7, 13, 396, 397, 399, 403, 411, 468, 469, 471, 473, 562, 563, 570
Sciuto, S., 2
Scoffone, E., 375
Scopes, P. M., 315
Scott, A. P., 470, 474
Scott, C., 35
Scott, C. F., 83
Scott, J. W., 14
Scott, M. E., 277
Scott, P. M., 418
Scott, V., 111
Scott, W. N., 500
Scouten, W. H., 361
Scozzafava, A., 285
Scratcherd, T., 528
Scrimgeour, K. G., 51
Scuro, L. A., 522
Seamon, K. B., 289
Seamons, L., 317
Seargeant, L. E., 42
Searle, G. F. W., 345, 358
Sears, D. W., 41
Seavey, B. K., 480
Sedmera, P., 29, 420
Seeburg, P. H., 481
Seela, F., 9
Seelig, A., 252
Seelig, G. F., 173
Seelig, J., 252
Seelig, S., 471
Seely, J. H., 371
Seeman, J. R., 265, 273
Seeman, N. C., 249
Seethalakshmi, S., 39
Segal, D. S., 579
Segawa, T., 403
Segel, I. H., 159
Segerbaeck, D., 28
Segnini, D., 153
Segre, G. V., 543, 545, 547
Sehlin, J., 177
Sehr, P., 262, 268, 279
Seib, C., 131
Seid, R. C., 33
Seidah, N. G., 476, 559
Seidel, J. C., 179
Seifert, W. E., 119
Seiler, N., 36
Seino, Y., 536
Seipke, G., 391
Seiter, C. H. A., 163
Sekar, V., 183
Seki, A., 527
Seki, K., 22
Seki, S., 6
Sekiguchi, T., 167, 173
Sekino, H., 150, 400, 511
Sekiyama, N., 518
Sekulic-Ivankovic, M., 303
Sekura, R. O., 51
Selby, D. J., 273
Seldin, D. C., 87
Selezneva, I. A., 301
Seliger, H. H., 347
Seligman, P. A., 60
Seligy, V. L., 112, 130
Selinger, Z., 524, 532
Sell, S. M., 110
Sellers, H. L., 23
Sellers, H. W., 191
Sells, B. H., 187
Selraggi, L., 519
Semenova, L. V., 5
Sempere, R., 289
Senaratne, P. A., 444
Senault, B., 564
Senbert, P. A., 159
Senegas-Balas, F., 524
Sengupta, S. K., 437
Seno, N., 40
Senyavina, L. B., 288
Seon, B. K., 149, 173
Seprodi, A., 517
Seprodi, J., 466
Serabjit-Singh, C. J., 272
Serafini, P., 465
Serban, F., 527
Serdyuk, I. N., 253, 290, 306
Serebryany, S. B., 376, 405
Sergot, P., 346
Serit, G. S., 55
Seshadri, T. P., 250
Sethuram, B., 24
Setlow, P., 113, 148
Seto, J., 407, 485
Setoguchi, M., 131
Settle, W. L., 287
Severin, E. S., 121, 126, 289
Sevilla, M. D., 31
Sewell, B. T., 234, 258
Seybert, D. W., 225
Seybold, P. G., 30
Seydoux, J., 502
Seyer, J. M., 135
Shaar, C. J., 533
Shafer, J. A., 40
Shah, A. H., 441
Shahani, K. M., 40
Shaltiel, S., 39, 183
Shamala, N., 276, 411, 453
Shamsuzzaman, K. M., 480
Shanker, G., 49
Shannon, L. M., 105, 271
Shanzer, A., 11
Shapiro, M., 475
Shapiro, R., 169
Shapovalenko, E. P., 312
Sharma, R. K., 49
Sharma, R. P., 25, 173, 197
Sharma, R. R., 322
Sharma, S. D., 27
Sharma, S. K., 39
Sharon, N., 200
Sharon, R., 256
Sharonov, Y. A., 316, 317, 318
Sharpe, R., 396, 405, 563
Sharpe, R. D., 406
Shatkin, A. J., 78
Shavit, N., 364
Shavitz, R., 455, 562
Shaw, B., 519
Shaw, E., 89, 181, 189, 191, 408
Shaw, I. M., 408, 453
Shaw, J. L., 64
Shaw, J. S., 561
Shaw, W. V., 122
Shea, R. G., 105
Shechter, Y., 404, 578
Sheehan, J. C., 447
Sheffer, M. G., 173, 506
Sheinkler, V. L., 271
Sheldrick, G. M., 460
Shelton, E., 51
Shelykh, G. I., 502
Shemyakin, M. F., 404
Shen, S. T., 287
Shen, T. Y., 407, 462
Sheorain, V. S., 124
Shepard, G. L., 546, 547, 548
Sheppard, R. C., 368, 385, 386, 401, 558
Sheridan, R. P., 256
Sherrell, B. J., 105
Sherry, A. D., 363
Sherwood, L. M., 147, 173
Shevach, E. M., 141
Shi, S., 518
Shiba, T., 1, 2, 403, 407, 423, 455, 462, 463
Shibahara, S., 6
Shibanova, T. A., 437
Shibata, S., 167, 515
Shibata, T., 73
Shibata, Y., 53
Shichida, Y., 312
Shields, D., 110
Shields, H. S., 450
Shiflett, M. A., 191
Shifrin, S., 103
Shiga, K., 313
Shih, D. H., 444
Shikano, E., 367
Shikita, M., 47
Shimada, H., 411
Shimada, K., 3
Shimada, S., 25
Shimakata, T., 47
Shimamura, S., 395
Shiman, R., 54
Shimanura, T., 155
Shimizu, A., 290
Shimizu, J., 57
Shimizu, K., 377, 410, 456
Shimizu, M., 569
Shimizu, O., 30, 351
Shimizu, S., 440
Shimizu, T., 163, 311, 533
Shimohigashi, J., 441
Shimohigashi, Y., 12, 20, 441

