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ISBN 0 85186 074 5
ISSN 0306-0004
Library of Congress Catalog No. 72-92548

A Specialist Periodical Report

Amino-acids, Peptides, and Proteins

Volume 8

A Review of the Literature Published during 1975

Senior Reporter

R. C. Sheppard, *MRC Laboratory of Molecular Biology, Cambridge*

ISBN: 0 85186 074 5

ISSN: 0306–0004

Library of Congress Catalog Card No. 72–92548

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Preface

This eighth Report reviews papers relevant to the chemistry of amino-acids, peptides, and proteins published in the main journals during 1975. Last year, Chapter 5 on Chemical Structure and Biological Activity dealt only with enzymes. This year it surveys peptide hormones and related compounds, and therefore covers the two-year period 1974—75. The biennial survey of metal derivatives of amino-acids, peptides, and proteins will appear in next year's volume.

As on previous occasions we have taken the opportunity of reprinting (in Chapter 6) new and revised recommendations of the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature (see also Volumes 2, 4, and 5). In the main, these recommendations are followed throughout these Reports, but not slavishly. One reporter has drawn attention (p. 294) to the irrelevance of Greek prefixes in naming partial sequences of natural peptides according to the I.U.P.A.C.–I.U.B. system. Indeed, it may be questioned whether the general and traditional practice of indicating numbers of amino-acid residues in peptides by Greek prefixes has outgrown its usefulness. Recent titles commencing 'Synthesis and immunological evaluation of an eicosapeptide . . . .',[1] and 'Synthesis of N^{α}-benzyloxycarbonyl-[Thr^{107}]-dotetracontapeptide . . . .',[2] and 'Synthesis of a pentekontapeptide . . . .' [3] place an unnecessary strain on the classical education of peptide and protein chemists. Accordingly, only limited use of Greek prefixes is made in the present volume, generally for rather short peptides.

Once again it is a pleasure to thank most warmly the many contributors to this volume who are listed opposite.

R. C. SHEPPARD

[1] C. H. Schneider, K. Blaser, Ch. Pfeuti, and E. Gruden, *F.E.B.S. Letters*, 1975, **50**, 272.
[2] L. Moroder, B. Filippi, G. Borin, and F. Marchiori, *Biopolymers*, 1975, **14**, 2061.
[3] D. Yamashiro and C. H. Li, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 4945.

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Abbreviations

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

Other abbreviations which have been used are;

Ac	acetyl
Acm	acetamidomethyl
Adoc	adamantyloxycarbonyl
Aoc	t-amyloxycarbonyl
Asu	α-aminosuberic acid
Asx	aspartic acid or asparagine (not yet determined)
ATP	adenosine 5′-triphosphate
Boc	t-butoxycarbonyl
Bpoc	2-(4-biphenylyl)-isopropoxycarbonyl
BSA	bovine serum albumin
Bu^t	t-butyl
Bzh	benzhydryl (diphenylmethyl)
$Bzh(OMe)_2$	4,4′-dimethoxybenzhydryl
Bzl	benzyl
$Bzl(NO_2)$	*p*-nitrobenzyl
$Bzl(2\text{-}NO_2)$	2-nitrobenzyl
c.d.	circular dichroism
Cha	cyclohexylamine
CIMS	chemical ionization mass spectrometry
Cm	carboxymethyl
Cmc	*S*-carboxymethylcysteine
Dce	2,2-diethoxycarbonyl
Dcha	dicyclohexylamine
Ddz	3,5-dimethoxy(α,α-dimethyl)benzyloxycarbonyl
DMF	*NN*-dimethylformamide
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
Dnp	2,4-dinitrophenyl
Dns	1-dimethylaminonaphthalene-5-sulphonyl (dansyl)
Dopa	3,4-dihydroxyphenylalanine
DP	degree of polymerization
DTNB	5,5′-dithiobis-(2-nitrobenzoic acid)
Ec	ethylcarbamoyl

edta	ethylenediaminetetra-acetate
e.p.r.	electron paramagnetic resonance
e.s.r.	electron spin resonance
Et	ethyl
Gal	galactose
GC–MS	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
i.r.	infrared
Mal=	maleoyl
Man	mannose
Me	methyl
Msc	2-(methylsulphonyl)ethoxycarbonyl
NAD	nicotinamide-adenine dinucleotide (NAD^+ oxidized: NADH, reduced)
NCA	*N*-Carboxyanhydride
Np	*p*-nitrophenyl
Np(*o*)	*o*-nitrophenyl
Nps	*o*-nitrophenylsulphenyl
NSu	succinimido
ONp	*p*-nitrophenoxy
ONp(*o*)	*o*-nitrophenoxy
ONSu	succinimido-oxy
OPcp	pentachlorophenoxy
OPfp	pentafluorophenoxy
OPic	4-picolyloxy
o.r.d.	optical rotatory dispersion
OTcp	2,4,5-trichlorophenoxy
Pcp	pentachlorophenyl
Ph(SMe)	p-methylthiophenyl
Picoc	4-picolyloxycarbonyl
Pipoc	piperidino-oxycarbonyl
Ppoc	phenylisopropoxycarbonyl
Pth-Gly	the phenylthiohydantoin derived from glycine, *etc.*
Pz	*p*-phenylazobenzyloxycarbonyl
Q(5-Cl)	5-chloroquinol-8-yl
RNA	ribonucleic acid
SBu^t	t-butylthio
Scm	carboxymethylsulphenyl
SDS	sodium dodecyl sulphate
Ser(P)	*O*-phosphorylserine
SPr^i	isopropylthio
Tac	toluene-*p*-sulphonylaminocarbonyl
Tcp	2,4,5-trichlorophenyl

Thp	tetrahydropyranyl
T.l.c.	thin layer chromatography
Tos	toluene-*p*-sulphonyl
Trt	triphenylmethyl
Tse	2-(toluene-*p*-sulphonyl)ethyl
U.v.	ultraviolet
Z	benzyloxycarbonyl
Z(OMe)	*p*-methoxybenzyloxycarbonyl
Ztf	1-benzyloxycarbonylamino-2,2,2-trifluoroethyl

1
Amino-acids

BY G. C. BARRETT

1 Introduction

The layout for this chapter follows that used in previous volumes. Coverage is intended to be thorough as far as the chemistry of the amino-acids is concerned, but excludes most of the biological literature. The patent literature is also excluded, but this is felt to be not a serious omission in a continuous series; the Sections 16 (Fermentations) and 34 (Amino-acids, Peptides, and Proteins) of *Chemical Abstracts* provide easy access to this aspect of the literature.

Nomenclature.—IUPAC–IUB Recommendations (1974) on the nomenclature of amino-acids [1] (reproduced in Chapter 6) include no drastic revision of current usage, but some opportunities have been taken to decide between rival systems. In particular, the imidazole nitrogen atoms of histidine should be distinguished as π and τ (*pros* and *tele*; the N atom closest to the side-chain CH_2 group, and that farthest from the side-chain CH_2 group, respectively).

Textbooks and Reviews.—Synthetic methods [2] and other specific topics [2, 3] are covered in recent textbooks. Reviews are cited elsewhere in this chapter.

2 Naturally Occurring Amino-acids

Occurrence of Known Amino-acids.—Implications of the occurrence of non-protein amino-acids in plants have been discussed.[4]

Sensitive g.l.c. assay techniques permit the identification of amino-acids in marine sediments [5] and in meteorites;[6] the NASA Viking programme leading to exploration on Mars will involve the use of these techniques for assessing the

[1] *Pure Appl. Chem.*, 1974, **40**, 315; *European J. Biochem.*, 1975, **53**, 1; *Biochem. J.*, 1975, **149**, 1; *Biochemistry*, 1975, **14**, 449; *Rev. Soc. Quim. Mexico*, 1975, **19**, 33.

[2] (*a*) 'Methodicum Chimicum', ed. F. Korte, Vol. 6, Thieme Verlag, Stuttgart and Academic Press, New York, 1975; (*b*) 'Houben-Weyl: Methoden der organischen Chemie', 4th edn., ed. E. Müller, Vol. XV, ed. E. Wunsch, Thieme Verlag, Stuttgart, 1974.

[3] 'Amino-acid Metabolism', ed. D. A. Bender, Wiley, Chichester, 1975; 'Critical Stability Constants', ed. A. E. Martell and R. M. Smith, Volume 1: 'Amino-acids', Plenum, New York, 1974.

[4] L. Fowden, *Recent Adv. Phytochem.*, 1974, **8**, 95; A. Kjaer and P. O. Larsen, in 'Biosynthesis', ed. T. Geissman (Specialist Periodical Reports), The Chemical Society, London, 1973, Vol. 2, p. 71.
1973, **2**, 71; E. A. Bell, in 'Chemotaxonomy of the *Leguminosae*', ed. J. B. Harborne, Academic Press, London, 1971, p. 179.

[5] J. K. Whelan, *J. Chromatog.*, 1975, **111**, 337.

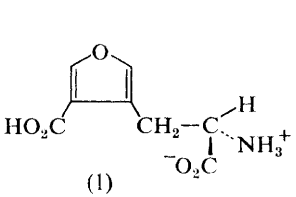
[6] (*a*) J. G. Lawless and M. P. Romiez, *Adv. Mass Spectrometry*, 1974, **6**, 143; (*b*) J. G. Lawless and E. Peterson, *Origins Life*, 1975, **6**, 3; (*c*) G. E. Pollock, C. N. Cheng, S. E. Cronin, and K. A. Kvenvolden, *Geochim. Cosmochim. Acta*, 1975, **39**, 1571; see also ref. 81*c*.

existence of life in former times, in case no evidence for surviving life forms is found.[7] At least 23 amino-acids are present in the Murchison meteorite, as shown using g.l.c.-m.s. techniques,[6b] and are thought to arise through extra-terrestrial abiotic synthesis[6b, 6c] since they are racemic; a clinching argument for their abiotic origin is the demonstration[6c] that isovaline from this source is also racemic.

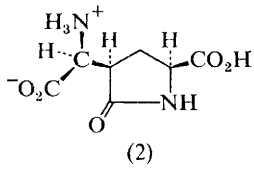
Seeds of *Combretum zeyheri* contain *N*-methyl-L-tyrosine,[8] while branches of *Limonium vulgare* contain 2-trimethylaminopropionic acid[9] and 2-trimethylamino-6-oxoheptanoic acid (as the choline ester).[10] L-Dopa found in *Hygrocybe conica* and *H. ovina* is responsible for the formation of red and black colours in these toadstools after bruising.[11]

Unusual amino-acids from microbial sources include 3-cyclohexenylglycine from *Streptomyces tendae*,[12] and δ-aminovaleric acid from rumen ciliate protozoa.[13] Elastatinal, a microbial elastase inhibitor, releases (2*RS*),(3*S*)-α-[2-iminohexahydro-4-pyrimidyl]glycine on acid hydrolysis, and is similar in this respect to the chymostatins (Vol. 6, p. 7).[14] The literature on microbial synthesis and production of amino-acids can only be represented generally here; recent reviews are available[15] and papers describing the fermentative production of L-proline or L-tryptophan[16] by auxotrophs of *Corynebacterium glutamicum* are typical of a substantial amount of current literature in this area.

New Natural Free Amino-acids.—L-3-(3-Carboxyfuran-4-yl)alanine (1) exists in the free state in *Phyllotopsis nidulans*[17] and in *Tricholomopsis rutilans* fruiting bodies.[18] Simultaneous independent investigation of the same species has occurred with *Pentaclethra macrophylla*,[19, 20] seeds of which contain penmacric



(1)



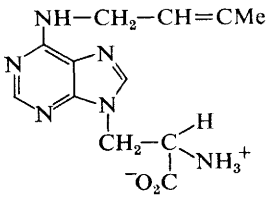
(2)

[7] P. H. S. Tsang, *Chemistry*, 1975, **48**, 15.
[8] K. Mwauluka, E. A. Bell, B. V. Charlwood, and J. M. Briggs, *Phytochemistry*, 1975, **14**, 1657.
[9] F. Larher and J. Hamelin, *Phytochemistry*, 1975, **14**, 205.
[10] F. Larher and J. Hamelin, *Phytochemistry*, 1975, **14**, 1789.
[11] W. Steglich and R. Preuss, *Phytochemistry*, 1975, **14**, 1119.
[12] W. Koenig, H. Hagenmaier, and U. Daehn, *Z. Naturforsch.*, 1975, **30b**, 626.
[13] W. Tsutsumi, R. Onodera, and M. Kandatsu, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 711.
[14] A. Okura, H. Morishima, T. Takita, T. Aoyagi, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1975, **28**, 337.
[15] W. M. Weigert, H. Offermanns, and P. Scherbereich, *Angew. Chem.*, 1975, **87**, 372; *Angew. Chem. Internat. Edn.*, 1975, **14**, 330; O. Tajima, *Hakko Kogaku Zasshi*, 1975, **53**, 482; R. Naumski, *Kem. Ind.*, 1975, **24**, 165.
[16] H. Hagino and K. Nakayama, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 343; K. Araki, Y. Takasawa, and J. Nakajima, *ibid.*, p. 1193.
[17] R. R. Doyle and B. Levenberg, *Phytochemistry*, 1974, **13**, 2813.
[18] S. Hatanaka and Y. Niimura, *Sci. Papers Coll. Gen. Educ., Univ. Tokyo*, 1975, **25**, 35 (*Chem. Abs.*, 1975, **84**, 40 800); *Phytochemistry*, 1975, **14**, 1436.
[19] A. Welter, M. Marlier, and G. Dardenne, *Bull. Soc. chim. belges*, 1975, **84**, 243; A. Welter, J. Jadot, G. Dardenne, M. Marlier, and J. Casimir, *Phytochemistry*, 1975, **14**, 1347.
[20] E. I. Mbadiwe, *Phytochemistry*, 1975, **14**, 1351.

acid [20] whose structure has been elucidated in full detail [19] as 3(*R*)-[1′(*S*)-amino-carboxymethyl]-2-pyrrolidone-5(*S*)-carboxylic acid (2). *X*-Ray and n.m.r. studies indicate a C_s-envelope conformation for (2) both in crystal and solution states.[19]

Of three unusual amino-acids found in *Mycena pura*,[21] *viz.* γ-methylene-, γ-ethylidene-, and γ-propylidene-L-glutamic acids, the third has not been found previously in Nature. A similar situation occurs for *Combretum zeyheri*,[22] seeds of which contain L-3-(3-aminomethylphenyl)alanine in addition to the 3-hydroxymethylphenyl and 3-carboxyphenyl analogues previously reported. New amino-acids have been isolated from fruit bodies of *Lactarius quietus* (L-2-amino-4-methylpimelic acid),[23] and from seeds of *Aleurites fordii* (L-3-carboxy-1,2,3,4-tetrahydro-β-carboline).[24] In addition to 13 known non-protein amino-acids, marine red algae contain pyrrolidine-2,5-dicarboxylic acid and *N*-methylmethionine sulphoxide.[25]

Lupinic acid (3), β-[6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purin-9-yl]-alanine, is the first reported example of a naturally occurring purine derivative



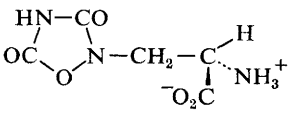
(3)

linked through one of its ring nitrogen atoms to an amino-acid moiety (although compounds of this type have been synthesized).[26] It is a novel zeatin metabolite, isolated from *Lupinus angustifolius* seedlings; the available 40 μg was insufficient to allow determination of its absolute configuration.

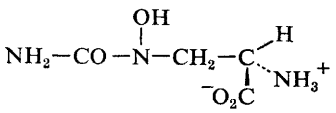
The structure β-(3,5-dioxo-1,2,4-oxadiazolidin-2-yl)-L-alanine (4) assigned [27] to quisqualic acid (from *Quisqualis fructus*) has been confirmed by synthesis;[27] hydrolysis by alkali gives the novel 2-amino-3-(1-hydroxyureido)propionic acid (5),[27] which might be expected to accompany quisqualic acid in the natural source.

New microbial metabolites include the anti-tumour agent (6) from *Streptomyces sviceus*;[28] the homologue (6; H in place of OH) was recently found in the same

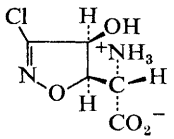
[21] S. Hatanaka and H. Katayama, *Phytochemistry*, 1975, **14**, 1434.
[22] K. Mwauluka, B. V. Charlwood, J. M. Briggs, and E. A. Bell, *Biochem. Physiol. Pflanz.*, 1975, **168**, 15.
[23] S. Hatanaka, H. Iizumi, A. Tsuji, and R. Gmelin, *Phytochemistry*, 1975, **14**, 1559.
[24] T. Okuda, T. Yoshida, N. Shiola, and J. Nobuhara, *Phytochemistry*, 1975, **14**, 2304.
[25] G. Impellizzeri, S. Mangiafico, G. Oriente, M. Piattelli, S. Sciuto, E. Fattorusso, S. Magno, C. Santacroce, and D. Sica, *Phytochemistry*, 1975, **14**, 1549.
[26] J. K. MacLeod, R. E. Summons, C. W. Parker, and D. S. Letham, *J.C.S. Chem. Comm.*, 1975, 809.
[27] T. Takemoto, K. Koike, T. Nakajima, and S. Arihara, *Yakugaku Zasshi*, 1975, **95**, 448 (*Chem. Abs.*, 1975, **83**, 97 875); *ibid.*, p. 326 (*Chem. Abs.*, 1975, **83**, 43 706).
[28] D. G. Martin, C. G. Chidester, S. A. Mizsak, D. J. Duchamp, L. Baczynskyj, W. C. Krueger, R. J. Wnuk, and P. A. Meulman, *J. Antibiotics*, 1975, **28**, 91; L. J. Hanka, S. A. Gerpheide, P. R. Spieles, D. G. Martin, P. A. Belter, T. A. Coleman, and H. F. Meyer, *Antimicrobial Agents and Chemotherapy*, 1975, **7**, 807.



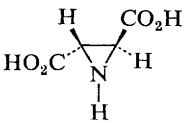
(4)



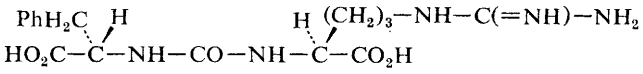
(5)



(6)



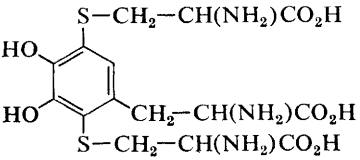
(7)



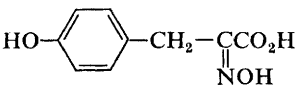
(8)

culture (Vol. 6, p. 6). L-*threo*-β-Hydroxyaspartic acid,[29] L-β-(5-hydroxy-2-pyridyl)alanine and L-β-(3-hydroxyureido)alanine,[30] L-*trans*-2,3-dicarboxyaziridine (7),[31] and the urea (8) derived from L-phenylalanine and L-arginine[32] have also been isolated from *Streptomyces* cultures. Cultures of *Claviceps fusiformis* deprived of oxygen accumulate N^{α}-methyl-4-dimethylallyl-L-tryptophan;[33] the non-methylated amino-acid was itself isolated from the same source previously.

Novel amino-acids isolated from higher organisms are 2,5-*S*,*S*-dicysteinyldopa (9) from the eye of the alligator *Lepisosteus spatula*,[34] and cystathionine sulphoxide and perhydro-1,4-thiazepine-3,5-dicarboxylic acid from the urine of a cystathioninuric patient.[35]



(9)



(10)

Although formally outside the scope of this chapter, the report of the isolation[36] of the tyrosine analogue (10) from the marine sponge *Hymeniacidon sanguinea* deserves mention.

[29] T. Ishiyama, T. Furuta, M. Takai, Y. Okimoto, S. Aizawa, A. Shimazu, and H. Yonehara, *J. Antibiotics*, 1975, **28**, 821.

[30] S. Inouye, T. Shomura, T. Tsuruoka, Y. Ogawa, H. Watanabe, J. Yoshida, and T. Niida, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2669.

[31] H. Naganawa, N. Usui, T. Takita, M. Hamada, and H. Umezawa, *J. Antibiotics*, 1975, **28**, 828.

[32] K. Fujimoto, K. Tatsuta, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiotics*, 1974, **27**, 685.

[33] K. D. Barrow and F. R. Quigley, *Tetrahedron Letters*, 1975, 4269.

[34] S. Ito and J. A. C. Nicol, *Tetrahedron Letters*, 1975, 3287.

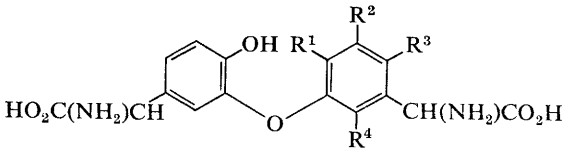
[35] H. Kodama, Y. Ishimoto, M. Shimomura, T. Hirota, and S. Ohmori, *Physiol. Chem. Phys.*, 1975, **7**, 147 (*Chem. Abs.*, 1975, **83**, 56 395).

[36] G. Cimino, S. De Stefano, and L. Minale, *Experientia*, 1975, **31**, 756.

New Amino-acids from Hydrolysates.—The outstanding new example under this heading is the discovery[37] of γ-carboxy-L-glutamic acid in several locations in Vitamin K-dependent prothrombin[37–40] and in mineralized tissue proteins.[38] It survives alkaline hydrolysis, but is quantitatively decarboxylated in 0.05M-HCl at 100 °C.[38]

The presence of an α-aminoadipic acid δ-semialdehyde residue in myelin basic protein from bovine brain has been established[41] through reduction with NaB^3H_4 and isolation of ^3H-labelled ε-hydroxynorleucine from alkaline hydrolysates. Further fascinating work on collagen cross-links has been reported,[42] leading to structure assignment to a new hydroxy-aldolhistidine trifunctional cross-link from cow skin collagen.

Peptide antibiotics and related compounds providing new derivatives of the protein amino-acids on acid hydrolysis are cerexins A and B (first appearance of L-*threo*-γ-hydroxylysine)[43] and actinomycin Z_1 (3-hydroxy-5-methylproline).[44] More complicated phenylglycine derivatives are released from ristocetin A,[45, 46] actinoidin, ristomycin, and vancomycin;[46] the derivative (11; R^1 = Me, R^2 = OH,



(11)

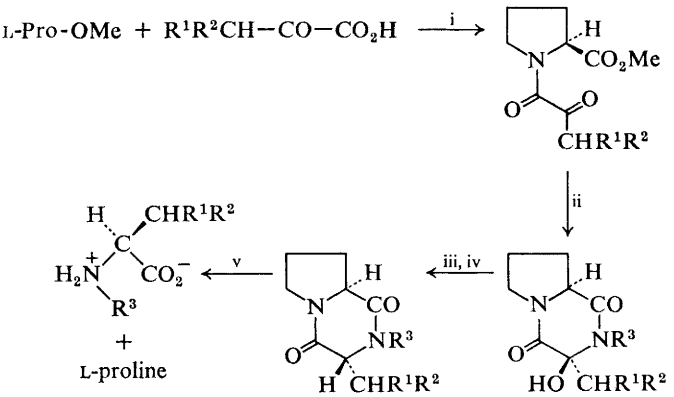
R^3 = R^4 = H) is present in hydrolysates of ristocetin A,[45] and structure (11; R^1 = R^2 = H, R^3 = R^4 = OH) is established[46] for actinoidinic acid, present in hydrolysates of the other antibiotics. Substantial progress towards elucidation of structure of vancomycin has been made;[46] it is thought to include three oxygenated phenylglycine units and two chloro-β-hydroxytyrosine units.

3 Chemical Synthesis and Resolution of Amino-acids

Asymmetric Synthesis.[47]—Further development of asymmetric hydrogenation using ruthenium(II) chiral phosphine catalysts has been reported, leading to moderate optical yields for synthesis of 2-aminoalkanoic acids from α-acylamidoacrylic acids.[48, 49]

[37] H. R. Morris, *Biochem. Soc. Trans.*, 1975, **3**, 465.
[38] P. V. Hauschka, J. B. Liau, and P. M. Gallop, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3925.
[39] H. R. Morris, M. R. Thompson, and A. Dell, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 856.
[40] P. Fernlund, J. Stenflo, P. Roepstorff, and J. Thomsen, *J. Biol. Chem.*, 1975, **250**, 6125; J. B. Howard, M. O. Fausch, and G. L. Nelsestuen, *ibid.*, p. 6178.
[41] E. Wada and T. Tsumita, *Jap. J. Exp. Med.*, 1975, **45**, 313.
[42] T. Housley, M. L. Tanzer, E. Henson, and P. M. Gallop, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 824.
[43] J. Shoji and H. Hinoo, *J. Antibiotics*, 1975, **28**, 60.
[44] E. Katz, K. T. Mason, and A. B. Mauger, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 502.
[45] T. M. Harris, J. R. Fehlner, A. B. Raabe, and D. S. Tarbell, *Tetrahedron Letters*, 1975, 2655.
[46] G. A. Smith, K. A. Smith, and D. H. Williams, *J.C.S. Perkin I*, 1975, 2108.
[47] For a review see J. W. Scott and D. Valentine, *Science*, 1974, **184**, 943.
[48] B. R. James, D. K. W. Wang, and R. F. Voigt, *J.C.S. Chem. Comm.*, 1975, 574.
[49] W. S. Knowles, M. J. Sabacky, B. D. Vineyard, and D. J. Weinkauff, *J. Amer. Chem. Soc.*, 1975, **97**, 2567.

While enzymic synthesis of L-amino-acids from DL-α-hydroxy-acids[50] and synthesis of L-aspartic acid derivatives from addition of (*S*)-PhCHMeNH$_2$ to dimethyl fumarate or maleate[51] illustrate familiar principles, asymmetric induction in the synthesis of *N*-benzoyl-*N*[(*R*)-α-ferrocenylisobutyl]-L- and -D-valine t-butylamide *via* a four-component condensation involving benzoic acid, (*R*)-α-ferrocenylamine, Me_2CHCHO, and Bu^tNC is particularly interesting because of its extremely high stereoselectivity.[52] Another highly selective example is the general synthesis of L-amino-acids and their *N*-methyl derivatives from corresponding α-keto-acids using L-proline as chiral agent (Scheme 1).[53]



Reagents: i, DCCI; ii, NH_3–$MeOCH_2CH_2OMe$; iii, TFA; iv, H_2 with Adams' catalyst; v, H_3O^+

Scheme 1

Asymmetric synthesis of threonine and allo-threonine from optically active *N*-salicylideneglycine cobalt(III) complexes has been reported.[102]

General Methods of Synthesis.—Isocyanides continue to appeal as starting materials in general syntheses of α-amino-acids. α-Isocyano-alkanoate esters $CNCHR^1CO_2R^2$ yield *N*-formylamino-acid esters on hydrolysis; they may be prepared by alkylation of alkyl isocyanoacetates (R^1 = H)[54–57] after metallation, though the nature of R^2 influences ratios of mono- and di-alkylated products.[54] Alkylation with a ketone gives β-branched amino-acids when reaction conditions causing dehydration of the β-hydroxyalkanoate are employed,[55] or threonine analogues when aldehydes are used.[57] t-Alkylglycines are obtained by addition of a Grignard reagent to an α-isocyanoacrylate [$R^1R^2C{=}C(NC)CO_2Et$ + $R^3MgBr \rightarrow R^1R^2R^3CCH(NHCHO)CO_2Et$].[58] Good yields of phenylglycines

[50] H. Matsushima, K. Murata, and Y. Mase, *Hakko Kogaku Zasshi*, 1975, **53**, 443 (*Chem. Abs.*, 1975, **83**, 129 947); *ibid.*, p. 450.
[51] Y. Nakajima, J. Oda, and Y. Inouye, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2065.
[52] R. Urban and I. Ugi, *Angew. Chem.*, 1975, **87**, 67.
[53] B. W. Bycroft and G. R. Lee, *J.C.S. Chem. Comm.*, 1975, 988.
[54] U. Schollkopf, D. Hoppe, and R. Jentsch, *Chem. Ber.*, 1975, **108**, 1580.
[55] H. J. Praetorius, J. Flossdorf, and M. R. Kula, *Chem. Ber.*, 1975, **108**, 3079.
[56] K. Bischofberger, R. H. Hall, and A. Jordaan, *J.C.S. Chem. Comm.*, 1975, 806.
[57] K. Matsumoto, Y. Urabe, Y. Ozaki, T. Iwasaki, and M. Miyoshi, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1869.
[58] U. Schollkopf and R. Meyer, *Angew. Chem.*, 1975, **87**, 624.

are obtained[59] by successive lithiation, carboxylation, and hydrolysis of $PhCH_2NC$.

Ogura and Tsuchihashi's extraordinary new synthesis (Vol. 7, p. 6) has been exemplified further[60] in a synthesis of *N*-lauroylvaline methyl ester from $MeSOCH_2SMe$ and Bu^tCN.

Methods employing Schiff bases as starting materials involve either metallation followed by alkylation[59, 61] [use of dithioacetals $(RS)_2C{=}NCH_2CO_2Et$[61] is noteworthy], or more novel procedures, *e.g.* $Cl_3CCH{=}NCO_2Et + RMgX \rightarrow Cl_3CCHRNHCO_2Et \rightarrow {}^-O_2CCHRNH_3{}^+$,[62] and $MeCH{=}NCHMePh + Me_3SiCN \rightarrow MeCH(CN)N(SiMe_3)CHMePh \rightarrow$ DL-alanine in 37% yield.[63]

α-Hydroxy-,[64, 65, 119] α-methoxy-,[66, 116] or α-alkanethio-hippuric acids[67, 116] may be employed in new α-amino-acid syntheses since they are amidoalkylating agents towards aromatic compounds (*e.g.* $PhCONHCH(OH)CO_2H + ArH \rightarrow PhCONHCHArCO_2H$),[64, 67] active methylene compounds,[65] and alkenes.[66]

Classical procedures of amino-acid synthesis continue to be methods of first choice in many areas. The hydantoin synthesis,[68, 69] Strecker synthesis,[215] acetamidomalonate synthesis,[70, 71] use of ethyl α-nitroacetate,[72] and extension of a side-chain through functionalized amino-acids (*e.g.* β-chloro-L-alanine[73] and *trans*-4-bromoproline[74]) and through αβ-dehydro-α-amino-acids,[26, 75] are representative examples of methods used for synthetic objectives described in later sections of this chapter. Use of 2-phenylimidazol-5-ones in synthesis of *N*-benzoylamino-acids (a relative of the azlactone synthesis) has been described.[76]

A new synthesis of amino-acids[77] is general in the sense that an excellent reagent (RuO_2) is available for oxidizing the aryl moiety of an aralkylamine to a carboxy-group, *e.g.* $ArCHR(CH_2)_nNH_2 \rightarrow {}^-O_2CCHR(CH_2)_nNH_3{}^+$; tyrosine can be oxidized to aspartic acid by this method.[77] A classical method for converting an α-amino-acid into its β-homologue is illustrated for the conversion of *Z*-L-Pro-OH into 'α-L-homoproline' by treatment with diazomethane (Wolff rearrangement) followed by de-protection.[78]

[59] T. Oguri, T. Shioiri, and S. Yamada, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 173.
[60] K. Ogura, I. Yoshimura, N. Katoh, and G. Tsuchihashi, *Chem. Letters*, 1975, 803.
[61] D. Hoppe, *Angew. Chem.*, 1975, **87**, 450.
[62] C. Kashima, Y. Aoki, and Y. Omote, *J.C.S. Perkin I*, 1975, 2511.
[63] Y. Nakajima, T. Makino, J. Oda, and Y. Inouye, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 571.
[64] D. Ben-Ishai, I. Satati, and Z. Berler, *J.C.S. Chem. Comm.*, 1975, 349.
[65] D. Ben-Ishai, Z. Berler, and J. Altman, *J.C.S. Chem. Comm.*, 1975, 905.
[66] J. Altman, R. Moshberg, and D. Ben-Ishai, *Tetrahedron Letters*, 1975, 3737.
[67] U. Zoller and D. Ben-Ishai, *Tetrahedron*, 1975, **31**, 863.
[68] M. Winn, R. Rasmussen, F. Minard, J. Kyncl, and N. Plotnikoff, *J. Medicin. Chem.*, 1975, **18**, 434.
[69] A. H. El Masry, S. E. El Masry, L. E. Hare, and R. E. Counsell, *J. Medicin. Chem.*, 1975, **18**, 16.
[70] T. Shiba, Y. Mukunoki, and H. Akiyama, *Bull. Chem. Soc. Japan*, 1975, **48**, 1902.
[71] J. W. Thanassi, *Bio-org. Chem.*, 1975, **4**, 132.
[72] A. Rosenthal and B. Cliff, *J. Carbohydrates, Nucleosides, and Nucleotides*, 1975, **2**, 263.
[73] H. Nishimura, A. Mizuguchi, and J. Mizutani, *Tetrahedron Letters*, 1975, 3201.
[74] F. M. Kaspersen and U. K. Pandit, *J.C.S. Perkin I*, 1975, 1617, 1798.
[75] W. Maerki and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 1471.
[76] G. M. Devasia and C. R. Pillai, *Tetrahedron Letters*, 1975, 4051.
[77] D. C. Ayres, *J.C.S. Chem. Comm.*, 1975, 440.
[78] L. Balaspiri, B. Penke, G. Papp, G. Dombi, and K. Kovacs, *Helv. Chim. Acta*, 1975, **58**, 969.

Prebiotic Synthesis; Model Reactions.—Reports of the synthesis of amino-acids from simple molecules under simulated prebiotic conditions now usually promote a feeling of indifference, but a report that no amino-acids are formed by low-pressure Hg lamp irradiation of a CH_4–N_2–H_2O mixture (1 : 1 : 1)[79] catches us in mid-yawn. Reviews of more productive experiments of this type are available,[80–83] and further results on direct carboxylation of aliphatic amines by formic acid under glow discharge electrolysis conditions,[81b, 84] and on the accumulation of urea, amino-acids, and u.v.-absorbing substances on alumino-silicates saturated with Ca^{2+}, NH_4^+, and Fe^{3+} salts when heated in an atmosphere of CO + NH_3,[85] have been published. Specific factors (metal ion catalysis) favouring formation of cystine in irradiated mixtures of NH_4SCN, HCHO, KH_2PO_4, and $Ca(OAc)_2$ have been studied (other amino-acids are also formed under such conditions).[86]

More evidence that α-aminonitriles are the primary condensation products in these processes, *e.g.* their formation behind shock waves in CH_4–C_2H_6–NH_3–H_2O mixtures,[87] is provided indirectly by the link between the difficulty of hydrolysis of α-alkyl-α-aminonitriles and the fact that α-alkyl-α-amino-acids are not formed in simulated prebiotic reaction mixtures.[88] Experiments with poly(α-cyanoglycine) and HCN suggest that the presence of α-amino-acids was not necessarily a prerequisite for the chance synthesis of the first proteins.[89]

Protein and Other Naturally Occurring Amino-acids.—Although general synthetic methods outlined in the preceding section are represented here in reports of new syntheses of natural amino-acids, another common approach, the use of the protein amino-acids as starting materials for the synthesis of related compounds, is also illustrated.