Shimomura, T., 167
Shimonishi, Y., 402
Shimoyama, A., 1
Shimura, Y., 300
Shin, C., 12, 421, 455
Shin, M., 439
Shinagawa, S., 535
Shinamura, S., 470
Shinar, S., 183
Shindo, H., 283, 408
Shindo, Y., 20
Shine, J., 481
Shinitzky, M., 503
Shinkai, W., 181
Shinke, R., 103
Shinoda, K., 254, 294, 409
Shinogi, T., 412
Shinozaki, K., 367
Shioiri, T., 6, 376
Shiomi, H., 559, 570
Shiozaki, M., 447
Shippey, P. M., 302
Shirafugi, H., 449
Shirai, T., 8
Shiraishi, K., 19
Shiraki, M., 541
Shirakihara, Y., 252
Shire, S. J., 332
Shiro, Y., 287
Shiromot , T., 453
Shishkino, A. A., 399
Shivaji, S., 573
Shizuta, Y., 73
Shlyapnikov, S. V., 121
Shmeleva, G. A., 405
Shnyrov, V. L., 295
Shoham, M., 224
Shoji, J., 451
Shoji, S., 125
Shome, B., 480
Shooter, E. M., 106
Shore, J. D., 354, 360
Shosenji, H., 25
Shoshan, V., 364
Shraiber, V. M., 21, 291
Shubin, V. V., 308
Shuey, D. K., 55
Shukuya, R., 57
Shulkes, A., 531
Shulmina, A. I., 308
Shutskever, N. E., 234
Shvachkin, Y. P., 374, 399, 405
Sibilli, L., 122
Sibley, C., 142
Sica, V., 39
Siddiqui, A. A., 6
Sidorova, M. V., 405
Sidorowicz, A., 20
Sieber, P., 397, 398, 469, 501, 512, 541
Siedel, J., 51
Siefring, G. E., 83
Siegel, J. S., 189
Siegel, S. R., 533
Sieker, L., 220
Sieker, L. C., 134, 171, 227, 229
Sielecki, A. R., 230, 233
Siemion, I. Z., 404, 408, 573
Sierra, M. G., 442
Sievers, G., 310
Sievertsson, H., 406
Siezen, R. J., 198, 328
Sifniades, S., 24
Sigler, P. B., 199, 249
Sigman, D. S., 277
Sigrist, P., 83
Siigur, J., 95
Sikakana, C. T., 279
Silaev, A. B., 5
Siler, P., 448
Silman, R. E., 474
Silver, J., 142, 324
Silver, L., 389
Silver, P., 284
Silverstein, R., 181
Sim, R. B., 81
Simmonds, A. D., 167
Simmonds, M., 167
Simmonds, R. J., 62
Simon, B., 528
Simon, C., 342
Simon, E. J., 559, 578, 579
Simon, J., 500
Simon, J.-P., 55
Simonetta, M., 222
Simonov, V. I., 437
Simons, S. S., 202
Simonsson, I., 284
Simpkin, D. J., 16
Simpson, R. C., 51
Simpson, R. T., 171
Sims, N. R., 65
Singer, M. V., 523, 524
Singer, S. J., 107
Singer, T. P., 187, 281
Singh, A. K., 442
Singh, B., 401
Singh, D. V., 13
Singh, M., 524
Singh, P., 40
Singh, R. N., 480
Sinha, B. K., 436
Sinha, D., 196
Sinha, N. K., 75
Sinha, S., 358
Sinohara, H., 84
Sinou, D., 6
Sint, L., 222
Siperstein, A., 510
Sips, H. J., 126
Sirinek, K. R., 534
Sizemore, G. W., 543
Sizov, S. Y., 5
Sjodin, T., 189
Sjölund, K., 510, 515
Sjoqvist, A., 533
Skaric, D., 403, 551
Skaric, V., 403, 551
Skehel, J. J., 136
Skenar, I., 421
Skoglund, U., 220
Škopková, J., 409
Skorna, G., 26
Skorov, R., 91
Skow, L. C., 99
Skrabal, P., 276, 409
Skrabanek, P., 521
Skuin'sh, A. A., 472
Skuratovskii, I. Y., 249
Slaby, I., 265
Sladic-Simic, D., 133
Slaninova, J., 407
Slater, E. E., 89
Slater, G. P., 422
Slater, N. A., 64
Slavin, W., 34
Slebe, J. C., 157, 167
Sliwkowski, M. X., 353
Slobodyanskaya, E. M., 312
Slopek, S., 408
Slotboom, A. J., 181, 283
Slotin, L. A., 405
Slyudkin, O. P., 301
Smale, T. C., 445
Small, E. D., 358
Smallridge, R. C., 36
Smeaton, L. A., 519
Smillie, L. B., 129
Smirnoff, P., 183
Smirnov, V. N., 161, 171
Smirnova, A. P., 399
Smirnova, E. A., 366
Smith, A., 136
Smith, A. F., 559
Smith, B. E., 287
Smith, C. J., 79
Smith, C. W., 386, 407, 409
Smith, E. L., 123
Smith, G. A., 460
Smith, G. D., 323, 574
Smith, G. M., 286
Smith, G. P., 523
Smith, H. J., 507
Smith, I., 474
Smith, I. C. P., 275, 278
Smith, J. C., 346
Smith, J. M., 548
Smith, J. R., 578
Smith, K. M., 285
Smith, L., 167
Smith, L. A., 73
Smith, L. T., 97
Smith, M. A., 139
Smith, M. E., 77
Smith, P., 140
Smith, P. H., 510
Smith, P. J., 15
Smith, P. K., 197
Smith, P. R., 249
Smith, R., 309
Smith, R. A. G., 189
Smith, R. E., 169
Smith, R. J., 197
Smith, S., 338
Smith, S. J., 306

Smith, S. L., 123
Smith, T. H., 406
Smith, W. D., 25
Smith, W. E., 301
Smith-Johannsen, H., 77
Smits, P., 149
Smolkova, E., 31
Smulkowska, E., 15
Smulkowski, M., 381
Smy, J. R., 538
Smyth, D., 278, 575
Smyth, D. G., 146, 478, 559
Smyth, M. J., 521
Snape, W. J., jun., 524
Snapir, N., 523
Snatzke, G., 298
Snitzer, R. L., 38
Snyder, S. H., 521, 559
Sobczak, A., 411, 456
Sobell, H. M., 249, 250
Sobolev, V. M., 256
Soda, K., 16
Soderling, T. R., 42, 124
Soeldner, J. S., 534
Sönksen, P. H., 499, 500
Sogami, M., 290
Sogawa, K., 85, 179
Soininen, R., 310
Sokolova, A. E., 438
Sokolowska, T., 411, 456
Sokolvsky, M., 40, 87, 344
Solcia, E., 518, 530
Soldatenkov, A. T., 1
Solheim, B. G., 315
Solomon, A. K., 280
Solomon, S., 61
Solomon, T. E., 524, 525, 528
Soltysik-Rasek, M., 290
Solum, N. O., 103
Somekh, L., 11
Somkuti, G. A., 149
Somorjai, R. L., 275
Sondheimer, J., 389
Sone, N., 159
Sonenberg, N., 78
Song, P.-S., 153, 357, 358
Sonne, O., 501
Soon-Shiong, P., 535
Sopata, I., 167
Soper, T. S., 155
Sorantoglou, V., 78
Sorensen, H., 2
Sorm, F., 492
Soroka, M., 15
Sorokin, M. M., 305
Southgate, R., 445
Southwick, P. L., 195
Spaapen, L. J. M., 311
Spach, G., 276, 280
Spacil, J., 420
Spadon, P., 254, 409
Spaeth, J. T., 534
Späth, P. J., 101, 338
Spanos, E., 543
Sparrow, J. T., 403
Spartalian, K., 319, 320
Spatola, A. F., 277, 465
Spear, S. R., 444
Spears, K. G., 345
Speck, S. H., 163
Speir, G. R., 519
Spek, A. L., 18
Spelsberg, T. C., 63
Spencer, M., 60
Spencer, R. D., 346
Speranzo, F., 515
Sperti, S., 361
Spiegelman, B. M., 109, 342
Spiess, J., 115, 128, 468, 513
Spikes, J. D., 24, 344
Spindler, C. D., 51
Spirin, A. S., 290, 306
Spisni, A., 280
Spitzer, W. A., 447
Spitzmiller, E. R., 410
Spoerel, N., 77
Spokes, E. G. S., 529
Spolter, L., 16
Sponar, J., 295, 304, 315
Sportelli, L., 283
Spragg, J., 87, 89
Sprang, S., 235, 245, 258
Springall, J. P., 316, 317
Springhorn, S. S., 64
Squire, P. G., 326
Srinivasan, R., 20
Srinivasan, Y., 38
Srivastara, S. K., 179
Srivastava, B., 8
Srivastava, M., 8
Srivastava, P. N., 59, 91
Stacey, G., 51
Stacher, G., 523
Stachowiak, K., 402, 555
Stachowski, A., 523
Stachulski, A. V., 28, 373, 407
Stachura, Z., 404, 573
Stackebrandt, E., 461
Stadler, P. A., 420
Stadtman, E. R., 285
Staehelin, T., 77, 103
Staffa, J. A., 18
Stafford, S. S., 555
Stahl, G. L., 278, 386, 407
Stahl, J., 187
Stallings, W., 250
Stalon, V., 55
Stamberg, J., 39
Stammer, C. H., 19, 25, 383, 455, 562
Stammers, D. K., 238
Stanbro, W. D., 25
Stanciu, C., 527
Standaert, M. L., 98, 325
Stange, H., 405
Stangl, A., 132, 147
Stanley, S. J., 55
Stanworth, D. R., 405
Stark, G., 437
Stark, G. R., 103
Starratt, A. N., 407
Staub, J. F., 541
Stearns, M. E., 342
Steckert, J. J., 332
Stedman, J. D., 171
Steele, J. C. H., 339
Steele, J. M., 341
Steen, G., 527
Steennis, P. J., 53
Steer, M. L., 523
Steer, W., 132
Steezko, J., 185
Stefani, M., 153
Steffen, E., 334
Steffens, G. C. M., 119, 123
Steffens, G. J., 119, 122, 123, 132
Steglich, W., 10
Steigemann, W., 227, 300
Stein, R., 518
Stein, S., 61, 103, 108, 113, 392, 475, 559, 560
Steinberg, D., 194
Steinberg, I. Z., 344, 346, 348, 357, 363
Steiner, D., 512
Steiner, D. F., 510
Steiner, L. A., 143
Steiner, R. F., 264, 329, 361
Steinfeld, A. S., 267, 275, 410
Steinmeier, R. C., 163, 335
Steinrauf, L. K., 434
Steinringer, H., 523
Steitz, T. A., 236, 264
Stellwagen, E., 53, 75, 99, 267
Stenflo, J., 139
Stengrevics, O. A., 265
Stenkamp, R. E., 171, 227
Stenzel, P., 132
Stepanov, V. M., 87, 126
Stepanyan, S. A., 293
Stephens, E. M., 284
Stephens, G., 177
Stephens, P. J., 317, 318
Stephenson, J. R., 137
Steppe, T. V., 421
Sterling, J., 423
Stern, A. S., 560
Stern, E. A., 224
Stern, M., 367
Stern, P. S., 256
Stern, S. J., 9
Sternberg, M. J. E., 221, 229, 260, 261, 263
Sternlicht, H., 343
Stevanato, R., 281
Stevens, C. M., 276, 449
Stevens, F. C., 308
Stevens, F. S., 189
Stevenson, J. C., 543
Stevenson, K. J., 47
Stewart, F. H. C., 378
Stewart, J. M., 263, 279, 466, 549, 551, 557, 562
Stewart, M., 251, 253, 334

Stewart, R. M., 450
Stiele, J. M., 198
Stimson, E. R., 21, 278, 435, 575
Stinson, R. A., 42
Stirewalt, W. S., 111
Stirpe, F., 91
Stob, S., 283
Stocker, K., 89, 189
Stocker, M., 549
Stockx, J., 95
Stoeckenius, W., 251
Stöffler, G., 131
Stoesz, J., 161, 282
Stoffel, W., 171, 281, 288
Stoffer, G., 187
Stoian, M., 527
Stokes, A. M., 79
Štokrová, S., 295, 304
Stollenwerk, U., 400
Stone, D., 123
Stoodley, R. J., 447
Storm, C. B., 179
Storm, D. R., 53, 424
Stout, R. W., 526
Stouthamer, A. H., 57
Strachen, R. G., 400
Stracher, A., 110
Strader, C. D., 144
Strader, D. J., 193
Straight, R., 24
Strange, R. C., 110
Strassburger, W., 258, 300
Straub, D. K., 322
Straub, J., 123
Straume, B. K., 527
Straus, E., 520, 521, 522, 523
Strauss, W. L., 65
Stravropoulos, G., 456
Streicher, W., 449
Streter, F. A., 201
Strewler, G. J., 181
Strickland, D. K., 149
Strickland, W. N., 129
Stridsberg, B., 7, 16
Strigunkova, T. F., 24
Strike, P. M., 116, 144
Strittmatter, P., 163, 355
Stroming, T. A., 61
Strominger, J. L., 112, 141, 171, 449
Strong, D. D., 137, 138
Strong, J. M., 32
Strongin, A. Y., 87, 126
Strosberg, A. D., 38, 93, 135, 144, 153, 484
Stroud, R. M., 222
Struck, H., 161
Strumwasser, F., 128
Strunz, U., 531
Strycharz, W. A., 185
Stryer, L., 221, 253, 264, 344, 354, 452
Stuart, D., 221
Stuart, D. I., 238
Stuart, D. K., 128
Stuart, J. D., 34
Stubbs, L., 356
Stuchburg, T., 153
Studer, R. O., 404, 521
Stukan, R. A., 325
Stunnenberg, H. G., 73
Stura, E. A., 236
Sturaro, R., 515
Stutz, P. L., 420
Suarato, A., 447
Suau, P., 250
Subbaraman, A. S., 27
Subbarao, B., 103
Subramanian, A. R., 131
Subramanian, V., 41
Suda, M., 171
Suda, T., 475
Sudgen, P. H., 125
Sudha, T. S., 573
Sudnick, D. R., 362, 363
Suehisa, E., 34
Suetter, C. H., 185, 193
Sugahara, K., 536
Sugai, S., 301
Sugano, H., 277, 428, 432, 434
Sugano, N., 73
Sugano, T., 514
Sugawara, S. I., 448
Sugi, Y., 6
Sugie, A., 557
Sugii, M., 36
Sugita, Y., 335
Sugiura, M., 75, 167
Sugiura, Y., 459
Sugiyama, M., 518
Sugiyama, T., 153
Sugiyama, Y., 163
Suhai, S., 22, 256
Sukumaran, C. P., 13
Sullivan, E. A., 304
Sumikawa, K., 151
Sun, M., 30, 171
Sun, T. T., 16
Sundaram, K., 438
Sundaram, P., 531
Sundaram, T. K., 47, 183
Sundberg, K. R., 300
Sundby, F., 510, 511
Sunderland, C. A., 60
Sundler, F., 510, 514, 515, 519, 520, 529, 530
Sung, W. L., 500
Sunita, M., 27
Sunshine, H. R., 340
Sur, S. S., 179, 356
Surkov, S., 317
Surolia, A., 187
Sussman, H. H., 101, 105
Sussman, J. L., 224, 249
Sutherland, J. W. H., 287
Sutherland, W. M., 155, 193
Suttnar, J., 83
Sutton, D. A., 173, 501
Sutton, D. W., 51
Suzukake, K., 450
Suzuki, A., 146, 418, 439
Suzuki, C., 526
Suzuki, E., 253, 254
Suzuki, H., 439
Suzuki, K., 125, 181
Suzuki, M., 12, 75, 146
Suzuki, S., 536
Suzuki, T., 421
Suzuki, Y., 84
Suzuta, Y., 451
Svanvik, J., 527
Svasti, J., 100, 150
Svedas, V. J. K., 25
Svenberg, T., 59
Svendsen, I., 126, 146
Svensmark, O., 38
Sverdlov, E. D., 404
Svoboda, M., 514, 524
Svoboda, P., 175
Swaab, D. F., 474
Swaisgood, H. E., 40, 353
Swales, J. D., 552
Swaminathan, S., 18
Swanson, R., 358
Swanson, S. M., 223
Swartz, H. M., 274
Sweeney, C. C., 62
Swenson, R. P., 147, 175
Swerts, J.-P., 560, 561, 564
Swift, H. W., 512
Switzer, R. C., 103
Switzer, R. L., 285
Switzer, S. J., 487
Sykes, B. D., 230, 289, 291, 332
Sytkowski, A. J., 155
Sykulev, Y. K., 173
Sylvester, S. R., 276
Symons, R. H., 103
Synowiec, J. A., 345, 362
Sytinskii, I. A., 1
Szabo, A., 286
Szabo, A. G., 347, 350, 351
Szabo, I., 522
Szajn, H., 283
Szarvas, T., 26
Szebeni, A., 574
Szejtli, J., 36
Székely, J. I., 562, 579
Szelke, M., 396, 563
Szilagyi, L., 27, 201
Szilasi, M., 36
Szirmai, M., 554
Szoka, F. C., 155
Szporny, L., 554
Sztaricskai, F., 4, 5, 461
Szuecs, M., 517
Szymaniec, S., 408
Szymanovicz, A., 33, 35

Tabachnick, M., 189
Tabita, F. R., 51, 187
Taboury, J., 298
Tabushi, I., 310
Tachi, K., 102