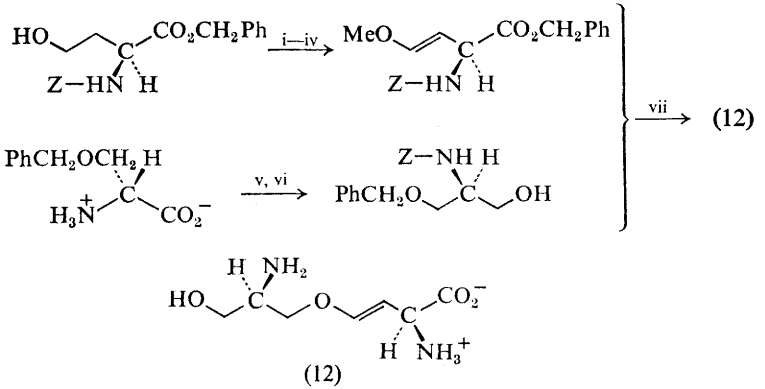
Several syntheses have been described for γ-carboxyglutamic acid,[39, 40, 75, 90] some[75, 90] yielding the γγ′-di-t-butyl ester α-methyl ester suitable for use in peptide synthesis. The pyroglutamic acid homologue 3,5-di(methoxycarbonyl)-pyrrolid-2-one has also been prepared.[75]

Longicatenamycin constituents *threo*-β-hydroxy-L-glutamic acid, L-2-amino-5-methylhexanoic acid, L-2-amino-6-methylheptanoic acid, L-2-amino-7-methyl-octanoic acid, and DL-5-chlorotryptophan,[70] DL-furanomycin,[91] an antibiotic α-amino-acid containing a 2,5-dihydrofuran moiety, have been synthesized.

Examples of the use of protein amino-acids in the synthesis of other natural amino-acids involve β-chloro-L-alanine in syntheses of *S-trans*-propenyl-L-

[79] J. P. Ferris and C. T. Chen, *J. Amer. Chem. Soc.*, 1975, **97**, 2962.
[80] K. Harada, in 'Chemistry and Biochemistry of Amino-acids, Peptides and Proteins', ed. B. Weinstein, Vol. 2, Dekker, New York, 1974.
[81] 'Origin of Life and Evolutionary Biochemistry', ed. K. Dose, S. W. Fox, and G. A. Deborin, Plenum, New York, 1974: (*a*) K. Dose, p. 69; (*b*) K. Harada, p. 183; (*c*) K. A. Kvenvolden, p. 301.
[83] K. Dose, *BioSystems*, 1975, **6**, 224.
[83] W. Groth, *BioSystems*, 1975, **6**, 229.
[84] K. Harada and T. Iwasaki, *Chem. Letters*, 1975, 185.
[85] G. Poncelet, A. T. van Assche, and J. J. Fripiat, *Origins Life*, 1975, **6**, 401.
[86] K. Bahadur and P. Sen, *Z. Mikrobiol.*, 1975, **15**, 143.
[87] A. Bar-Nun, *Origins Life*, 1975, **6**, 109.
[88] M. Béjaud, L. Mion, J. Taillades, and A. Commeyras, *Tetrahedron*, 1975, **31**, 403.
[89] R. Minard, W. Yang, P. Varma, J. Nelson, and C. Matthews, *Science*, 1975, **190**, 387.
[90] N. T. Boggs, R. E. Gawley, K. A. Koehler, and R. G. Hiskey, *J. Org. Chem.*, 1975, **40**, 2850.
[91] T. Masamune and M. Ono, *Chem. Letters*, 1975, 625.

cysteine [73] and of quisqualic acid (4),[27] use of *O*-tosyl-L-serine methyl ester in the synthesis of γ-carboxy-L-glutamic acid,[90] and use of D-serine and L-homoserine in the synthesis of rhizobitoxine [(12) in Scheme 2].[92] L-Histidine is used for the

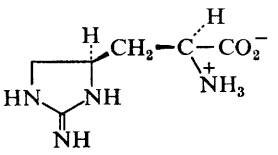


(12)

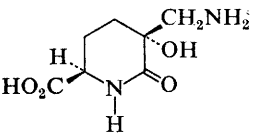
Reagents: i, CrO_3–py–CH_2Cl_2; ii, MeOH–$CH(OMe)_3$; iii, Ac_2O/cation exchange resin; iv, 180 °C; v, $LiAlH_4$; vi, Z-Cl; vii, dichlorobis(benzonitrile)palladium(II)

Scheme 2

synthesis [93] of enduracididine (13), a component amino-acid of enduracidin; Bamberger cleavage of L-histidine methyl ester, followed by catalytic hydrogenation, gives a mixture of (2*S*,4*R*)- and (2*S*,4*S*)-2,4,5-triaminopentanoic acid methyl esters, from which both natural (2*S*,4*R*) and allo-enduracididines were prepared by de-protection and guanidination.[93] 2,5-*S*,*S*-Dicysteinyldopa (9) is synthesized from L-cysteine, L-dopa, and O_2 using mushroom tyrosinase.[34]



(13)



(14)

Synthesis of higher homologous amino-acids of natural origin is represented by (−)-(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid, present in pepstatins,[94] and for tabtoxinine-δ-lactam (14).[95]

α-Alkyl- and αα-Dialkyl-amino-acids.—Further details of routes reported last year (Vol. 7, p. 10) leading to α-alkyl-substituted ornithines [96] and to 2-amino-4-chloroalkanoic acids [97] have been published. Chlorinolysis of methionine

[92] D. D. Keith, J. A. Tortora, K. Ineichen, and W. Leimgruber, *Tetrahedron*, 1975, **31**, 2633.
[93] S. Tsuji, S. Kusumoto, and T. Shiba, *Chem. Letters*, 1975, 1281.
[94] M. Kinoshita, A. Hagiwara, and S. Aburaki, *Bull. Chem. Soc. Japan*, 1975, **48**, 570.
[95] D. L. Lee and H. Rapoport, *J. Org. Chem.*, 1975, **40**, 3491.
[96] M. M. Abdel-Monem, N. E. Newton, B. C. Ho, and C. E. Weeks, *J. Medicin. Chem.*, 1975, **18**, 600.
[97] Y. Urabe, M. Miyoshi, and K. Matsumoto, *Agric. and Biol. Chem. (Japan)*,, 1975, **39**, 1085.

derivatives yields 2-amino-3,4,4,4-tetrachlorobutanoic acid derivatives in addition to the 4,4,4-trichloro analogues previously reported;[98] *N*-aryl substituents are not cleaved.[97] Electrochemical reduction of 2-amino-4,4,4-trichlorobutanoic acid[99] gives the 4,4-dichloro-analogue, *alias* armentomycin, a powerful antibacterial agent isolated in 1967 from *Streptomyces armentosus*. Electrochemical reduction of *N*-benzyloxycarbonyl 2-amino-3,4,4,4-tetrachlorobutanoic acid methyl ester gives the armentomycin analogue $Cl_2C{=}CHCH(NH_3^+)CO_2^-$ after removal of protecting groups.[99]

N-Protected esters of 3,4-dehydro-DL-proline give the corresponding stereoisomeric 3,4-epoxy-proline derivatives with peroxytrifluoroacetic acid.[100]

α-Hydroxy-α-amino-acids and α-Amino-acids with Hydroxy-groups in the Side-chain.—Novel applications of α-hydroxyhippuric acid and analogues in amino-acid synthesis[64–67] are particularly promising because of the ready availability of these starting materials using standard methods of synthesis. Oxygenation at pH 11 of optically active *N*-salicylidenealanine cobalt(III) complexes gives the corresponding diastereoisomeric α-hydroxyalanine complexes.[101] Corresponding *N*-salicylideneglycine complexes react with acetaldehyde at pH 11 to give a threonine–allothreonine mixture after electrochemical decomposition and removal of Co^{2+} ions, constituting a new asymmetric synthesis when the starting material is one of the two possible enantiomers.[102] More conventional syntheses of *threo*-4,4,4-trichlorothreonine,[57] and DL-*threo*- and DL-*erythro*-3-(*p*-fluorophenyl)-,[103] -3-(*o*-tolyl)-,[104] -3-(3,4-dihydroxyphenyl)-,[105] and -3-(3′,4′-methylenedioxyphenyl)serines[106] using corresponding aldehydes as starting materials have been reported.

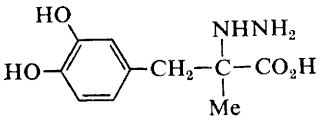
Aromatic and Heterocyclic Amino-acids.—Continuing improvements in the methods available for the synthesis of L-dopa, *e.g.* from 3-aminotyrosine[107] or from *N*-benzoyl-3-(4-hydroxy-3-methoxyphenyl)alanine,[108] have been described. 2,3,4-Trihydroxy-L-phenylalanine has been synthesized from *S*-methyl-L-cysteine and pyrogallol in the presence of L-tyrosine phenol-lyase.[109] Several combinations of carboxy-, hydroxy-, and amino-methylated derivatives are obtained by reaction of a suspension of L-dopa in MeOH–Et_2O containing diazomethane during 24 h.[110] 6-Chloro-D-tryptophan (a non-nutritive sweetening agent) can be prepared by nitration, reduction, diazotization, and Sandmeyer reactions applied to D-tryptophan.[111]

[98] Y. Urabe, T. Okawara, K. Okukura, M. Miyoshi, and K. Matsumoto, *Synthesis*, 1974, 440.
[99] Y. Urabe, T. Iwasaki, K. Matsumoto, and M. Miyoshi, *Tetrahedron Letters*, 1975, 997.
[100] C. B. Hudson, A. V. Robertson, and W. R. J. Simpson, *Austral. J. Chem.*, 1975, **28**, 2479.
[101] N. G. Faleev, Y. N. Belokon, V. M. Belikov, and L. M. Melnikova, *J.C.S. Chem. Comm.*, 1975, 85.
[102] Y. N. Belokon, V. M. Belikov, S. V. Vitt, M. M. Dolgaya, and T. F. Saveleva, *J.C.S. Chem. Comm.*, 1975, 86.
[103] N. Blazevic and F. Zymalkowski, *Arch. Pharm.*, 1975, **308**, 541.
[104] A. Hajos, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 471.
[105] B. Hegedues, A. F. Krasso, K. Noack, and P. Zeller, *Helv. Chim. Acta*, 1975, **58**, 147.
[106] K. Eisele, *Z. Naturforsch.*, 1975, **30c**, 538.
[107] B. Rzeszotarska and K. Pawelczak, *Farm. Pol.*, 1975, **31**, 137 (*Chem. Abs.*, 1975, **83**, 79 563).
[108] E. O. Renth, *Angew. Chem.*, 1975, **87**, 379; *Angew. Chem. Internat. Edn.*, 1975, **14**, 361.
[109] P. Rapp, H. Kumagai, H. Yamada, T. Ueno, and H. Fukami, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 241.
[110] J. F. Suida, *J. Org. Chem.*, 1975, **40**, 3611.
[111] T. Moriya, K. Hagio, and N. Yoneda, *Bull. Chem. Soc. Japan*, 1975, **48**, 2217.

Nucleotide analogues of α-amino-acids with variously substituted purinyl and pyrimidinyl side-chains are attracting increasing interest (see also List, p. 12). Standard methods yield *cis*-4-adeninyl-, guaninyl-, hypoxanthinyl-, uracilyl-, and thyminyl-L-prolines [74] and DL-β-[6-(4-hydroxy-3-methylbut-*trans*-2-enylamino)purin-9-yl]alanine.[26]

Monosaccharide-based α-amino-acids are also important synthetic objectives (sugars linked to amino-acid moieties *via* a C^1—C^α bond are represented in the polyoxins), and recent syntheses include L-2-(β-D-mannofuranosyl)- and -(β-D-lyxofuranosyl)glycines [56] and L- and D-2-(1,2:5,6-di-*O*-isopropylidene-α-D-allofuranos-3-yl)glycines [72] (see also List, p. 12).

N-Substituted Amino-acids.—Excluding N-protected amino-acids prepared for use in peptide synthesis, and work relevant to the coverage of this section mentioned elsewhere in this chapter,[229] recent papers have described the conversion of BOC-dehydroamino-acid methyl esters into *N*-methyl homologues using MeI–K_2CO_3 in DMF or Me_2SO_4–K_2CO_3 in MeCN;[112] also, the synthesis of N^β-oxalyl-L-αβ-diaminopropionic acid (a neurotoxin),[113] and the synthesis of 'Carbidopa' (15), a hydrazine analogue of α-methyldopa which is a potent



(15)

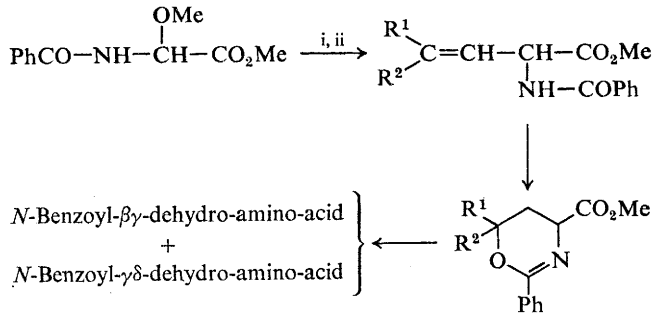
inhibitor of extra-cerebral aromatic amino-acid decarboxylase and enhances the efficiency of dopa *in vivo*.[114]

α-Aza-amino-acids.—In anticipation of an increasing volume of work under this heading, this new section is launched with one representative citation, describing the synthesis of *N*-benzoyl-α-aza-ornithine phenyl ester, a potent trypsin inhibitor.[115]

Amino-acids with Unsaturated Functional Groups in Side-chains.—Novel procedures have been devised, or have arisen from work with other objectives, for the synthesis of unsaturated amino-acids. α-Methoxy-α-amino-acids, prepared from amino-acid esters or amides by *N*-chlorination (Bu^tOCl) and dehydrochlorination followed by base-catalysed addition of MeOH to the resulting α-iminocarboxylic acid, give αβ-dehydroamino-acids on treatment with DABCO.[116] Synthesis of dehydrophenylalanine from phenylalanine *via* 2-trifluoromethyl-4-benzyloxazolone exemplifies an alternative route for the conversion of an α-amino-acid into its αβ-dehydro-analogue.[117] Conversion of 2-amino-3,4,4,4-tetrachlorobutanoic acid into 2-amino-4,4-dichlorobut-3-enoic acid by electrochemical reduction [99] cannot be developed into a general synthetic method for

[112] D. H. Rich, J. Tam, P. Mathiaparanam, and J. Grant, *Synthesis*, 1975, 402.
[113] S. L. N. Rao, *Biochemistry*, 1975, **14**, 5218.
[114] S. Vickers, E. K. Stuart, H. B. Hucker, and W. J. A. Vanden Heuvel, *J. Medicin. Chem.*, 1975, **18**, 134.
[115] C. J. Gray and R. C. Parker, *Tetrahedron*, 1975, **31**, 2940.
[116] H. Poisel and U. Schmidt, *Chem. Ber.*, 1975, **108**, 2547, 2917.
[117] E. G. Breitholle and C. H. Stammer, *Tetrahedron Letters*, 1975, 2381.

αβ-dehydroamino-acids (and requires protection of both NH_2 and CO_2H groups), but alkylation of alkenes with methyl α-methoxyhippurate [66] gives 2-phenyloxazines (Scheme 3) which give mixtures of βγ- and γδ-dehydroamino-acids, as their *N*-benzoyl derivatives, on hydrolysis.[66]



Reagents: i, $R^1R^2C{=}CH_2$; ii, BF_3

Scheme 3

Amino-acids containing Sulphur.—Straightforward syntheses of cysteine derivatives [34, 73, 118] have been reported. Protein amino-acids modified by substitution of the α-hydrogen atom by a sulphur grouping are of interest because the trifunctional moiety —NH—CR(S—)—CO— exists in gliotoxins and aranotins, and amino-acids may be modified in this way by N-protection, conversion into an α-imino-acid derivative, and addition of thioacetic acid,[116, 119] or from an α-hydroxy-α-benzamido-acid,[116, 120] prepared similarly from the α-imino-acid intermediate [116] or by the addition of an amide to an α-keto-acid.[120]

A List of Amino-acids which have been Synthesized for the First Time.—

Compound [a]	*Ref.*
β-Fluoro-L-alanine	121
β-Fluoro-DL-phenylalanine	121
D- and L-Homohistidine	122
N^{β}-[*p*-(Fluorosulphonyl)benzyl]-L-asparagine	123
N^{β}-[*p*-(Fluorosulphonyl)benzyl]-L-glutamine	123
4′-Azido-2′-nitro-L-phenylalanine	124
2-Amino-4-(3,4-dihydroxyphenyl)butyric acid ('homo-dopa')	68
2-Amino-4-(3,4,5-trihydroxyphenyl)butyric acid	68
2-Amino-2-methyl-4-(3,4-dihydroxyphenyl)butyric acid ('α-methyl-homo-dopa')	68
α-Methyl-(3-allyl-4-hydroxyphenyl)alanine	69
α-Methyl-(3-methallyl-4-hydroxyphenyl)alanine	69
α-Methyl-(3-propenyl-4-hydroxyphenyl)alanine	69
α-Methyl-(3-propyl-4-hydroxyphenyl)alanine	69

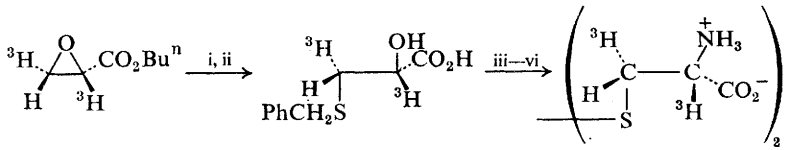
[118] R. T. Borchardt and Y. S. Wu, *J. Medicin. Chem.*, 1975, **18**, 300.
[119] R. K. Olsen and A. J. Kolar, *Tetrahedron Letters*, 1975, 3579.
[120] H. C. J. Ottenheijm, A. D. Potman, and T. Van Vroonhoven, *Rec. Trav. chim.*, 1975, **94**, 135.
[121] J. Kollonitsch, S. Marburg, and L. M. Perkins, *J. Org. Chem.*, 1975, **40**, 3808.
[122] W. Bloemhoff and K. E. T. Kerling, *Rec. Trav. chim.*, 1975, **94**, 182.
[123] M. Mokotoff, S. Brynes, and J. F. Bagaglio, *J. Medicin. Chem.*, 1975, **18**, 888.
[124] F. Fahrenholz and G. Schimmack, *Z. physiol. Chem.*, 1975, **356**, 469.

Compound [a]	*Ref.*
α-Methyl-(3-isobutyl-4-hydroxyphenyl)alanine	69
α-Methyl-[3-(2′,3′-epoxypropyl)-4-hydroxyphenyl]alanine	69
α-Methyl-[3-(2′-substituted)-5′-dihydrobenzofuryl]alanines	69
o- and *p*-Substituted phenylglycines	64
L-Canavaninosuccinic acid	125
S-Adenosyl-L-homocysteines with modified ribose moiety	118
N^{ϵ}-(4,6-Dimethyl-2-pyrimidinyl)lysine	126
N^{ϵ}-(4-Methyl-6-oxo-1,6-dihydro-2-pyrimidinyl)lysine	126
N^{ϵ}-(5-Methyl-6-oxo-1,6-dihydro-2-pyrimidinyl)lysine	126
N^{δ}-(5-Methyl-2-pyrimidinyl)ornithine	126
N^{δ}-(5-Propyl-2-pyrimidinyl)ornithine	126
N^{δ}-(5-Butyl-2-pyrimidinyl)ornithine	126
N^{δ}-(5-Pentyl-2-pyrimidinyl)ornithine	126
N^{δ}-(4,6-Dimethyl-2-pyrimidinyl)ornithine	126
O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-L-threonine *N*-methylamide	127
O-[2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl] L-serine *N*-methylamide	127
O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetyl-DL-serine *N*-methylamide	128
O-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-acetyl-DL-threonine *N*-methylamide	128

[a] Other new amino-acids, and labelled analogues of known amino-acids, mentioned elsewhere in this chapter, are not repeated in this list.

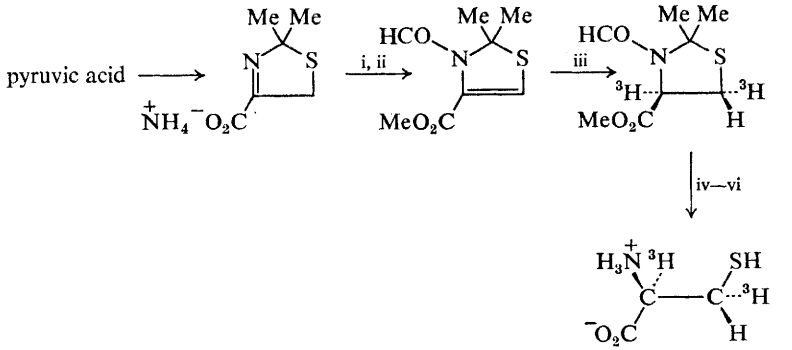
Labelled Amino-acids.—Considerable ingenuity is involved in devising routes to strategically labelled compounds, and the routes in themselves are often of outstanding interest in the general context of organic synthesis. ^{3}H-Labelled cysteine,[129] cystine,[130, 131] and valine,[132] as well as ^{15}N,^{2}H-labelled valine,[133] have been synthesized to assess aspects of penicillin biosynthesis. The routes to L-[α-^{3}H]cystine [131] and D-[α-^{3}H]valine [132] involve conventional α-tritiation (Ac_2O–$MeCO_2{}^3H$) of the isotopically natural amino-acids, while the synthesis [133] of (2*S*,3*S*)-[4,4,4-$^{2}H_3$]valine-^{15}N involving treatment of mesaconic acid-[*methyl*-$^{2}H_3$] with $^{15}NH_3$ and β-methylaspartase follows the route established earlier (Vol. 6, p. 22) for the synthesis of the [4-^{13}C]-analogue. The synthesis of (3*R*,3′*R*)-[3,3′-$^{3}H_2$]cystine and its (3*S*,3′*S*)-isomer outlined in Scheme 4 starts with labelled n-butyl glycidate,[130] and is related to a route established earlier (Vol. 6, p. 21) for the synthesis of labelled valines. The α-^{3}H in the resulting amino-acid was replaced by ^{1}H by equilibration. An alternative route (Scheme 5) starting with pyruvic acid [129] has been employed in the synthesis of (2*R*,3*R*)-[2,3-$^{3}H_2$]cysteine and its (2*R*,3*S*)-[3-^{3}H] analogue.[129]

[125] G. A. Rosenthal, *Analyt. Biochem.*, 1975, **65**, 60.
[126] F.-S. Tjoeng, E. Kraas, E. Stark, E. Breitmaier, and G. Jung, *Chem. Ber.*, 1975, **108**, 862.
[127] V. A. Derevitskaya and O. S. Novikova, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1436.
[128] M. N. Mirzayanova, I. V. Medvedeva, and A. Y. Khorlin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 697.
[129] D. J. Morecombe and D. W. Young, *J.C.S. Chem. Comm.*, 1975, 198.
[130] D. J. Aberhardt, L. J. Lin, and J. Y.-R. Chu, *J.C.S. Perkin I*, 1975, 2517.
[131] B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 123.
[132] B. W. Bycroft, C. M. Wels, K. Corbett, A. P. Maloney, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 923.
[133] F. C. Huang, J. A. Chan, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, 1975, **97**, 3858.



Reagents: i, $PhCH_2S^-Na^+$; ii, H_3O^+; iii, resolve; iv, substitution of OH by NH_2 *via* Br; v, $Na-NH_3$; vi, O_2

Scheme 4



Reagents: i, Me_2SO_4; ii, HCO_2COMe; iii, $^3H_2$/10% Pd–C; iv, NaOH; v, resolve; vi, N-HCl

Scheme 5

[4′-^{3}H]-L-Phenylalanine has been prepared from *p*-chloro-L-phenylalanine,[134] and the [2,3-$^3H_2$] analogue from 2-(acetamido)cinnamoyl-L-glutamic acid by $^3H_2$–Pd reduction;[135] [2,3-$^3H_2$]-L-tyrosine has been synthesized in a similar way.[135] The distribution of the ^{3}H label in L-[2,3-$^3H_2$]-phenylalanine and -tyrosine is approximately equal in the two positions, with the C-3 label predominantly in the *pro-S* position but of lower configurational purity than expected from the presumed stereospecificity of the method of synthesis.[135] Related degradative studies for establishing ^{3}H-distribution have been reported for [2,3-$^3H_2$]-L-valine [136] and for DL-[G-^{3}H]phenylalanine.[137] [5-^{3}H]-L-Ornithine and [6-^{3}H]-L-lysine may be prepared from γ-cyano-L-α-aminobutyric acid and from δ-cyano-L-norvaline, respectively, using NaB^3H_4 [138] as an alternative to catalytic tritiation (Vol. 6, p. 21).

^{14}C-Labelled amino-acid synthesis is illustrated for N^4-[*ethyl*-^{14}C]-L-asparagine [139] and L-4-azaleucine-[*dimethyl*-^{14}C].[140] While ^{3}H- and ^{14}C-labelling offers advantages in biosynthetic and physiological studies, ^{13}N-labelling (glutamic acid

[134] J. Kovacs, I. Teplan, and I. Mezo, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 109.
[135] G. W. Kirby, S. Narayanaswami, and P. S. Rao, *J.C.S. Perkin I*, 1975, 645.
[136] P. Adriaens, B. Meesschaert, and H. Vanderhaeghe, *Analyt. Biochem.*, 1975, **69**, 297.
[137] M. C. Clifford, A. E. Evans, A. E. Kilner, and D. C. Warrell, *J. Labelled Compounds*, 1975, **11**, 435.
[138] I. Mezo, M. Havranek, I. Teplan, J. Benes, and B. Tanacs, *Acta Chim. Acad. Sci. Hung.*, 1975, **85**, 201.
[139] R. W. Dineen and D. O. Gray, *Radiochem. Radioanalyt. Letters*, 1975, **22**, 205.
[140] L. I. Harrison, H. N. Christensen, M. E. Handlogten, A. L. Oxender, and S. C. Quay, *J. Bacteriol.*, 1975, **122**, 957.

and glutamine),[141] ^{15}N-labelling (glutamic acid,[142] pyroglutamic acid,[142] ornithine,[143] and valine [133]), and ^{18}F-labelling (DL-[4-^{18}F]-phenylalanine) [144] continue to be practised. Selective α-deuteriation of amino-acids $NH_2(CH_2)_n$-CO_2H (n = 2, 3, or 5) may be accomplished [145] by treatment with 5N-^{2}HCl at 117 °C during 20—60 h, and Pt-catalysed ^{2}H exchange of the aromatic protons of L-phenylalanine has been studied.[146] Amino-acids 85% enriched with ^{13}C have been prepared for ^{13}C–^{13}C coupling constant studies.[147, 148]

Resolution of Amino-acids.—Work continues on the applicability of chiral macroheterocycles in resolution of hexafluorophosphate salts of DL-amino-acid esters through differential complexation (Vol. 6, p. 21; Vol. 7, p. 15);[149–151] current studies deal with the testing of several macrocyclic ethers containing 2,2′-substituted-1,1′-binaphthyl units connected through various aliphatic ether chains,[150] and chromatographic resolution using the macrocycle covalently bonded to silica gel.[151]

More conventional procedures are illustrated in papers describing papain-catalysed acylation of arylamines [152] and carboxyhydrazides [153] by Z-DL-Ala-OH, and the use of carboxypeptidase A for resolution of α-methylphenylalanine and α-methylvaline as their *N*-TFA derivatives, based on the same enantiomeric selectivity principle.[154] *N*-Acetyl derivatives of L-dopa and α-methyldopa may be resolved as di-n-butylamine and hydrazine salts, respectively, through preferential crystallization of one enantiomer.[155]

N-Thiobenzoyl-α-amino-acids may be resolved in the conventional way, using a chiral amine,[156] their particular merit being their long-wavelength absorption (*ca.* 370 nm in aqueous media) which generates easily measured circular dichroism in partially resolved salts, from which the degree of resolution achieved by their crystallization may be assessed. 2-Phenylthiazol-5-ones obtained by cyclization of *N*-thiobenzoyl-DL-arginine and -lysine are substrates for trypsin, giving corresponding *N*-thiobenzoyl-L-amino-acids as hydrolysis products.[157] This provides a novel method for converting a D- or DL-α-amino-acid quantitatively

[141] A. S. Gelbard, L. P. Clarke, J. M. McDonald, W. G. Monahan, R. S. Tilbury, T. Y. T. Kuo, and J. S. Laughlin, *Radiology*, 1975, **116**, 127.
[142] W. Greenaway and F. R. Whatley, *J. Labelled Compounds*, 1975, **11**, 395.
[143] K. W. Hindmarsh, N. W. Hamon, and W. J. Ostapovich, *Canad. J. Pharm. Sci.*, 1975, **10**, 73.
[144] R. W. Goulding and S. W. Gunasekera, *Internat. J. Appl. Radiat. Isotopes*, 1975, **26**, 561.
[145] V. P. Kumarev and G. A. Almanov, *Zhur. fiz. Khim.*, 1975, **49**, 1361.
[146] R. S. Norton and J. H. Bradbury, *J. Catalysis*, 1975, **39**, 53.
[147] S. Fermandjian, D. S. Tran, J. Savrda, E. Sala, R. Mermet-Bouvier, E. Bricas, and P. Fromageot, *Biochem. Biophys. Acta*, 1975, **399**, 313.
[148] D. S. Tran, S. Fermandjian, E. Sala, R. Mermet-Bouvier, and P. Fromageot, *J. Amer. Chem. Soc.*, 1975, **97**, 1267.
[149] D. J. Cram, R. S. Helgeson, L. R. Sousa, J. M. Timko, M. Newcomb, P. Moreau, F. De Long, G. W. Gokel, and D. H. Hoffman, *Khim. geterotsikl. Soedinenii*, 1975, 1299.
[150] G. W. Gokel, J. M. Timko, and D. J. Cram, *J.C.S. Chem. Comm.*, 1975, 394, 444.
[151] G. Dotsevi, Y. Sogah, and D. J. Cram, *J. Amer. Chem. Soc.*, 1975, **97**, 1259.
[152] J. L. Abernethy, F. G. Howell, A. Ledesma, D. Doose, and R. Everett, *Tetrahedron*, 1975, **31**, 2659.
[153] J. L. Abernethy, D. Srulevitch, and M. J. Ordway, *J. Org. Chem.*, 1975, **40**, 3445.
[154] J. Turk, G. T. Panse, and G. R. Marshall, *J. Org. Chem.*, 1975, **40**, 953.
[155] S. Yamada, M. Yamamoto, and I. Chibata, *J. Org. Chem.*, 1975, **40**, 3360.
[156] G. C. Barrett and P. R. Cousins, *J.C.S. Perkin I*, 1975, 2313.
[157] M. A. Coletti-Previero and C. Axelrud-Cavadore, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 844.

into its L-enantiomer; an *in vivo* equivalent of this process is the conversion of D-lysine into L-lysine *via* L-pipecolic acid in *Neurospora crassa*.[158]

'Resolution' of a DL-amino-acid by destruction of one enantiomer is a well-known application of enzyme specificity, but the equivalent process, involving the more rapid destruction of one enantiomer of a DL-amino-acid by chiral longitudinally polarized electrons produced by β-decay processes, still appears to be unsubstantiated. The notion that L-amino-acids predominated in prebiotic times through secondary effects of chiral radiation[159] has been tested[160–163] through subjecting DL-leucine as a film to irradiation during 1.34 years with left-circularly polarized electrons; after this time, the residual amino-acid comprised 50.8% L- and 49.2% D-leucine.[160–162] However, this is still within experimental error,[162] and similar studies indicated no difference in absorption of right- or left-circularly polarized γ-radiation by D- or L-tryptophan.[163] A reasoned assessment of stereospecific discrimination expected in such high-energy processes as the interaction of elementary particles with pure crystalline chiral media suggests that effects will be very small,[164] though the expectation that higher discrimination could be found in solutions involving inherently chiral solvated electrons could not be confirmed in studies with simple chiral organic compounds.[164] An alternative effect of chiral electrons, that of favouring the crystallization of one enantiomer from a racemic melt, was demonstrated for sodium ammonium tartrate.[165]

Analytical resolution of DL-amino-acids by g.l.c. and by high-pressure liquid chromatography is discussed in Section 6.

4 Physical and Stereochemical Studies of Amino-acids

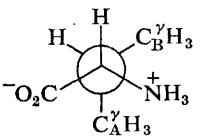
Crystal Structures of Amino-acids.—Neutron diffraction analysis of L-cysteine[166] and of glycine hydrochloride[167] provides definitive hydrogen locations and conformational features.

X-Ray crystal analysis of naturally occurring amino-acids and related compounds has been reported for L-cystine dihydrobromide dihydrate,[168] *S*-methyl-lanthionine dihydrate,[169] DL-tyrosine hydrochloride,[170] 3,5,3′-tri-iodo-L-thyronine methyl ester,[171] and *N*-(5-*O*-phosphopyridoxyl)-L-tyrosine hepta-hydrate.[172]

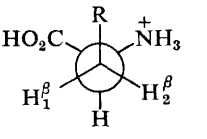
[158] W. U. Mueller and E. Leistner, *Z. Naturforsch.*, 1975, **30c**, 253.
[159] T. L. V. Ulbricht, *Nature*, 1975, **258**, 383; *Origins Life*, 1975, **6**, 303; *New Scientist*, 1975, **67**, 479.
[160] W. A. Bonner, *J. Mol. Evol.*, 1974, **4**, 23.
[161] W. A. Bonner, M. A. van Dort, and M. R. Yearian, *Nature*, 1975, **258**, 419.
[162] W. A. Bonner and J. J. Flores, *Origins Life*, 1975, **6**, 187.
[163] L. Keszthelyi and I. Vincze, *Radiat. Environ. Biophys.*, 1975, **12**, 181.
[164] M. M. Ulrich and D. C. Walker, *Nature*, 1975, **258**, 418.
[165] K. L. Kovacs and A. S. Garay, *Nature*, 1975, **254**, 538.
[166] K. A. Kerr, J. P. Ashmore, and T. F. Koetzle, *Acta Cryst.*, 1975, **B31**, 2022.
[167] A. R. Al-Karaghouli, F. E. Cole, M. S. Lehmann, C. F. Miskell, J. J. Verbist, and T. F. Koetzle, *J. Chem. Phys.*, 1975, **63**, 1360.
[168] R. E. Rosenfield and R. Parthasarathy, *Acta Cryst.*, 1975, **B31**, 816.
[169] J. R. Knox and P. C. Keck, *Acta Cryst.*, 1975, **B31**, 2698.
[170] B. Khawas, *Indian J. Phys.*, 1975, **49**, 403.
[171] V. Cody, *J. Medicin. Chem.*, 1975, **18**, 126.
[172] A. Mangia, M. Nardelli, G. Pelizzi, C. B. Volrattorni, A. Orlacchio, and C. Turano, *J.C.S. Perkin II*, 1975, 60.

Absolute configuration assigned to 3(*R*)-[1′(*S*)-aminocarboxymethyl]-2-pyrrolidone-5(*S*)-carboxylic acid (2) on the basis of o.r.d. and c.d. data has been confirmed by X-ray crystal analysis.[19]

N.M.R. Spectroscopy.—The major applications of n.m.r. spectroscopy of amino-acids continue to be the establishment of solution conformations and of protonation equilibria. Detailed interpretation of n.m.r. data for β-functional α-amino-acids in terms of rotamer populations in solution has been reported from several laboratories,[173–178] including further details of work [174] reported in preliminary communication form last year (Vol. 7, p. 18). Valine, isoleucine, and allo-isoleucine were shown, on the basis of ^1H–^1H and ^1H–^{13}C coupling constant data, to adopt similar conformations around their C^α—C^β bond, *e.g.* (16) for L-valine, and comparisons of data for these three amino-acids indicate that the upfield ^{13}C-resonance for valine can be assigned to C^γ_A.[175] L-Aspartic acid is common to the other studies of conformational preferences involving the



(16)



(17)

C^α—C^β bond in β-functional amino-acids,[174, 176–178] and the situation for serine,[173, 176] cysteine,[177] phenylalanine,[173, 176] histidine,[173] and tyrosine [176] has also been considered. The aliphatic amino-acids of this family adopt the rotamer (17) in which the three bulky groups are preferentially gauche in acidic solutions,[176, 177] primarily as a result of the structure of the surrounding solvent,[176] but the aromatic analogues show smaller preference for this rotamer indicating that steric effects exert a dominant influence.[176] These results have been obtained through 220 MHz ^1H n.m.r. studies [177] and the use of selectively deuteriated amino-acids to exploit the vicinal coupling constant between the carbonyl carbon atom and a β-proton.[176] N.m.r. data have been reported for diastereoisomers of β-hydroxyaspartic acid.[178]

Conformational studies of a more conventional type have been reported for Boc-amino-acids (using ^{13}C n.m.r. and i.r. data),[179] and for a variety of proline derivatives.[180–185] Rotameric equilibria in *N*-acyl-prolines,[181, 182] and ring conformations for L-proline [184] and derivatives [183] and their correlation with

[173] S. Fujiwara, H. Ishizuka, and S. Fudano, *Chem. Letters*, 1974, 1281.
[174] P. E. Hansen, J. Feeney, and G. C. K. Roberts, *J. Magn. Resonance*, 1975, **17**, 249.
[175] J. G. Batchelor and J. Feeney, *J.C.S. Chem. Comm.*, 1975, 503.
[176] M. Kainosho and K. Ajisaka, *J. Amer. Chem. Soc.*, 1975, **97**, 5630.
[177] B. J. Dale and D. W. Jones, *Spectrochim. Acta*, 1975, **31A**, 83.
[178] E. Luboch and J. F. Biernat, *Roczniki Chem.*, 1974, **48**, 2181.
[179] M. Branik and H. Kessler, *Chem. Ber.*, 1975, **108**, 2176, 2722.
[180] I. Z. Siemion, T. Wieland, and K. H. Pook, *Angew. Chem. Internat. Edn.*, 1975, **14**, 702.
[181] H. Nishihara, K. Nishihara, T. Uefuji, and N. Sakota, *Bull. Chem. Soc. Japan*, 1975, **48**, 553.
[182] E. W. B. de Leer and J. M. van der Toorn, *Rec. Trav. chim.*, 1975, **94**, 119.
[183] L. Pogliani, *Spectroscopy Letters*, 1975, **8**, 37.
[184] L. Pogliani, M. Ellenberger, and J. Valat, *Org. Magn. Resonance*, 1975, **7**, 61.
[185] G. V. Fazakerly and G. E. Jackson, *J.C.S. Perkin II*, 1975, 567.

long-range coupling constant data,[183] are described. Related work covers *N*-acetyl-*N*-methyl-L-alanine-[*dimethylamide*-$^2H_6$],[186] and β- and γ-amino-acids.[187]

Geminal ^2J(C,C) and vicinal ^3J(C,C) ^{13}C–^{13}C coupling constants are small (0—5 Hz) and only observable in ^{13}C-enriched amino-acids if the enrichment factor is substantially greater than 50%, and if Fourier transform acquisition times are greater than about 0.8 s.[147, 148] Vicinal coupling between CO and C^γ is shown by amino-acids with a four-carbon sequence (Asp, Thr, and Val), while C^α–C^δ coupling is shown by higher homologues.[148] Information on torsion angles should be contained in ^{13}C–^{13}C coupling constant data,[148] and further studies are under way to see what possibilities exist for conformational studies of amino-acids using this approach.[148]

N.m.r. studies of histidine deal with pH-dependence of ^{13}C chemical shift data and ^1H–^{13}C coupling constants for the imidazole moiety.[188] A splendid example of the merits of ^{15}N n.m.r. describes the use of pH-shift data to show that the τ-H tautomer of L-histidine predominates in alkaline solution.[189] ^{15}N n.m.r. data of a more fundamental type (relaxation times and chemical shifts) as a function of pH have been reported for glycine in aqueous solutions,[190] and ^{13}C n.m.r. data are available for the same system.[191] ^1H and ^{19}F n.m.r. titration curves (in ^2H_2O and H_2O, respectively), have been measured for 2- and 4-fluoro-histidines.[192]

^{13}C-Resonances for the ε- and *N*-methyl carbon atoms are shifted progressively downfield through the series lysine, N^ε-methyl-lysine, and NN^ε-dimethyl-lysine.[193]

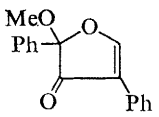
Binding of metal ions to lysine and histidine,[194] or to thiaproline,[185] has been reported, representing an application of n.m.r. of increasing importance. A novel application, dipolar splitting in ^1H n.m.r. shown for the nematic lyotropic phase formed between optically active sodium dodecyl sulphate and D- and L-alanine, respectively, reveals that the D-alanine complex is some 6% less ordered than that formed with L-alanine.[195]

O.R.D. and C.D. Spectra.—Aliphatic L-α-amino-acids show a weak Cotton effect in the 240—250 nm wavelength range in addition to the diagnostic 215 nm Cotton effect. The longer-wavelength features are best studied by c.d., which shows the presence of the long-wavelength Cotton effect in spectra of L-leucine and its esters, as well as corresponding *N*-methanesulphonyl derivatives and *N*-hydroxy-analogues.[196] Previous assignments of the long-wavelength c.d. of aliphatic amino-acids, in terms of a charge-transfer transition of a non-bonding

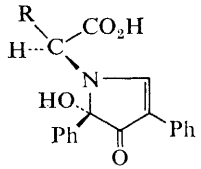
[186] Y. Imanishi, K. Kugimiya, and T. Higashimura, *Polymer*, 1975, **16**, 345.
[187] N. S. Ham, '7th Jerusalem Symposium on Quantum Chemistry and Biochemistry', 1974, 261 (*Chem. Abs.*, 1975, **83**, 39 073).
[188] R. E. Wasylishen and G. Tomlinson, *Biochem. J.*, 1975, **147**, 605.
[189] K. Kawano and Y. Kyogoku, *Chem. Letters*, 1975, 1305.
[190] T. K. Leipert and J. H. Noggle, *J. Amer. Chem. Soc.*, 1975, **97**, 269.
[191] H. Pearson, D. Gust, I. M. Armitage, H. Huber, J. D. Roberts, R. E. Stark, R. R. Vold, and R. L. Vold, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1599.
[192] H. J. C. Yeh, K. L. Kirk, L. A. Cohen, and J. S. Cohen, *J.C.S. Perkin II*, 1975, 928.
[193] C. S. Baxter and P. Byvoet, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 514.
[194] W. Voelter, G. Sokolowski, U. Weber, and U. Weser, *European J. Biochem.*, 1975, **58**, 159.
[195] A. S. Tracey and P. Diehl, *F.E.B.S. Letters*, 1975, **59**, 131.
[196] T. Polonski, *Tetrahedron*, 1975, **31**, 347.

electron on nitrogen to a π^*-antibonding orbital of the carboxy chromophore, are supported.[196]

Further examples of the use of c.d. spectra in assignments of absolute configuration to amino-acids after introducing a suitable chromophore have been reported for [Cu(succinimidato)$_2$(amino-acidato)$_2$] complexes,[197] ketimines,[198] *N*-thiobenzoyl [156] and *N*-(2,4-dinitrophenyl) derivatives,[199] and pyrrolinones (19)



(18)



(19)

obtained through condensation of α-amino-acids with 2-methoxy-2,4-diphenylfuran-3(2*H*)-one (18).[200] Although (19) is formed together with its diastereoisomer by reaction of (18) with an L-α-amino-acid, this is apparently not a drawback to the use of the pyrrolinones (19) for the chirospectral assignment of absolute configuration to amino-acids since the longest-wavelength Cotton effect (centred at *ca.* 380 nm) is positive in every case for reaction products (19) from 24 L-α-amino-acids.[200] On this basis, these are more reliable than most of the many other chromophoric derivatives proposed for the same purpose in recent years; assignment of absolute configuration to rhizobitoxine [(12) in Scheme 2] using this method required careful use of analogues of known absolute configuration,[201] and the chirospectral assignment was proved to be correct through stereochemically unambiguous total synthesis.[92] Like any other empirical method, however, the underlying reasons for the relationship between sign of longest-wavelength Cotton effect and absolute configuration need to be established so that this method may be used with full confidence.

An important observation, relevant to the interpretation of the c.d. behaviour of *N*-acyl-L-amino-acids in different solvents, has emerged from n.m.r. and c.d. study of *trans*- and *cis*-isomers of *N*-acyl-L-prolines;[181] the $n \rightarrow \pi^*$ c.d. developed in the *cis*-amide chromophore in these compounds is opposite in sign to that of the *trans*-isomer.

Further data on factors determining the c.d. developed in the chiral disulphide chromophore are provided through study of *S*-alkylthio-L-cysteines and glutathione.[202]

Absolute configurational assignments to penmacric acid (2) made with the help of c.d. data have been confirmed by *X*-ray crystal analysis.[19]

Mass Spectrometry.—The mass spectrum of methionine can be interpreted to indicate the formation of EtS^+ by successive elimination of CO_2H and $CH_2{=}CHNH_2$.[203]

[197] F. Kerek and G. Snatzke, *Angew. Chem.*, 1974, **87**, 133.
[198] G. Bettoni, V. Tortorella, A. Hope, and B. Halpern, *Tetrahedron*, 1975, **31**, 2383.
[199] M. Kawai, U. Nagai, and M. Katsumi, *Tetrahedron Letters*, 1975, 2845.
[200] V. Toome, S. de Bernardo, and M. Weigele, *Tetrahedron*, 1975, **31**, 2625; V. Toome and G. Reymond, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 75.
[201] D. D. Keith, S. de Bernardo, and M. Weigele, *Tetrahedron*, 1975, **31**, 2629.
[202] M. Ottnad, C. Ottnad, P. Hartter, and G. Jung, *Tetrahedron*, 1975, **31**, 1155.
[203] C. G. Van den Heuvel and N. M. M. Nibbering, *Org. Mass Spectrometry*, 1975, **10**, 250.

This section does not attempt a comprehensive coverage of mass spectrometry of amino-acid derivatives since most papers describing this topic are linked with peptide sequence methodology. A representative paper[204] describes the identification of amino-acids and peptides after reaction with fluorescamine or with 2-methoxy-2,4-diphenylfuran-3(2*H*)-one (18).

Other Physical and Theoretical Studies.—MO calculations leading to predicted conformations for amino-acid derivatives have been reported for *N*-acetyl *N*-methylamides of L-lysine,[205] L-leucine,[206] and glycine.[207] Laser-Raman spectra and depolarized Rayleigh scattering data for the *N*-methylamide of pyroglutamic acid reveal a change in torsion angle ψ_1 from $+169°$ to $-20°$ from crystal to aqueous solution states.[208]

I.r. spectroscopic studies of ^{14}C-labelled amino-acids[209] and of L- and DL-alanine[210] have been reported; complementary n.m.r. and i.r. studies of amino-acid derivatives are cited in the n.m.r. section.[178,179] Molar magnetic susceptibilities of alkali-metal salts of amino-acids have been determined.[211]

Physical properties at surfaces and interfaces, with particular reference to prebiotic events concerning amino-acids, have been studied for simple systems. Partitioning of amino-acids between water and aqueous micellar sodium dodecanoate and between hexane and water trapped in dodecylammonium propionate has been investigated.[212] Although asymmetric adsorption of phenylalanine enantiomers and asymmetric polymerization of DL-aspartic acid on kaolin has been ruled out,[162] (+)-quartz in powdered form is claimed to be capable of preferential adsorption of the D-enantiomer from anhydrous DMF solutions of DL-alanine,[213] leading to solutions with D : L ratio 49.5 : 50.5.

Determination of Absolute Configuration of Amino-acids.—Chemical correlation allows allotment of (*S*)-configuration to (+)-α-ethylphenylglycine,[214] and use of amino-acid oxidases leads to allotment of the L-configuration to the alternamic acid residue in alternariolide, a novel toxin reported last year (Vol. 7, p. 2).[215]

5 Chemical Studies of Amino-acids

Racemization.—Amino-acids present in fossils found in temperate regions (average temperature 25 °C) are completely racemized after *ca.* 100 000 years, but L-aspartic acid (racemization rate constant 8.29×10^{-4} year^{-1}) undergoes

[204] D. G. Pritchard, W. C. Schnute, and C. W. Todd, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 312.
[205] B. S. Zhorov, E. M. Popov, and V. A. Govyrin, *Mol. Biol.* (*Moscow*), 1975, **9**, 415 (*Chem. Abs.*, 1975, **83**, 73 844).
[206] I. S. Maksumov, S. F. Arkhipova, G. M. Lipkind, and E. M. Popov, *Khim. prirod. Soedineniya*, 1975, **11**, 211.
[207] R. Rein, V. Renugopalakrishnan, S. Niv, and T. J. Swissler, *Internat. J. Quantum Chem., Quantum Biol. Symp.*, 1975, **2**, 99.
[208] M. Allard, M. Avignon, and A. M. Bellocq, *Biopolymers*, 1975, **14**, 1565.
[209] L. Zilka, *Radioisotopy*, 1975, **16**, 215.
[210] N. Kaneko, H. Takahashi, and K. Higasi, *Bull. Chem. Soc. Japan*, 1975, **48**, 1961.
[211] M. A. Bernard, N. Bois, and M. Daireaux, *Canad. J. Chem.*, 1975, **53**, 3167.
[212] J. H. Fendler, F. Nome, and J. Nagyvary, *J. Mol. Evol.*, 1975, **6**, 215.
[213] W. A. Bonner, P. R. Kavasmaneck, F. S. Martin, and J. J. Flores, *Origins Life*, 1975, **6**, 367.
[214] H. Mizuno and S. Yamada, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 527.
[215] T. Okuno, Y. Ishita, A. Sugawara, Y. Mori, K. Sawai, and T. Matsumoto, *Tetrahedron Letters*, 1975, 335.

relatively rapid racemization in fossils and in biological cul-de-sacs such as tooth enamel. An interesting consequence is that *ca.* 8% of the aspartic acid content of tooth enamel of a warm-blooded animal (*e.g.* man) would be the D-enantiomer after 60 years; furthermore, aspartic acid residues in proteins with a long *in vivo* lifetime might undergo racemization sufficient to form 'mutant' proteins with some involvement in the ageing process.[216] An immediate application is suggested,[216] the checking of controversial claims to longevity by certain Georgians (if still equipped with their own teeth).

Among several reports of pyridoxal-mediated reactions of amino-acids (see also p. 23) a study of the racemization of L-glutamic acid by various pyridoxal–copper(II) systems [217] is eligible for citation in this section. An interesting example of enantiomeric differentiation is provided by the faster racemization of L-alanine by the (*S*)-2′-nitro-5-nitroso-6,6′-dimethylbiphenyl-2-ol–copper(II) system compared with that of D-alanine.[218] A thorough mechanistic study of base-catalysed racemization of *NN*-di(carboxymethyl)-D-phenylglycine, in the presence of metal ions, indicates removal of the α-proton by HO^- in all systems studied.[219]

The α-epimerization step in the conversion of isoleucine into allo-isoleucine may be effected using isobutyric anhydride.[220]

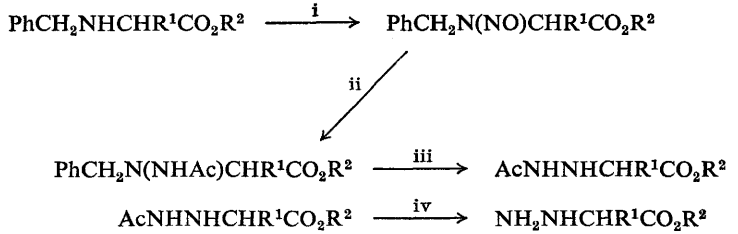
General Reactions.—α-Amino-acids are converted into corresponding carbonyl derivatives on treatment with *N*-sulphinylaniline, analogous to the Strecker degradation;[221] glycine is exceptional in being oxidized to formic acid in this reaction.[221] Oxidation of amino-acids with H_2O_2 or H_2O_2–$CuSO_4$ has been studied, leading to a number of unexpected observations (ornithine gives β-alanine *via* 4-aminobutyric acid; proline gives 3-hydroxyproline).[222] Direct addition of α-amino-acids to alkenes in Ac_2O gives *N*-acetyl-2-pyrrolines or isomeric acetamido-cyclobutanones.[223] A limited study of thermal condensation reactions occurring in mixtures of six amino-acids (including glutamic acid in excess) has been reported; three peptides were isolated from the glass-like melt.[224] Mannich reactions involving amino-acids have been surveyed.[225]

ATP-activated amino-acids become paramagnetic in a solution containing an acyl-tRNA-synthetase, the unpaired electron being located on the carboxy-group as a rule (but on the thiol group in the exceptional case of cysteine).[226] Further details of free-radical formation during reactions of amino-acids with sugar derivatives have been published.[227]

Full details are now available [228] of synthesis of α-substituted α-diazo-esters from amino-acid esters (Vol. 6, p. 30). Yamada's group [229] has also established

[216] P. M. Helfman and J. L. Bada, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2891.
[217] M. Ando and S. Emoto, *Bull. Chem. Soc. Japan*, 1975, **48**, 1655.
[218] K. Hirota, *Bull. Chem. Soc. Japan*, 1975, **48**, 2509.
[219] L. G. Stadtherr and R. J. Angelici, *Inorg. Chem.*, 1975, **14**, 925.
[220] G. Flouret and S. H. Nakagawa, *J. Org. Chem.*, 1975, **40**, 2635.
[221] T. Taguchi, S. Morita, and Y. Kawazoe, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 2654.
[222] H. A. Gruber and E. F. Mellon, *Analyt. Biochem.*, 1975, **66**, 78.
[223] F. Texier and O. Yebdri, *Tetrahedron Letters*, 1975, 855.
[224] P. Melius and J. Y. P. Sheng, *Bio-org. Chem.*, 1975, **4**, 385.
[225] J. H. Short and C. W. Ours, *J. Heterocyclic Chem.*, 1975, **12**, 869.
[226] C. Courty, *Compt. rend.*, 1975, **281**, *D*, 583.
[227] M. Namiki and T. Hayashi, *J. Agric. Food Chem.*, 1975, **23**, 487.
[228] N. Takamura, T. Mizoguchi, K. Koga, and S. Yamada, *Tetrahedron*, 1975, **31**, 227.
[229] K. Achiwa and S. Yamada, *Tetrahedron Letters*, 1975, 2701.

a convenient route to L-hydrazino-acids (Scheme 6) for use in their newly introduced asymmetric synthesis of α-amino-acids (Vol. 7, p. 6).



Reagents: i, $NaNO_2$–aq. HCl; ii, Zn–AcOH–Ac_2O; iii, H_2–Pd–C; iv, 6N-HCl

Scheme 6

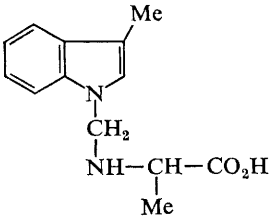
Amino-acids can be recovered from their hydrochlorides by treatment in DMF with $(Me_3Si)_2NH$ and hydrolysis of the resulting *NO*-bis(trimethylsilyl) derivatives.[230] *NO*-Bis(dimethylphenylsilyl)amino-acids are conveniently prepared in THF using dimethylphenylsilane and $NiCl_2$.[231] A useful procedure for synthesis of esters from N-protected amino-acids employs a hydrazone and peracetic acid at 0—10 °C.[232] De-protection procedures are augmented by two recent studies, one providing delicate selectivity in removal of Boc-groups in the presence of t-butyl esters [233] and the other providing means (FSO_3H or $MeSO_3H$) for stripping most of the commonly used amino-acid protecting groups.[234]

Specific Reactions.—The uses of readily available amino-acids as starting materials for synthesis of terpenes, carbohydrates, and alkaloids have been reviewed.[235]

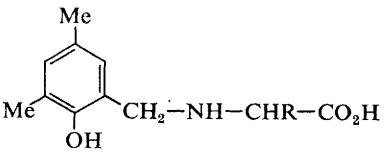
Oxidation studies (see also preceding section) based on aliphatic amino-acids [manganese(III) as oxidant] [236] and products from tryptophan (peroxyacetic acid as oxidant) [237] have been reported. *N*-Acetyltyrosine ethyl ester and N^{α}-acetyllysine react with formaldehyde in EtOH at 37 °C during several days to give complex mixtures, but identifiable products (20) and (21) are formed when the amino-acid derivatives are replaced by 2,4-dimethylphenol or 3-methylindole together with glycine, alanine, or valine.[238]

Nitrosation at the indole nitrogen atom of *N*-acetyltryptophan has been confirmed [239] by the appearance of new signals in the 500—550 p.p.m. region of the ^{15}N n.m.r. spectrum of the product obtained using $Na^{15}NO_2$, and independent

[230] A. I. Yurtanov, Y. A. Davidovich, and S. V. Rogozhin, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 1420; *Synthesis*, 1975, 113.
[231] M. Abe, K. Adachi, T. Takiguchi, Y. Iwakura, and K. Uno, *Tetrahedron Letters*, 1975, 3207.
[232] R. Bywood, G. Gallagher, G. K. Sharma, and D. Walker, *J.C.S. Perkin I*, 1975, 2019.
[233] J. Goodacre, R. J. Ponsford, and I. Stirling, *Tetrahedron Letters*, 1975, 3609.
[234] H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1164.
[235] S. Yamada and K. Koga, *Yuki Gosei Kagaku Kyokai Shi*, 1975, **33**, 535.
[236] A. M. Beg and Kamaluddin, *Acta Chim. Acad. Sci. Hung.*, 1975, **86**, 65.
[237] W. E. Savage, *Austral. J. Chem.*, 1975, **28**, 2275.
[238] M. K. Dewar, R. B. Johns, D. P. Kelly, and J. F. Yates, *Austral. J. Chem.*, 1975, **28**, 917.
[239] R. Bonnett, R. Holleyhead, B. L. Johnson, and E. W. Randall, *J.C.S. Perkin I*, 1975, 2261.



(20)

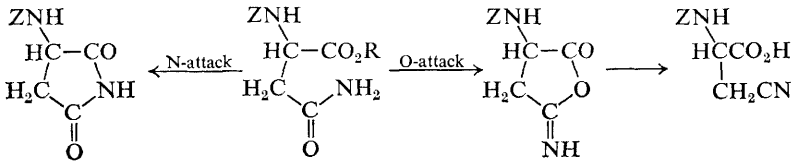


(21) R = H, Me, or Pr^i

evidence for the same site of nitrosation in *N*-acetyltryptophan esters is provided by the susceptibility of the products to chymotrypsin-catalysed hydrolysis.[240]

Studies with basic amino-acids cover synthesis of *N*-DNP-ornithine and lysine,[241] identification of the unprotonated ε-amino-group as the site of reaction between lysine and *p*-nitrophenyl acetate at pH 8.1—10.1,[242] periodate oxidation of δ-hydroxylysine to Δ^1-pyrroline-5-carboxylic acid *via* α-aminoglutaric acid γ-semi-aldehyde,[243] and synthesis of τ- and π-carboxymethyl-histidines.[244]

Asparagine active esters, *e.g.* (22; R = C_6F_5), provide the first[245] demonstration of competitive O- and N-attack at a carbonyl group under neutral conditions (Scheme 7); however, neither intramolecular process is fast enough to



Scheme 7

compete with the intermolecular aminolysis reactions applied to (22) when used in peptide synthesis.[245] High-yield syntheses of β-aspartate and γ-glutamate monoesters by copper(II)-catalysed hydrolysis of corresponding diesters represent improvements of a well-known procedure.[246]

Specific Reactions of Amino-acids Related to Biochemical Processes.—Several reports of pyridoxal-mediated processes have appeared, dealing with β-elimination from *S*-(*p*-substituted phenyl)cysteines and *O*-phosphothreonine,[247] β-decarboxylation of L-aspartic acid,[248] retro-aldol cleavage of threonine and β-hydroxyvaline,[249] monodecarboxylation of α-amino-α-alkylmalonate esters,[71] and racemization of L-glutamic acid.[217]

[240] T. B. Brown and M. F. G. Stevens, *J.C.S. Perkin I*, 1975, 2357.
[241] J. L. Brooks, *Analyt. Biochem.*, 1975, **66**, 290.
[242] D. S. Kristol, P. Krautheim, S. Stanley, and R. C. Parker, *Bio-org. Chem.*, 1975, **4**, 299.
[243] G. Y. Wu and S. Seifter, *Analyt. Biochem.*, 1975, **67**, 413.
[244] T. Weighardt and H. J. Gorne, *Bio-org. Chem.*, 1975, **4**, 30.
[245] L. Kisfaludy, I. Schon, M. Renyei, and S. Gorog, *J. Amer. Chem. Soc.*, 1975, **97**, 5588.
[246] R. L. Prestidge, D. R. K. Harding, J. E. Battersby, and W. S. Hancock, *J. Org. Chem.*, 1975, **40**, 3287.
[247] Y. Murakami and H. Kondo, *Bull. Chem. Soc. Japan*, 1975, **48**, 125, 541.
[248] N. Y. Sakkab and A. E. Martell, *Bio-inorg. Chem.*, 1975, **5**, 67.
[249] J. A. Marcello, A. E. Martell, and E. H. Abbott, *J.C.S. Chem. Comm.*, 1975, 16.

Further confirmation of specific interactions between inosine and L-lysine [250a] or D- or L-tryptophan [250b] (Vol. 6, p. 31) has been provided. Cationic micelles (cetyltrimethylammonium bromide) catalyse nucleophilic aromatic substitution of 2,6-dinitro-4-trifluoromethylbenzenesulphonate by amino-acids with hydrophobic side-chains.[251]

A recent structural assignment to the *p*-benzoquinone-cysteine ethyl ester condensation product has been corrected so as to conform with earlier conclusions.[252]

Effects of Electromagnetic Radiation on Amino-acids.—U.v. photochemistry of amino-acids has been reviewed for the areas of photosensitized reactions [253] and their quenching of singlet oxygen.[254] Photo-oxidation of methionine [255] and reactions of photolytically produced hydrogen atoms with cysteine and penicillamine have been studied.[256] Luminescence properties of aromatic amino-acids,[257, 258] products of laser flash photolysis [259] of tryptophan in aqueous solution, and radical-anion formation from u.v.-irradiated tryptophan amides [260] have been reported.

Ultrasound (800 kHz) irradiation of representative amino-acids in dilute aqueous solutions at isoelectric points (histidine also at pH 2 and 10) during 6 h gives NH_3, primary amines, HCHO, and other carbonyl compounds, in simple cases.[261] Conversion into other α-amino-acids is also observed (*e.g.* cysteine give cystine and serine) but between 39 and 92% of the starting material survives the irradiation.[261]

Radiolysis studies of cysteine,[262, 264] *N*-acetylcysteine,[263] and *N*-acetylmethionine,[264] *N*-acetylalanine,[265] tryptophan,[266] proline,[267] and histidine,[268] and broader studies of the same type,[269] have been reported.

[250] (*a*) Y. Suzuki, K. Hara, and T. Hirahara, *Bull. Chem. Soc. Japan*, 1975, **48**, 2149; (*b*) Y. Suzuki, K. Hara, and T. Hirahara, *ibid.*, p. 2342.
[251] C. A. Bunton and J. L. Wright, *Tetrahedron*, 1975, **31**, 3013.
[252] F. McCapra and Z. Razari, *J.C.S. Chem. Comm.*, 1975, 42; *corrigendum, ibid.*, p. 492; G. Prota and E. Ponsiglione, *Tetrahedron Letters*, 1972, 1327.
[253] G. Jori, *Photochem. and Photobiol.*, 1975, **21**, 463.
[254] I. B. C. Matheson, R. D. Etheridge, N. R. Kratowich, and J. Lee, *Photochem. and Photobiol.*, 1975, **21**, 165.
[255] S. G. Cohen and S. Ojanpera, *J. Amer. Chem. Soc.*, 1975, **97**, 5633.
[256] J. T. Wu and R. R. Kuntz, *Radiation Res.*, 1975, **64**, 662.
[257] D. V. Bent and E. Hayon, *J. Amer. Chem. Soc.*, 1975, **97**, 2599, 2606, 2612.
[258] C. Helene, T. Montenay-Garestier, and M. Charlier, *An. Acad. Bras. Cienc.*, 1975, **45** (Supplement), 59.
[259] F. D. Bryant, R. Santus, and L. I. Grossweiner, *J. Phys. Chem.*, 1975, **79**, 2711.
[260] V. B. Ilyasova, C. A. Azizova, and L. P. Kayushin, *Stud. Biophys.*, 1975, **49**, 143.
[261] W. H. Staas and L. A. Spurlock, *J.C.S. Perkin I*, 1975, 1675.
[262] G. C. Goyal and D. A. Armstrong, *Canad. J. Chem.*, 1975, **53**, 1475.
[263] G. Saxebol and O. Herskedal, *Radiation Res.*, 1975, **62**, 395.
[264] J. H. Hadley and W. Gordy, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3486.
[265] G. Saxebol, *Internat. J. Radiation Biol. Related Studies in Phys., Chem., Med.*, 1975, **27**, 293.
[266] J. L. Redpath, R. Santus, J. Ovadia, and L. I. Grossweiner, *Internat. J. Radiation Biol. Related Studies in Phys., Chem., Med.*, 1975, **27**, 201.
[267] J. Kopoldova and M. Voracek-Hubsch, *Z. Naturforsch.*, 1975, **30c**, 474.
[268] E. Westhof, W. Flossman, and A. Mueller, *Internat. J. Radiation Biol. Related Studies in Phys., Chem., Med.*, 1975, **27**, 51.
[269] T. Gejvall and G. Lofroth, *Radiation Effects*, 1975, **25**, 187; Y. Tal and M. Farragi, *Radiation Res.*, 1975, **62**, 337, 347; J. E. Aldrich, K. Y. Lam, P. C. Shragge, and J. W. Hunt, *ibid.*, 1975, **63**, 42.

6 Analytical Methods

This section is sub-divided as in previous volumes; there is some overlap in sub-divisions dealing with colorimetric procedures and specific amino-acid assays, and with the preceding section dealing with chemical reactions of amino-acids.

Gas–Liquid Chromatography.—G.l.c. techniques for amino-acid analysis are now widely used; general techniques have been reviewed [270] and the use of g.l.c.–m.s. techniques for trace amino-acid analysis is well illustrated for studies of meteorites [6, 7] and of bacterial cultures.[12]

Volatile derivatives used for the g.l.c. identification of amino-acids, as reported in the 1975 literature, are *N*-trifluoroacetyl butyl esters,[271, 272] (of guanidino-acids [272]), 1,1,1,3,3,3-hexafluoropropyl esters,[273] *N*-pentafluoropropionyl 2,2,3,3,3-pentafluoropropyl esters,[274] *N*-heptafluorobutyryl n-propyl esters,[275] isobutyl esters,[276] and isoamyl esters.[277] Alternative approaches described in recent years are further illustrated for acetylated methylthiohydantoins [278] and 2-trifluoromethyloxazol-3-in-5-ones,[279] these heterocyclic compounds being readily prepared from amino-acids and in the latter case providing a method for the quantitative analysis of phenylalanine in blood serum.[279] L-Tryptophan condensed with formaldehyde gives 2,3,4,5-tetrahydro-β-carboline-4-carboxylic acid, whose trimethylsilyl derivative is suitable for g.l.c.–m.s. analysis.[280]

The g.l.c. technique lends itself particularly well to the determination of optical purity of amino-acids (for reviews see refs. 281, 282; for applications in meteorite studies and in aspartic acid analysis of fossils and tooth enamel see refs. 6*c* and 216, respectively). This is achieved either by conversion of the amino-acid into a pair of volatile diastereoisomers (*N*-trifluoroacetyl 2-butyl esters,[5] and *l*-menthyl esters,[283, 284] or *N*-trifluoroacetyl-L-prolyl methyl [285] and butyl esters [286] have been employed; the optical purity of aspartic acid has been determined by reaction with L-leucine-*N*-carboxyanhydride followed by conversion of the diastereoisomeric dipeptides into volatile derivatives [216]), or by resolving a volatile derivative of the amino-acid on an optically active stationary phase [161, 283, 287–289] (*N*-trifluoroacetyl-L-methionyl-L-methionine cyclo-

[270] B. Kolb, in ref. 2*b*, Vol. 1B, p. 1020.
[271] P. Morard, M. Garcia, and J. P. Cabassy, *Analusis*, 1975, **3**, 451.
[272] H. Patel and B. D. Cohen, *Clin. Chem.*, 1975, **21**, 838.
[273] J. D. M. Pearson and D. F. Sharman, *J. Neurochem.*, 1975, **24**, 1225.
[274] S. Wilk and M. Orlowski, *Analyt. Biochem.*, 1975, **69**, 100.
[275] J. F. March, *Analyt. Biochem.*, 1975, **69**, 420.
[276] S. L. MacKenzie and D. Tenaschuk, *J. Chromatog.*, 1975, **104**, 176; **111**, 413.
[277] P. Felker and R. Bandurski, *Analyt. Biochem.*, 1975, **67**, 245.
[278] J. C. Cavadore and J. Derancourt, *Biochimie*, 1974, **56**, 1151.
[279] O. Grahl-Nielsen and B. Moerik, *Biochem. Med.*, 1975, **12**, 143.
[280] B. S. Middleditch, *Analyt. Letters*, 1975, **8**, 397.
[281] E. Gil-Av, *J. Mol. Evol.*, 1975, **6**, 131.
[282] K. D. Haegele, P. Y. Howard, and W. Parr, *Origins Life*, 1975, **6**, 195.
[283] H. Iwase, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1604, 1608.
[284] M. Hasegawa and I. Matsubara, *Analyt. Biochem.*, 1975, **63**, 308.
[285] J. D. Kemp, *Physiol. Plan.*, 1975, **35**, 53.
[286] H. Iwase, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 217.
[287] F. Andrawes, R. Brazell, W. Parr, and A. Zlatkis, *J. Chromatog.*, 1975, **112**, 197.
[288] W. A. Koenig and G. J. Nicholson, *Analyt. Chem.*, 1975, **47**, 951.
[289] R. Charles, U. Beitler, B. Feibush, and E. Gil-Av, *J. Chromatog.*, 1975, **112**, 121.

hexyl ester[287] and related dipeptide derivatives,[288] and *N*-lauroyl-L-valine t-butylamide[161] or 2-methyl-2-heptadecylamide,[289] or *N*-docosanoyl-L-valine t-butylamide,[289] have been used successfully as stationary phases).