Tachibana, S., 560
Tachikawa, S., 542
Tack, B. F., 81, 139
Tack, L. O., 171
Tae, H., 197
Täljedal, I. B., 177
Tagaki, T., 338
Tager, H. S., 105, 503, 510, 512
Tahara, H., 229
Tai, H., 171
Tai, H.-H., 526, 530
Taii, S., 475, 476
Taillandier, E., 298
Taira, N., 533
Taiwo, A., 169
Tajima, S., 133
Takabe, T., 362
Takada, S., 157
Takagaki, H., 12
Takagi, H., 542, 559, 570
Takagi, S., 3
Takagi, T., 100, 101, 138
Takahara, Y., 119, 148, 440, 526
Takahashi, I., 536
Takahashi, K., 85, 148, 179, 297
Takahashi, N., 4, 81, 119, 149, 169, 451
Takahashi, S., 185
Takaishi, Y., 453
Takano, K., 17
Takasaki, S., 93
Takashi, R., 361
Takashima, K., 10, 451
Takasu, S., 518
Takatsu, K., 157
Takeda, H., 411
Takeda, K., 377
Takei, H., 12
Takei, T., 440
Takemoto, K., 409
Takemoto, T., 3
Takenaka, K., 362
Takeno, H., 445
Takeo, K., 38
Taketani, Y., 294
Taketomi, H., 260
Takeuchi, F., 53
Takeuchi, H., 410, 456
Takeuchi, K., 57, 515, 519
Takeuchi, S., 536
Takeuchi, T., 450
Takeuchi, Y., 31, 119, 148, 440
Takeyama, M., 409, 528
Takiguchi, H., 428
Takikawa, S., 183
Takita, T., 402, 451, 458, 459, 460
Takizawa, T., 518
Takruri, I., 134
Talalay, P., 47
Talamantes, F., 480
Talbot, S., 465
Talgoy, M. M., 161
Taljaard, N., 130
Tallant, E. A., 53
Talupphet, N., 100
Tam, J. P., 373, 383, 384, 385
Tam, J. W. O., 169
Tamagaki, S., 383
Tamaka, A., 470, 473
Tamegai, T., 34
Tamiya, N., 181
Tamm, L. K., 252
Tamplin, C. B., 98
Tamura, C., 439
Tamura, M., 6
Tamura, S., 4, 119, 149, 418, 451
Tan, C., 77
Tan, E. L., 57
Tan, Y. H., 77
Tanabe, S., 12
Tanaka, A., 301, 395
Tanaka, F., 349
Tanaka, H., 89, 167
Tanaka, M., 123
Tanaka, N., 228, 229
Tanaka, S., 297
Tanaka, T., 51, 448
Tanase, S., 121, 153
Tancrede, P., 278
Tancredi, T., 276, 289
Tanford, C., 255, 256
Tang, J., 87
Tani, M., 536
Tani, N., 536
Tani, S., 406
Tanimura, T., 93
Taniuchi, H., 263
Tanokura, M., 19
Tao, T., 360
Tapia-Arancibia, L., 533
Tapuhi, Y., 35
Tarentino, A. L., 54
Tarnawski, J., 411
Tas, J., 98
Tashima, Y., 53
Tashiro, Y., 67
Tasumi, M., 284
Taszner, E., 381
Tate, S. S., 59
Tatemoto, K., 526, 538
Tatischeff, I., 352
Tattam, F. G., 347
Tau, E., 569
Tawada, K., 360
Taylor, A., 408, 419, 436, 440, 453
Taylor, B. J., 104
Taylor, D. P., 531, 577
Taylor, F. R., 66
Taylor, G., 250
Taylor, G. A., 546
Taylor, I. L., 518
Taylor, P., 151, 264, 358
Taylor, S. S., 183
Taylor, W. L., 57
Teale, J. M., 273
Tebling, F., 173
Tedro, S. M., 134
Teherani, J., 143
Teichberg, V. I., 558
Teichmann, R. K., 518
Teisner, B., 102
Teitei, T., 15
Tellam, R., 30, 337
Teller, D. C., 331
Teller, R. G., 432
Telzer, B. R., 342
Tempst, P., 133
Temussi, P. A., 289
Tenaschuk, D., 31
Teng, T.-L., 157, 327, 361
ten Heggeler, B., 249
Ten Kate, J. W., 81
Tennakone, K., 18
Tenner, T. E., jun., 548
Téplan, I., 466, 517, 558
Tepperman, F. S., 523
Terabe, S., 61
Terada, S., 407, 428
Terahara, A., 439
Terao, T., 161
Teraoka, H., 71
Terachi, K., 523
Terashima, S., 10
Terato, K., 526
Terau, K., 185
Terayama, K., 183
Terbojevich, M., 276
Terenius, L., 529, 530
Tereshchenko, V. P., 527
Termine, J. D., 291
Terra, I. C. M., 189
Terra, W. R., 189
Terrier, F., 22
Teschemacher, H., 145
Teshima, T., 423
Tesser, G. I., 15, 266, 393, 471, 521
Teuchner, K., 345
Teuscher, A., 500
Thamm, P., 28, 374, 498, 520
Thanassi, J. W., 177
Thanei, P., 7, 396, 562
Thang, D. C., 71, 77
Thang, M. N., 71, 77
Thatcher, D. R., 121
Thelander, L., 75
Thelen, M., 163
Theobald, C. J., 441
Theodoropoulos, D., 403, 407, 456, 555, 558
Theodoropoulos, G., 519
Theoharides, A. D., 289
Thész, J., 155
Thiele, H., 300
Thiele, R., 26
Thielens, N., 60
Thiery, C., 470
Thiery, J. M., 470
Thiessen, W. E., 428, 432
Thireos, G., 105

Thistlethwaite, P. J., 350
Thody, A. J., 474
Thom, E., 16
Thomas, D. D., 344
Thomas, E. J., 447
Thomas, E. W., 95
Thomas, H., 310
Thomas, J. O., 171
Thomas, J. W., 505
Thomas, P. A., 514, 536
Thomas, R. M., 265
Thomas, S. E., 2
Thomas, S. H. L., 500
Thomassett, B., 167
Thomaz, M. P. F., 350
Thomford, N. R., 534
Thompson, E. B., 202
Thompson, E. O. P., 119, 133
Thompson, J. C., 514, 515, 518, 533
Thompson. R. C., 233
Thompson, S. T., 53
Thomsen, J., 127
Thomson, A. J., 316, 317
Thomson, W. D., 575
Thornburg, R. W., 155
Thorburn, G. D., 474
Thorell, J., 520
Thoresen, F., 302
Thornton, E. W., 324
Thorpe, A., 505
Thorpe, R., 342
Thorpe, S. R., 155, 194
Thu, B. T., 30
Thulin, E., 286, 288, 289
Thulin, L., 525
Thurmayr, G. R., 523
Thusius, D., 330
Tibbelin, G., 95
Tibbs, V. L., 107
Tichá, M., 58
Tickle, I. J., 574
Tiezzi, E., 20
Tijhuis, M. W., 419, 420
Tijssen, P., 103, 191
Tikhomirov, M. M., 32, 111
Tikhonov, V. E., 17, 24
Tilley, B. E., 122
Tilley, J. W., 14
Tilzer, L., 69
Timasheff, S. N., 255, 341
Timko, J. M., 18
Timmer, K., 18
Timofeev, V. P., 173
Timpl, R., 135
Tingling, N. L., 2
Tinney, F. J., 406
Tinoco, I., 299
Tipping, E., 169
Tirpak, A., 51
Tishchenko, G. N., 438, 456
Tisse, C., 411
Titani, K., 125, 137
Titlestad, K., 430
Tixier-Vidal, A., 533
Tjian, R., 71
Tjoeng, F.-S., 373, 384, 385, 390
Tobari, J., 134
Tobe, H., 402, 451
Tobe, T., 409, 528
Tobin, J. D., 535
Tochino, Y., 393, 500
Todaro, G. J., 107
Toepfer-Petersen, E., 138
Toft, D., 181
Tokunoga, F., 312
Tokura, K., 457
Tokura, S., 254
Tokushige, M., 175, 181, 191
Tolbert, N. E., 47
Tolle, J. C., 368, 374
Toma, F., 275, 421, 470
Tomas, A., 221
Tomasi, M., 161
Tomasic, J., 461
Tomasic, L., 253, 302
Tomasselli, A. G., 51
Tomassetti, P., 519
Tomatis, R., 278, 374, 404, 562, 575
Tometsko, A. M., 111, 194
Tomida, 28, 411
Tomita, G., 30
Tomita, M., 146
Tomlinson, E., 34
Tomoeda, M., 34
Tomova, N., 305
Tondello, E., 18
Tonegawa, F., 367
Tonelli, A. E., 423
Tonetti, H., 71
Toniolo, C., 19, 290, 292, 303, 308, 389, 400
Tonnelle, C., 142
Tonomura, B., 187
Tonomura, Y., 179
Toome, V., 20
Topic-Bulic, M., 403, 551
Toraya, T., 191
Torbet, J., 248, 249
Torchia, D. A., 287, 291
Torchiana, M., 512
Torchilin, V. P., 66, 161, 171
Torchinsky, Y. M., 121
Torea, F., 456
Toren, E. C., 61
Tori, K., 448, 457
Torikata, T., 353
Torosis, J. D., 474
Torres, A. R., 62
Tosabayashi, Y., 391
Toscano, W. A., 321
Toste, A. P., 53
Toth, G., 517
Toulme, F., 275
Toulme, J. J., 351
Towbin, H., 103
Towers, G. H. N., 1
Townshend, G. S., 148
Toyama, S., 16
Traber, R., 425
Trachsel, H., 77
Track, N. S., 518, 519, 531, 536
Tracy, H. J., 517
Tracy, M. J., 405
Tracy, P. S., 44
Tracy, R. P., 338
Trägårdh, L., 69, 112, 141
Trampisch, H. J., 239, 507
Tran, C. D., 18
Tran, C. H., 349
Tratschin, J. D., 111, 245, 263
Traub, W., 224
Traut, R. R., 187
Trautwein, A., 319, 320
Travis, J., 183
Trayer, I. P., 167, 171
Traylor, T. G., 286, 321
Treadway, W. J., 161
Tredwell, C. J., 358
Treffot, M. J., 515
Tregear, G. W., 365, 545, 546, 547
Tremper, A. W., 448
Trepmann, E., 35, 356
Treszd, L., 26
Trevor, A. J., 287
Trewhella, J., 287
Tria, J. J., 31
Triandorf, I., 527
Tribe, D. E., 418
Trifonova, Z. P., 5
Trimble, R. B., 54
Tristram, S., 159
Trites, D. H., 437
Tritsch, D., 131
Trivellone, E., 290
Troitskaya, V. B., 536
Troll, W., 89
Trotta, P. P., 95
Trotter, J., 18
Trout, G. E., 36
Troxler, R. F., 134
Trtik, B., 420
True, R. J., 171
Truffy, J., 333
Truppe, W., 91
Tryggvason, K., 97
Trzeciak, A., 404, 521
Tsai, I.-H., 189
Tsakova, T. N., 268
Tsao, C.-P., 502
Tscherter, H., 420
Tschesche, H., 191, 268, 279
Tschesche, R., 441
Tseng, C. C., 10
Tseng, L.-F., 397, 404, 564, 573, 574
Tseng, Y.-M., 49
Tsernoglou, D., 225, 261, 264
Tsetlin, V. I., 288
Tsin, T. B., 319
Tsiolakis, D., 534
Ts'o, P. O. P., 538