Ion Exchange and Partition Column Chromatography.—Improvements to amino-acid analyser techniques[290, 291] including a simplified buffer system for gradient elution analysis of the protein amino-acids[292, 293] have been described. A system for single-column amino-acid analysis which also copes with amino-sugars has been developed,[294] and progress towards sub-nanomole automated amino-acid analysis has been discussed.[295] The high-pressure partition chromatography technique has been advocated for ion-exchange chromatographic analysis of amino-acids,[296] allowing 16 protein amino-acids to be separated in 45 min, and analysed in the picomole range using fluorescamine as colour reagent.

Analysis of basic amino-acids by ion-exchange methods calls for modified amino-acid analyser techniques; recent papers[297–304] describe resolutions of lysine, thialysine, and selenalysine,[298] techniques for hydroxylysine[299] and 3-methylhistidine,[300] and the separation of the various basic amino-acids and their *N*-methyl derivatives.[301–304]

Chromatographic analysis of cysteine and related sulphur-containing amino-acids has been reviewed,[305] and determination of *S*-adenosyl-methionine and -homocysteine in tissue has been described.[306] Separation of tri-iodothyronine, thyroxine, and various iodinated tyrosines and histidines can be achieved on Biogel P-2.[307]

The possibility of interaction between an amino-acid and components of buffer systems must be borne in mind, and the formation of double or asymmetric peaks for L-*trans*-3-hydroxyproline has been traced to reversible complex formation with hydroxy-acids.[308]

Analysis of β-aminoisobutyric acid[309] and γ-aminobutyric acid[310] in physiological fluids using the autoanalyser has been described.

High-pressure liquid chromatography has already proved its value in analytical chemistry, and recent papers in the amino-acid field describe the separation of

[290] S. J. Potashner and J. D. Knowles, *Analyt. Biochem.*, 1975, **65**, 435.
[291] R. W. Giese and J. F. Riordan, *Analyt. Biochem.*, 1975, **64**, 588.
[292] D. G. Redman, *J. Chromatog.*, 1975, **104**, 178.
[293] Y. Houpert, P. Tarallo, and G. Siest, *J. Chromatog.*, 1975, **115**, 33.
[294] A. G. Georgiadis, J. W. Coffey, J. G. Hamilton, and O. N. Miller, *Analyt. Biochem.*, 1975, **67**, 453.
[295] R. L. Niece, *J. Chromatog.*, 1975, **103**, 25.
[296] W. Voelter and K. Zech, *J. Chromatog.*, 1975, **112**, 643; *Chromatographia*, 1975, **8**, 350.
[297] R. S. Ersser, *J. Chromatog.*, 1975, **115**, 612.
[298] C. De Marco, A. Rinaldi, S. Dernini, P. Cossu, and D. Cavallini, *J. Chromatog.*, 1975, **114**, 291.
[299] N. Blumenkrantz and G. Asboe-Hansen, *Clin. Biochem.*, 1975, **8**, 177.
[300] C. C. Long and J. W. Geiger, *Biochem. Med.*, 1975, **12**, 267.
[301] H. W. Lange and K. Hempel, *J. Chromatog.*, 1975, **107**, 389.
[302] A. Beckerton, P. J. Buttery, F. J. Bailey, and N. Bolton, *J. Chromatog.*, 1975, **104**, 170.
[303] R. T. Markiw, *Biochem. Med.*, 1975, **13**, 23.
[304] C. G. Zarkadas, *Canad. J. Biochem.*, 1975, **53**, 96.
[305] M. Friedman and A. T. Noma, *Nutr. Clin. Nutr.*, 1975, **1**, 521.
[306] J. Hoffmann, *Analyt. Biochem.*, 1975, **68**, 522.
[307] P. Thomopoulos, *Analyt. Biochem.*, 1975, **65**, 600.
[308] M. Man, R. M. Gryder, and E. Adams, *Analyt. Biochem.*, 1975, **63**, 513.
[309] E. Solem, D. P. Agarwal, and H. W. Goedde, *Clin. Chim. Acta*, 1975, **59**, 203.
[310] B. S. Glaeser and T. A. Hare, *Biochem. Med.*, 1975, **12**, 274.

phenylthiohydantoins[311] and the estimation of optical purity of amino-acids after conversion into diastereoisomeric *N*-[(+)-10]camphorsulphonyl *p*-nitrobenzyl esters.[312]

Thin-layer Chromatography.—Suitable conditions have been found[313] for the separation of protein amino-acids in hydrolysates, using electrophoresis followed by t.l.c. on cellulose. A novel technique, t.l.c. on strong acid cation exchange resin with part of the layer in the Na^+ form, the remainder in the Li^+ form, shows its worth in the separation of asparagine from glutamine.[314] Double spots for L-3,3′,5-tri-iodothyronine in certain eluents are due to the use of 50% aqueous propyleneglycol as solvent for placing the sample on the chromatogram.[315]

The already extensive literature on quantitative t.l.c. of phenylthiohydantoins has been augmented further;[316–319] fluorescein-thiohydantoins can be detected on micro-polyamide layers at picomole levels.[320]

Dansylamino-acids can be detected on thin layers at extremely low concentrations, quantitative estimation of leucine at $\leqslant 10^{-11}$ mol l^{-1} being typical;[321] cyclohepta-amylose increases the fluorescence intensity of these derivatives 10-fold, and stabilizes the fluorescence[322] (see also Vol. 7, p. 29). No improvement is obtained using 4-dimethylaminoazobenzene-4′-sulphonyl chloride for chromophoric labelling of amino-acids since 10^{-10}—10^{-11} mol l^{-1} are lower limits.[323] Extraordinary sensitivity is provided by use of radioactively labelled materials;[324] use of ^{3}H-labelled dansyl chloride leads to the possibility of detection of femtomolar amounts of amino-acids, and further development of the double isotope dansylation technique (^{3}H-dansyl chloride and ^{14}C-labelled amino-acids as internal standards; Vol. 6, p. 37) permits the analysis of putative amino-acid transmitters glutamic acid and glutamine, aspartic acid, glycine, alanine, serine, taurine, and γ-aminobutyric acid, in *ca.* 50 μg of pigeon optic nerve.[325]

Colorimetric Procedures.—Mention has been made elsewhere in this chapter of colorimetric analysis procedures, and the papers collected together here describe fundamental or comparative studies. Although quantitative colorimetric assays of amino-acids, using the fluorescent pyrrolinones obtained on reaction with fluorescamine,[326] have much merit, modified conditions for the *o*-phthalaldehyde

[311] J. X. De Vries, R. Frank, and C. Birr, *F.E.B.S. Letters*, 1975, **55**, 65.
[312] H. Furukawa, E. Sakikibara, A. Kamei, and K. Ito, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1625.
[313] R. L. Munier and B. Longchambon-Faivre, *Compt. rend.*, 1975, **280**, *D*, 919.
[314] A. Varadi, *J. Chromatog.*, 1975, **110**, 166.
[315] F. G. Stanford and M. L. Golder, *J. Chromatog.*, 1975, **104**, 474.
[316] D. A. Walz and J. Reuterby, *J. Chromatog.*, 1975, **104**, 180.
[317] G. K. Zwolinski and L. R. Treiber, *J. Chromatog.*, 1975, **107**, 311.
[318] M. Kubota, N. Takahashi, K. Goto, and T. Murachi, *Analyt. Biochem.*, 1975, **64**, 494.
[319] K. D. Kulbe and Y. M. Nogueira-Hattesohl, *Analyt. Biochem.*, 1975, **63**, 624.
[320] K. Muramoto, H. Kawauchi, and K. Tuzimura, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 2241.
[321] B. Loessner, R. Jork, and H. Matthies, *Acta Biol. Med. Ger.*, 1975, **34**, 1.
[322] T. Kinoshita, F. Iinuma, K. Atsumi, Y. Kanada, and A. Tsuji, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1166.
[323] J.-K. Lin and J.-Y. Chang, *Analyt. Chem.*, 1975, **47**, 1634.
[324] S. R. Burzynski, *Analyt. Biochem.*, 1975, **65**, 93.
[325] P. M. Beart and S. R. Snodgrass, *J. Neurochem.*, 1975, **24**, 821.
[326] A. M. Felix, V. Toome, S. De Bernardo, and M. Weigele, *Arch. Biochem. Biophys.*, 1975, **168**, 601.

procedure show that it can be made some 5—10 times more sensitive than the fluorescamine or ninhydrin procedures, permitting quantitative analysis of picomole amounts.[327] Similar sensitivity is claimed for the fluorimetric assay of pyridoxal derivatives of amino-acids.[328]

Successive treatment of tryptophan or dopa with formaldehyde and glyoxylic acid gives fluorescent products permitting the identification of these amino-acids in tissue by microspectrofluorimetry.[329]

Other Analytical Methods.—An electrophoretic method is suitable for the determination of *S*-methylmethionine from plant sources;[330] the technique is represented in publications cited elsewhere in this chapter.[313]

Determination of Specific Amino-acids.—Several papers in other sections deal with techniques for the determination of specific amino-acids, but the papers collected here use well-established methods or tailor-made modifications.

Determination of specific amino-acids present in physiological fluids has been reviewed.[331] Most of the literature cited here deals with clinical assays, with methods for proline and hydroxyproline,[332–336] and methods for aromatic amino-acids,[337–346] being particularly numerous. Among the latter group of papers are methods for determination of tyrosine,[337, 338, 342] thyroxine and triiodothyronine,[339–341] tryptophan,[342–344] 5-hydroxytryptophan,[345] and histidine.[346] Enzymic assays are presented for arginine[347, 348] and for asparagine[348] as well as for some of the aromatic amino-acids.[337, 344, 346] A method has been developed for the analysis of diaminopimelic acid in urine,[349] and a new colorimetric procedure for the determination of cystine has been announced.[350]

[327] J. R. Benson and P. E. Hare, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 619.
[328] K. Hempel, H. W. Lange, and N. Lustenberger, *Instrum. Forsch.*, 1974, **2**, 13.
[329] O. Lindvall, A. Bjorklund, B. Falck, and L. A. Svensson, *Histochemistry*, 1975, **46**, 27.
[330] G. N. Khuchna and A. B. Stasyak, *Priklady Biokhim. Mikrobiol.*, 1975, **11**, 914.
[331] F. A. Ibbott, in 'Clinical Chemistry: Principles and Techniques', ed. R. J. Henry, D. C. Cannon, and J. W. Winkelman, Harper and Row, New York, 1974, 2nd edn., p. 565.
[332] N. Blumenkrantz and G. Asboe-Hansen, *Analyt. Biochem.*, 1975, **63**, 331.
[333] R. Nagel and M. Keysser, *Deutsch. Gesundheitswes.*, 1975, **30**, 2289.
[334] B. T. Nobbs, A. W. Walker, and T. J. Davies, *Clin. Chim. Acta*, 1975, **64**, 219.
[335] P. Vratny, M. Stefl, and I. Trcka, *Chem. Listy*, 1975, **69**, 379.
[336] T. Tepper and L. De Vos, *Clin. Chim. Acta*, 1975, **59**, 373.
[337] A. Kumar and G. D. Christian, *Clin. Chem.*, 1975, **21**, 325.
[338] J. Chrastil, *Analyt. Chem.*, 1975, **47**, 2293.
[339] P. S. Zavadskii, A. V. Negovskaya, and A. S. Ametov, *Patol. Fiziol. Eksp. Ter.*, 1975, 83.
[340] M. I. Surks and J. H. Oppenheimer, *Methods Enzymol.*, 1975, **36A**, 537.
[341] A. Burger, C. Sakaloff, V. Staeheli, M. B. Vallotton, and S. H. Ingbar, *Acta Endocrinol.*, 1975, **80**, 58; V. Chan, T. Merrett, J. Landon, A. M. Linden, and M. Joustra, *Ann. Clin. Biochem.*, 1975, **12** (Part 4), 173.
[342] S. S. Hassan, *Analyt. Chem.*, 1975, **47**, 1429.
[343] A. Dalby and C.-Y. Tsai, *Analyt. Biochem.*, 1975, **63**, 283.
[344] K. Shikata, H. Obata, and T. Tokuyama, *Technol. Rep. Kansai Univ.*, 1975, **16**, 103 (*Chem. Abs.*, 1975, **83**, 189 981).
[345] K. Shimomura, T. Fukushima, and T. Danno, *J. Pharm. Pharmacol.*, 1975, **27**, 197.
[346] T. T. Ngo, *Internat. J. Biochem.*, 1975, **6**, 371.
[347] G. Gaede and M. Grieshaber, *Analyt. Biochem.*, 1975, **66**, 393.
[348] T. T. Ngo, *Internat. J. Biochem.*, 1975, **6**, 663.
[349] J. Krysciak, *Chem. Analysis*, 1975, **20**, 549, 635.
[350] M. Horiuchi and T. Chiba, *J. Pharm. Soc. Japan*, 1975, **95**, 1263.

2
Structural Investigations of Peptides and Proteins

BY I. D. WALKER, G. WINTER, D. J. WORTHINGTON, H. MUIRHEAD, AND R. H. PAIN

PART I: Primary Structure and Chemical Modification *by I. D. Walker, G. Winter, and D. J. Worthington*

1 Introduction

The purpose of this section is to present a summary of work published in 1975 relevant to the purification, chemical modification, and sequence analysis of proteins. Priority in the text has been given to interesting and novel approaches, whereas the tables are more comprehensive. We have attempted, where possible, to evaluate the usefulness of new techniques and ideas.

2 Protein Isolation and Characterization

Many of the protein purifications reported in 1975 utilize the well-tried and tested properties of ion-exchange resins, gel filtration, salting-out phenomena, and precipitation by organic solvents. Space limitations preclude discussion of the more routine procedures and this section will concentrate on novel methods of isolation and recent advances in the chemical characterization of proteins.

Two new volumes of 'Methods in Enzymology' were published in 1975 relevant to the isolation of proteins.[1] Both are concerned with enzymes involved in carbohydrate metabolism, and form extremely useful reference books, containing the purification details of a host of enzymes that are classed as having carbohydrate substrates. Most of the purifications have appeared previously in the biochemical literature, but to have them all under one (or two) covers is valuable.

Membrane Proteins.—With the recent upsurge of interest in the structure and function of membranes there has been a corresponding increase in the number of reports concerned with the techniques of isolating proteins from membranes. Most of these deal with the use of different detergents for the initial solubilization of the membrane, before their purification by conventional techniques of ion-exchange chromatography, gel filtration, and affinity chromatography. The binding of Triton X100 to several membrane proteins has been reported.[2] Sucrose density gradient centrifugation was used to measure the binding of Triton X100 to opsin, plasma low-density lipoprotein, sodium–potassium dependent ATPase, and human red-cell major sialoglycoprotein. The diffusion coefficients of Triton-protein complexes were estimated from gel filtration

[1] 'Methods in Enzymology', Academic Press, New York, 1975, Vols. 41 and 42.
[2] S. Clarke, *J. Biol. Chem.*, 1975, **250**, 5459.

experiments, and a value for the partial specific volume of these complexes was determined by the variation between sedimentation coefficients in H_2O and D_2O. The parathyroid hormone receptor from bovine kidney cortex plasma membrane has been solubilized with some success by Triton X100, and the detergent removed afterwards by the use of Bio-beads SM2.[3]

^{35}S-labelled carboxyatractylate was used to monitor the specific binding protein for this ligand in mitochondrial membranes.[4] The extent of solubilization was investigated using various detergents including Brij, sodium dodecylsulphate (SDS), cholate, and deoxycholate. Eventually 4% cholate was used to extract the most soluble proteins, followed by Triton X100 to solubilize the binding protein in the presence of carboxyatractylate.[5] The ligand stabilizes the protein presumably by preventing denaturation. The technique of preloading receptors with their binding ligand will probably be a useful asset for stabilizing membrane proteins during their isolation.

The major sialoglycopeptides of the human red-cell membrane have been fractionated into two distinct components by gel filtration, in the presence of the non-ionic detergent Ammonyx-LO.[6] Similarly the zwitterionic detergent Empigen BB has been used in low concentrations to solubilize the proteins of the milk fat globule membrane, in a form suitable for gel filtration experiments.[7]

Another approach to the solubilization of membrane proteins has been to increase the negative charge on the proteins by chemical modification. Certain proteins appear to be selectively released from human erythrocyte membranes by treatment with citraconic or maleic anhydride, resulting in the solubilization of up to 20% of the membrane proteins that were not released by normal washing techniques.[8] Another report suggests that up to 50% of the erythrocyte membrane protein may be solubilized by maleic anhydride under certain conditions.[9] With the addition of 8M urea this may be increased to 95% of the total membrane proteins. The extra proteins solubilized by maleic anhydride, after the addition of urea, are presumed to be the integral membrane proteins, as opposed to the peripheral proteins released in the absence of urea. The increase in negative charge by the introduction of these carboxy-groups obviously causes greater electrostatic repulsion forces between the membrane proteins, increasing their hydrophilicity. This technique has the advantage that under certain circumstances the blocking group may be removed and the native protein regained, thus facilitating reconstruction experiments.

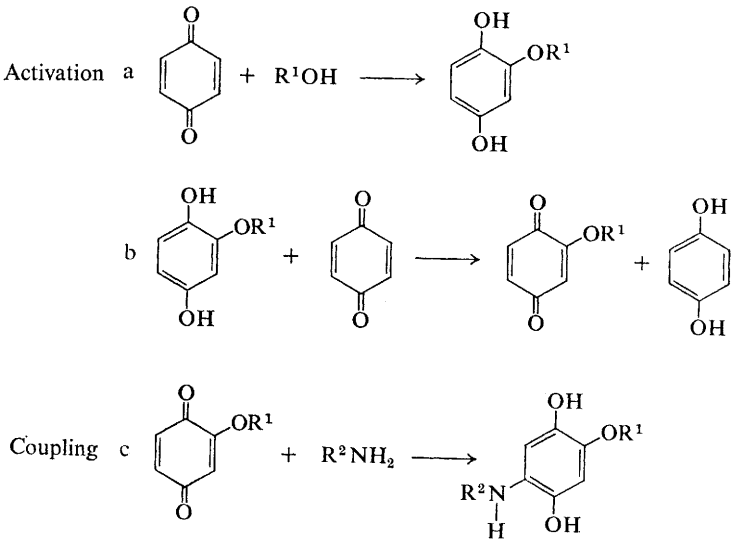
Affinity Chromatography.—Each year sees refinements and modifications to the methodology of affinity chromatography, either in new activation reactions, new supports, or new applications for old methods. It is clear from Table 1 (see p. 43) that affinity techniques are being used increasingly for the purification of proteins. The data presented in the Table are quite comprehensive but the

[3] C. C. Malbon and J. E. Zull, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 179.
[4] P. Riccio, H. Aquila, and M. Klingenberg, *F.E.B.S. Letters*, 1975, **56**, 129.
[5] P. Riccio, H. Aquila, and M. Klingenberg, *F.E.B.S. Letters*, 1975, **56**, 133.
[6] H. Furthmayr, M. Tomita, and V. T. Marchesi, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 113.
[7] J. C. Allen and C. Humphries, *F.E.B.S. Letters*, 1975, **57**, 158.
[8] P. Lundahl, *Biochim. Biophys. Acta*, 1975, **379**, 304.
[9] B. P. Vadlamadi, J. D. Dzandu, and P. F. Larway, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 64.

text has to be selective in nature, and can pretend to give no more than an indication of current trends in this active field of research.

Attachment of Ligands. An improved procedure for cyanogen bromide (CNBr) activation has been reported.[10] CNBr dissolves in polar solvents such as *N*-methyl-2-pyrrolidone, dioxan, or dimethylformamide, which are miscible with water, allowing more accurate and convenient addition of reagent in the activation step. The chemistry of the CNBr activation step and of subsequent reactions with amine groups is discussed in a report dealing with the quantitation of alkylamino side-chains coupled to Agarose beads.[11]

A method of covalently attaching ligands to polysaccharide matrices using benzoquinone has been described.[12] This method was used successfully to couple the proteins ribonuclease, chymotrypsin, and serum albumin to Agarose and dextran gels, and there is no reason why small ligands containing amino-groups could not be coupled using the same technique. The advantage claimed for this method is the high degree of coupling over a relatively broad pH range. The mechanism proposed for this reaction is given in Scheme 1.



Scheme 1

A novel method has been described for reductively alkylating proteins to aldehyde matrices.[13] After oxidizing Sepharose, dextran-coated porous glass, or glycerylsilane-coated glass with sodium periodate, proteins could be reductively alkylated to the support using sodium borohydride. Although this technique has found an initial application in coupling proteins to matrices, it is possible that smaller molecular weight ligands could be attached by a similar mechanism.

[10] A. H. Nishikawa and P. Bailon, *Analyt. Biochem.*, 1975, **64**, 268.
[11] M. Sharma and W. R. Slaunwhite, jun., *Analyt. Biochem.*, 1975, **68**, 79.
[12] J. Brandt, L.-O. Andersson, and J. Porath, *Biochim. Biophys. Acta*, 1975, **386**, 196.
[13] G. P. Royer, F. A. Liberatore, and G. M. Green, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 478.

The quantitation of the amount of ligand bound to a matrix remains a problem, and often the only method that is used is to estimate the amount of unbound ligand remaining after the coupling reaction. Spectrophotometric methods to determine the amount of proteins bound to Sepharose have been described,[14] and these might find a use for estimating the amount of antibody attached to a resin when attempting to purify proteins and peptides by immunoabsorbent chromatography. Similarly the distribution of proteins covalently attached to Sepharose has been directly visualized by ferritin-antibody labelling of the protein and electron microscopy.[15]

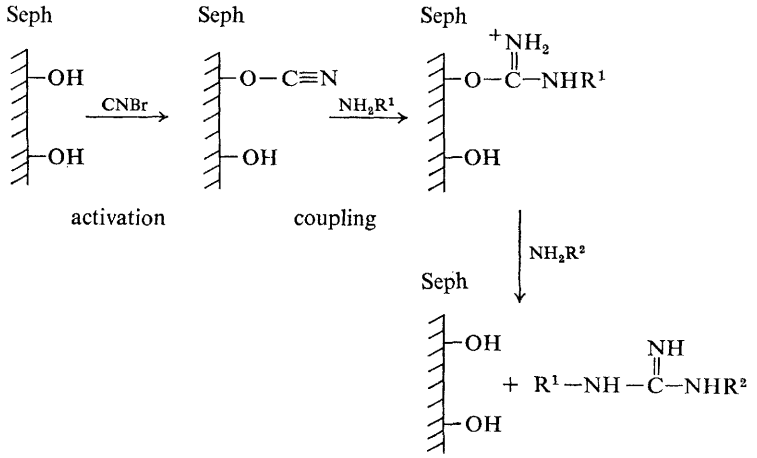
The application of supports other than insoluble carbohydrate polymers is rather limited, though there have been several reports of the use of hydroxyalkyl methacrylate gels for the covalent attachment of mercury derivatives [16, 17] and pepsin,[18] and polyacrylamide gels have been used for the attachment of lysine [19] and a glucopyranoside.[20] It is claimed that these gels are more resistant to degradation, there is more control over the gel porosity and easier control over the concentration of ligand attached. It is probable that such gels will find an application for the attachment of a variety of ligands suitable for the purification of proteins by affinity chromatography. A cross-linked copolymer of maleinic anhydride and butanediol-divinyl ether has been used for the covalent attachment of acetyl cholinesterase and cholinesterase,[21] and the cross-linking of concanavalin A by glutaraldehyde has enabled the purification of a carcinoembryonic antigen.[22] Another support that could prove useful to affinity methodology is nylon tubing,[23] although its applications to date have been mainly restricted to the attachment of enzymes to the nylon surface.[24, 25]

Release of Ligands. One of the problems often encountered in affinity chromatography is the slow leakage of the ligand from the column. This may be due to unstable attachment of the ligand to the matrix, and a recent report has shown that commercial Sepharose beads are non-uniform in structure.[26] Microscopic examination has shown vacuoles of varying size and structure inside Sepharose beads. Obviously diffusion of ligand trapped in such vacuoles, but not covalently bound, could account for some examples of its leakage. Leakage rates may be monitored using radioactive ligands, and this has been demonstrated using isoproterenol [27] and growth hormone.[28]

[14] R. Koelsch, J. Lasch, I. Marquardt, and H. Hanson, *Analyt. Biochem.*, 1975, **66**, 556.
[15] J. Lasch, M. Iwig, R. Koelsch, H. David, and I. Marx, *European J. Biochem.*, 1975, **60**, 163.
[16] S. Vavreinová and J. Turková, *Biochim. Biophys. Acta*, 1975, **403**, 506.
[17] J. Turková, S. Vavreinová, M. Krivakova, and J. Čoupek, *Biochim. Biophys. Acta*, 1975, **386**, 503.
[18] O. Valentová, J. Turková, R. Lapka, J. Zima, and J. Čoupek, *Biochim. Biophys. Acta*, 1975, **403**, 192.
[19] E. E. Rickli and W. I. Otavsky, *European J. Biochem.*, 1975, **59**, 441.
[20] R. L. Schnaar and Y. C. Lee, *Biochemistry*, 1975, **14**, 1535.
[21] C. Alsen, U. Bertram, T. Gertseuer, and F. K. Ohnesorge, *Biochim. Biophys. Acta*, 1975, **377**, 297.
[22] M. G. Brattain, C. M. Jones, J. M. Pittman, and T. G. Pretlow, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 63.
[23] D. L. Morris, J. Campbell, and W. E. Hornby, *Biochem. J.*, 1975, **147**, 593.
[24] J. Campbell, W. E. Hornby, and D. L. Morris, *Biochim. Biophys. Acta*, 1975, **384**, 307.
[25] T. T. Ngo and K. J. Laidler, *Biochim. Biophys. Acta*, 1975, **377**, 317.
[26] T. C. J. Gribnau, C. Stumm, and G. I. Tesser, *F.E.B.S. Letters*, 1975, **57**, 301.
[27] G. Vavquelin, M. L. Lacombe, J. Hanoune, and A. D. Strosberg, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 1076.
[28] F. F. Bolander, jun., and R. E. Fellows, *Biochemistry*, 1975, **14**, 2938.

The problems associated with the release of ligands from matrices has been highlighted by work with insolubilized insulin. It has been suggested that insolubilized insulin could be used to show whether its hormonal effects were mediated solely by attachment to membrane receptors or whether it needed to penetrate the membrane and gain access to components inside the cell. It has now been shown that less than 1% of the bound insulin is biologically active and this low activity may be accounted for by non-covalently bound ligand.[29] This tends to cast doubt on the original suggestions that insulin could not penetrate the membrane, based on similar insolubilization experiments.

Another interesting report has demonstrated that insulin may be released from a Sepharose support by bovine serum albumin, giving a product with increased insulin activity.[30] The adduct formed between CNBr-activated Sepharose and insulin is thought to be an *O*-Sepharose-N-substituted isourea. This may be susceptible to nucleophilic attack by amine groups in the bovine serum albumin, releasing an N^1,N^2-disubstituted guanidine (Scheme 2). Such a mechanism would



Scheme 2

explain the release of insulin from a Sepharose matrix. The possibility of ligand detachment by this type of mechanism should be borne in mind when designing experiments using CNBr-activated Sepharose.

A novel method of releasing protein bound to affinity resin, which has been used successfully for the purification of a hydroxysteroid dehydrogenase, is to include a disulphide bond in the spacer arm.[31] After the binding of specific protein to the ligand, and the removal of other proteins by washing, the ligand–protein complex may be removed by reducing the disulphide bridge with thiols. This technique may find an application for the recovery of labile membrane proteins.

[29] H. J. Kolb, R. Renner, K. D. Hepp, L. Weiss, and O. H. Wieland, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 248.
[30] M. Wilchek, T. Oka, and Y. J. Topper, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1055.
[31] F. Sweet and N. K. Adair, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 99.

Another gentle method of removing a protein from an insolubilized ligand utilizes a temperature-labile linkage.[32] After attaching cobalamin-binding proteins to a hydroxycobalamin support, raising the temperature of the column causes dissociation of the amine–cobalt interaction responsible for binding, and thus releases those proteins that bind to this ligand.

Nucleotide Binding Proteins. Several applications of blue dextran, covalently attached to carbohydrate matrices, for the purification of proteins containing the so-called 'dinucleotide fold' have been described. Numerous dehydrogenase and kinase enzymes which possess binding sites of this type may be bound to blue dextran–Sepharose at low ionic strength, and eluted either by increasing the salt concentration, or by low concentrations of the nucleotide substrate.[33–35] The protein kinase of bacteriophage T_7 was purified by attachment to blue dextran and subsequent gel filtration, when the protein–blue dextran complex elutes with the void volume of the column.[36]

The preparation of new analogues of ATP, ADP, and AMP suitable for attachment to insoluble matrices has been reported.[37] These differ from previous C^8 analogues in being substituted at the N^6-position forming (6-aminohexyl)-carbamoylmethyl derivatives. It has been shown that the N^6 derivative of ATP could be dephosphorylated with soluble hexokinase and then rephosphorylated by acetate kinase. The same N^6-derivative of NADH has been bound to Sephadex together with lactate dehydrogenase, and the enzyme can be shown to catalyse its normal reaction with no requirement for a soluble cofactor.[38] Another NAD derivative, nicotinamide-6-(2-hydroxy-3-carboxypropylamino)purine di-nucleotide, has been synthesized and shown to be reducible when bound to a Sephadex support.[39] Obviously these nucleotide derivatives can be used for the purification of enzymes that possess a suitable binding site.

Covalent and Hydrophobic Chromatography. The purification of proteins by covalent attachment to insoluble matrices is often used for thiol-containing enzymes (see Table 1 and last year's report). The method is either based on thiol–disulphide exchange,[40–42] or the covalent binding of thiol groups to mercury derivatives.[16, 17, 43–46] In most cases, the protein can be eluted by addition of thiols to the elution buffer.

Another type of attachment reported utilizes the formation of a covalent enzyme–substrate intermediate on a solid support.[47] Acetoacetyl-coenzyme A

[32] N. Ebba, *Biochim. Biophys. Acta*, 1975, **379**, 189.
[33] S. T. Thompson, K. H. Cass, and E. Stellwagen, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 669.
[34] R. Roskoski, jun., C.-T. Lim, and L. M. Roskoski, *Biochemistry*, 1975, **14**, 5105.
[35] M. Silink, R. Reddel, M. Bethel, and P. B. Rowe, *J. Biol. Chem.*, 1975, **250**, 5982.
[36] S.-H. Pai, H. Ponta, M.-J. Rahmsdorf, M. Hirsch-Kauffmann, P. Herrlich, and M. Schweiger, *European J. Biochem.*, 1975, **55**, 299.
[37] M. Lindberg and K. Mosbach, *European J. Biochem.*, 1975, **53**, 481.
[38] S. Gestrelius, M.-O. Månsson, and K. Mosbach, *European J. Biochem.*, 1975, **57**, 529.
[39] P. Zappelli, A. Rossodivita, and L. Re, *European J. Biochem.*, 1975, **54**, 475.
[40] L. J. Lin and J. F. Foster, *Analyt. Biochem.*, 1975, **63**, 485.
[41] J. Carlsson, R. Axén, and T. Unge, *European J. Biochem.*, 1975, **59**, 567.
[42] C.-B. Laurell, J. Pierce, U. Persson, and E. Thulin, *European J. Biochem.*, 1975, **57**, 107.
[43] K. Otto and H. Riesenkönig, *Biochim. Biophys. Acta*, 1975, **379**, 462.
[44] V. Madelian and W. A. Warren, *Analyt. Biochem.*, 1975, **64**, 517.
[45] R. J. Boegman, *F.E.B.S. Letters*, 1975, **53**, 99.
[46] J. Bode and K. G. Wagner, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 868.
[47] A. Fenselau and K. Wallis, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 350.

may be linked to an Agarose support *via* the coenzyme moiety. Addition of succinyl CoA–acetoacetyl CoA transferase to this substituted matrix produces a covalent enzyme–CoA–Agarose complex. The enzyme may be eluted by adding 10 mM acetoacetate to the buffer. Similar types of support could be designed for enzymes that form a covalent attachment with their substrate as part of their reaction mechanism.

Hydrophobic chromatography depends on the interaction of a hydrophobic ligand with hydrophobic sites on the protein molecule. Although the mechanism behind this technique is not fully understood, it has been used with good effect for the purification of several proteins (see Table 1). The choice of conditions for a specific separation is still highly empirical, and most methods rely upon trial and error to determine optimal conditions. The length of the alkyl chain, and the number and type of charged substituents, may have profound effects on whether a particular protein becomes bound. This type of information may be used to indicate the nature of the accessible hydrophobic groups in proteins.[48, 49]

Applications of Affinity Techniques. The numerous proteins purified in 1975 using affinity column techniques are given in Table 1.

A new method of protein purification termed 'affinity partitioning' has been described.[50] The use of a specific polymer ligand that partitions predominantly in one phase of an aqueous polymer two-phase system, will shift the protein to which it binds into that phase. Countercurrent distribution in the presence of a polymer ligand can separate a particular protein from a heterogeneous mixture. This method has been used successfully to purify concanavalin A and a specific myeloma protein.

Biospecific affinity chromatography continues to expand in directions other than the isolation of proteins. In a broad sense, affinity chromatography may include the specific interaction of small molecules with insolubilized proteins, and the attachment of enzymes and immunoglobins to intact matrices to study cofactor and antigen binding are good examples of this. The binding of NADH to lactate dehydrogenase has been studied after attaching oxamate to a Sepharose matrix,[51] and nucleotide binding to *Staphylococcal* nuclease has been estimated by attachment of thymidine to a Sepharose matrix.[52] Similarly pyridoxal phosphate binding to insolubilized apotryptophanase has been studied kinetically.[53] The isolation of selected cofactors may be effected using the same approach of binding apoenzyme to a solid support and has found success in the isolation of NAD using alcohol or lactate dehydrogenase,[54] and of FMN using apoflavodoxin.[55] The analysis of refolding of denatured, immobilized trypsin and trypsinogen,[56] and the interaction between immobilized actin and myosin have both been helped by minimizing the polymerization found in free solution.[57, 58] Immobilized proteases

[48] B. H. J. Hofstee, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 618.
[49] A. H. Nishikawa and P. Bailon, *Analyt. Biochem.*, 1975, **68**, 274.
[50] S. D. Flanagan and S. H. Barondes, *J. Biol. Chem.*, 1975, **250**, 1484.
[51] R. I. Brinkworth, C. J. Masters, and D. J. Winzor, *Biochem. J.*, 1975, **151**, 631.
[52] B. M. Dunn and I. M. Chaiken, *Biochemistry*, 1975, **14**, 2343.
[53] S.-I. Ikeda, Y. Sumi, and S. Fukui, *Biochemistry*, 1975, **14**, 1464.
[54] K. Das, P. Dunnill, and M. D. Lilly, *Biochim. Biophys. Acta*, 1975, **397**, 277.
[55] S. G. Mayhew and M. J. J. Strating, *European J. Biochem.*, 1975, **59**, 539.
[56] N. K. Sinha and A. Light, *J. Biol. Chem.*, 1975, **250**, 8624.
[57] R. C. Bottomley and I. P. Trayer, *Biochem. J.*, 1975, **149**, 365.
[58] E. S. Elgart, T. Gusovsky, and M. D. Rosenberg, *Biochim. Biophys. Acta*, 1975, **410**, 178.

have been used to probe the topography of RNA polymerase [59] and to investigate the thermally induced unfolding of ribonuclease.[60] Similarly an immobilized protein kinase has found a use in the radioactive labelling of proteins, overcoming the problems associated with the incorporation of the phosphate label into the kinase itself in free solution.[61] The fact that immobilization of enzymes and proteins probably produces some degree of altered polypeptide conformation must not be overlooked in such experiments.[62–66]

Electrophoresis.—*Isoelectric Focusing.* The use of electrophoresis for protein purification is still rather limited, possibly due to the difficulties in adapting analytical systems to cope with the amount of protein needed for a preparative scale. The most promising technique seems to be isoelectric focusing, whereby proteins are subjected to electrophoresis in a gradient of pH, maintained by a suitable ampholyte mixture in a relatively inert, anticonvective medium, such as polyacrylamide, Sepharose, or sucrose density gradients. The proteins migrate to a position corresponding to their isoelectric point in the pH gradient, where they often precipitate and appear as visible bands.

One problem associated with this technique is the removal of the matrix after the electric field has been disconnected without diffusion of the ampholytes and subsequent disturbance of the protein bands. A recent report describes a modified commercial apparatus for evacuating a sucrose gradient column, whilst still applying an electric field.[67] The lower electrode compartment is physically separated from the column by a suitable membrane, allowing fractionation of the column without diffusion of the ampholytes.

Preparative isoelectric focusing has also been used in layers of granulated gels, such as Sephadex or Biogel, with protein loadings from 0.1 g to 10.0 g.[68] To increase the capacity of the system, the gel layer is increased in size. Sperm whale myoglobin and pronase E have been studied by this method. Fractionation of horseradish peroxidase,[69] and a heat-labile enterotoxin from *E. coli* [70] have been aided by the inclusion of a preparative isoelectric focusing step in their purification procedure. However, despite these attempts to incorporate isoelectric focusing in preparative procedures, this technique still remains basically an analytical tool.

An interesting use of ampholyte mixtures has been described in the resolution of β-*N*-acetyl hexosaminidase isoenzymes.[71] Although the isoenzymes are not separated by conventional isoelectric focusing, they can be eluted selectively from a column of CM cellulose by a pH 8—10 ampholyte gradient. This method does

[59] H. R. Lill and G. R. Hartmann, *European J. Biochem.*, 1975, **54**, 45.
[60] A. W. Burgess, L. I. Weinstein, D. Gabel, and H. A. Scheraga, *Biochemistry*, 1975, **14**, 197.
[61] V. Kinzel, A. Alonso, and D. Kübler, *European J. Biochem.*, 1975, **55**, 361.
[62] J. Visser and M. Strating, *F.E.B.S. Letters*, 1975, **57**, 183.
[63] A. O. Barel and J.-P. Prieels, *European J. Biochem.*, 1975, **50**, 463.
[64] A. S. Levi, *F.E.B.S. Letters*, 1975, **52**, 278.
[65] M. Don and C. J. Masters, *Biochim. Biophys. Acta*, 1975, **384**, 25.
[66] J. Campbell and W. E. Hornby, *Biochim. Biophys. Acta*, 1975, **403**, 79.
[67] M. Boddin, H. J. Hilderson, A. Lagrou, and W. Dierick, *Analyt. Biochem.*, 1975, **64**, 293.
[68] B. J. Radola, *Biochim. Biophys. Acta*, 1975, **386**, 181.
[69] H. Delincée and B. J. Radola, *Biochem. J.*, 1975, **52**, 321.
[70] R. Möllby, S. G. Hjalmarsson, and T. Wadström, *F.E.B.S. Letters*, 1975, **56**, 30.
[71] D. H. Leaback and H. K. Robinson, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 248.

not vary greatly from the normal pH gradient method of eluting proteins from ion-exchange resins, but the use of an ampholyte gradient is unusual.

A word of warning has been expressed about staining artefacts in certain batches of commercial ampholytes used for isoelectric focusing.[72] As always, the need to use careful control experiments to validate staining patterns is demonstrated.

Polyacrylamide Gels. The use of polyacrylamide gel electrophoresis, both in the presence and absence of dissociating agents, is now so widespread that a full account of the technique is not presented here. Polyacrylamide gels containing the detergent sodium dodecylsulphate (SDS) are used almost universally to determine the subunit composition of proteins and as a purity check during isolation procedures. However, a certain amount of caution should be exercised when this method is the only one used to determine the molecular weight of protein subunits. The basic assumption that all proteins bind SDS to the same degree may not be valid for glycoproteins and highly hydrophobic proteins.

A delightful set of scanning electron micrographs of freeze-dried polyacrylamide gels has been presented.[73] These pictures confirm the general ideas that have been formulated about the porosity and meshwork nature of polyacrylamide gels, and show convincingly the 'leaflet' nature of the gel when no cross-linking agent is included. Freeze drying is not thought significantly to modify the gel structure.

The problem with using preparative SDS gel electrophoresis, besides that of the capacity of the gel for proteins, is the localization of the protein after electrophoresis and the subsequent removal of the detergent. Some of these problems may be overcome by using fluorescent dansyl-labelled proteins,[74] and eluting the proteins by electrophoresis into dialysis bags.[74, 75] The removal of SDS once bound to protein is a difficult job, although it may be removed by equilibrium dialysis over long periods of time, and a recent report suggests that electrophoretic dialysis under low ionic strength conditions removes the SDS several fold faster.[76] The complex formed between methylene blue and SDS can be used as the basis for rapid determination of SDS in solutions.[77]

It seems unlikely that anionic SDS will be replaced as the detergent of choice for gel separations, but the use of a cationic detergent, *N*-hexadecylpyridinium bromide, has been reported.[78] Unfortunately the correlation between molecular weight and relative mobilities for various proteins is not as good as with SDS. Varying the amount of SDS relative to proteins has been investigated, and low ratios of SDS to protein have been found to affect the relative mobility of the protein–dodecylsulphate complexes.[79]

The retention of trypsin and chymotrypsin activity in 1% SDS solutions has demonstrated the resistance of these proteases to denaturation by mild SDS treatment.[80] Heating at 100 °C for 25 min destroys the activity. The normal

[72] W. I. Otavsky and J. W. Drysdale, *Analyt. Biochem.*, 1975, **65**, 533.
[73] R. Rüchel and M. D. Brager, *Analyt. Biochem.*, 1975, **68**, 415.
[74] R. E. Stephens, *Analyt. Biochem.*, 1975, **65**, 369.
[75] N. Weliky, D. H. Leaman, jun., and B. J. Kallman, *Analyt. Biochem.*, 1975, **67**, 507.
[76] G. P. Tuszynski and L. Warren, *Analyt. Biochem.*, 1975, **67**, 55.
[77] K. Hayashi, *Analyt. Biochem.*, 1975, **67**, 503.
[78] M. Schick, *Analyt. Biochem.*, 1975, **63**, 345.
[79] J. T. Stoklosa and H. W. Latz, *Analyt. Biochem.*, 1975, **68** 358.
[80] W. H. Porter and J. L. Preston, *Analyt. Biochem.*, 1975, **66**, 69.

practice of heating protein samples with SDS and 2-mercaptoethanol has been shown to cause artifacts when applied to myelin proteins.[81] A major proteolipid protein is apparently excluded from the gel, possibly because of incomplete denaturation. However, mild conditions of solubilization using 1% SDS and ultrasonication, before addition of the thiol, were shown to produce a better gel pattern.

Detection of Protein. The detection and quantitation of proteins in acrylamide gels remains a problem due to differential staining properties. To overcome this problem it has been suggested that polyacrylamide gels, without detergents, can be scanned at one wavelength to detect unstained protein, and at another wavelength, where the protein does not absorb, to compensate for the gel background.[82] If suitable substrates can be diffused into the gel and converted enzymatically into products which absorb at a suitable wavelength, the localization of enzyme activity relative to the protein bands may be achieved.

A novel method of locating enzymes by activity staining in polyacrylamide gels has been used to locate the soluble nucleases of tobacco leaves.[83] Polyuridine was included in the gel before polymerization, and the proteins subjected to electrophoresis under conditions where the nucleases were not active. Subsequent immersion of the gels in 7% acetic acid restores enzyme activity, and degradation of the polyuridine occurs at the nuclease bands. Staining with 1% pyronin visualizes those areas that have remaining polynucleotide, enabling the nucleases to be seen as clear areas against a coloured background.

The use of Coomassie Brilliant Blue G250 in perchloric acid to stain proteins in ordinary polyacrylamide gels may receive a wider currency because of the colour change when the dye becomes bound to proteins, the best conditions being 0.04% Coomassie Brilliant Blue G250 and 3.5% perchloric acid.[84] The authors do not indicate the applicability of this method to gels containing detergents. The densitometric scanning of Amido Black [85] or Coomassie Blue stained polyacrylamide gels is popular but suffers the drawbacks of differential dye binding by proteins, inaccuracy in the integration of small areas, and the non-linearity of response of absorbance with respect to protein concentration. These effects can be nullified to a certain extent by cutting out the stained protein band and extracting the dye in 25% pyridine–water (v/v).[86] Absorbance measurements of the extracted dye may then be carried out in a spectrophotometer against calibrated standards. Better quantitation may be achieved when measuring submicrogram amounts of protein by using slab gels rather than tube gels.[87] This method decreases the amount of protein that needs to be applied, and thin slab gels have been claimed to give better staining characteristics than tube gels.

Another method of visualizing membrane glycoproteins in SDS gels makes use of the affinity between the carbohydrate moiety and certain lectins.[88] After

[81] P. Morell, R. C. Wiggins, and M. J. Gray, *Analyt. Biochem.*, 1975, **68**, 148.
[82] E. Fries and S. Hjerten, *Analyt. Biochem.*, 1975, **64**, 466.
[83] L. C. van Loon, *F.E.B.S. Letters*, 1975, **51**, 266.
[84] A. H. Reisner, P. Nemes, and C. Bucholtz, *Analyt. Biochem.*, 1975, **64**, 509.
[85] E. B. Wright and D. E. Olins, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 642.
[86] C. Fenner, R. R. Traut, D. T. Mason, and J. Wikman-Coffelt, *Analyt. Biochem.*, 1975, **63**, 595.
[87] R. Kahn and R. W. Rubin, *Analyt. Biochem.*, 1975, **67**, 347.
[88] P. J. Robinson, F. G. Bull, B. H. Anderton, and I. M. Roitt, *F.E.B.S. Letters*, 1975, **58**, 330.

fixing the gels with glutaraldehyde, staining as normal with a general protein stain, and blocking excess aldehyde groups, the gels are incubated with ^{131}I lectin conjugates. After appropriate washing, the glycoproteins can be visualized by autoradiography. In this system wheatgerm agglutinin, concanavalin A, and phytohaemagglutinin were used as lectins.

An elegant method for the quantitation of protein in SDS polyacrylamide gels has been used to determine the subunit stoicheiometry of the pyruvate dehydrogenase multi-enzyme complex.[89] The protein was amidinated by [^{14}C]methyl acetimidate in 5M guanidine hydrochloride and the subunits were separated using SDS polyacrylamide gels. Subsequent removal of the stained bands and radioactive counting gave an estimate of the amount of each subunit present, giving an overall stoicheiometry for the complex. This method depends upon a knowledge of the lysine content of the protein subunits and assumes that all subunits are modified to the same extent. This technique may find a useful role in confirming results obtained by densitometric scanning.

Autoradiography of polyacrylamide gels has normally been used for purely qualitative estimations of labelling. A recent report suggests that quantitative measurements of ^{3}H and ^{14}C may be made if the gels are impregnated with the scintillant PPO to convert the β-particle energy into visible light, and the *X*-ray film is presensitized by exposure to a brief flash of light.[90] This evidently corrects for the non-linear relationship between the radioactivity of the sample and the absorbance of the film image.

Subunit Cross-linking and Two-dimensional Polyacrylamide Gels. The use of 2-D polyacrylamide gel electrophoresis is increasing. This type of system can be used either to analyse complex mixtures of proteins that are not separable by electrophoresis in one dimension, or to investigate the subunit structure of oligomeric proteins using cleavable cross-linkers. The scope of the 2-D gel system is admirably demonstrated by the identification of 1100 proteins from *E. coli* using isoelectric focusing in the first dimension and SDS electrophoresis in the second.[91] The sensitivity of this system was enhanced by autoradiography of the gels using ^{14}C- or ^{35}S-labelled proteins. Similar 2-D gels have been used to characterize the soluble guinea-pig exocrine pancreatic enzymes,[92] the proteins of the human erythrocyte membrane,[93] rabbit muscle microsomal proteins,[94] and ribosomal proteins from rabbit,[95] chicken eggs,[96] and *Bacillus stearothermophilus*.[97]

The use of cross-linking agents to determine the near neighbours in multi-subunit proteins, together with polyacrylamide gel electrophoresis to separate the cross-linked products, has proved an ideal match when analysing several oligomeric proteins. The identification of several neighbouring proteins in

[89] D. L. Bates, R. A. Harrison, and R. N. Perham, *F.E.B.S. Letters*, 1975, **60**, 427.
[90] R. A. Laskey and A. D. Mills, *European J. Biochem.*, 1975, **56**, 335.
[91] P. H. O'Farrell, *J. Biol. Chem.*, 1975, **250**, 4007.
[92] G. A. Scheele, *J. Biol. Chem.*, 1975, **250**, 5375.
[93] V. Anselstetter and H. J. Horstmann, *European J. Biochem.*, 1975, **56**, 259.
[94] E. J. Barrett and D. R. Headon, *F.E.B.S. Letters*, 1975, **51**, 121.
[95] O. H. W. Martini, R. Temkin, A. Jones, K. Riley, and H. J. Gould, *F.E.B.S. Letters*, 1975, **56**, 205.
[96] P. G. Dondi, *Biochem. J.*, 1975, **149**, 475.
[97] S. Isono and K. Isono, *European J. Biochem.*, 1975, **50**, 483.

E. coli ribosomes has been reported using dimethyl suberimidate and 2-D gel electrophoresis.[98] A general investigation of the subunit symmetry of several enzymes including catalase, β-galactosidase, aldolase, yeast alcohol dehydrogenase, fumarase, and pyruvate kinase has been reported using di-imido-esters of various lengths.[99] Similarly, dimaleimides and di-imido-esters have been used to determine the arrangements of the α- and β-subunits in *E. coli* succinic thiokinase.[100]

The current interest in histone proteins and their relevance to chromatin structure has prompted several groups to examine the associations between the various protein subunits. Several non-cleavable cross-linkers have been used to covalently cross-link the histone subunits. These include di-imido-esters,[101, 102] water-soluble carbodi-imides,[103] and formamide.[104] Use of the cleavable disulphide cross-linker 3,3′-dithiobisproprionimidate allows the cross-linked histone proteins to be separated and identified by 2-D polyacrylamide gels.[105] The cross-linked molecule may be cleaved by reduction between the first and second dimension. This enables the separate protein subunits to move independently and facilitates the identification of the subunits which make up the high molecular weight bands. Obviously, such experiments will be invaluable in complementing more rigorous structural work such as *X*-ray crystallography.

The same technique of cleavable disulphide cross-linkers has been used to probe the structures of phage Qβ RNA replicase,[106] aldolase,[107] and concanavalin A.[107] Similar reduction of the disulphide bridges between the constituent polypeptides of IgA has demonstrated that there is no disulphide between the light and heavy chains.[108] Troponin structure has been analysed by 2-D gel electrophoresis using a diazide cross-linker that may be cleaved by virtue of containing vicinal hydroxy-groups.[109] Cleavage by periodate between the first and second dimensions has shown that the troponin constituents T, I, and C may all be cross-linked to each other. The length of the cross-linking molecule is 0.6 nm and so all of the subunits must be within this distance of each other. A similar diazide derivative of tartaric acid has been used to study the protein neighbours of the 30S ribosome from *E. coli*.[110]

A report about staining artefacts has indicated that dimethyl suberimidate in SDS–mercaptoethanol–triethanolamine buffer systems produces a band with apparent molecular weight of 40 000 after staining with Coomassie Blue, when no protein is included in the gel.[111]

[98] D. Barritault, A. Expert-Bezançon, M. Milet, and D. H. Háyes, *F.E.B.S. Letters*, 1975, **50**, 114.
[99] F. Hucho, H. Müllner, and H. Sund, *European J. Biochem.*, 1975, **59**, 79.
[100] J. A. Teherani and J. S. Nishimura, *J. Biol. Chem.*, 1975, **250**, 3883.
[101] J. O. Thomas and R. D. Kornberg, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2626.
[102] R. Chalkey, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 587.
[103] W. M. Bonner and H. B. Pollard, *Biochem. Biophys. Res. Chem.*, 1975, **64**, 282.
[104] J. E. Hyde and I. O. Walker, *F.E.B.S. Letters*, 1975, **50**, 150.
[105] J. O. Thomas and R. D. Kornberg, *F.E.B.S. Letters*, 1975, **58**, 353.
[106] R. A. Young and T. Blumenthal, *J. Biol. Chem.*, 1975, **250**, 1829.
[107] A. Ruomo, P. A. Bartlett, A. Dutton, and S. J. Singer, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 417.
[108] B. N. Manjula and F. F. Richards, *Analyt. Biochem.*, 1975, **66**, 412.
[109] S. E. Hitchcock and L. C. Lutter, *F.E.B.S. Letters*, 1975, **57**, 172.
[110] L. C. Lutter, C. G. Kurland, and G. Stoffler, *F.E.B.S. Letters*, 1975, **54**, 144.
[111] W. F. Burke and H. C. Reeves, *Analyt. Biochem.*, 1975, **63**, 267.

Removal of Denaturants and Detergents.—Although the removal of Triton and SDS has been referred to previously [3, 76] (see pp. 30, 37), there are still many problems encountered when trying to recover proteins, especially those of low molecular weight, from detergents and denaturants such as urea and guanidine hydrochloride. The use of ion-exchange resins to remove Triton X100 is not yet widespread, and because of this the success of the method is difficult to assess.[3] The extraction of Triton from the carboxyatractylate binding protein was achieved with diethyl ether,[5] but again it is difficult to assess how much Triton was removed and if the protein regains any of its native conformation.

A method for removing urea and guanidine hydrochloride from proteins has been described,[112] utilizing the solubility properties of these denaturants in organic solvents. Initially the protein–denaturant solution is treated with a solvent of high dielectric permittivity, such as propanol, when a small amount of protein precipitation occurs. The slow addition of a second solvent of low dielectric permittivity, such as ethyl acetate, causes additional protein precipitation. This method has given reasonable yields of protein when used with glucagon, insulin, or RNase.

Protein Determination.—The increasing use of detergents to solubilize membrane proteins has necessitated modifying the Lowry technique for determination of protein concentration in the presence of detergents. The addition of SDS to the alkaline copper reagent has been reported to stop the precipitate formation that occurs when the phenol Folin–Ciocalteu reagent is added to a protein solution containing other detergents.[113, 114] Manganous, cobaltous, and mercuric chlorides have been reported to interfere with the Lowry method.[115] A modified version of the Lowry technique has been employed to determine protein amounts after the deposition of insoluble protein on filter paper strips.[116] This enables interfering low molecular weight substances to be removed by successive washing with aqueous and non-aqueous solvents. It is also claimed that radioactive proteins that have been previously counted by scintillation methods can be estimated using this technique.

Another method involving the assay of proteins on membrane filters uses a cyclopeptamylose–dansyl chloride complex.[117] The protein is spotted onto a filter membrane and treated with the complex in aqueous urea solution. This overcomes the normal problems of the solubility of dansyl chloride in aqueous solution. Using fluorimetry detection, a linear response from 0.05 to 4 μg of proteins is claimed for this method, which suffers no problems from interfering substances as in other colorimetric procedures.

An improved, automated biuret procedure has been described which uses a modified reagent formulation and a range expander, giving sensitivity to the range 0.01—0.3 mg ml^{-1}.[118]

[112] P. S. Pollock and G. G. Glenner, *Analyt. Biochem.*, 1975, **68**, 341.
[113] J. R. Dulley and P. A. Grieve, *Analyt. Biochem.*, 1975, **64**, 136.
[114] C.-S. Wang and R. L. Smith, *Analyt. Biochem.*, 1975, **63**, 414.
[115] M. Higuchi and F. Yoshida, *Analyt. Biochem.*, 1975, **65**, 591.
[116] M. R. V. Murthy and T. Leroux, *Analyt. Biochem.*, 1975, **64**, 18.
[117] T. Kinoshita, F. Iinuma, and A. Tsuji, *Analyt. Biochem.*, 1975, **66**, 104.
[118] K. V. Honn and W. Chavin, *Analyt. Biochem.*, 1975, **68**, 230.

The absorption spectrum of pure proteins can often be used to estimate the amount of protein present in solution, and Kirschenbaum has extended his compilations of the molar absorptivity values for proteins beginning with the letters M to S.[119, 120] He has also tabulated the available data on the amino-acid compositions of proteins beginning with the letters F to H,[121, 122] including the human and non-human haemoglobins,[123, 124] thus supplementing the earlier tables of data (see previous years' Reports).

Molecular Weight Determination.—The methods for the determination of molecular weights and subunit structures of proteins have remained basically unchanged for several years, and 1975 saw very few modifications to the standard procedures of analytical centrifugation and gel filtration for the estimation of molecular weights. SDS gel electrophoresis and gel filtration in the presence of denaturants are now used almost universally to estimate the subunit composition.

An automated, differential scanning dilatometer has been described, which is able to measure changes in the partial specific volume of proteins, and which, it is claimed, can detect changes in volume as small as 0.02 μl.[125] A theoretical treatment of the difference sedimentation analysis method has appeared,[126] and a theoretical interpretation of the dependence of elution volume as a function of applied protein concentration in gel filtration has been presented.[127]

The use of analytical ultracentrifugation has been illustrated by experiments on spectrin from human erythrocyte membranes.[128] This protein is thought to form an aggregated meshwork on the inside surface of the erythrocyte membrane. The centrifuge has shown that, at pH 5.0—5.5, the protein does precipitate to form filaments, which can be seen in the electron microscope. However, under physiological conditions the protein appears to be a dimer of 460 000 molecular weight, with an S value of 9.75, and is considerably asymmetric. Under these conditions, it is not precipitated by salts.

Another example of the use of analytical ultracentrifugation is shown by work on human erythrocyte glutathione reductase.[129] This protein exists as a dimer of 100 000 molecular weight in the presence of reduced glutathione or 2-mercaptoethanol. On removal of the thiol the protein forms aggregates that can be detected by sedimentation velocity centrifugation. Sedimentation equilibrium analysis confirms that integral polymers of the native enzyme are formed by disulphide bridge cross-linking, giving species with molecular weights of 200 000 and 300 000.

[119] D. M. Kirschenbaum, *Analyt. Biochem.*, 1975, **64**, 186.
[120] D. M. Kirschenbaum, *Analyt. Biochem.*, 1975, **68**, 465.
[121] D. M. Kirschenbaum, *Analyt. Biochem.*, 1975, **66**, 123.
[122] D. M. Kirschenbaum, *Analyt. Biochem.*, 1975, **66**, 303.
[123] D. M. Kirschenbaum, *Analyt. Biochem.*, 1975, **65**, 466.
[124] D. M. Kirschenbaum, *Analyt. Biochem.*, 1975, **66**, 590.
[125] J. F. Blazyk, D. L. Melchior, and J. M. Steim, *Analyt. Biochem.*, 1975, **68**, 586.
[126] R. J. Skerrett, *Analyt. Biochem.*, 1975, **66**, 1.
[127] P. A. Baghurst, L. W. Nichol, A. G. Ogston, and D. J. Winzor, *Biochem. J.*, 1975, **147**, 575.
[128] W. B. Gratzer and G. H. Beaven, *European J. Biochem.*, 1975, **58**, 403.
[129] D. J. Worthington and M. A. Rosemeyer, *European J. Biochem.*, 1975, **60**, 459.

Table 1 *Proteins purified using affinity chromatography techniques*

Protein	*Ligand*	*Matrix*	*Eluent*	*Ref.*
Acetylcholine esterase				
(human)	trimethyl (*o*-aminophenyl)-ammonium ion	Sepharose	decamethonium	130
(electrophorus)	trimethyl (*o*-aminophenyl)-ammonium ion	Sepharose	decamethonium	131
Acetyl CoA carboxylase	biotin	Agarose	sodium chloride	132
N-Acetyl-β-hexosaminidase				
(human)	concanavalin A	Sepharose	α-methyl glucoside	133
(bovine)	2-acetamido-2-deoxy-D-glucono-1,4-lactone	Sepharose	high pH or Triton X100	134
(trichomonas)	2-acetamido-2-deoxy-β-gluco-pyranosylamine	Sepharose	*N*-acetylglucosamine	135
Adenylate cyclase	cholera toxin subunit	Agarose	—	136
Agglutinin				
(solanum)	*p*-aminobenzyl-1-thio-*N*-acetyl-β-D-glucosaminidine	Sepharose	acetic acid	137
(soybean)	galactosamine	Sepharose	galactose	138
D-Alanine carboxypeptidase	*p*-aminobenzyl penicillin	Sepharose	sodium chloride	139
Alcohol dehydrogenase	AMP	Sepharose	NADH	140
(*Bacillus stearothermophilus*)				
(rat)	NADP	Sepharose	potassium chloride	141

[130] P. Ott, B. Jenny, and V. Brodbeck, *European J. Biochem.*, 1975, **57**, 469.
[131] P. J. Morrod, A. G. Marshall, and D. G. Clark, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 335.
[132] J. S. Wolpert and M. L. Ernst-Fonberg, *Biochemistry*, 1975, **14**, 1095.
[133] D. K. Banerjee and D. Basu, *Biochem. J.*, 1975, **145**, 113.
[134] M. Pokorny and C. P. J. Glaudermans, *F.E.B.S. Letters*, 1975, **50**, 66.
[135] R. G. Edwards, P. Thomas, and J. M. Westwood, *Biochem. J.*, 1975, **151**, 145.
[136] V. Bennett, E. O'Keefe, and P. Cuatrecasas, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 33.
[137] F. Delmonte, C. Kieda, and M. Monsigny, *F.E.B.S. Letters*, 1975, **53**, 324.
[138] A. K. Allen and A. Neuberger, *F.E.B.S. Letters*, 1975, **50**, 362.
[139] M. Gorecki, A. Bar-eli, Y. Burstein, A. Patchornik, and E. B. Chain, *Biochem. J.*, 1975, **147**, 131.
[140] M. J. Comer, D. B. Craven, M. J. Harvey, A. Atkinson, and P. D. G. Dean, *European J. Biochem.*, 1975, **55**, 201.
[141] B. Tabakoff and J. P. Von Wartburg, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 957.

Table 1 (*cont.*)

Protein	*Ligand*	*Matrix*	*Eluent*	*Ref.*
Aldehyde reductase	NADP	Sepharose	potassium chloride	141
Amine oxidase	concanavalin A	Sepharose	α-methyl-D-glucoside	142
Aminoacyl tRNA synthetases				
(Val, Trp, Ser)	aminohexane	Sepharose	potassium chloride	143
(Phe)	*p*-aminophenyl mercury acetate	Sepharose	guanidine hydrochloride and mercaptoethanol	144
4-Aminobutyrate-2-oxoglutarate transaminase	4-aminobutyrate	Sepharose	retarded only	145
α-Amylase	glycogen	Sepharose	glycogen	146
Anthranilate synthetase	anthranilate	Sepharose	high pH	147
Antibodies:				
Cell surface	fetuin	Sepharose	low pH	148
Streptococcal carbohydrate	*N*-acetyl-α-galactosamine	Sepharose	*N*-acetylgalactosamine	149
Carbohydrate specific	various glycosides	Sepharose	free sugars	150
Cytochrome *c* oxidase	cytochrome *c*	Sepharose	guanidine hydrochloride	151
Glutamate dehydrogenase	glutamate dehydrogenase	Sepharose	high or low pH	152
Sickle cell haemoglobin	synthetic peptide	Sepharose	guanidine hydrochloride	153
Anti-B haemagglutinin	gum arabic	—	D-galactose	154
Anti-T lectin	β-D-galactopyranosylamine	Sepharose	galactose	155
α_1-Antitrypsin	5,5′-dithiobis-(2-nitrobenzoate)	Sepharose	thiols	42
L-Arabinose kinase	ATP	Sepharose	sodium chloride	156
Arginase				
(chicken)	arginine	Sepharose	sodium chloride	157
(rat)	antiarginase	Sepharose	arginine	158
L-Arginine de-iminase	L-arginine	Sepharose	arginine	159
Arylsulphatase A (sheep)	concanavalin A	Sepharose	α-methyl-D-glucoside	160
Arylsulphatase impurities	antialbumin	Sepharose	—	161
Aspartokinase-I-homoserine dehydrogenase-I	alkylamine	Sepharose	low ionic strength	162
ATPase coupling factor	ADP	Sepharose	potassium chloride and pyrophosphate	163
Binding proteins:				
Cobalamin	hydroxycobalamin	Sepharose	temperature increase	32

Cytokinin	benzyl adenine	Sepharose	high pH	164
Folate	concanavalin A	Sepharose	methyl-α-mannoside	165
β-D-Galactoside	—	Agarose	retarded only	166
Progesterone	deoxycorticosteroid	Sepharose	progesterone	167
Testosterone-oestradiol (human)	5-α-dihydrotestosterone	Sepharose	dihydrotestosterone	168
(human)	androstanediol	Sepharose	dihydrotestosterone	169
Carbamyl phosphate synthetase	glutamine	Sepharose	potassium chloride	170

142 J. J. Shiem, R. Tamaye, and K. J. Yasunobu, *Biochim. Biophys. Acta*, 1975, **377**, 229.
143 H. Jakubowski and J. Panelkiewicz, *European J. Biochem.*, 1975, **52**, 301.
144 A. Murayama, J. P. Raffin, P. Remy, and J. P. Ebel, *F.E.B.S. Letters*, 1975, **53**, 23.
145 M. Maître, L. Ciesielski, C. Cash, and P. Mandel, *European J. Biochem.*, 1975, **52**, 157.
146 R. Tkachuk, *F.E.B.S. Letters*, 1975, **52**, 66.
147 T. H. Grove and H. R. Levy, *Biochim. Biophys. Acta*, 1975, **397**, 80.
148 B.-A. Sela, J. L. Wang, and G. M. Edelman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1127.
149 K. C. S. Chen, T. J. Kindt, and R. M. Krause, *J. Biol. Chem.*, 1975, **250**, 3280.
150 A. M. Jeffrey, D. A. Zopf, and V. Ginsburg, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 608.
151 C. R. Hackenbrock and K. M. Hammon, *J. Biol. Chem.*, 1975, **250**, 9185.
152 G. di Prisco and L. Casola, *Biochemistry*, 1975, **14**, 4679.
153 N. S. Young, J. G. Curd, A. Eastlake, B. Furie, and A. N. Schechter, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4759.
154 Y. Fujita, K. Oishi, K. Suzuki, and K. Imahori, *Biochemistry*, 1975, **14**, 4465.
155 R. Lotan, E. Skutelsky, D. Danon, and N. Sharon, *J. Biol. Chem.*, 1975, **250**, 8518.
156 P. H. Chan and W. Z. Hassid, *Analyt. Biochem.*, 1975, **64**, 372.
157 S. Traniello, R. Barsacchi, E. Magri, and E. Grazi, *Biochem. J.*, 1975, **145**, 153.
158 R. Tarrab, B. Perez, and C. Lopez, *Analyt. Biochem.*, 1975, **65**, 26.
159 T. Shibatani, T. Kakimoto, and I. Chibata, *J. Biol. Chem.*, 1975, **250**, 4580.
160 K. A. Balasubramanian and B. K. Bachhawat, *Biochim. Biophys. Acta*, 1975, **403**, 113.
161 R. L. Stevens, A. L. Flumarty, M. H. Skokut, and H. Kihara, *J. Biol. Chem.*, 1975, **250**, 2495.
162 O. Raibaud, A. Högburg-Raibaud, and M. E. Goldberg, *F.E.B.S. Letters*, 1975, **50**, 130.
163 T. Higashi, V. K. Kalra, S.-H. Lee, E. Bogin, and A. F. Brodie, *J. Biol. Chem.*, 1975, **250**, 6541.
164 T. Takegami and K. Yoshida, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 782.
165 S. Waxman and C. Schreiber, *Biochemistry*, 1975, **14**, 5422.
166 V. I. Teichberg, I. Silman, D. D. Beitsch, and G. Resheff, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1383.
167 R. W. Kuhn, W. T. Schrader, R. G. Smith, and B. W. O'Malley, *J. Biol. Chem.*, 1975, **250**, 4220.
168 K. E. Michelson and P. H. Petra, *Biochemistry*, 1975, **14**, 957.
169 W. Rosner and R. N. Smith, *Biochemistry*, 1975, **14**, 4813.
170 A. T. H. Abdelal and J. L. Ingraham, *J. Biol. Chem.*, 1975, **250**, 4410.