Tsong, T. Y., 260, 269
Tsuboi, M., 29
Tsubota, T., 294
Tsuchihashi, G., 9
Tsuchiya, D., 406
Tsuchiya, E., 119, 149, 451
Tsuchiya, S., 518
Tsuda, K., 526
Tsuda, M., 91, 312
Tsuda, Y., 402
Tsudu, M., 157
Tsuge, H., 183
Tsugita, A., 249
Tsui, H. W., 482
Tsuji, A., 189
Tsuji, T., 448
Tsujita, T., 84, 175
Tsukada, K., 73
Tsukamoto, I., 155
Tsukerblat, B. S., 299
Tsukihara, T., 229
Tsukiyama, K., 524
Tsunoda, S., 518
Tsuru, D., 95, 163, 167, 181
Tsushima, K., 42
Tsusue, M., 183
Tsuzuki, H., 394, 498, 500
Tsuzukida, Y., 143
Tu, A. T., 130, 179, 314, 496
Tu, S., 159, 177
Tu, S.-C., 359
Tubokawa, M., 127, 480, 559
Tudos, F., 36
Tuengler, P., 98, 326
Tufariello, J. J., 444
Tuley, J., 361
Tulinsky, A., 222, 337, 362
Tunggal, B. D., 281, 288
Tun-Kyi, A., 374, 396, 411, 562
Turini, G. A., 556
Turkey, J., 159
Turner, B. M., 93
Turner, D. H., 298, 344
Turner, G. L., 251
Turner, J. L., 374
Turnowsky, F., 449
Turtle, J. R., 69
Tussell, J., 31
Tustanoff, E. R., 403, 551
Tuszynski, G. P., 102
Twitchin, B., 6
Tyagi, R. S., 438
Tyagi, S., 318
Tyihak, E., 26
Tyler, D. D., 65
Tyler, G. A., 547, 548
Tyler, J. A., 87
Tyrrell, N. D., 447
Tyson, R. G., 371, 401
Tzartos, S. J., 272
Tzougraki, C., 407

Ubom, G. A., 285
Uchida, H., 155
Uchida, S., 73
Uddman, R., 530
Udenfriend, S., 61, 103, 108, 113, 392, 475, 559, 560
Ue, K., 179
Ueda, H., 559, 570
Ueda, K., 371, 428
Uedaira, H., 280
Uehara, H., 140
Uehara, Y., 450
Uekara, K., 165
Ueki, M., 367
Ueki, R., 301
Ueki, T., 251
Uematsu, H., 169
Ueng, S.-N., 10
Ueno, A., 304, 409
Ueno, T., 280
Ueyama, M., 448
Ugarova, N. N., 171
Ugi, I., 26, 27, 369
Uhl, G. R., 521
Uhlemann, E. R., 514, 531
Uhmann, R., 525
Ui, N., 99, 115, 329
Uitzetter, J. H., 15
Ujita, K., 403
Ulbrich, N., 41
Ullman, A., 271
Ullma, E. F., 175, 272
Ullrich, A., 481
Ulrichsen, H., 533
Umeda, Y., 157
Umezawa, H., 402, 450, 451, 458, 459, 460
Umezawa, Y., 458
Unger, K. K., 61
Unger, R., 512
Unger, R. H., 510
Unger, T., 161
Unoki, K., 12, 455
Untucht-Grau, R., 123, 124
Unwin, P. N. T., 252, 253, 333
Uotila, L., 45
Upson, D. A., 277, 407, 496
Urdea, M. S., 159
Urry, D. W., 277, 280, 315, 428, 432, 492, 575
Usmani, A. A., 455
Utkin, Y. N., 288
Utrobin, N. P., 5
Utter, M. F., 183, 333, 334
Uvarova, N. N., 428
Uvnaes-Wallensten, K., 518, 530
Uyemura, K., 146
Uyeo, S., 444, 448

Vacher, M., 149
Vadali, G., 388
Vagne, M., 127, 515, 517
Vaillant, C., 518, 520
Vale, W., 115, 128, 468, 512, 513
Valente, L., 457
Valeur, B., 347, 352
Valinger, Z., 461
Vallee, B. L., 87, 155, 159
Vallet, B., 97
Vamos, E., 93
Van Beeumen, T., 133
van Berkel, W. J. H., 53
Van Binst, G., 277
van Boom, J. H., 249
Van Brouwershaven, J., 149
Van Bruggen, E. F. J., 134, 333
Van Calker, D., 512
Vance, D. E., 57
Van Dam, R. H., 542
Van Deenen, L. L. M., 148, 202 288
Vandekerckhove, J. S., 128, 136
Vandenbark, A. A., 61
Vandenbelt, J. M., 406
Van den Besselaar, A. M. H. P., 288
Van den Bosch, H., 288
Van den Broek, H. W. J., 73
Van den Broek, R., 249
Vandenbunder, B., 364
van den Dries, C. L., 15
Vander, A. J., 548
van der Baan, J. L., 2, 450
Van der Branden, C., 133
Van Der Donk, J. A., 542
Vanderharghen, J. J., 521
van der Helm, D., 433
van der Heyden, J. A. M., 35
Vander Jagt, D. L., 44, 53
Vanderkooi, J. M., 357
Vanderlaan, W. P., 480
Van der Lijn, P., 364
van der Marel, G., 249
van der Molen, H. J., 66
Vander Onderaa, F., 149
Van Der Sluys Veer, J., 542
van der Voort, F. R., 98
Van der Wel, H., 149
Vandlen, R. L., 151
van Dorsselaer, V., 11
Van Es, T., 157, 181
Van Etten, R. L., 151
van Fossen Bravo, R., 441
Van Helden, P. D., 129
Van Hoek, A., 345
van Holde, K. E., 336
Van Hoorn-Hickman, R., 519
Van Houten, M., 503
van Kamp, G. J., 79
van Keuren, M. L., 103
van Meerssche, M., 440
Van Metter, R. L., 350
Van Nispen, J. W., 435, 471
van Noorden, S., 513, 529, 543
Van Obberghen, E., 500
van Peppen, J. F., 24
van Poucke, L. C., 22
van Praag, H. M., 579
Vanquelin, G., 38, 153
Van Ree, J. M., 472, 579
Van Reitschoten, J., 545