Table 1 (*cont.*)

Protein	*Ligand*	*Matrix*	*Eluent*	*Ref.*
Carbonic anhydrase				
(human)	sulphanilamide	Sepharose	sodium chloride or sodium perchlorate	171
(human)	sulphanilamide	Sepharose	potassium iodide or potassium cyanide	172
Catalase inhibitor	catalase	Sepharose	sodium chloride	173
Catechol-*o*-methyl transferase	3,4-dimethoxy-5-hydroxyphenyl ethylamine	Agarose	high ionic strength	174
Cathepsin B_1 and B_2	mercury derivative	Agarose	dithiothreitol	43
Choline acetyl transferase	blue dextran	Sepharose	potassium chloride	34
Chorismate synthase	phosphate	cellulose	phosphate	175
Citrate synthase	ATP	Sepharose	ATP	37
Collagenase	collagen	Sepharose	sodium chloride	176
Collagenase impurities	elastin	—	—	177
Creatine kinase				
(human)	*p*-mercuribenzoate	Sepharose	2-mercaptoethanol	44
(avian)	*p*-mercuribenzoate	Sepharose	cysteine	45
Cytochrome P450				
(bovine)	aniline derivative	Sepharose	KCl	178
(rat)	amino-octane	Sepharose	Triton	179
Dehydroquinase	anti-dehydroquinase	Sepharose	guanidine hydrochloride	180
Deoxyribosylase	N^6-(*p*-aminobenzyl)adenine	Sepharose	deoxyinosine or guanidine hydrochloride	181
Dihydrofolate reductase				
(bovine)	pteroyl-L-lysine	Sepharose	sodium folate	182
(*E. coli*)	methotrexate	Sepharose	folic acid	183
DNA binding non-histone chromatin proteins	DNA	cellulose	sodium chloride	184
DNA polymerase				
(*E. coli*)	DNA	Sepharose	sodium chloride	185
(mouse Pol III)	DNA	cellulose	potassium chloride	186
(viral)	pyran derivative	Sepharose	potassium chloride	187
(kilham rat virus)	DNA	cellulose	sodium chloride	188

(human polycythemia)	oligo-deoxythymidine	cellulose	potassium chloride	189
Elastase	elastin	cellulose	high ionic strength	190
Endonuclease (rat)	DNA	cellulose	sodium chloride	191
Enterokinase (porcine)	*p*-aminobenzamidine	Sepharose	sodium chloride and ethylene glycol	192
Fatty acid synthetase (pigeon)	pantetheine	Sepharose	high pH and ionic strength	193—195
α-Fetoprotein	oestradiol	Sepharose	oestrone	196
2-Keto-3-deoxy-L-fuconate-NAD-oxidoreductase	NAD	Sepharose	NAD	197

[171] P. J. Wistrand, S. Lindahl, and T. Wåhlstrand, *European J. Biochem.*, 1975, **57**, 189.
[172] W. R. A. Osborne and R. E. Tashian, *Analyt. Biochem.*, 1975, **64**, 297.
[173] J. C. Sorenson and J. G. Scandalois, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 239.
[174] R. T. Borchardt, C.-F. Cheng, and D. R. Thakker, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 69.
[175] K. W. Cole and F. H. Gaertner, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 170.
[176] C.-C. Huang and M. Abramson, *Biochim. Biophys. Acta*, 1975, **384**, 484.
[177] A. Serafini-Fracassini, J. M. Field, G. W. Rodger, and M. Spina, *Biochim. Biophys. Acta*, 1975, **368**, 80.
[178] S. Takemori, K. Sumara, S. Hashimoto, M. Hashimoto, H. Sato, T. Gomi, and M. Katagiri, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 588.
[179] B. Jernstrom, J. Capdevila, and S. Jakobsson, *Biochem. Biophys. Res. Comm.*, 1974, **64**, 814.
[180] J. A. Hautala, J. W. Jacobson, M. E. Case, and N. Giles, *J. Biol. Chem.*, 1975, **250**, 6008.
[181] J. Holguin and R. Cardinaud, *European J. Biochem.*, 1975, **54**, 505.
[182] D. L. Peterson, J. M. Gleisner, and R. L. Blakley, *Biochemistry*, 1975, **14**, 5261.
[183] D. Baccanari, A. Phillips, S. Smith, D. Sinski, and J. Burchall, *Biochemistry*, 1975, **14**, 5267.
[184] N. C. Kostraba, R. A. Montagna, and T. Y. Wang, *J. Biol. Chem.*, 1975, **250**, 1548.
[185] D. J. Arndt-Jovin, T. M. Jovin, W. Bähr, A.-H. Frischauf, and M. Marquardt, *European J. Biochem.*, 1975, **54**, 411.
[186] A. Matsukage, E. W. Bohn, and S. H. Wilson, *Biochemistry*, 1975, **14**, 1006.
[187] J. G. Chirikjian, L. Rye, and T. S. Papas, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1142.
[188] L. A. Salzman and L. McKerlie, *J. Biol. Chem.*, 1975, **250**, 5583.
[189] B. J. Weimann, J. Schmidt, N. Kluge, W. Ostertag, and D. I. Wolfrum, *European J. Biochem.*, 1975, **59**, 581.
[190] Y. Legrand, G. Pignaud, J. P. Caen, B. Robert, and L. Robert, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 224.
[191] M. Mechali and A.-M. De Recondo, *European J. Biochem.*, 1975, **58**, 461.
[192] D. A. W. Grant and J. Hermon-Taylor, *Biochem. J.*, 1975, **147**, 363.
[193] A. A. Qureshi, R. A. Jenik, M. Kim, F. A. Lornitzo, and J. W. Porter, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 344.
[194] A. A. Qureshi, M. Kim, F. A. Lornitzo, R. A. Jenik, and J. W. Porter, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 836.
[195] F. A. Lornitzo, A. A. Qureshi, and J. W. Porter, *J. Biol. Chem.*, 1975, **250**, 4520.
[196] J. Uriel, D. Bouillon, and M. Dupiers, *F.E.B.S. Letters*, 1975, **53**, 305.
[197] N. A. Nwokoro and H. Schachter, *J. Biol. Chem.*, 1975, **250**, 6185.

Table 1 (*cont.*)

Protein	*Ligand*	*Matrix*	*Eluent*	*Ref.*
α-L-Fucosidase	fucosamine	Agarose	fucose or sodium chloride	198
D-Galactose kinase	ATP	Sepharose	sodium chloride	156
β-Galactosidase				
(*E. coli*)	alkylamines	Sepharose	decrease ammonium sulphate	162
(Jack bean)	D-galactopyranoside	Sepharose	retarded only	199
endo-1,4-β-Glucanases	concanavalin A	Sepharose	α-methyl-D-mannoside	200
exo-1,4-β-Glucanases	concanavalin A	Sepharose	α-methyl-D-mannoside	201
Glutamine synthetase	aminopentane	Sepharose	potassium chloride	202
Glyceraldehyde phosphate dehydrogenase	NAD	Sepharose	NADH	140
γ-Glutamyl hydrolase	polyglutamate	Sepharose	sodium chloride	35
Glycogen debranching enzyme	aminobutane	Sepharose	sodium chloride	203
Glycogen phosphorylase	AMP	Sepharose	AMP	204
Glycogen synthetase	alkylamines	Sepharose	various salts	205
Glycoproteins:				
Bovine tendon	concanavalin A	Sepharose	methyl α-D-glucopyranoside	206
Rat Thy I	concanavalin A	Sepharose	methyl α-D-glucopyranoside	207
Saccharomyces cerevisiae	L-valine or L-norleucine	Sepharose	low salt concentration	208
Glyoxylase	glutathione	Sepharose	high pH	209
Histaminase	cadaverine	Sepharose	heparin	210
Histidine decarboxylase	alkylamines	Agarose	low ionic strength	211
L-Histidine-2-oxoglutarate amino-transferase	histidine	Sepharose	sodium chloride	212
Histone proteins	*p*-mercuribenzoate	Agarose	urea	46
Hyaluronidase	concanavalin A	Sepharose	α-methyl-D-glucoside	213
D-β-Hydroxybutyrate apo-dehydrogenase	—	glass beads	lithium bromide	214
15-Hydroxyprostaglandin dehydrogenase	NAD	Agarose	NADH	215
20β-Hydroxysteroid dehydrogenase	11α-hydroxyprogesterone	Sepharose	2-mercaptoethanol	31
Hypoxanthine guanine phosphoribosyl transferase	GMP	Sepharose	potassium chloride	216
Initiation factor				
(Rabbit reticulocyte)	heparin	Sepharose	potassium chloride	217
Interferon (human)	carboxypentane	Agarose	ethylene glycol	218

α-Isopropylmalate isomerase				
(yeast)	valine	Sepharose	retarded	219
(yeast)	leucine	Sepharose	glycerol and decrease ammonium sulphate	219
Lactate dehydrogenase	AMP	Sepharose	NAD	220
Lectins:				
Abrus precatorius	—	Sepharose	D-galactose	221
Dictyostelium discoideum	—	Sepharose	D-galactose	222
Dolichos biflorus	concanavalin A	Sepharose	methyl α-D-glucopyranoside	223
Various	fetuin	Sepharose	sodium chloride	224

[198] J. A. Alhadeff, A. L. Miller, H. Wenaas, T. Vedvick, and J. S. O'Brien, *J. Biol. Chem.*, 1975, **250**, 7106.
[199] S.-C. Li, M. Y. Mazzotta, S.-F. Chien, and Y.-T. Li, *J. Biol. Chem.*, 1975, **250**, 6786.
[200] K.-E. Eriksson and B. Pettersson, *European J. Biochem.*, 1975, **51**, 193.
[201] K.-E. Eriksson and B. Pettersson, *European J. Biochem.*, 1975, **51**, 213.
[202] S. Shaltiel, S. P. Adler, D. Purich, C. Caban, P. Senior, and E. R. Stadtman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3397.
[203] C. Taylor, A. J. Cox, J. C. Kernohan, and P. Cohen, *European J. Biochem.*, 1975, **51**, 105.
[204] N. B. Sørenson and P. Wang, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 883.
[205] H. P. Jennissen and L. M. G. Heilmeyer, jun., *Biochemistry*, 1975, **14**, 754.
[206] J. C. Anderson, *Biochim. Biophys. Acta*, 1975, **379**, 444.
[207] M. Letarte-Muirhead, A. N. Barclay, and A. F. Williams, *Biochem. J.*, 1975, **151**, 685.
[208] H. Bussey, R. A. Rimerman, and G. W. Hatfield, *Analyt. Biochem.*, 1975, **64**, 380.
[209] M. V. Kester and S. J. Norton, *Biochim. Biophys. Acta*, 1975, **391**, 212.
[210] S. B. Baylin and S. Margolis, *Biochim. Biophys. Acta*, 1975, **397**, 294.
[211] L. Mammar, S. Påhlman, and S. Hjerten, *Biochim. Biophys. Acta*, 1975, **403**, 554.
[212] A. J. Hacking and H. Hassall, *Biochem. J.*, 1975, **147**, 327.
[213] C.-H. Yang and P. N. Srivistava, *Biochim. Biophys. Acta*, 1975, **391**, 382.
[214] H.-G. Bock and S. Fleischer, *J. Biol. Chem.*, 1975, **250**, 5774.
[215] S. S. Braithwaite and J. Jarabak, *J. Biol. Chem.*, 1975, **250**, 2315.
[216] S. H. Hughes, G. M. Wahl, and M. R. Capecchi, *J. Biol. Chem.*, 1975, **250**, 120.
[217] A. A. Waldman, G. Marx, and J. Goldstein, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2352.
[218] M. W. Davey, J. W. Huang, E. Sulkonski, and W. A. Carter, *J. Biol. Chem.*, 1975, **250**, 348.
[219] R. Bigelis and H. E. Umbarger, *J. Biol. Chem.*, 1975, **250**, 4315.
[220] C. R. Lowe and K. Mosbach, *European J. Biochem.*, 1975, **52**, 99.
[221] C. H. Wei, C. Koh, P. Pfuderer, and J. R. Einstein, *J. Biol. Chem.*, 1975, **250**, 4790.
[222] W. A. Frazier, S. D. Rosen, R. W. Reitherman, and S. H. Barondes, *J. Biol. Chem.*, 1975, **250**, 7714.
[223] W. G. Carter and M. E. Etzler, *J. Biol. Chem.*, 1975, **250**, 2756.
[224] B.-A. Sela, J. L. Wang, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 7535.

Table 1 (*cont.*)

Protein	*Ligand*	*Matrix*	*Eluent*	*Ref.*
Linulin	mucin	Sepharose	sodium chloride	225
Lipoamide dehydrogenase				
(porcine)	lipoamide	Sepharose	high ionic strength	226
(porcine)	NAD	Sepharose	high ionic strength	62
Lipoprotein lipase				
(human)	heparin	Sepharose	sodium chloride	227
(porcine)	heparin	Sepharose	sodium chloride	228
Lysozyme				
(various)	*N*-acetyl-β-D-glucosamide	Sepharose	sodium chloride	229
(*Asterius rubens*)	chitotetraose	Sepharose	sodium acetate	230
Maltodextrin phosphorylase	alkylamines	Sepharose	sodium chloride	231
α-D-Mannosidase	mannosylamine	Sepharose	mannose	232
Myosin	ADP	Sepharose	ATP	233
NADPH–Adrenodoxin reductase	adrenodoxin	Sepharose	sodium chloride	234
Neuraminidase	tyrosyl-*p*-nitrophenyl oxamic acid	Agarose	high pH	235
Nuclease T_1	thymidine diphosphate	Sepharose	guanidine hydrochloride	236
Nucleotide phosphodiesterase	polyhistidine	Sepharose	increase pH	237
Nucleotide pyrophosphatase	AMP	Sepharose	sodium chloride	238
Ovoflavoprotein	FMN	Agarose	sodium chloride	239
Phenylalanine hydroxylase	pteridine	Sepharose	phenylalanine and high pH 11.3	240
Phosphofructokinase				
(*B. stearothermophilus*)	AMP	Sepharose	ATP or potassium chloride	140
(*Thermus aquaticus*)	AMP	Sepharose	ATP	241
Phospholipase A_2				
(*Crotalus adamanteus*)	Glycero-3-phosphocholine	Sepharose	edta	242
Phospholipase				
(human)	heparin	Sepharose	sodium chloride	243
(human)	concanavalin A	Sepharose	sodium chloride or methyl-D-pyranoside	243
Phospholipase C				
(*Bacillus cereus*)	egg yolk lipoprotein	Sepharose	urea	244
Phosphoprotein phosphatase impurities	ATP	Sepharose	—	245

Phosphorylase *b*	alkylamines	Sepharose	various salts	205
Phosphorylase kinase	alkylamines	Sepharose	various salts	205
Phosphorylase phosphate				
(rabbit)	aminohexane	Sepharose	sodium chloride	246
(rabbit)	alkylamines	Sepharose	various salts	205
Plasmin (human)	lysine	poly-acrylamide	6-aminohexanoic acid	19
Plasminogen (rabbit)	lysine	Sepharose	6-aminohexanoic acid	247, 248
Proconvertin				
(bovine)	benzamidine	Agarose	guanidine hydrochloride	249
(bovine)	heparin	Agarose	sodium chloride	249

[225] A.-C. Roche, R. Schauer, and M. Monsigny, *F.E.B.S. Letters*, 1975, **57**, 245.
[226] J. Visser and M. Strating, *Biochim. Biophys. Acta*, 1975, **384**, 69.
[227] D. Ganesan and H. B. Bass, *F.E.B.S. Letters*, 1975, **53**, 1.
[228] C. Enholm, P. K. J. Kinnunen, J. K. Huttunen, E. A. Nikkilä, and H. Ohta, *Biochem. J.*, 1975, **149**, 649.
[229] E. Junowicz and S. E. Charm, *F.E.B.S. Letters*, 1975, **57**, 219.
[230] J. Jollès and P. Jollès, *European J. Biochem.*, 1975, **54**, 19.
[231] F. Thanner, D. Palm, and S. Shaltiel, *F.E.B.S. Letters*, 1975, **55**, 178.
[232] D. Robinson, N. C. Phillips, and B. Winchester, *F.E.B.S. Letters*, 1975, **53**, 110.
[233] H. R. Trayer and I. P. Trayer, *F.E.B.S. Letters*, 1975, **54**, 291.
[234] T. Sugiyama and T. Yamano, *F.E.B.S. Letters*, 1975, **52**, 145.
[235] B. Venerando, G. Tettamanti, B. Cestara, and V. Zambotti, *Biochim. Biophys. Acta*, 1975, **403**, 461.
[236] J. L. Bohnert and H. Taniuchi, *J. Biol. Chem.*, 1975, **250**, 2388.
[237] N. Miki, J. M. Baraban, J. J. Keirns, J. J. Boyce, and M. W. Bitensky, *J. Biol. Chem.*, 1975, **250**, 6320.
[238] E. Bischoff, T.-A. Tran-Thi, and K. F. A. Decker, *European J. Biochem.*, 1975, **51**, 353.
[239] G. Blankenhorn, D. T. Osuga, H. S. Lee, and R. E. Feeney, *Biochim. Biophys. Acta*, 1975, **386**, 470.
[240] R. G. H. Cotten and P. J. Grattan, *European J. Biochem.*, 1975, **60**, 427.
[241] H. Hengartner and J. I. Harris, *F.E.B.S. Letters*, 1975, **55**, 282.
[242] C. O. Rock and F. Snyder, *J. Biol. Chem.*, 1975, **250**, 6564.
[243] C. Ehnholm, N. Shaw, H. Greten, and W. V. Brown, *J. Biol. Chem.*, 1975, **250**, 6756.
[244] C. Little, B. Aurebekk, and A. B. Otnaess, *F.E.B.S. Letters*, 1975, **52**, 175.
[245] B. Ullman and R. L. Perlman, *Biochim. Biophys. Acta*, 1975, **403**, 393.
[246] H. Brandt, Z. L. Capulong, and E. Y. C. Lee, *J. Biol. Chem.*, 1975, **250**, 8038.
[247] M. W. C. Hatton and E. Regoeczi, *Biochim. Biophys. Acta*, 1975, **379**, 504.
[248] M. W. C. Hatton and E. Regoeczi, *Biochim. Biophys. Acta*, 1975, **386**, 451.
[249] W. Kisiel and E. W. Davie, *Biochemistry*, 1975, **14**, 4928.

Table 1 (*cont.*)

Protein	*Ligand*	*Matrix*	*Eluent*	*Ref.*
Prolyl hydroxylase	poly(L-proline)	Agarose	poly-(L-lysine)	250, 251
Proteases:				
Human	4-phenylbutylamine	acrylamide	sodium chloride	252
Phaseolus vulgaris	mercury derivative	methyl-acrylate	mercuric chloride	16
Sea urchin	soybean trypsin inhibitor	Sepharose	urea	253
Protein kinase				
(human)	histone	Sepharose	sodium chloride	254
(rabbit)	cyclic-AMP	Sepharose	cyclic-AMP	255
(rabbit)	alkylamines	Sepharose	various salts	205
Protein phosphatase	histone	Sepharose	sodium chloride	254
Proteoglycans	collagen	Sepharose	sodium chloride	256
Prothrombin	antiprothrombin	Sepharose	sodium bromide	257
Prothrombin precursor				
(bovine)	antiprothrombin	Sepharose	sodium chloride and urea	258
(rat)	heparin	acrylamide	ammonium chloride	259
Pyruvate-uridine diphospho-*N*-acetyl glucosamine transferase	UDP derivative	Sepharose	sodium chloride	260
Receptor proteins:				
Acetylcholine				
(feline)	trimethyl (*p*-aminophenyl)-ammonium ion	Sepharose	flaxedil	261
(rat)	cobrotoxin	Sepharose	carbamylcholine	262
Cholinergic	trimethyl (*p*-aminophenyl)-ammonium ion	Sephadex	acetylcholine	263
Glucocorticoid	oxycorticosterone	Agarose	—	264
Gonadotropin	chorionic gonadotropin	Sepharose	acetic acid	265
Progesterone	ATP	Sepharose	potassium chloride	266
Cyclic purine monophosphates	cyclic AMP	Sepharose	urea and methylamine	267
Steroid	antisteroid antibodies	Sepharose	—	268
Renin	pepstatin	Agarose	low pH	269
Ribonucleotide reductase	dGTP	Sepharose	dGTP or urea	270

RNA binding proteins:				
Bacteriophage	poly A, poly U, or poly C	cellulose	sodium chloride or urea	271
Ribonuclear proteins				
(mouse)	RNA	Sepharose	urea	272
(rat)	poly U	Sepharose	urea or formamide	273
ribosomal (*E. coli*)	RNA	Agarose	potassium chloride	274
RNA polymerase				
(*E. coli*)	DNA	cellulose	sodium chloride	275
(*E. coli*)	rifamycin	Sepharose	urea	276
(*E. coli*)	heparin	Sepharose	ammonium chloride	277

[250] L. Tuderman, E.-R. Kuutti, and K. I. Kivirikko, *European J. Biochem.*, 1975, **52**, 9.
[251] E.-R. Kuutti, L. Tuderman, and K. I. Kivirikko, *European J. Biochem.*, 1975, **57**, 181.
[252] G. Feinstein and A. Janoff, *Biochim. Biophys. Acta*, 1975, **403**, 477.
[253] E. J. B. Fodor, H. Ako, and K. A. Walsh, *Biochemistry*, 1975, **14**, 4923.
[254] P.-K. Tsung, T. Sakamoto, and G. Weissmann, *Biochim. J.*, 1975, **145**, 437.
[255] W. L. Dills, jun., J. A. Beavo, P. J. Bechtel, and E. G. Krebs, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 70.
[256] R. A. Greenwald, C. E. Schwartz, and J. O. Cantor, *Biochem. J.*, 1975, **145**, 601.
[257] R. Wallin and H. Prydz, *F.E.B.S. Letters*, 1975, **51**, 191.
[258] R. Wallin and H. Prydz, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 398.
[259] C. T. Esmon, G. A. Grant, and J. W. Suttie, *Biochemistry*, 1975, **14**, 1595.
[260] R. I. Zemell and R. A. Anwar, *J. Biol. Chem.*, 1975, **250**, 3185.
[261] J. O. Dolly and E. A. Barnard, *F.E.B.S. Letters*, 1975, **57**, 267.
[262] J. P. Brockes and Z. W. Hall, *Biochemistry*, 1975, **14**, 2092.
[263] F. J. Barrantes, S. Arbilla, N. C. L. de Carlin, and E. de Robertis, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 194.
[264] D. Failla, G. M. Tomkins, and D. V. Santi, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3849.
[265] M. L. Dufau, D. W. Ryan, A. J. Baukal, and K. J. Catt, *J. Biol. Chem.*, 1975, **250**, 4822.
[266] V. K. Moudgil and D. O. Toft, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 901.
[267] G. N. Gill and C. B. Kanstein, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 1113.
[268] E. Castañeda and S. Liao, *J. Biol. Chem.*, 1975, **250**, 883.
[269] K. Murakami and T. Inagami, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 757.
[270] P. J. Hoffmann and R. L. Blakley, *Biochemistry*, 1975, **14**, 4804.
[271] C. G. Carmichael, *J. Biol. Chem.*, 1975, **250**, 6160.
[272] J. M. Egly, O. Krieger, and T. Kempf, *F.E.B.S. Letters*, 1975, **53**, 64.
[273] A. Schweiger and G. Mazur, *F.E.B.S. Letters*, 1975, **54**, 39.
[274] H. R. Burrell and J. Horowitz, *F.E.B.S. Letters*, 1975, **49**, 306.
[275] R. R. Burgess and J. J. Jendrisak, *Biochemistry*, 1975, **14**, 4634.
[276] K. H. Scheit and A. Stutz, *F.E.B.S. Letters*, 1975, **50**, 25.
[277] H. Sternbach, R. Engelhardt, and A. G. Lezius, *European J. Biochem.*, 1975, **60**, 51.

Table 1 (*cont.*)

Protein	*Ligand*	*Matrix*	*Eluent*	*Ref.*
Sialidase	α_1-acid glycoprotein	Sepharose	high pH	278
Succinyl-CoA-acetoacetyl-CoA transferase	acetoacetyl CoA	Agarose	acetoacetate	47
Thermolysin	phosphoramidon	Sepharose	high pH	279
Threonine deaminase	valine or isoleucine	Sepharose	potassium phosphate or potassium chloride	280
Transcarboxylase	avidin	Sepharose	high pH	281
Triglyceride lipase	heparin	Sepharose	sodium chloride	228
Trypsin inhibitor	trypsin	Sepharose	low pH	282
Tryptophanase				
(*E. coli*)	pyridoxal phosphate	Sepharose	pyridoxal phosphate	283
(*E. coli*)	alkylamines	Sepharose	low ionic strength	162
Tyrosine phenol lyase	pyridoxal phosphate	Sepharose	pyridoxal phosphate	283

[278] M. J. Geison, *Biochem. J.*, 1975, **151**, 181.
[279] T. Komiyama, T. Aoyagi, T. Takeuchi, and H. Umezana, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 352.
[280] K. Koerner, I. Rahimi-Laridjani, and H. Grimminger, *Biochim. Biophys. Acta*, 1975, **397**, 220.
[281] M. Berger and H. G. Wood, *J. Biol. Chem.*, 1975, **250**, 927.
[282] A. Pineiro, F. Ortega, and J. Uriel, *Biochim. Biophys. Acta*, 1975, **379**, 201.
[283] S. Ikeda, H. Hara, S. Sugimoto, and S. Fukui, *F.E.B.S. Letters*, 1975, **56**, 307.

Table 2 *Chemical modification of proteins*

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
α-Acetohydroxy-acid isomeroreductase	*Salmonella typhimurium*	dimethylsebacimidate	Lys	tetramer	284
Acetylcholinesterase	*Aplysia californica*	[^{3}H]di-isopropyl fluoro-phosphate	Ser?	only one subunit labels	285
Acetylcholinesterase	*Electrophorus electricus*	glutaraldehyde	Lys	coupled to nylon tube	286
Acetylcholinesterase	*E. electricus*	[^{125}I]iodide, lactoperoxidase [^{125}I]-3-(4-hydroxyphenyl)-propionic acid *N*-hydroxy-succinimide ester	Tyr		287
Acid phosphatase	bovine brain	DTNB	Cys	two Cys per mol	289
Active transport systems	*Neurospora crassa*	PCMB, NEM, iodoacetamide	Cys	inactivation	290
Actin	rabbit skeletal muscle	[^{14}C]-NEM	Cys-373		291
Actin	rabbit skeletal muscle	CNBr-activated Sepharose	Lys		291
Actin-like protein	chicken skin fibroblasts	[^{125}I]iodide, chloramine T	Tyr	fingerprints of iodinated peptides similar to actin	292
Actomyosin	rabbit skeletal muscle	FDNB		calcium sensitivity affected	293
Acyl carrier protein	*E. coli*	acetic anhydride, butane-2,3-dione	Lys Arg	no loss of activity for modification of the single Arg	294

284 J. G. Hofler, C. J. Decedue, G. H. Luginbuhl, J. A. Reynolds, and R. O. Burns, *J. Biol. Chem.*, 1975, **250**, 877.
285 F. A. Bevelaque, K. S. Kim, M. H. Kumarasiri, and J. H. Schwartz, *J. Biol. Chem.*, 1975, **250**, 731.
286 T. T. Ngo and K. J. Laidler, *Biochim. Biophys. Acta*, 1975, **377**, 317.
287 P. J. Morrod and D. G. Clark, *F.E.B.S. Letters*, 1975, **59**, 316.
289 C. E. M. Baldijao, E. Guija, H. M. S. Bitten-Court, and H. Chaimovich, *Biochim. Biophys. Acta*, 1975, **391**, 316.
290 S. O. Nelson, G. I. Glover, and C. W. Magill, *Arch. Biochem. Biophys.*, 1975, **168**, 483.
291 M. Elzinga and J. H. Collins, *J. Biol. Chem.*, 1975, **250**, 5897.
292 R. C. Bottomley and I. P. Trayer, *Biochem. J.*, 1975, **149**, 365.
293 D. Bray and C. Thomas, *Biochem. J.*, 1975, **147**, 221.
294 G. Bailin, *Biochim. Biophys. Acta*, 1975, **400**, 167.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Acylphosphatase	horse muscle	pyridoxal 5′-phosphate–sodium borohydride	Lys	irreversible inhibition	295
Adenylate kinase	pig heart	phenylglyoxal	Arg	only one of 11 Arg modified substrate protects	296
Adenylate kinase	pig muscle	7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl)	Cys-25 Cys-187	fast slow slow transfer of NBD from thiol to Lys-31	
		N-(1-oxyl-1,2,2,5,5-tetramethyl-3-pyrrolidinyl)iodoacetamide	Cys	spin label	297
Adenyl cyclase	rat fat pad membranes	5-guanylyliminodiphosphonate, 5-guanylylmethylene-diphosphonate		covalently bound to GTP regulatory site?	298
Adenyl cyclase	rat liver plasma membranes	[^{14}C]iodoacetamide [^{14}C]iodoacetic acid [^{3}H]iodoacetic acid	Cys	double-label technique: 240 000 and 10 000, components labelled more extensively in presence of glucagon	299
Agglutinins	wheatgerm	various reagents		electrophoretic variants for hybridization	300
Albumin	bovine serum	nitroxide free radical separated from iodoacetamide by various chain lengths	Cys	e.s.r. spectra as function of chain length: molecular dipstick approach	301
Albumin	bovine serum	1-nitroguanyl-3,5-dimethyl-pyrazole	Lys		302
Albumin	bovine serum	*N*-hydroxysuccinimido-ester of β-amanitin	Lys	toxicity studies	303
Pre-albumin	human serum	DNS-Cl	Lys-15	thyroxine binding domain labelled	304
Alcohol dehydrogenase	horse liver	*p*-chloromercuriphenyl sulphonate	Cys	fluorescence quenched	305

Alcohol dehydrogenase	horse liver	iodoacetic acid	Cys-46	enzyme in crystal-marker for correlation of primary and tertiary structure	306
Alcohol dehydrogenase	horse liver	iodoacetic acid	Cys-46	ligand binding	307
Alcohol dehydrogenase	horse liver	5-bromoacetyl-4-methyl-imidazole dinucleotide	Cys-174	NAD analogue enzyme inactivated	308
Alcohol dehydrogenase	horse liver	3-(4-bromoacetylpyridinio)-propyl adenosine triphosphate	Cys-174	NAD analogue	309
Alcohol dehydrogenase	horse liver	pyridoxal 5′-phosphate–sodium borohydride	Lys	irreversibly inhibited	310
Alcohol dehydrogenase	horse liver	i, ethyl acetimidate	Lys	active site protected by NAD, pyrazole	311
		ii, pyridoxal 5′-phosphate	Lys-228		
Alcohol dehydrogenase	horse liver	imido-esters	Lys	*N*-alkylimidates formed	312
Alcohol dehydrogenase	horse liver	i, ethyl acetimidate	Lys	active site protected by NAD, pyrazole	313
		ii, [^{14}C]ethyl acetimidate	Lys-228		

[295] H. Schulz, *J. Biol. Chem.*, 1975, **250**, 2299.
[296] G. Ramponi, G. Manao, G. Camici, and G. F. White, *Biochim. Biophys. Acta*, 1975, **391**, 486.
[297] J. Berghauser, *Biochim. Biophys. Acta*, 1975, **397**, 370.
[298] N. C. Price, M. Cohen, and R. H. Schirmer, *J. Biol. Chem.*, 1975, **250**, 644.
[299] P. Cuatrecasas, S. Jacobs, and V. Bennett, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1739.
[300] D. R. Storm and R. A. Chase, *J. Biol. Chem.*, 1975, **250**, 2539.
[301] R. H. Rice and M. E. Etzler, *Biochemistry*, 1975, **14**, 4093.
[302] H. H. Hull, R. Chang, and L. J. Kaplan, *Biochim. Biophys. Acta*, 1975, **400**, 132.
[303] C. H. C. M. Buys, A. S. H. de Jong, J. M. W. Bouma, and M. Gruber, *Biochim. Biophys. Acta*, 1975, **392**, 95.
[304] S. Y. Cheng, H. J. Cahnmann, M. Wilchek, and R. N. Ferguson, *Biochemistry*, 1975, **14**, 4132.
[305] H. Faulstich, H. Trischmann, and S. Zobeley, *F.E.B.S. Letters*, 1975, **56**, 312.
[306] K. Tatemoto, *Arch. Biochem. Biophys.*, 1975, **166**, 8.
[307] E. Zeppezauer, H. Jörnvall, and I. Ohlsson, *European J. Biochem.*, 1975, **58**, 95.
[308] C. H. Reynolds and J. S. Mckinley-McKee, *Arch. Biochem. Biophys.*, 1975, **168**, 145.
[309] H. Jörnvall, C. Woenckhaus, and G. Johnscher, *European J. Biochem.*, 1975, **53**, 71.
[310] H. Jörnvall, C. Woenckhaus, E. Schattie, and R. Jeck, *F.E.B.S. Letters*, 1975, **54**, 297.
[311] S. S. Chen and P. C. Engel, *Biochem. J.*, 1975, **149**, 627.
[312] D. C. Sogin and B. V. Plapp, *J. Biol. Chem.*, 1975, **250**, 205.
[313] D. T. Browne and S. B. H. Kent, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 133.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Alcohol dehydrogenase	horse liver	halogenated carboxy-acids and amides		activation and inhibition; imido-esters formed in solution	314
Alcohol dehydrogenase	horse liver	CNBr–Sepharose	Lys	co-immobilized with NAD analogue	315
Alcohol dehydrogenase	horse liver	butanedione	Arg	reduces [^{14}C]iodoacetic incorporation into Cys-46	316
Alcohol dehydrogenase	yeast	5-bromoacetyl-4-methyl-imidazole dinucleotide	Cys-43	inactivates enzyme	308
Alcohol dehydrogenase	yeast	[3-(3-bromoacetylpyridinio)-propyl] adenosine pyro-phosphate and [3-(4-bromo-acetylpyridinio)propyl] adenosine pyrophosphate	Cys-43 Cys-43	inactivates enzyme	309
Alcohol dehydrogenase	yeast	diazotized 3-aminopyridine adenine dinucleotide	Cys	*S*(3-pyridyl)cysteine found after acid hydrolysis	317
Alcohol dehydrogenase	yeast	[^{3}H]styrene oxide	Cys	two per mol subunit	318
Alcohol dehydrogenase	yeast	diethyl pyrocarbonate, DTNB	His Cys	His and Cys close at substrate binding site	319
Alcohol dehydrogenase	yeast	diethyl pyrocarbonate	His	1 mol *N*-carboxyethyl-histidine per mol subunit	320
Alcohol dehydrogenase	yeast	AE-cellulose/glutaraldehyde DEAE-cellulose/2-amino-4,6-dichloro-*sym*-triazine CNBr/agarose	 Lys	immobilization studies	321
Aldolase	rabbit muscle	dimethyl 3,3-dithiobis-propionimidate	Lys		322
Aldolase	rabbit muscle	*p*-carboxybenzenediazonium chloride	Cys	modified on phospho-cellulose	323
Aldolase	rabbit muscle	*p*-carboxybenzenediazonium chloride diazotized 5-aminotetrazole		oxidation of tryptophans by free-radical mechanism?	324

Aldolase	rabbit muscle	[^{14}C]phenylglyoxal	Arg	*ca.* one Arg per mol subunit	325
Aldolase	bovine, whale, *Loligo vulgaris*	dihydroxy[^{14}C]acetone phosphate	Lys		326
Alkaline phosphatase	*E. coli*	succinic anhydride TNM/sodium dithionate	Lys Tyr	activity of hybrid of (native + modified) dimers studied	327
Amidase	*Pseudomonas acidovorans*	PCMB	Cys	inhibits	328
Amine oxidase	*Aspergillus niger*	DTNB	Cys	10/25 titrate	329
Amine oxidase	bovine plasma	diethyl pyrocarbonate, photo-oxidation	His	inactivates enzyme, kynuramine protects	330
Amine oxidase	bovine plasma	β-bromoethylamine		substrate and irreversible inhibitor; one per mol subunit	331
δ-Aminolevulinic acid dehydratase	bovine liver	photo-oxidation	His	inactivated	332
Amylase	pig pancreas	DTNB	Cys	two per mol	333