van Rooijen, P. J., 53
Van Scharrenburg, G. J. M., 283
Van Steelandt, J., 317
Vantini, I., 522
Vantrappen, G., 536
Van Vugt, D. A., 559
van Wartburg, A., 425
Van Wassenaar, P. D., 149
Van Wauwe, J., 400
Van Zile, J., 194
Varadi, A., 33, 155
Varenne, P., 436
Varga, J. M., 473
Varga, L., 518, 519
Varga, S. L., 410, 434
Vargha, E., 17
Varimo, K., 153
Varma-Nelson, P., 161
Varmus, H. E., 105
Varner, H. H., 57
Varner, H. S., jun., 175
Varro, V., 517, 518, 522, 524
Varshavskaya, M. Y., 66
Vasak, M., 282, 355
Vasil, A., 98
Vasilev, A. D., 437
Vasilieva, G. A., 404
Vasiloff, J., 194
Vasquez, B., 47
Vatieri, A., 83
Vatteroni, B., 515
Vauclin, N., 533, 538
Vaughan, M., 64
Vavra, I., 496
Vaysse, N., 521
Vázquez, D., 185, 187
Vdovykin, G. P., 1
Veatch, W., 280, 452
Veber, D. F., 400, 410, 421, 434, 455, 464, 468, 511, 512
Vecchini, P., 41, 538
Vedel, M., 399, 468
Vederas, J. C., 191
Veeger, C., 183, 339, 357, 361
Vegner, R. E., 367
Vegners, R., 407
Veilis, B., 579
Veldink, G. A., 311
Velmurugan, D., 219
Vemura, H., 75
Venkatachalapathi, Y. V., 253, 267, 456
Venkataraman, K., 377
Venkatesan, K., 421
Venter, J. C., 65
Ventura, M. M., 308
Venyaminov, S. Y., 265, 290, 306
Verdini, A. S., 275, 430
Verdini, S., 391
Verduin, B. J. M., 357
Veres, K., 29
Verfürth, C., 165, 339
Vergues, J. P., 33, 111
Verheij, H. M., 125
Verhoef, H., 181
Verhoef, J., 561
Verhoeven, J. W., 280
Verhoeven, W. M. A., 579
Verrall, M. S., 444
Versteeg, D. H. G., 498
Vertuani, G., 405
Verwilghen, R. L., 98
Verzele, M., 32
Vessey, D. A., 95
Vestling, C. S., 47
Vettermann, W., 339
Vetto, R. M., 61
Vevert, J. P., 11
Vezzadini, P., 519
Vicar, J., 275, 430
Vickery, L. E., 169, 317
Vickovic, I., 220
Viggiano, G., 287
Viglino, P., 281
Vigna, S. R., 525
Vignais, P. V., 157
Vijayan, E., 524, 532
Vijayan, M., 245, 258, 456
Vilchez-Martinez, J. A., 465, 466
Viljoen, C. C., 130
Villa, T. G., 93
Villafranca, J. J., 279, 282, 285
Villa-Komaroff, L., 499
Villanueva, J. R., 93
Villemoes, P., 388
Villiers, M. B., 60
Villiger, A., 533
Vilu, R., 317
Vincent, M. G., 219
Vining, L. C., 440
Vinogradov, S. N., 19, 245, 256
Viola, R. E., 279
Virden, R., 265, 273
Virudachalam, R., 256, 462
Visintainer, J., 276, 404
Visser, A. J. W. G., 345, 357, 361
Visser, M., 474
Visser, T. J., 543
Vissner, S., 53
Viswanatha, T., 192
Viswanatha, V., 16, 278, 392, 407
Viswanathan, G., 185
Viswanathan, T. S., 282
Vita, C., 265, 273
Vitale, S., 322
Viterbo, D., 220
Viti, V., 283
Vito, C. C., 66
Vizet, A., 394
Vlaovic, M., 51
Vlasov, G. P., 367, 436, 502
Vliegen, J., 22
Vliegenthart, J. F. G., 311
Vo, K.-P., 339
Vodrážka, Z., 83
Voelter, W., 32, 111, 314, 518
Vogel, C.-W., 39
Vogel, H., 251
Vogt, H. P., 258
Voigt, K. H., 510, 543
Vokoun, J., 420
Volkenstein, M. V., 256
Volkert, W. A., 30, 352
Volkova, L. I., 249
Volkovitskaya, O. E., 189
Volpin, D., 254
Volshtein, L. M., 301
Vol'skaya, N. E., 404
Von Holt, C., 129
von Kapel, J., 41
von Wartburg, J.-P., 155
Voordouw, G., 53
Voorma, H. O., 77
Vorlickova, M., 315
Vorontsov, E. D., 288
Voskuyl-Holtkamp, I., 401
Voss, E. W., jun., 359, 361
Vovelle, F., 256
Voyles, N. R., 469
Vrana, M., 144
Vrobel, T. R., 556
Vuento, M., 83
Vukovick, R. A., 556
Vuk-Pavlovic, S., 171, 290, 355
Vuksan, S., 406

Wache, H., 370
Wachtel, E. J., 248
Wachter, E., 117, 135, 146
Wachtmeister, C. A., 28
Wackerle, L., 381
Wada, H. G., 101
Wada, K., 229
Wadano, A., 71, 192
Wade, J. D., 405
Wade, R., 395, 472
Wade, T. N., 11
Wade-Jardetzky, N., 264, 288, 290
Waeder, B., 556
Wagle, D. R., 377
Wagniere, G., 303, 316, 433
Wagner, D. D., 165
Wagner, F., 45
Wagner, F. W., 40
Wagner, G., 191, 262, 268, 279, 394, 533
Wagner, H., 531
Wagner, J. R., 33
Wagner, K. G., 391
Wahl, P., 30, 347, 350, 360
Wajcman, H., 340
Wajda, S., 318
Wakabayashi, K., 252
Wakabayashi, S., 134
Wakabayashi, T., 252
Wakamiya, T., 423
Waki, M., 12, 20, 381, 387
Wakimasu, M., 511
Wakselman, M., 25

Walckhoff, B., 364
Walder, J. A., 198, 341
Walder, R. Y., 198, 341
Waldman, A. A., 61
Waldum, H. L., 515, 526, 527
Waley, S. G., 173, 285, 446
Walker, J. E., 144, 149, 251
Walker, J. M., 145, 146, 147
Walker, L. E., 142
Walker, N., 221
Walker, R. J., 518
Walker, T. E., 281
Wall, K. A., 121
Wallace, B. A., 289, 452
Wallace, C. J. A., 266, 393
Wallace, J. C., 334
Wallace, L. J., 155, 177
Wallace, R. W., 53
Wallenfels, B., 103
Waller, C. A., 125
Waller, J.-P., 71
Waller, B. W., 345
Walsh, C., 16
Walsh, J. H., 515, 517, 522, 531, 557
Walsh, K. A., 137
Walsh, M., 308
Walsh, M. A. R., 462
Walsh, M. P., 97
Walsh, R. G., 4, 179
Walsh, S., 537
Walsh, T., 317
Walter (Nave), B., 105
Walter, D. E., 185
Walter, I., 272
Walter, J. A., 432
Walter, R., 181, 278, 356, 386, 407, 409, 492, 496, 498, 579
Walter, R. W., 275
Walters, F. H., 34
Walters, J. A. L. I., 64
Walters, K. L., 159
Walton, E., 444
Wampler, J. E., 346, 347
Wan, T. S., 449
Wan, W.-P., 406
Wan, Y.-P., 400
Wang, A. H.-J., 243, 249
Wang, B.-C., 222, 223
Wang, C.-C., 133, 143
Wang, C.-H., 33, 523
Wang, D., 163, 185
Wang, F. C., 284
Wang, J. H., 163
Wang, S. M., 287
Wang, S.-S., 400
Warach, J. B., 57
Warbinek, R., 138
Ward, C. W., 136, 137
Ward, D. N., 148, 482, 483
Ward, P., 443
Ward, W. W., 358
Ware, W. R., 347, 348
Warme, P. K., 266, 393
Warning, K. 8
Warr, G. W., 140
Warren, L., 102
Warshawsky, A., 367
Wartofsky, L., 36
Warwick, R., 183
Wasada, T., 512
Washburn, L. C., 16
Wasiak, T., 406, 466
Wasielewski, C., 411, 456
Wasko, M. L., 200
Wass, J. A. H., 465
Wasserman, G. F., 393
Wasserman, H. H., 445, 448
Wasserman, R. H., 108, 289
Wasteson, Å., 44
Wasylewski, Z., 298
Wat, C.-K., 1
Watanabe, H., 10, 185, 412, 451, 470, 473
Watanabe, J., 30, 351
Watanabe, K., 148, 283, 393, 500, 518, 531
Watanabe, S., 539
Watanabe, T., 448, 450
Watanabe, Y., 191, 232, 376
Watari, H., 313
Watenpaugh, K. D., 220, 229
Waterfield, A. A., 561, 577
Waterfield, M. D., 136
Waters, M. J., 69
Watkin, D. J., 221, 222
Watkins, W. B., 543
Watson, B. K., 14
Watson, C., 431
Watson, D. C., 147
Watson, D. G., 223
Watson, D. H., 43
Watson, J. L., 45, 244
Watson, N. S., 443, 447
Watt, K. W. K., 137, 138
Watt, R. M., 359, 361
Watter, T. J., 47
Watters, L. M., 519
Watts, D. A., 133
Watts, D. C., 163
Waxdal, M. J., 141, 484
Waxman, D. J., 449
Weare, J. A., 175
Weatergaard, J. G., 102
Weatherford, D. W., 246, 257
Weaver, D. L., 260, 269
Webb, N. L., 60
Webb, P. R., 65
Weber, B. H., 43
Weber, E., 227, 510, 543
Weber, G., 346, 352
Weber, I. T., 236
Weber, K., 128, 290, 343
Weber, L. D., 337, 362
Weber, W., 39, 300
Weedon, A. C., 418
Wegrzynski, B., 20
Wehlin, L., 527
Wehrstedt, K.-D., 404
Wehry, E. L., 347
Wei, G. J., 98, 330, 331
Wei, Y.-H., 123
Weichardt, H., 32
Weideman, M. M., 309
Weiel, J., 360
Weigert, M., 142
Weil, J. H., 78
Weinberger, S. B., 579
Weinblum, D., 81
Weiner, J. H., 291
Weinger, R. E., 283
Weinkam, R. J., 21
Weinstein, S., 452
Weintraub, B. D., 484
Weir, G., 519
Weiser, M. W., 55
Weiser, U., 89
Weisner, H., 132
Weiss, H., 70, 251
Weiss, J., 118
Weiss, L., 53
Weiss, L. C., 300
Weitzman, S., 111
Welbergen, J., 87, 181
Welberry, T. R., 220
Welinder, K. G., 125
Wellems, T. E., 227, 340
Wellner, D., 18
Wellner, V. P., 51
Wells, B., 361
Wells, E., 177, 187
Wells, J. W., 285
Wells, M. A., 57
Wells, R. J., 417
Wells, S. A., jun., 518
Welsh, E. J., 288
Welter, A., 3
Weltzien, H. U., 65
Wemple, J., 13
Wendel, A., 241
Wengert-Müller, M., 388
Wenkert, E., 442
Wennekes, L. M. J., 73
Werham, R., 117
Wermuth, B., 155
Werner, S., 122
Werner, T. C., 351
West, S. C., 149
Westcott, K. R., 53
Westenbrink, F., 58
Westerhuis, L. W., 15
Westermann, P., 159, 177, 187
Westermark, B., 44
Westheimer, F. H., 97
Westheimer, S. H., 200
Westler, W. M., 183, 284
Westman, S., 219
Westphal, U., 83
Wetlaufer, D. B., 273
Wetz, K., 159
Whalen, R. G., 110
Wharton, J., 529
Wheeldon, S. G., 527
Wheeler, F. B., 500
Wheeler, W. J., 442