[314] R. Dworschack, G. Tarr, and B. V. Platt, *Biochemistry*, 1975, **14**, 200.
[315] R. W. Fries, D. P. Bohlken, R. T. Blakeley, and B. V. Platt, *Biochemistry*, 1975, **14**, 5233.
[316] S. Gestrellius, M. O. Mansson, and K. Mosbach, *European J. Biochem.*, 1975, **57**, 529.
[317] L. G. Lange, J. F. Riordan, B. L. Vallee, and C.-I. Bränden, *Biochemistry*, 1975, **14**, 3497.
[318] J. K. Chan and B. M. Anderson, *J. Biol. Chem.*, 1975, **250**, 67.
[319] J. P. Klinman, *Biochemistry*, 1975, **14**, 2568.
[320] V. Leskovac and D. Pavkov-Pericin, *Biochem. J.*, 1975, **145**, 581.
[321] C. J. Dickenson and F. M. Dickenson, *European J. Biochem.*, 1975, **52**, 595.
[322] K. Das, P. Dunnill, and M. D. Lilly, *Biochim. Biophys. Acta*, 1975, **397**, 277.
[323] R. Felicioli, L. Nannicini, E. Balestreri, and G. Montagnoli, *European J. Biochem.*, 1975, **51**, 467.
[324] S. Monti, G. Montagnoli, L. Nannicini, and R. Feliciolo, *F.E.B.S. Letters*, 1975, **58**, 175.
[325] R. R. Lobb, A. M. Stokes, H. A. O. Hill, and J. F. Riordan, *F.E.B.S. Letters*, 1975, **54**, 70.
[326] J. C. Mareschal, E. Schonne, R. R. Crighton, and D. A. Strosberg, *F.E.B.S. Letters*, 1975, **54**, 97.
[327] E. Meighen and R. Yue, *Biochim. Biophys. Acta*, 1975, **412**, 262.
[328] J. Alt, E. Heymann, and K. Krisch, *European J. Biochem.*, 1975, **53**, 357.
[329] H. Susuki, Y. Ogura, H. Yamada, and K. Arima, *Biochim. Biophys. Acta*, 1975, **404**, 23.
[330] S. Tsureshiin, A. Hiramatsu, M. Inamasu, and K. T. Yasunobu, *Biochim. Biophys. Acta*, 1975, **400**, 451.
[331] R. Neumann, R. Hevey, and R. M. Abeles, *J. Biol. Chem.*, 1975, **250**, 6362.
[332] I. Tsukamoto, T. Yoshinaga, and S. Sano, *Biochem. Biophys. Res. Comm.*, 1974, **67**, 294.
[333] M. Granger, B. Abadie, Y. Mazzei, and G. Marchis-Mouren, *F.E.B.S. Letters*, 1975, **50**, 276.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Apamin	bee venom	various reagents		structure–function study	334
Apoliprotein A-I	human plasma	[^{125}I]iodine monochloride	Tyr	immunoassay	335
Arginase	*Saccharomyces cerevisiae*	L-2-amino-4-oxo-5-chloro-pentanoic acid		L-ornithine analogue	336
Aspartase	*E. coli*	NEM, DTNB	Cys	inactivates, two SH per mol subunit; aspartate protects against NEM inactivation	337
Aspartate aminotransferase		acylazide-activated collagen	Lys	bound to collagen films	338
Aspartate aminotransferase	bovine kidney	butanedione	Arg	reversible inactivation	339
	mitochondria, pig heart	[^{14}C]phenylglyoxal	Arg	irreversible inactivation two Arg per mol subunit	
Aspartate aminotransferase	pig heart	4-*N*-(2,4-dinitro-5-fluorophenyl)-pyridoxamine-5′-phosphate	Lys	inactive enzyme	340
Aspartate aminotransferase	pig heart	4-ethynyl pyridoxal 5-phosphate analogue	Lys		341
Aspartate aminotransferase	pig heart	photo-oxidation	His?	inactive in trans-amination with L-aspartic, active with L-alanine	342
Aspartate aminotransferase	bovine kidney mitochondria	tetranitromethane	Tyr	two Tyr per mol subunit	343
Aspartate transcarbamylase	*Bacillus subtilis*	dimethyl suberimidate	Lys	trimer	345
Aspartate transcarbamylase regulatory subunits	*E. coli*	[^{14}C]succinic anhydride	Lys	hybridization studies	346
Aspartate transcarbamylase	*E. coli*	pyridoxal 5′-phosphate/sodium borohydride	Lys-80		347
Aspartokinase I–homoserine dehydrogenase I (threonine sensitive)	*E. coli*	2-amino-4-oxo-5-chloropentanoic acid		one per mol subunit homoserine dehydrogen-ase inactivated, aspartokinase still active	348
ATPase	bovine heart mitochondria	[^{14}C]-NEM, [^{3}H]-NEM	Cys	location of S—H and S—S bonds	349

ATPase	bovine heart mitochondria	i, 4-chloro-7-nitrobenzofurazan ii, 4-chloro-7-nitrobenzofurazan, followed by SDS	Tyr Cys	one per mol transfer to Cys from Tyr	350
ATPase	bovine heart mitochondria	4-chloro-7-nitrobenzofurazan	Tyr	transfer from Tyr at pH 9.0	351
ATPase	bovine heart mitochondria	a mercury-substituted nitrophenol	Cys?	inhibits	352
ATPase sodium potassium activated	bovine brain	*S*-(2,4-dinitrophenyl)-6-mercaptopurine riboside-5′-triphosphate and 6-mercaptopurine riboside-5′-triphosphate	Cys	inactivates enzyme at ATP site	353
ATPase-DCCD reactive protein	*E. coli*	[^{14}C]dicyclohexylcarbodi-imide		mol. wt. on gels 12 000	354

[334] J. P. Vincent, H. Schweritz, and M. Lazdunski, *Biochemistry*, 1975, **14**, 2521.
[335] M. Fainaru, M. C. Glangeaud, and S. Eisenberg, *Biochim. Biophys. Acta*, 1975, **386**, 432.
[336] M. Penninck, *European J. Biochem.*, 1975, **58**, 533.
[337] K. Mitzuta and M. Tokushige, *Biochim. Biophys. Acta*, 1975, **403**, 221.
[338] P. R. Coulet, C. Godinot, and D. C. Gautheron, *Biochim. Biophys. Acta*, 1975, **391**, 272.
[339] J. R. Riordan and R. Scandurra, *Biochem. Biophys. Res. Commun.*, 1975, **66**, 417.
[340] F. Riva, A. Giartosio, C. Voltattornia, A. Orlachio, and C. Turano, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 863.
[341] I. Yang, C. M. Harris, D. E. Metzler, W. Korytnyk, B. Lachmann, and P. P. G. Potti, *J. Biol. Chem.*, 1975, **250**, 2947.
[342] M. Yamasaki, S. Tanase, and Y. Morino, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 652.
[343] R. Scandurra, G. Polidoro, D. Di Cola, L. Politi, and J. F. Riordan, *Biochemistry*, 1975, **14**, 3701.
[344] A. Dutton and S. J. Singer, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2568.
[345] J. S. Brabson and R. L. Switzer, *J. Biol. Chem.*, 1975, **250**, 8664.
[346] G. M. Nagel and H. K. Schachman, *Biochemistry*, 1975, **14**, 3195.
[347] T. D. Kempe and G. R. Stark, *J. Biol. Chem.*, 1975, **250**, 6861.
[348] C. G. Hirth, M. Veron, C. Villar-Palasi, N. Hurion, and G. N. Cohen, *European J. Biochem.*, 1975, **50**, 425.
[349] A. E. Senior, *Biochemistry*, 1975, **14**, 660.
[350] S. J. Ferguson, W. J. Lloyd, M. H. Lyons, and G. K. Radda, *European J. Biochem.*, 1975, **54**, 117.
[351] S. J. Ferguson, W. J. Lloyd, and G. K. Radda, *European J. Biochem.*, 1975, **54**, 127.
[352] A. D. Vinogradov, Z. S. Gyurova, and A. F. Fitin, *F.E.B.S. Letters*, 1975, **54**, 230.
[353] R. Patzelt-Wenczler, H. Pauls, E. Erdmann, and W. Schoner, *European J. Biochem.*, 1975, **53**, 301.
[354] K. Altendorf and W. Zitzmann, *F.E.B.S. Letters*, 1975, **59**, 268.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
ATPase sodium potassium activated	human erythrocytes	*N*-acetylimidazole	Tyr	inhibits	355
ATPase sodium potassium activated	rat brain	2-(β-D-ribofuranosyl)maleimide	Cys?	irreversibly inhibits	356
ATPase	*Rhodopseudomonas capsulata*	NEM		irreversibly inhibits only if preincubated in light	357
ATPase subunit 9	*Saccharomyces cerevisiae* mitochondria	diazidoethidium bromide (u.v.)		photoaffinity label	358
ATP: L-Arginine phosphotransferase	*Solen ensisensis* *Sipunculus nudus*	DTNB diethyl pyrocarbonate	Cys His	six His modified on each enzyme	359
Basic protein	bovine brain	NBS, 2-hydroxy-5-nitrobenzyl bromide	Trp	affects dye binding	360
Benzoate-4-hydroxylase	*Aspergillus niger*	PCMB, NEM	Cys	inactivated	361
Bromelain	pineapple stem	photo-oxidation	His, Met, Trp		362
Capsids	turnip yellow mosaic virus	tetranitromethane trinitrobenzenesulphonate	Tyr Lys	chemical accessibility probed	363
Carbamyl phosphate synthetase	*E. coli*	[^{14}C]cyanate	Cys	one per mol	364
Carbonic anhydrase B	bovine	bromoacetazolamide	His-64	kinetics of modified enzyme	365
3-Carboxy-*cis*,*cis*-muconate cyclase	*Aspergillus niger*	diethyl pyrocarbonate photo-oxidation	His?		366
Carboxylesterases	various	bis(*p*-nitrophenyl) methyl phosphate		2 mol nitrophenol per active site	367
Carboxylesterases	bovine liver	α-bromoacetophenone	His		368
Procarboxypeptidase A	bovine	dimethylmaleic anhydride		mild dissociation	369
Carboxypeptidase A		diazotized arsanilic acid	Tyr-248	conformation sensitivity of chromophore	370
Carboxypeptidase Y	yeast	*p*-hydroxymercuribenzoate	Cys	retains activity toward poor substrates	371
Carboxypeptidase Y	yeast	benzyloxycarbonyl-L-phenylalanylchloromethane	His	inactivates enzyme	372

Cardiotoxin	cobra	tetranitromethane	Tyr	two or three Tyr nitrated, activity reduced	373
Catechol *O*-methyltransferase	rat liver	*N*-halogenoacetyl derivatives of: 3,5-dimethoxy-4-hydroxy-phenylethylamine 3,4-dimethoxy-5-hydroxy-phenylethylamine		kinetics of inactivation; substrate protection profiles	374
Citrate lyase	*Klebsiella aerogenes*	iodoacetic		conditions for Cys labelling	375
Cholinesterase	horse serum	maleinic anhydride–butanediol-divinylether copolymer		covalently bound; active	376
Chromatin nonhistone protein	calf thymus			binding to DNA studied	377
Chymotrypsinogen A and α-chymotrypsin		tetranitromethane	Tyr-146, Tyr-171		378

[355] S. J. Masiak and G. D'Angelo, *Biochim. Biophys. Acta*, 1975, **382**, 83.
[356] T. Tobin and T. Akera, *Biochim. Biophys. Acta*, 1975, **389**, 126.
[357] A. Baccarini-Melandri, E. Fabbri, E. Firstater, and B. A. Melandri, *Biochim. Biophys. Acta*, 1975, **376**, 72.
[358] R. N. Bastos, *J. Biol. Chem.*, 1975, **250**, 7739.
[359] N. V. Thiem, G. Lacombe, and N. V. Thoai, *Biochim. Biophys. Acta*, 1975, **377**, 95.
[360] M. B. Feinstein and H. Felsenfeld, *Biochemistry*, 1975, **14**, 3049.
[361] C. C. Reddy and C. S. Vaidyanathan, *Biochim. Biophys. Acta*, 1975, **384**, 46.
[362] T. Murachi, T. Tsudzuki, and K. Okumura, *Biochemistry*, 1975, **14**, 249.
[363] G. G. Re and J. M. Kaper, *Biochemistry*, 1975, **14**, 4492.
[364] P. M. Anderson and J. D. Carlson, *Biochemistry*, 1975, **14**, 3688.
[365] J. W. Wells, S. I. Kandel, M. Kandel, and A. G. Gornall, *J. Biol. Chem.*, 1975, **250**, 3522.
[366] D. R. Thatcher and R. B. Cain, *European J. Biochem.*, 1975, **56**, 193.
[367] S. E. Hamilton, N. P. B. Dudman, J. De Jersey, J. K. Stoops, and B. Zerner, *Biochim. Biophys. Acta*, 1975, **377**, 282.
[368] E. Heymann and G. Marcussen-Wulff, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 887.
[369] A. Puigserver and P. Desnuelle, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2442.
[370] J. T. Johansen and B. L. Vallee, *Biochemistry*, 1975, **14**, 649.
[371] Y. Bai and R. Hayashi, *F.E.B.S. Letters*, 1975, **56**, 43.
[372] R. Hayashi, Y. Bai, and T. Hata, *J. Biol. Chem.*, 1975, **250**, 5221.
[373] W. M. Keung, W. W. Leung, and Y. C. Kong, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 383.
[374] R. T. Borchardt and D. R. Thakker, *Biochemistry*, 1975, **14**, 4543.
[375] P. Dimroth and H. Eggerer, *European J. Biochem.*, 1975, **53**, 227.
[376] C. Alsen, U. Bertram, T. Gersteuer, and F. K. Ohnesorge, *Biochim. Biophys. Acta*, 1975, **377**, 297.
[377] G. H. Goodwin, K. V. Shooter, and E. W. Johns, *European J. Biochem.*, 1975, **54**, 427.
[378] K. B. Svensson, J. M. Imhoff, and B. Keil, *European J. Biochem.*, 1975, **58**, 493.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
α-Chymotrypsin		octyl isocyanate		*X*-ray map; n-octyl chain bound in tosyl pocket	379
α-Chymotrypsin		1-fluoro-2,4-dinitro[^{3}H]benzene	His-40 His-57	competitive labelling, binding of indole decreases His reactivity	380
δ-Chymotrypsin		*p*-nitrophenacyl bromide hydrogen peroxide	Met-192 Met-192	involvement of Met-192 in conformational oscillation	381
Citrate synthase	pig heart	citraconic anhydride, diethyl-pyrocarbonate	His		382
Coat protein	bacteriophage PM2	*p*-phenylenedimaleimide	Cys	cross-linked to spike protein	383
Collagen	bovine	fluorescein isothiocyanate	Lys hydroxy-lysine	4 per helical region 3 per telopeptide region	384
Collagen	bovine	i, potassium [^{3}H]borohydride ii, Smith degradation		dihydroxylysinonorleucine derived from hydroxy-lysino-5-oxonorleucine	385
Collagen	calf bone	i, sodium [^{3}H]borohydride ii, alkaline hydrolysis		isolation of a cross-linked tripeptide	386
Collagen	calf	various reagents		effect of modification on *in vitro* transformation of cross-links	387
Collagen	chick bone	sodium [^{3}H]borohydride		ratio of dihydroxy- to hydroxy-lysino-norleucine changes in vitamin D deficiency	388
Collagen	rabbit skin	CNBr			389
Collagen	guinea-pig insoluble skin and insoluble scar	CNBr		insoluble scar contains more hydroxylysine in both helical and non-helical regions	390
Collagen	rat	sodium [^{3}H]borohydride		ratio of hydroxy- to dihydroxy-lysino-norleucine ratio altered in uraemic rats	391

Collagen	rat tail tendon	CNBr-activated sepharose			392
Concanavalin A		[^{125}I]iodide, lactoperoxidase		iodinated version effective in bioassays	393
Concanavalin A		fluorescein isothiocyanate	Lys	mosaicism in organization of concanavalin A receptors on mouse egg membrane	394
Concanavalin A		dimethyl 3,3-dithiobis-propionimidate	Lys		322
Corticosteroid receptors	rat	9-bromo-21-diazo-21-deoxy-[^{3}H]corticosterone (u.v.)		photoaffinity label	395
Coupling factor	spinach chloro-plasts	7-chloro-4-nitrobenzo-2-oxa-1,3-diazole	Cys?	β-subunit labelled	396
Creatin kinase	rabbit muscle	butanedione–borate, phenyl-glyoxal	Arg	one Arg per mol subunit modified, nucleotide binding abolished	397
Creatin kinase	rabbit muscle	potassium tri-iodide	Tyr	4—5 Tyr, loss of enzyme activity	398

[379] W. E. Brown, *Biochemistry*, 1975, **14**, 5079.
[380] W. H. Cruickshank and H. Kaplan, *Biochem. J.*, 1975, **147**, 411.
[381] C. Ghelis, J. Labouesse, and B. Labouesse, *European J. Biochem.*, 1975, **59**, 159.
[382] A. Mahlen, *F.E.B.S. Letters*, 1975, **51**, 294.
[383] P. Schafer, U. Huber, R. M. Franklin, and J. Seelig, *European J. Biochem.*, 1975, **58**, 291.
[384] F. S. Steven, A. Torre-Blanco, and J. A. A. Hunter, *Biochim. Biophys. Acta*, 1975, **405**, 188.
[385] S. P. Robins and A. J. Bailey, *Biochem. J.*, 1975, **149**, 381.
[386] K. Fujii, D. Corcoran, and M. L. Tanzer, *Biochemistry*, 1975, **14**, 4409.
[387] N. R. Davis, O. M. Risen, and G. A. Pringle, *Biochemistry*, 1975, **14**, 2031.
[388] G. L. Mechanic, S. U. Toverud, W. K. Ramp, and W. A. Gonnerman, *Biochim. Biophys. Acta*, 1975, **393**, 419.
[389] U. Becker, P. P. Fietzek, H. Furthmayr, and R. Timpl, *European J. Biochem.*, 1975, **54**, 359.
[390] C. A. Shuttleworth, L. Forrest, and D. S. Jackson, *Biochim. Biophys. Acta*, 1975, **379**, 207.
[391] J. E. Russel, L. V. Avioli, and G. Mechanic, *Biochem. J.*, 1975, **145**, 119.
[392] R. A. Greenwald, C. E. Schwartz, and J. O. Cantor, *Biochem. J.*, 1975, **145**, 601.
[393] G. Weeks, *J. Biol. Chem.*, 1975, **250**, 6706.
[394] M. H. Johnson, D. Eagler, A. Muggleton-Harris, and H. M. Grave, *Nature*, 1975, **257**, 321.
[395] M. E. Wolff, D. Feldman, P. Catsoulacos, J. W. Funder, C. Hancock, Y. Amano, and I. S. Edelman, *Biochemistry*, 1975, **14**, 1750.
[396] D. W. Deters, E. Racker, N. Nelson, and H. Nelson, *J. Biol. Chem.*, 1975, **250**, 1041.
[397] C. L. Borders and J. F. Riordan, *Biochemistry*, 1975, **14**, 4699.
[398] A. Fattoum, R. Kassab, and L. A. Pradel, *Biochim. Biophys. Acta*, 1975, **405**, 324.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Cystathione γ-synthetase	*Salmonella typhimurium*	2-aminopent-4-ynoate	Lys?	active site directed cystathione protects	399
Cytochrome *c*	horse heart	bromoacetate	Met-65 Met-80	change in half reduction potential	400
Cytochrome *c*	horse heart	bromoacetate	Met-65 Met-80	Raman spectra: transition between two low-spin states?	401
Cytochrome *c*	horse heart	tetranitromethane, potassium tri-iodide	Tyr	separation of homogeneously modified tyrosine species	402
Cytochrome *c*	horse heart	2-hydroxy-5-nitrobenzyl bromide	Trp	reduction of modified enzyme by chromous ion	403
Cytochrome *c*	horse heart	2-hydroxy-5-nitrobenzyl bromide	Trp	separation of modified from unmodified species	404
Cytochrome *c*	horse heart	NBS,	Trp-59 Met-65, Met-80		
		formic acid–hydrochloric acid	Trp-59	conformational study	405
Cytochrome *c*	horse heart	fluorescein isothiocyanate	Lys	pH probe in submitochondrial particles	406
Cytochrome *c*	horse heart	*O*-methyl isourea ethylthiotrifluoroacetate	Lys	pH-dependent displacement of Met ligand from iron	407
Cytochrome *c*	horse heart	[^{13}C]bromoacetate		n.m.r. shows N^{ε}-monocarboxymethyl lysine and glycolate esters of aspartic and glutamic acids	408
Cytochrome *c*	horse heart	ethylthiotrifluoroacetate	Lys	abolishes binding of anionic iron hexacyanides	409

Cytochrome *c*	horse heart	2,4-dinitro-5-fluorophenyl azide (u.v.)	Lys?	links cytochrome *c* to cytochrome oxidase in 1 : 1 complex in cytochrome *c* depleted mitochondria	410
Cytochrome oxidase in membrane	bovine heart mitochondria	*p*-diazonium benzene [^{35}S]sulphonate		subunits asymmetric and transmembranous	411
Cytochrome oxidase in membrane	yeast	iodide/lactoperoxidase *p*-diazonium benzene sulphonate BSA carrying isocyanate group		topography of subunits	412
Cytochrome oxidase	*Pseudomonas*	[^{14}C]succinic anhydride		dissociates enzyme	413
Dihydrofolate reductase	*E. coli*	NBS	His, Met		414
Dihydrofolate reductase	*Streptococcus faecium*	DTNB	Cys	inactivation of enzyme	415
Dihydrofolate reductase	*Streptococcus faecium*	[^{14}C]iodoacetate	Met	inactivation of enzyme aminopterin protects	416

[399] P. Marcotte and C. Walsh, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 677.
[400] S. C. Cooper, J. Lambek, and M. Erecinska, *F.E.B.S. Letters*, 1975, **59**, 241.
[401] M. Ikeda-Saito, T. Kitagawa, T. Iizuka, and Y. Kyogoku, *F.E.B.S. Letters*, 1975, **50**, 233.
[402] P. K. Pal, B. Verma, and Y. P. Myer, *Biochemistry*, 1975, **14**, 4325.
[403] T. Brittain and C. Greenwood, *Biochem. J.*, 1975, **149**, 179.
[404] T. Brittain and C. Greenwood, *Biochem. J.*, 1975, **147**, 253.
[405] D. J. O'Hearn, P. K. Pal, and Y. P. Meyer, *Biochemistry*, 1975, **14**, 382.
[406] J. A. Thomas and D. L. Johnson, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 931.
[407] E. Stellwagen, J. Babul, and H. Wilgus, *Biochem. Biophys. Acta*, 1975, **405**, 115.
[408] R. T. Eakin, L. O. Morgan, and N. A. Matwiyoff, *Biochemistry*, 1975, **14**, 4538.
[409] E. Stellwagen and R. D. Cass, *J. Biol. Chem.*, 1975, **250**, 2095.
[410] M. Erecinska, J. M. Vanderkooi, and D. F. Wilson, *Arch. Biochem. Biophys.*, 1975, **171**, 108.
[411] G. D. Eytan, R. C. Carroll, G. Schatz, and E. Racker, *J. Biol. Chem.*, 1975, **250**, 8598.
[412] G. D. Eytan and G. Schatz, *J. Biol. Chem.*, 1975, **250**, 767.
[413] T. Kuronen, M. Saraste, and N. Ellfork, *Biochim. Biophys. Acta*, 1975, **393**, 48.
[414] M. N. Williams, *J. Biol. Chem.*, 1975, **250**, 322.
[415] P. E. Warwick and J. H. Freisheim, *Biochemistry*, 1975, **14**, 664.
[416] J. M. Gleisner and R. L. Blakley, *J. Biol. Chem.*, 1975, **250**, 1580.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Dihydrofolate reductase	*Streptococcus faecium*	[^{14}C]iodoacetate	Met-36, -163, -28	inactive	417
		[^{14}C]iodoacetate/aminopterin	Met-36, -163	active	
Diol dehydrogenase	*Aerobacter aerogenes*	CNBr-activated Sepharose		retains activity	418
DNA polymerase	avian myeloblastosis virus	[^{125}I]iodide, chloramine T	Tyr	radioactive peptide fingerprints indicate β cleaved to α subunit	419
Elongation factor Tu	*E. coli*	NEM	Cys	sequence round thiol essential for GTP, GDP binding	420
Elongation factor Tu	*E. coli*	DTNB	Cys	GTP, GDP, GMP protect	421
Estradiol 17β-dehydrogenase	human placenta	[^{3}H]-16α-bromoacetoxyestradiol 3-methyl ether	His	inactivates enzyme; on amino-acid analysis His (3-Cm) and His[1,3-$(Cm)_2$]	422
Estradiol 17β-dehydrogenase	human placenta	3-bromoacetoxyestrone		1 mol label per subunit; covalently bound steroid can be oxidized and reduced *in situ*	423
Estrogen binding protein	rat uterine cytosol	photoinactivated		protective effect of estrogen ligand at 315 nm	424
Estrogen receptor	calf uterus	2,4-bis(bromomethyl)-17β-estradiol 3-methyl ether	Cys	reaction prevented by prebinding of 17β-estradiol	425
Fibrinogen		[^{14}C]formaldehyde–sodium borohydride	Lys	unaltered in clotting ability, antigenicity	426
Fibrinogen	human	monodansyl cadaverine/activated Factor XIII		cross-linker acceptor sites labelled	427
Flagellae	*Proteus mirabilis*	TNM	Tyr	monomeric–$\frac{2}{5}$ Tyr modified polymeric–all Tyr modified	428
Flavoprotein	egg yolk	2-hydroxy-5-nitrobenzyl bromide, NEM, hydrogen peroxide	Trp	*ca.* $\frac{3}{4}$ Trp modified; cannot bind riboflavin	429
Fructose 1,6-bisphosphatase	pig kidney	butane-2,3-dione	Arg	loss of univalent cation activation	430

Fructose 1,6-bisphosphatase	rabbit liver, kidney, muscle	CNBr	Met	two genes in rabbit for this enzyme	431
β-Fructosidase	yeast	NBS	Trp	complete inactivation when $\frac{3}{17}$ Trp oxidized	432
β-Galactosidase	*E. coli*	coupled to 11-hydroxy-4-pregnene-3,20-dione hemi-succinate *via* carbodi-imide		enzyme immunoassay	433
β-Galactoside transport system	*E. coli*	2′-*N*-(2-nitro-4-azidophenyl)-aminoethyl-1-thio-β-D-galacto-pyranoside (u.v.)		photoinactivates lac transport system	434, 435
Gene 5 protein	bacteriophage fd	*N*-acetyl-imidazole	Tyr, Lys		436
		TNM	Tyr		
Glucagon	pig	CNBr	Met	Des-Asn-28, Thr-29 homoserine lactone 27 glucagon	
		Edman reaction on glucosaminol-isothiocyanate polystyrene		Des-His-1 glucagon	437

[417] J. M. Gleisner and R. L. Blakley, *European J. Biochem.*, 1975, **55**, 141.
[418] T. Toraya, K. Ohashi, and S. Fukui, *Biochemistry*, 1975, **14**, 4255.
[419] H. M. Rho, D. P. Grandgenett, and M. Green, *J. Biol. Chem.*, 1975, **250**, 5278.
[420] M. Wade, R. A. Laursen, and D. L. Miller, *F.E.B.S. Letters*, 1975, **53**, 37.
[421] R. C. March, G. Chinali, and A. Parmeggiani, *J. Biol. Chem.*, 1975, **250**, 8344.
[422] C. C. Chin and J. C. Warren, *J. Biol. Chem.*, 1975, **250**, 7682.
[423] E. V. Groman, R. M. Schultz, and L. L. Engel, *J. Biol. Chem.*, 1975, **250**, 5450.
[424] J. A. Katzenellenbogen, T. S. Ruh, K. E. Carlson, H. S. Iwamoto, and J. Gorski, *Biochemistry*, 1975, **14**, 2310.
[425] N. R. Kanamarlapudi and J. C. Warren, *J. Biol. Chem.*, 1975, **250**, 6484.
[426] L. J. Crane and D. L. Miller, *Analyt. Biochem.*, 1975, **64**, 60.
[427] M. Furlan, T. Seelich, and E. A. Beck, *Biochim. Biophys. Acta*, 1975, **400**, 112.
[428] W. Schalch and W. Bode, *Biochim. Biophys. Acta*, 1975, **405**, 292.
[429] J. Steczko and W. Ostrowski, *Biochim. Biophys. Acta*, 1975, **393**, 253.
[430] F. Marcus, *Biochemistry*, 1975, **14**, 3916.
[431] B. Abrams, T. Sasaki, A. Datta, E. Melloni, S. Pontremoli, and B. L. Horecker, *Arch. Biochem. Biophys.*, 1975, **169**, 116.
[432] V. Leskovac, S. Trivic, D. Pavkov-Pericin, and V. Prodanov, *Biochim. Biophys. Acta*, 1975, **405**, 482.
[433] F. Dry, J. M. Andrieu, and F. Ranaud, *Biochim. Biophys. Acta*, 1975, **403**, 131.
[434] G. Rudnick, H. R. Kaback, and R. Weil, *J. Biol. Chem.*, 1975, **250**, 6847.
[435] G. Rudnick, H. R. Kaback, and R. Weil, *J. Biol. Chem.*, 1975, **250**, 1371.
[436] R. A. Anderson, Y. Nakashima, and J. E. Coleman, *Biochemistry*, 1975, **14**, 907.
[437] M. C. Lin, D. E. Wright, V. R. Hruby, and M. Rodbell, *Biochemistry*, 1975, **14**, 1559.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Glucagon	pig, bovine	iodine monochloride	Tyr, His		438
Glucocorticoid receptor	mouse, hamster	NEM, iodoacetate	Cys	dexamethasone protects	439
Glucose 6-phosphate dehydrogenase		coupled to amino-substituted nylon tube through bisimidates or glutaraldehyde	Lys	co-immobilized with hexokinase for automated glucose assay	440
Glucose 6-phosphate dehydrogenase	*Leuconostoc mesenteroides*	pyridoxal 5′-phosphate–sodium borohydride	Lys	one Lys per subunit, irreversible inactivation	441
Glucose oxidase	*Aspergillus niger*	dimethyl sulphate activated nylon	Lys	active and stable	442
Glucose transport carrier in membrane	human erythrocytes	[^{14}C]FDNB with glucose [^{3}H]FDNB without glucose		glucose enhances reactivity of 180 000 protein to FDNB	443
β-Glucosidase	*Botryodiplodia theobromae*	iodoacetamide	Cys?	irreversible dissociation of octamer	444
β-Glucosidase	*Flavobacterium*	NEM, PCMB	Cys?	inhibited	445
Glutamate aspartate transaminase	pig heart	DTNB/[^{13}C]cyanide	Cys-390	n.m.r.	446
Glutamate aspartate transaminase	pig heart	AE-cellulose diethyladipimidate	Lys	active, stable	447
Glutamate dehydrogenase	*Neurospora*	cyclohexane-1,2-dione	Arg	activity reduced; one or two Arg modified per mol subunit	448
Glutamate dehydrogenase	*Neurospora*	tetranitromethane	Tyr-168	modification prevented by NADP	449
Glutamate dehydrogenase	bovine liver	pyridoxal 5′-phosphate–sodium borohydride	Lys	partial irreversible inactivation	450, 451
Glutamate dehydrogenase	bovine liver	[^{14}C]iodoacetate, or DTNB in Tris buffer	Cys	proteolytic digestion shows order in which Cys react as 89, 55, 197, 115, 270, 319	452
		dimethyl adipimidate dimethyl suberimidate diethyl pyrocarbonate		SDS gels of cross-linked products indicate subunits arranged as two groups of three	

Glutamate dehydrogenase	bovine liver	5′-fluorosulphonylbenzoyl adenosine	not Cys	NADH regulatory site lost; 1 mol per mol subunit	453
Glutamate dehydrogenase	bovine liver	3′-*p*-fluorosulphonylbenzoyl-adenosine		1 mol bound per subunit	454
Glutamine synthetase	sheep brain	phenylglyoxal	Arg	inactivated: ATP protects	455
Glutamine synthetase (PII regulatory protein)	*E. coli*	[^{125}I]iodide, chloramine T	Tyr	two radioactive peptides from P11A, one from P11B; UMP must be attached to hydroxyl of a Tyr	456
Glyceraldehyde 3-phosphate dehydrogenase	pig muscle	iodoacetamide, iodoacetic acid	Cys-149	pH profile of alkylation; ion pair at active site	457
Glyceraldehyde 3-phosphate dehydrogenase	pig muscle	iodoacetic acid	Cys-149	hybridization expts of native and modified subunits	458

[438] B. Desbuquois, *European J. Biochem.*, 1975, **53**, 569.
[439] H. A. Young, W. P. Parks, and E. M. Scolnick, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3060.
[440] D. L. Morris, J. Campbell, and W. E. Hornby, *Biochem. J.*, 1975, **147**, 593.
[441] M. Milhausen and H. R. Levy, *European J. Biochem.*, 1975, **50**, 453.
[442] J. C. Campbell, W. E. Hornby, and D. L. Morris, *Biochim. Biophys. Acta*, 1975, **384**, 307.
[443] C. Y. Jung and L. M. Carlson, *J. Biol. Chem.*, 1975, **250**, 3217.
[444] G. M. Umezurike, *Biochem. J.*, 1975, **145**, 361.
[445] K. Sand, A. Amemura, and T. Harada, *Biochim. Biophys. Acta*, 1975, **377**, 410.
[446] B. Boettcher and M. Martinez-Carrion, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 28.
[447] J. E. Campbell and W. E. Hornby, *Biochim. Biophys. Acta*, 1975, **403**, 79.
[448] K. M. Blumenthal and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 6555.
[449] K. M. Blumenthal and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 6560.
[450] S. S. Chen and P. C. Engel, *Biochem. J.*, 1975, **147**, 351.
[451] S. S. Chen and P. C. Engel, *F.E.B.S. Letters*, 1975, **58**, 202.
[452] F. Hucho, I. Rasched, and H. Sund, *European J. Biochem.*, 1975, **52**, 221.
[453] P. K. Pal, W. J. Wechter, and R. F. Colman, *J. Biol. Chem.*, 1975, **250**, 8140.
[454] P. K. Pal, W. J. Wechter, and R. F. Colman, *Biochemistry*, 1975, **14**, 707.
[455] S. G. Powers and J. F. Riordan, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2616.
[456] S. P. Adler, D. Purich, and E. R. Stadtman, *J. Biol. Chem.*, 1975, **250**, 6264.
[457] L. Polgar, *European J. Biochem.*, 1975, **51**, 63.
[458] D. S. Markovich and L. D. Krapivinskaya, *F.E.B.S. Letters*, 1975, **58**, 138.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Glyceraldehyde 3-phosphate dehydrogenase	pig muscle	trinitroglycerin	Cys-149	oxidized to sulphenic acid; acquires acylphosphatase activity	459
Glyceraldehyde 3-phosphate dehydrogenase	rabbit muscle	*p*-azidophenacyl bromide (bifunctional reagent)		inhibits enzyme	460
Glyceraldehyde 3-phosphate dehydrogenase	rabbit muscle	1,5-difluoro-2,4-dinitrobenzene	Cys-149 Lys-183	interprotomerial cross-link	461
Glyceraldehyde 3-phosphate dehydrogenase	rabbit muscle	3,3,3-trifluorobromoacetone	Cys-149	alkylation biphasic	462
Glyceraldehyde 3-phosphate dehydrogenase	rabbit muscle	3-pyridinealdehyde NAD	Cys-149?	thio-hemiacetal bond in active site?	463
Glyceraldehyde 3-phosphate dehydrogenase	rabbit muscle	4-(2-iodoacetamido)-2,2,6,6-tetramethylpiperidino-oxyl	Cys-149	spin label	464
Glyceraldehyde 3-phosphate dehydrogenase	yeast	2-nitro-5-thiocyanobenzoic acid, β-(2-furyl)acryloyl phosphate, iodoacetic acid	Cys-149	communication between subunits	465
Glyceraldehyde 3-phosphate dehydrogenase	yeast	butanedione	Arg	activity lost	466
Glyceraldehyde 3-phosphate dehydrogenase	yeast	dimethyl (2-hydroxy-5-nitro-benzyl)sulphonium bromide	Trp-193	activity lost	467
Glycogen phosphorylase *b*	rabbit muscle	*N*-(1-oxyl-2,2,6,6-tetramethyl piperidin-4-yl)iodoacetamide	Cys	spin label	468
Glyoxalase I	pig erythrocytes yeast	FDNB, 2,4,6-trinitrobenzene sulphonate	Lys	inactivates	469
Growth hormone	ox, sheep pituitary	CNBr	Met	split up biological activity	471
GMP synthetase	Ehrlich ascites cells	PCMB	Cys?	inhibits	472
Haemoglobin A	human	cyanate	*N*-terminal, Lys	preparation of each of the amino-terminally carbamylated derivatives	473
Haemoglobin A	human	cyanate	*N*-terminal	determination of pK's for α-amino-groups	474