Whitaker, J. M., 61, 115, 329
White, D. K., 321
White, D. N. J., 431
White, H. D., 342
White, M., 352
White, R. C., 33
White, S. W., 254
White, T. J., 104, 124
Whitley, R. J., 484
Whitman, W. B., 187
Whitney, J. B., 99
Whittman, H. G., 197
Wichman, A., 40
Wide, L., 465
Widmer, U., 302, 391
Wiebauer, K., 175, 201
Wiecek, C., 32
Wieder, K. J., 181
Wiegand, G., 244
Wiegand, H., 51
Wieland, F., 165, 339
Wieland, O. H., 504
Wieland, S. J., 66
Wieland, T., 253, 426, 427, 428
Wieloch, T., 285
Wieneke, H. J., 498
Wierenga, R. K., 242
Wierenga, W., 9
Wietzes, P., 125
Wiget, P., 354
Wijngaards, G., 87, 181
Wilcox, W. C., 487
Wild, U. P., 345
Wilder, R. L., 103
Wildonger, K. J., 444
Wilgus, H., 99
Wilhelmi, A. E., 479
Wilkinson, J. M., 34
Wilkinson, P. C., 404
Wilkinson, S., 404, 578
Will, P. C., 177
Willard, J. J., 148
Willcox, P., 43
Willeford, K., 43
Williams, A. F., 60, 144
Williams, B. J., 132, 368, 385
Williams, B. R., 330
Williams, D. H., 4, 438, 460, 461
Williams, D. L., 106
Williams, H. R., 161
Williams, J. A., 521, 524
Williams, J. W., 98
Williams, L. C., 129, 130, 171
Williams, M. V., 53
Williams, P. F., 69
Williams, R., 279
Williams, R. C., 342
Williams, R. E., 201, 405, 578
Williams, R.H., 510, 514, 535
Williams, R. J. P., 258, 278, 283, 288, 289, 507, 575
Williams, R. M., 127, 419
Williams, S. C. 556
Williams, T. H., 271, 279
Williamson, K. L., 275, 278, 431, 507
Williamson, M. P., 4, 461
Willick, G. E., 149
Willis, I., 175
Willis, R. A., 33
Wills, C., 121, 239
Willshire, I. R., 537
Wilschowitz, L., 374, 520
Wilson, A. C., 104, 124
Wilson, C. M., 103
Wilson, C. S., 151
Wilson, J. F., 169
Wilson, J. M., 320
Wilson, K., 221
Wilson, K. J., 113, 117, 118
Wilson, K. S., 236
Wilson, L., 63
Wilson, M. F., 2
Wilson, M. T., 163
Wilson, R. M., 526
Wilson, S. H., 73
Wilson, S. M., 62
Wilson, T.D., 34
Wilting, J., 309
Wilton, D. C., 173
Wiman, K., 112, 141
Winchester, B., 93
Winer, F. B., 191
Wingfield, P., 251
Winkler, F. K., 220
Winkler, H., 321, 323
Winter, D. P., 400
Winter, W., 19
Winterbourn, C. C., 169
Winterhalter, K. H., 113, 118
Winzor, D. J., 30, 328, 337
Wirtz, K. W. A., 148, 202
Wise, A., 33
Wisher, M. H., 500
Wisnieski, B. J., 161, 177, 191
Wissmann, H., 525
Withers, S. G., 332
Witt, G. S., 49
Witte, L., 119
Wittebort, R. J., 286
Witter, A., 561
Wittinghofer, A., 165
Wittle, E. L., 406
Wittmann-Liebold, B., 131, 146
Wittschieber, E., 260, 266, 407
Witty, M. J., 24, 26, 381
Witzel, L., 519
Witzemann, V., 200
Wize, J., 167
Woenckhaus, C., 155
Wohlrab, F., 57
Wohlrab, H., 177, 179
Woiwode, W., 32, 390
Wojciechowska, H., 404
Wojtowicz, A. J., 299
Wold, F., 47
Wolf, B., 179, 183
Wolf, C. R., 272
Wolf, G., 399
Wolf, H. U., 100, 338
Wolf, O., 142
Wolf, W., 399
Wolfe, R. G., 98, 175
Wolfe, S., 8, 27, 449
Wolfe, T. M., 498
Wolfensberger, M., 31
Wolff, T. E., 323
Wollemann, M., 517, 574
Wollert, U., 155
Wollin, G., 8
Wollmer, A., 258, 300
Wolosin, J. M., 151
Wolters, M., 134
Wolynes, P. G., 221, 261
Wong, C., 153
Wong, C.-H., 235
Wong, F., 421
Wong, G. B., 281
Wong, H. C., 557
Wong, J. Y., 38
Wong, K.-P., 258
Wong, N. C. W., 147
Wong, R. S. C., 148
Wong, S. T., 83
Wong, T.-W., 373, 383
Wong, Y., 191
Woo, N. H., 249
Wood, E. J., 325, 334
Wood, H. G., 127
Wood, J. L., 185
Wood, L., 35
Woodard, E. J., 311
Woodford, T. A., 478
Woodrooft, M. N., 155
Woodrow, S., 169
Woods, K. R., 115, 146
Woods, V. L., 103
Woodward, C. K., 264, 279
Woodward, F. J., 83
Woodward, R. B., 420, 446
Woodworth, R, C., 16
Woody, R. W., 44
Wool, I. G., 41
Woolfe, G. J., 350
Woolfson, M. M., 219, 438
Wooten, J. B., 284
Worning, H., 527
Wrange, Ö., 69
Wray, J. S., 250
Wright, D. E., 511
Wright, D. J., 146
Wright, I. G., 447
Wright, I. P., 47
Wright, J. K., 140
Wright, J. L. C., 432, 440
Wright, J. W., 534
Wright, K. A., 286
Wright, P. E., 287
Wrigley, N. G., 183, 333
Wu, C. S., 299
Wu, C.-S. C., 576
Wu, C.-W., 355, 359
Wu, H.-L., 155
Wu, J. S., 448

Wu, P. H., 33
Wu, R., 500
Wu, Z. C., 533
Wünsch, E., 28, 374, 514, 518, 520, 525, 535
Wüthrich, K., 191, 262, 268, 273, 274, 279, 282, 286, 288, 355, 366, 394, 424
Wyatt, J. R., 22
Wyatt, R. J., 542
Wybrandt, G., 112, 122
Wydro, R., 131
Wyman, J., 259, 336
Wyss, O., 51
Wyssbrod, H. R., 277, 496
Wystup, G., 71

Xavier, A. V., 134
Xuong, N.-H., 241, 339

Yabana, T., 518
Yabe, Y., 406
Yagi, N., 250
Yagi, T., 121
Yagishita, K., 3
Yaguchi, M., 112, 130, 131, 149
Yajima, H., 28, 29, 373, 375, 382,395, 397, 403, 409, 451, 470, 473, 528
Yalow, R. S., 520, 475, 521, 522, 523
Yam, C. F., 570, 574
Yamada, H., 155
Yamada, K., 25
Yamada, S., 17, 517
Yamada, T., 10, 412, 451
Yamada, Y., 198
Yamagami, N., 412
Yamagawa, H., 8
Yamaguchi, I., 408, 557
Yamaguchi, K., 535
Yamaguchi, M., 153, 175, 376, 448
Yamaguchi, T., 228
Yamaizumi, Z., 4, 253
Yamamoto, D. M., 277, 407
Yamamoto, H., 302, 304
Yamamoto, K., 87, 462
Yamamoto, M., 542
Yamamoto, R., 62
Yamamoto, T., 317
Yamamoto, Y., 17
Yamamura, H., 49
Yamamura, H. I., 579
Yamamura, K., 310
Yamamura, Y., 463
Yamanaka, K., 518
Yamanchi, H., 539, 541
Yamane, T., 254
Yamano, T., 153
Yamaoka, K., 302
Yamasaki, N., 175
Yamasaki, T., 526
Yamasawa, K., 45
Yamashiro, D., 392, 397, 404, 470, 472, 474, 564, 574, 576
Yamashiro, Y., 378
Yamashita, J., 62
Yamashita, O., 254
Yamashita, S., 254
Yamashita, T., 179, 254
Yamato, S., 339
Yamauchi, T., 53
Yamazaki, N., 376
Yameshita, S., 30
Yampolskaya, G. P., 305
Yamaskov, I. A., 17, 24, 25
Yanagida, T., 350, 363
Yanagisawa, S., 8
Yanagisawa, T., 153
Yanaihara, C., 498, 535
Yanaihara, N., 498, 532, 535, 536
Yancey, M. R., 556
Yang, C.-H., 256, 366, 432
Yang, C. S., 57
Yang, C.-Y., 34, 117, 143
Yang, D., 245, 258
Yang, I., 153
Yang, I.-H., 235
Yang., J. T., 299, 576
Yang, M., 548
Yang, P.-H., 256
Yang, S. F., 2
Yang, Y. C. S., 406, 473
Yankeelov, J. A., 465
Yano, T., 444, 448
Yano, Y., 61, 101, 153
Yanonis, V. V., 87, 126
Yansura, D. G., 481, 499
Yarbrough, L. R., 364
Yariv, J., 274, 323
Yarranton, G. T., 73
Yasuda, H., 532
Yasuhara, T., 127, 558
Yasui, T., 288
Yasumura, K., 395
Yasunobu, K. T., 123, 312
Yasutake, A., 431
Yauchihara, N., 513
Ybarra, D. M., 26, 195
Ycas, M., 260
Yeates, D., 221
Yee, J. A., 548
Yeh, W. K., 41
Yeung, C. W. T., 394, 502
Yeung, H.-W., 574
Yielding, K. L., 179
Ying, S.-Y., 465, 478
Yip, C. C., 394, 502
Yocum, R. R., 428, 449
Yoda, K., 87
Yokosuwa, H., 57
Yokosuwa, N., 81
Yokoyama, M., 99
Yokoyama, S., 253
Yon, R. T., 62
Yonaha, K., 16
Yonath, A., 224
Yoneda, M., 449
Yoneda, N., 12
Yonehara, H., 458
Yonehara, Y., 526
Yoneta, T., 6
Yonetani, T., 220, 319, 320
Yoneyama, M., 301
Yoneyama, Y., 311, 337
Yonezawa, Y., 12, 455
Yoo, C. S., 222, 223
York, J. L., 165
Yoshida, A., 446
Yoshida, H., 153, 181
Yoshida, M., 159, 167
Yoshida, R. A., 175, 272
Yoshida, T., 303
Yoshihara, S., 67
Yoshikawa, K., 153
Yoshikawa, M., 148
Yoshima, H., 93
Yoshimoto, T., 95, 163, 167, 181
Yoshimura, H., 67
Yoshimura, J., 12, 421, 455
Yoshimura, S., 402
Yoshimura, Y., 457
Yoshinaga, T., 155, 181
Yoshino, M., 42
Yoshioka, M., 448
Yoshitake, S., 167, 198
Yoshizawa, T., 312
Young, G. T., 369, 401, 403, 551
Young, I. M., 474
Young, J. D., 406
Young, J. G., 34
Young, J. L., 153
Young, M. C. K., 328
Young, M. E. M., 404
Younker, D. R., 31
Yu, B. P., 177
Yu, C.-A., 123
Yu, E. H., 279
Yu, F., 100
Yu, L., 123
Yu, L. C., 250
Yudman, B. H., 247, 258
Yuen, C., 103, 229
Yuki, H., 294, 391
Yun, S., 185, 193
Yuu, H., 523, 526

Zaal-de-Jong, M., 87
Zaccai, G., 251, 252, 253
Zackroff, R. V., 330
Zahedi-Asl, S., 519
Zahn, H., 394, 399, 412, 498, 501, 504
Zahn, R. K., 81
Zaidenzaig, Y., 122
Zaidi, Z. H., 132
Zak, Z., 297
Zakarian, S., 559
Zakut, R., 179
Zalkin, H., 51, 121

Zalut, 81, 139
Zalutsky, M., 17
Zama, M., 302
Zaman, Z., 98
Zamcheck, N., 99
Zamierowski, M. M., 181
Zampighi, G., 253, 333
Zanetti, G., 49, 165
Zanotti, G., 18, 254, 428
Zantena, A., 167, 280
Zaoral, M., 409
Zappelli, P., 5
Zarandi, M., 517
Zaretskii, Z. V. I., 119
Zaroulis, C. G., 579
Zaslavsky, B. Y., 63
Zaugg, R. H., 198, 341
Zechner, J., 352
Zeeberg, B., 343
Zeiri, L., 307
Zeitler, H.-J., 140
Zelenik, M., 132
Zemb, T., 352
Zemell, R., 142
Zentsova, N. K., 366
Zeppezauer, M., 280
Zermatten, A., 538
Zerner, B., 193
Zervas, L., 413
Zervis, C., 18
Zetler, G., 521, 524
Zetta, L., 278, 575
Zevely, E. M., 177
Zfass, A. M., 524, 526
Zgierski, M. Z., 299
Zheltukhina, G. A., 405
Zhuchkova, L. Y., 17
Ziegler, M. M., 124
Ziemer, J. N., 119
Zigman, S., 30, 171
Zilversmit, D. B., 57
Zimm, B. H., 349
Zimmer, G., 157
Zimmer, H.-G., 98, 103
Zimmerman, C. L., 33
Zimmerman, R. A., 187
Zimmerman, S. B., 249
Zimmermann, A., 87
Zimmermann, G., 275, 431
Zimmermann, K., 295, 304
Zinnecker, M., 179
Zinsli, P. E., 347
Zinsmeister, H. D., 24
Zisblatt, S., 196
Zlokazov, V. B., 221
Zobrocki, J., 10
Zoller, M. J., 183
Zomaya, I. I., 443
Zoon, K. C., 77
Zounes, M. C., 109
Zuber, H., 97, 111, 245, 263, 296, 539
Zucker, F. H., 236
Zuckerman, M., 163, 263
Zukin, R. S., 165, 196, 359, 576, 578
Zumberge, J. E., 23
Zurcher, J. A., 165
zur Nedden, D., 77
Zusman, D. R., 334
Zsadon, B., 36
Zweibaum, A., 532
Zweig, S. E., 107
Zwieb, C., 199
Zwilling, R., 87, 122
Zwolinski, G. K., 127