Haemoglobin A	human	cyanate	*N*-terminal	no effect on O_2 linked binding of chloride or phosphate	475
Haemoglobin A	human	methyl acetimidate	Lys-40(α)	oxy-conformation favoured, allosteric equilibrium shifted	476
Haemoglobin A	human	2-nor-2-formylpyridoxal 5′-phosphate–sodium borohydride	Lys	bridge between β-chains across polyphosphate binding site	477
Haemoglobin A (apo)	human	diborane	Asp, Glu	reduced to corresponding alcohols	478
Haemoglobin A	human	3-bromo-1,1,1-trifluoro-propan-2-one		does not react with β Cys-93	479
Haemoglobin A, F	human	iodoacetic, iodoacetamide, PCMB	Cys	effect on mid-point potentials	480

[459] K. S. You, L. V. Benitez, W. A. McConachie, and W. S. Allison, *Biochim. Biophys. Acta*, 1975, **384**, 317.
[460] S. H. Hixson and S. S. Hixson, *Biochemistry*, 1975, **14**, 4251.
[461] E. Reisler, M. Tauber-Finkelstein, and S. Shaltiel, *F.E.B.S. Letters*, 1975, **54**, 315.
[462] J. Bode, M. Blumenstein, and M. A. Raftery, *Biochemistry*, 1975, **14**, 1146.
[463] E. J. Hill, T. Chou, M. C. Shih, and J. M. Park, *J. Biol. Chem.*, 1975, **250**, 1734.
[464] R. A. Dwek, H. R. Levy, G. K. Radda, and P. J. Seeley, *Biochim. Biophys. Acta*, 1975, **377**, 26.
[465] L. D. Byers and D. E. Koshland, *Biochemistry*, 1975, **14**, 3661.
[466] N. K. Nagradova and R. A. Asryants, *Biochim. Biophys. Acta*, 1975, **386**, 365.
[467] H. Heilmann and G. Pfleiderer, *Biochim. Biophys. Acta*, 1975, **384**, 331.
[468] J. R. Griffiths, N. C. Price, and G. K. Radda, *Biochem. J.*, 1975, **147**, 609.
[469] B. Mannervik, E. Marmstal, E. Ekwall, and B. Gorna-Hall, *European J. Biochem.*, 1975, **53**, 327.
[470] E. I. Budowsky, N. A. Simukova, M. F. Turchinsky, I. V. Boni, and Y. M. Skoblov, *Nucleic Acids Res.*, 1976, **3**, 261.
[471] L. A. Holladay, J. H. Levine, W. E. Nicholson, D. N. Orth, W. D. Salmon, and D. Puett, *Biochim. Biophys. Acta*, 1975, **381**, 47.
[472] T. Spector, *J. Biol. Chem.*, 1975, **250**, 7372.
[473] R. C. Williams, L. L. Chung, and T. M. Schuster, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 118.
[474] M. M. Garner, R. A. Bogardt, and F. R. N. Gurd, *J. Biol. Chem.*, 1975, **250**, 4398.
[475] A. M. Nigen and J. M. Manning, *J. Biol. Chem.*, 1975, **250**, 8248.
[476] D. T. Zolock and W. G. Niehaus, *J. Biol. Chem.*, 1975, **250**, 3080.
[477] R. Benesch, R. E. Benesch, S. Yung, and R. Edalji, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 1123.
[478] M. Z. Atassi and E. T. Nakhleh, *Biochim. Biophys. Acta*, 1975, **379**, 1.
[479] F. C. Knowles, *Biochim. Biophys. Res. Comm.*, 1975, **65**, 1155.
[480] E. C. Abraham and J. F. Taylor, *J. Biol. Chem.*, 1975, **250**, 3929.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Haemoglobin A, S	human	[^{14}C]bis(β-chloroethylmethyl-amine hydrochloride)		binding of Hb to red cell membranes quantitated	481
Haemoglobin S	human	bis(β-chloroethyl)methylamine hydrochloride		n.m.r., polymerization of deoxy-HbS inhibited	482
Haemoglobin S	human	bis(*N*-maleimidomethyl)ether	Cys-93 His-97	linked together: prevents sickling reaction	483
Haemocyanin	*Helix pomatia*	dimethyl suberimidate	Lys	can fix in low or high O_2 affinity state	484
Hexokinase	ox brain	DTNB	Cys	inactivation	485
Hexokinase	yeast	iodoacetate, iodoacetamide	Cys	two of four Cys per subunit are essential	486
Hexokinase	yeast	*N*-bromoacetyl-D-galactosamine	Cys	two Cys per subunit: protection by glucose	487
High density lipoprotein	human serum	1,5-difluoro-2,4-dinitrobenzene		apo I and apo II lie close together	488
Histone	rat sperm	[^{14}C]iodoacetamide	Cys	S—S bonds form during maturation	489
Histone (lysine rich)	Ehrlich ascites tumour cells	NBS	Trp	two fragments formed; phosphate only associated with *C*-terminal fragment	490
Histone (in chromatin)	calf thymus	formaldehyde		F2A1-F3, (F2A1-F3)n F2A2-F2B, (F2A2-F2B)n cross-links	491
Histone (in chromatin)	calf thymus	formaldehyde		(F1)n cross-links	492
Histone (in chromatin)	calf thymus	TNM as cross-linking agent	Tyr?	F2A1-F2B cross-links; can exclude F1 and F3 from chromatin without effect on formation of dimer	493
Histone (in chromatin)		dimethyl suberimidate	Lys	(F1)n, (F2B-F2A2)n cross-links	494
Histone (in chromatin)	rat liver	dimethyl suberimidate, dithiobis-(succinimidyl propionate)	Lys	chain of octamers (F2A1)2, (F3)2, (F2A2)2, (F2B)2	495

Histone (in chromatin)	rat liver	methyl 3-mercaptopropionimidate	Lys	(F3)2, F2A2-F2B, F2A1-F3, F2A1-F2B, F2A1-F2A2 cross-links	496
Histone (in chromatin)	rat liver	a carbodi-imide		(F1)n, F2A1-F3 cross-linked histones identified from ^{125}I peptide fingerprints	497
HLA antigens		[^{125}I]iodide, lactoperoxidase	Tyr	first, second, and third HLA loci may have substantial homology	498
HLA antigens		formaldehyde–potassium [^{3}H]borohydride	Lys	radiolabelled	499
20β-Hydroxysteroid dehydrogenase	*Streptomyces hydrogenans*	2-α-bromoacetoxyprogesterone	Met	inactivates enzyme one Met per subunit	500
Hypoxanthine phosphoribosyl transferase	human	[^{125}I]iodide, lactoperoxidase	Tyr	determination of CRM in erythrocytes of Lesch–Nyhan patients	501

[481] S. Fischer, R. L. Nagel, R. M. Bookchin, E. F. Roth, and I. Tellez-Nagel, *Biochem. Biophys. Acta*, 1975, **375**, 422.
[482] L. W. M. Fung, C. Ho, E. F. Roth, and R. L. Nagel, *J. Biol. Chem.*, 1975, **250**, 4786.
[483] S. J. Zak, G. R. Gelher, B. Finkel, D. P. Tukey, M. K. McCormack, and W. Krivit, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4153.
[484] R. van Driel and E. J. van Bruggen, *Biochemistry*, 1975, **14**, 730.
[485] V. D. Redkar and U. W. Kenkare, *Biochemistry*, 1975, **14**, 4704.
[486] J. G. Jones, S. Otieno, E. A. Barnard, and A. K. Bhargava, *Biochemistry*, 1975, **14**, 2396.
[487] S. Otiend, A. K. Bhargava, E. A. Barnard, and A. H. Ramel, *Biochemistry*, 1975, **14**, 2403.
[488] T. E. Grow and M. Friend, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 352.
[489] Y. Marushige and K. Marushige, *J. Biol. Chem.*, 1975, **250**, 39.
[490] D. Sherod, G. Johnson, R. Balhorn, V. Jackson, R. Chalkley, and D. Granner, *Biochim. Biophys. Acta*, 1975, **381**, 337.
[491] J. E. Hyde and I. O. Walker, *F.E.B.S. Letters*, 1975, **50**, 150.
[492] R. Chalkley and C. Hunter, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1304.
[493] H. G. Martinson and B. J. McCarthy, *Biochemistry*, 1975, **14**, 1073.
[494] R. Chalkley, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 587.
[495] J. O. Thomas and R. D. Kornberg, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2626.
[496] J. O. Thomas and R. D. Kornberg, *F.E.B.S. Letters*, 1975, **58**, 353.
[497] W. M. Bonner and H. B. Pollard, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 282.
[498] C. Cunningham-Rundles, C. Jersild, A. Svejgaard, B. Dupont, and R. A. Good, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 5081.
[499] P. Parham, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1594.
[500] R. C. Strickler, F. Sweet, and J. C. Warren, *J. Biol. Chem.*, 1975, **250**, 7656.
[501] G. S. Ghangas and G. Milman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4147.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Isoleucine-tRNA synthetase	*E. coli*	photochemically cross-linked to yeast Phe-tRNA^{Phe}		regions of tRNA identified	502
Isoleucine-tRNA synthetase	*E. coli*	photochemically cross-linked to (*E. coli*) tRNA^{Ile}		regions of tRNA identified	503
Immunoglobulin A (secretory)	human	CNBr	Met	secretory component disulphide bonded to only one of the monomer subunits of IgA dimer	504
Immunoglobulin A	human myeloma	mercaptoethylamine, iodo-[^{14}C]acetamide	Cys	mechanism of polymerization	505
Immunoglobulin A secretory component	human	DTT, iodo[^{14}C]acetic acid	Cys	reactive thiol peptide sequenced	506
Immunoglobulin G	rabbit	[^{125}I]iodide, lactoperoxidase	Tyr	remove IgG inactivated by labelling by passing down immunoadsorbent column	507
Immunoglobulin (anti blood group A antibodies)		fluorescein isothiocyanate	Lys	clustering of fluorescent label on surface of human erythrocytes	508
Immunoglobulin (anti mouse lymphoma cells)	rabbit	coupled to poly-L-glutamic acid heavily substituted with *N*-mustard		suppression of tumour growth in mice	509
Insulin	ox	[^{125}I]iodide, lactoperoxidase	Tyr	mono-iodo form purified	510
Insulin	pig	[^{3}H]Phe inserted through *N*-α-Boc-Phe-hydroxysuccinimido ester into des-Phe insulin		preparation of semi-synthetic insulin of high specific activity	511
Invertase	*Neurospora crassa*	iodine		protein not iodinated; activity lost	512
Iodopsin	chicken	sodium borohydride	Schiff base?	bleaches iodopsin in dark	513
Isocitrate dehydrogenase (NAD)	ox heart	*N*-acetylimidazole	Tyr	activity reduced; one Tyr per mol subunit	514
Isocitrate dehydrogenase (NAD)	pig heart	potassium [^{14}C]cyanate	Lys	activity lost, one Lys per mol subunit	515
Isocitrate dehydrogenase (NADP)	pig heart	various reagents		effect on NADP binding	516

Isomaltase–sucrase complex	rabbit intestine	citraconic anhydride	Lys	dissociates subunits which can be isolated in catalytically active form	517
2-Keto-3-deoxy-6-phospho-galactonate aldolase	*Pseudomonas saccharophila*	β-bromopyruvate		inactivates enzyme	518
3-Ketosteroid isomerase	*Pseudomonas testosteroni*	air, 3-oxo-4-estren17β-yl acetate, u.v.	Asx His	oxygen independent oxygen dependent	519
Lactalbumin	ox	NBS, 2-hydroxy-5-nitrobenzyl-bromide	Trp-118, -26		520
Lactalbumin	ox	iodoacetate	Met, His-107, -68, -32	selective carboxymethyl-ation of His used to assign ^{1}H n.m.r. resonances to particular His residues	521
β-Lactamase I	*Bacillus cereus*	carbodi-imide, triethyloxonium fluoroborate, Woodwards reagent K		inactivated by these carboxyl reagents	522

[502] H. J. P. Schoemaker, G. P. Budzik, R. Giege, and P. R. Schimmel, *J. Biol. Chem.*, 1975, **250**, 4440.
[503] G. P. Budzik, S. S. S. Lam, H. J. P. Schoemaker, and P. R. Schimmel, *J. Biol. Chem.*, 1975, **250**, 4433.
[504] B. J. Underdown, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 54.
[505] S. P. Hauptman and T. B. Tomasi, *J. Biol. Chem.*, 1975, **250**, 3891.
[506] C. Cunningham-Rundles and M. E. Lamm, *J. Biol. Chem.*, 1975, **250**, 1987.
[507] J. M. Pages, D. Louvard, and C. Lazdunski, *F.E.B.S. Letters*, 1975, **59**, 32.
[508] J. A. Gordon and M. D. Marquardt, *Nature*, 1975, **258**, 346.
[509] G. F. Rowland, G. T. O'Neill, and D. A. L. Davies, *Nature*, 1975, **225**, 487.
[510] J. C. Sodoyez, F. Sodoyez-Goffaux, M. M. Goff, A. E. Zimmerman, and E. R. Arquilla, *J. Biol. Chem.*, 1975, **250**, 4268.
[511] P. A. Halban and R. E. Offord, *Biochem. J.*, 1975, **151**, 219.
[512] C. H. Bigger and H. D. Braymer, *Biochim. Biophys. Acta*, 1975, **397**, 418.
[513] H. Matsumoto, F. Tokunaga, and T. Yoshizawa, *Biochim. Biophys. Acta*, 1975, **404**, 300.
[514] C. C. Fan, L. A. Tomcho, and G. W. Plaut, *J. Biol. Chem.*, 1975, **250**, 6197.
[515] W. C. Shen and R. F. Colman, *J. Biol. Chem.*, 1975, **250**, 2973.
[516] R. S. Ehrlich and R. F. Colman, *Biochemistry*, 1975, **14**, 5008.
[517] H. Braun, A. Cogoli, and G. Semenza, *European J. Biochem.*, 1975, **52**, 475.
[518] H. P. Meloche and C. T. Monti, *Biochemistry*, 1975, **14**, 3682.
[519] R. J. Matyr and W. F. Benisek, *J. Biol. Chem.*, 1975, **250**, 1218.
[520] J. E. Bell, F. J. Castellino, I. P. Trayer, and R. L. Hill, *J. Biol. Chem.*, 1975, **250**, 7579.
[521] J. H. Bradbury and R. S. Norton, *European J. Biochem.*, 1975, **53**, 387.
[522] S. G. Waley, *Biochem. J.*, 1975, **149**, 547.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
β-Lactamase I	*Bacillus cereus*	NBS	Trp	one Trp modified	523
Lactate dehydrogenase	dogfish	pyridoxal 5′-phosphate–sodium borohydride	Lys	enzyme inactivated	524
Lactate dehydrogenase	pig	pyridoxal 5′-phosphate–sodium borohydride	Lys	one Lys per four subunits	525
Lactose synthetase	ox	dimethyl pimelimidate	Lys	cross-linking of components	526
Lactotransferrin	human	diethyl pyrocarbonate	His		527
Lectin	*Dolichos biflorus*	CNBr	Met	only one of the two fragments contains carbohydrate	528
Leucine aminopeptidase	ox lens	diethyl pyrocarbonate	His		529
Leucine aminopeptidase	ox lens	2,2,6,6-tetramethyl-4-(2-iodo-acetamido)piperidine-1-oxyl	Cys	two conformational states in environment of label	530
Lipase	ox pancreas	DTNB	Cys	one Cys buried, one Cys free	531
Lipid acyl-hydrolase	potato tuber	di-isopropyl fluorophosphonate diethyl pyrocarbonate	Ser, His	enzyme inactivated	532
Lipoamide dehydrogenase	pig heart	copper(II) ions	Cys	insertion of a second disulphide bridge	533
Luteinizing hormone	ox	carbodi-imide–glycine methyl ester	carboxy-groups	modified α-subunit does not recombine with native β-subunit	534
Lutotropin β-subunit	sheep	acetic anhydride, methyl acetimidate	Lys	less active in bioassays	535
Lysine decarboxylase	*Bacillus cadaveris*	coupled to albumin by glutaraldehyde	Lys	membrane for pCO_2 measurement	536
Lysine mono-oxygenase	*Pseudomonas fluorescens*	various thiol reagents	Cys	inactivates oxygenase; induces oxidase activity	537
Lysozyme	egg white	2-hydroxy-5-nitrobenzyl bromide	Trp-62	reversibly activated	538
Lysozyme	egg white	iodine	Trp-108, Glu-35	*X*-ray diffraction map of internal cross-link	539
Lysozyme	egg white	iodine	Trp-108, Glu-35	thermodynamic measure-ments	540
		NBS	Trp-62		

Lysozyme (antigenic peptide)	egg white	i, 2,3-dioxo-5-indolinesulphonic acid; ii, succinic anhydride	Trp Lys	antigenic reactivity destroyed	541
Lysozyme	egg white	[^{14}C]succinic anhydride	Lys	derivatives separated; modified residues identified	542
Lysozyme	egg white	pentane-2,4-dione	Lys, Arg	new reagent	543
Lysozyme	egg white	[^{3}H, ^{14}C]FDNB	His	competitive labelling	544
Lysozyme	egg white	diborane	Asp-119, *C*-terminal Leu	these residues are not part of antigenic reactive site	546
Lysozyme	egg white	[^{32}P]-ATP/protein kinase	Ser-24 Ser-50		
Lysozyme	egg white	2-acetamido-1-amino-3,4,6-tri-*O*-acetyl-1,2-dideoxy-β-D-glucose, carbodi-imide		*N*-acetylglucosamine derivative of lysozyme	547

[523] H. Ogawara and H. Umezawa, *Biochim. Biophys. Acta*, 1975, **391**, 435.
[524] S. S. Chen and P. C. Engel, *Biochem. J.*, 1975, **151**, 447.
[525] S. S. Chen and P. C. Engel, *Biochem. J.*, 1975, **149**, 107.
[526] K. Brew, J. H. Shaper, K. W. Olsen, I. P. Trayer, and R. L. Hill, *J. Biol. Chem.*, 1975, **250**, 1434.
[527] M. A. Krysteva, J. Mazurier, G. Spik, and J. Montreuil, *F.E.B.S. Letters*, 1975, **56**, 337.
[528] W. G. Carter and M. E. Etzler, *Biochem. J.*, 1975, **14**, 5118.
[529] M. Ludewig, M. Frohne, I. Marquardt, and H. Hanson, *European J. Biochem.*, 1975, **54**, 155.
[530] G. Lassman, W. Damerau, G. Sklenar, D. Schwarz, M. Frohne, M. Ludwig, and H. Hanson, *European J. Biochem.*, 1975, **54**, 453.
[531] R. Julien, J. Rathelot, P. Canioni, L. Sarda, and T. H. Plummer, *Biochim. Biophys. Acta*, 1975, **379**, 157.
[532] O. Hirayama, H. Matsuda, M. Takeda, K. Maenaka, and H. Takatsuka, *Biochim. Biophys. Acta*, 1975, **384**, 127.
[533] C. Thorpe and C. H. Williams, *Biochemistry*, 1975, **14**, 2419.
[534] M. R. Faith and J. G. Pierce, *J. Biol. Chem.*, 1975, **250**, 6923.
[535] W. K. Piu and D. N. Ward, *Biochim. Biophys. Acta*, 1975, **405**, 522.
[536] C. Calvot, A. M. Berjonneau, G. Gellf, and D. Thomas, *F.E.B.S. Letters*, 1975, **59**, 258.
[537] T. Yamauchi, S. Yamamoto, and O. Hayaishi, *J. Biol. Chem.*, 1975, **250**, 7127.
[538] A. Reiss and A. Lukton, *Bioorg. Chem.*, 1975, **4**, 1.
[539] C. R. Beddell, C. C. F. Blake, and S. J. Oatley, *J. Mol. Biol.*, 1975, **97**, 643.
[540] S. K. Banerjee, A. Pogolotti, and J. A. Rupley, *J. Biol. Chem.*, 1975, **250**, 8260.
[541] C. Lee and M. Z. Atassi, *Biochim. Biophys. Acta*, 1975, **405**, 464.
[542] C. Lee, M. Z. Atassi, and A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1975, **400**, 423.
[543] H. F. Gilbert and M. H. O'Leary, *Biochemistry*, 1975, **14**, 5194.
[544] R. G. Duggleby and H. Kaplan, *Biochemistry*, 1975, **14**, 5168.
[545] M. Zatassi, A. M. Suliman, and A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1975, **405**, 452.
[546] D. B. Bylund and E. G. Krebs, *J. Biol. Chem.*, 1975, **250**, 6355.
[547] E. Moczar and J. Leboul, *F.E.B.S. Letters*, 1975, **50**, 300.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
α-Lytic protease β-Lytic protease	*Sorangium* sp.	NBS, hydrogen peroxide, 2-hydroxy-5-nitrobenzyl bromide	Trp	α $\frac{1}{2}$ Trp modified β $\frac{1}{4}$ Trp and $\frac{4}{4}$ Trp modified	548
Malate dehydrogenase	pig heart mitochondria	pyridoxal 5′-phosphate–sodium borohydride	Lys	irreversible inactivation one residue per subunit	549, 550
Malate dehydrogenase	pig heart mitochondria	pyridoxal 5′-phosphate	Lys, X	biphasic inactivation; two residues react?	551
Malate dehydrogenase	pig heart cytoplasmic	butanedione	Arg	four Arg per mol enzyme inactivated, two Arg protected by NADH	552
Malate dehydrogenase	ox	iodoacetamide NEM	His Cys	one His per 2 mol subunit one Cys per 2 mol subunit	553
Membrane proteins	cat spinal cord	[^{125}I]iodide, lactoperoxidase	Tyr	outer high mol. wt. proteins	554
Membrane proteins	Chinese hamster ovary	[^{125}I]iodide, lactoperoxidase	Tyr	subcellular fractionation	555
Membrane proteins	dog gastric mucosa	[^{125}]iodide, lactoperoxidase, and also glutaraldehyde	Tyr, Lys		556
Membrane proteins	guinea-pig intestine	[^{125}I]iodide, lactoperoxidase	Tyr	subcellular fractionation	557
Membrane proteins	human erythrocytes	[^{125}I]iodide, lactoperoxidase	Tyr	protein E spans membrane twice	558
Membrane proteins	human erythrocytes	[^{125}I]iodide, [^{131}I]iodide, lactoperoxidase	Tyr	^{125}I/^{131}I	559
Membrane proteins	human erythrocytes	[^{125}I]iodide, [^{131}I]iodide, lactoperoxidase	Tyr	^{125}I/^{131}I, resealed vesicles	560
Membrane proteins	human platelets	[^{125}I]iodide, [^{131}I]iodide, lactoperoxidase	Tyr	^{131}I normal patients ^{125}I patients with thrombosthenia ^{125}I/^{131}I very different	561
Membrane proteins	human lymphocytes and platelets	[^{125}I]iodide, lactoperoxidase	Tyr	major components are not the same	562
Membrane proteins	human tumour cells	[^{125}I]iodide, lactoperoxidase	Tyr	most of iodinated proteins are glycosylated	563

Membrane proteins	horse erythrocytes	[^{125}I]iodide, lactoperoxidase	Tyr	major sialoglycoprotein not labelled though at surface	564
Membrane proteins	mouse BALB cells	[^{125}I]iodide, lactoperoxidase	Tyr	LETS protein disappears on temperature drop	565
Membrane proteins	mouse T and B cells	[^{125}I]iodide, lactoperoxidase	Tyr	differences in pattern of cell surface proteins	566
Membrane proteins	mouse L cells	[^{125}I]iodide, lactoperoxidase	Tyr	plasma membrane markers	567
Membrane proteins	mouse L cells	[^{125}I]iodide, lactoperoxidase	Tyr	high mol. wt. protein exposed during G1 phase	568
Membrane proteins	mouse L cells	[^{125}I]iodide, lactoperoxidase	Tyr	method for identifying transmembrane protein	569
Membrane proteins	ox mitochondria	[^{125}I]iodide, lactoperoxidase	Tyr	major 29 000 component on outer surface inner membrane	570

[548] V. Leskovac, *Biochim. Biophys. Acta*, 1975, **393**, 563.
[549] S. S. Chen and P. C. Engel, *Biochem. J.*, 1975, **151**, 297.
[550] M. J. Wimmer and J. H. Harrison, *J. Biol. Chem.*, 1975, **250**, 8768.
[551] M. J. Wimmer, T. Mo, D. L. Sawyer, and J. H. Harrison, *J. Biol. Chem.*, 1975, **250**, 710.
[552] D. M. Bleile, M. Foster, J. W. Brady, and J. H. Harrison, *J. Biol. Chem.*, 1975, **250**, 6222.
[553] E. M. Gregory, *J. Biol. Chem.*, 1975, **250**, 5470.
[554] J. F. Poduslo and P. E. Braun, *J. Biol. Chem.*, 1975, **250**, 1099.
[555] R. L. Juliano and M. Behar-Bannelier, *Biochemistry*, 1975, **14**, 3816.
[556] G. Saccomani, G. Shan, J. G. Spenny, and G. Sachs, *J. Biol. Chem.*, 1975, **250**, 4802.
[557] B. A. Lewis, A. Elkin, R. H. Michell, and R. Coleman, *Biochem. J.*, 1975, **152**, 71.
[558] R. E. Jenkins and M. J. Tanner, *Biochem. J.*, 1975, **147**, 393.
[559] E. Reichstein and R. Blostein, *J. Biol. Chem.*, 1975, **250**, 6256.
[560] G. W. Litman, R. T. Litman, and D. C. Merz, *Biochim. Biophys. Acta*, 1975, **394**, 348.
[561] D. R. Phillips, C. S. P. Jenkins, E. F. Luscher, and M. J. Larrieu, *Nature*, 1975, **257**, 599.
[562] R. E. Gates, D. R. Phillips, and M. Morrison, *Biochem. J.*, 1975, **147**, 373.
[563] T. D. Butters and R. C. Hughes, *Biochem. J.*, 1975, **50**, 59.
[564] K. L. Carraway, D. G. Colton, B. C. Shin, and R. B. Triplett, *Biochim. Biophys. Acta*, 1975, **382**, 181.
[565] P. S. Rudland, E. Pearlstein, D. Kamely, M. Nutt, and W. Eckhart, *Nature*, 1975, **256**, 43.
[566] I. S. Stowbridge, P. Ralph, and M. J. Bevan, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 157.
[567] C. C. Tsai, K. Y. Chen, and E. S. Canellakis, *Biochim. Biophys. Acta*, 1975, **401**, 196.
[568] R. C. Hunt, E. Gold, and J. C. Brown, *Biochim. Biophys. Acta*, 1975, **413**, 453.
[569] R. C. Hunt and J. C. Brown, *J. Mol. Biol.*, 1975, **97**, 413.
[570] D. H. Boxer, *F.E.B.S. Letters*, 1975, **59**, 149.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Membrane proteins	rabbit bone marrow and blood cells	[^{125}I]iodide, lactoperoxidase	Tyr	surface proteins change in process of red blood cell formation	571
Membrane proteins	*Streptomyces albus*	[^{125}I]iodide, lactoperoxidase	Tyr	protoplasts and cytoplasmic membranes	572
Membrane proteins	*Acinetobacter*	carbodi-imide/ethylene diamine	carboxy-groups	interferes with reattachment of external protein subunits to outer membrane	573
Membrane proteins	spinach chloroplasts	carbodi-imide/methylamine, glycine ethyl ester		inhibits calcium binding	574
Membrane proteins	human erythrocytes	[^{14}C]-DTNB	Cys	reacts with 100 000 band; water permeability inhibited	575
Membrane proteins	pig intestine brush borders	[^{203}Hg]-*p*-chloromercuribenzene sulphonic acid		D-glucose or phlorhizin increases labelling of 31 500 band	576
Membrane proteins	*Spirillum serpens*	di-imido esters	Lys	cross-links	577
Membrane proteins	human erythrocytes	dimethyl 3,3-dithiobis-propionimidate	Lys	cleavable cross-linker; penetrates membranes linking membrane proteins to internal haemoglobin	578
Membrane proteins	chicken erythrocytes	*p*-nitrophenyl *NNN*-trimethyl [^{125}I]iodotyrosinate	amino-groups	cationic, impermeable, labels mainly 100 000 and 55 000 components	579
Membrane proteins	human erythrocytes	4,4-di-isothiocyano-2,2-stilbene disulphonic acid, pyridoxal 5′-phosphate–sodium borohydride			580
Membrane proteins	human erythrocytes	citraconic anhydride, maleic anhydride		selective solubilization of membrane proteins	581, 582
Membrane proteins	ox heart mitochondria	2-bromo-2,4-dinitro[^{14}C]-acetanilide	Cys	selective labelling of 44 000 and 31 000 proteins	583

Membrane proteins	human erythrocytes	*N*-(4-azido-2-nitrophenyl)-2-aminoethyl sulphonate		photolabile nitrene precurser used to label only inner face of membrane	584
Membrane proteins	human erythrocytes	bilirubin/light		proteins modified	585
Membrane proteins	human erythrocytes	various reagents		amino-group modification inhibits interaction with sheep erythrocytes	586
Membrane proteins	human erythrocytes	formyl[^{35}S]methionyl sulphone methyl phosphate	Lys	MN-glycoprotein, *C*-terminal region lies in cell cytoplasm	587
β_2-Microglobulin	human	DTT/iodoacetic acid	S—S	carboxymethylation prevents refolding	588
Monoamine oxidase	ox liver mitochondria	diethyl pyrocarbonate, amino-1-*H*-tetrazole, photo-oxidation	His	inactivates	589
Myoglobin	sperm whale	methyl acetimidate, [^{13}C]t-butoxycarbonylglycine *N*-hydroxysuccinimide ester	Lys *N*-terminal	preparation of specifically enriched Nα-[1-^{13}C]-glycine adducts; n.m.r.	590

[571] M. Fehlmann, L. Lafleur, and N. Marceau, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 322.
[572] V. Larraga and E. Munoz, *European J. Biochem.*, 1975, **54**, 207.
[573] K. J. I. Thorne, M. J. Thornley, P. Naisbitt, and A. M. Glauert, *Biochim. Biophys. Acta*, 1975, **389**, 97.
[574] L. J. Prochaska and E. L. Gross, *Biochem. Biophys. Acta*, 1975, **376**, 126.
[575] P. A. Brown, M. B. Feinstein, and R. I. Shaafi, *Nature*, 1975, **254**, 523.
[576] M. W. Smith, D. R. Ferguson, and K. A. Burton, *Biochem. J.*, 1975, **147**, 617.
[577] M. Schweizer, I. Sonntag, and U. Henning, *J. Mol. Biol.*, 1975, **93**, 11.
[578] K. Wang and F. M. Richards, *J. Biol. Chem.*, 1975, **250**, 6622.
[579] R. C. Jackson, *J. Biol. Chem.*, 1975, **250**, 617.
[580] Z. I. Cabantchik, M. Balshin, W. Breuer, H. Markus, and A. Rothstein, *Biochim. Biophys. Acta*, 1975, **382**, 621.
[581] P. Lundahl, *Biochim. Biophys. Acta*, 1975, **379**, 304.
[582] B. P. Vadlamudi, J. D. Ozandu, and P. F. Larway, *Biochim. Biophys. Res. Comm.*, 1975, **64**, 64.
[583] C. C. Chen, M. Yang, H. D. Durst, D. R. Saunders, and J. H. Wang, *Biochemistry*, 1972, **14**, 4122.
[584] J. V. Staros, F. M. Richards, and B. E. Haley, *J. Biol. Chem.*, 1975, **250**, 8174.
[585] A. W. Girotti, *Biochemistry*, 1975, **14**, 3377.
[586] P. Lalezari, S. L. Nehlsen, J. Novodoff, and I. Lalezuri, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 697.
[587] M. S. Bretscher, *J. Mol. Biol.*, 1975, **98**, 831.
[588] D. E. Isenman, R. H. Painter, and K. J. Dorrington, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 548.
[589] A. Hiramatsu, S. Tsurushin, and K. Y. Yasunobu, *European J. Biochem.*, 1975, **57**, 587.
[590] W. H. Garner and F. R. N. Gurd, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 262.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Myosin	cat	[^{14}C]iodoacetate, 2-nitro-5-thiocyanobenzoic acid	Cys	identification of heavy chains in fast/slow twitch cross-re-inervation expts	591
Myosin	rabbit, fast, slow, and heart muscle	[^{14}C]-NEM	Cys	three classes of reactive thiol; stoicheiometry determined	592
Myosin	rabbit	[^{14}C]-NEM	Cys	changes in reactive thiols during hydrolytic cycle	593
Myosin (HMM)	rabbit	NEM	Cys	interaction of actin with NEM-HMM	594
Myosin	rabbit skeletal, pig cardiac	DTNB	Cys	complete dissociation of skeletal muscle myosin, partial dissociation of cardiac muscle myosin	595
Myosin	rabbit	6,6′-dithiobis(inosinyl imidodiphosphate)	Cys	analogue of ATP binds covalently at regulatory site;	596, 597
				4/HMM (displace mixed disulphide with ^{14}C-cyanide)	597
Myosin	rabbit	*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)iodoacetamide	Cys	saturation transfer e.s.r.; great flexibility of subfragment relative to rest of chain	598
Myosin and HMM	rabbit	*N*-(1-oxyl-2,2,6,6-tetramethyl-4-piperidinyl)iodoacetamide	Cys	SH groups, e.s.r. study	599
Myosin	rabbit	trinitrophenol	Lys	active site Lys: both ATPase sites behave in same way w.r.t. reagent	600
Myosin	rabbit	di-imidates, cleavable and non-cleavable	Lys	B protein associated with myosin	601
Nerve growth factor receptor	rabbit superior cervical ganglia	2-hydroxy-5-nitrobenzyl bromide		nerve growth factor protects	602

Neurotoxin III	cobra	sodium borohydride–iodoacetic acid	Cys	Cys-30–Cys-34 bridge alkylated; structure–function study	603
Nitrate reductase complex	*Spinacea oleracea*	thiol reagents	Cys	inactivated	604
Nitrate respiration complex (membrane)	*E. coli*	[^{125}I]iodide lactoperoxidase	Tyr	vectorial orientation; complex spans the membrane	605
p-Nitrophenyl phosphase (intact cells)	Ehrlich ascites tumour cells	various thiol reagents	Cys	affects activity	606
Nuclease	*Staphylococcus aureus*	TNM	Tyr-115	c.d. study	607
Opiate receptor	rat brain	NEM	Cys	protection in presence of an opiate or an antagonist	608
Ovalbumin	egg white	acetic anhydride	Lys		609
Papain		diazo-1*H*-tetrazole	His-159? Tyr, Trp		610
Paramyosin	*Mercenaria*	2-nitro-5-cyanobenzoic acid	Cys	cleavage; separation of fragments	611

[591] A. G. Weeds and K. Burridge, *F.E.B.S. Letters*, 1975, **57**, 203.
[592] M. Pfister, M. C. Schaub, M. Knecht, J. G. Watterson, and P. G. Waser, *Biochim. Biophys. Acta*, 1975, **410**, 193.
[593] J. G. Watterson, M. C. Schaub, R. Locher, S. Di Pierri, and M. Kutzer, *European J. Biochem.*, 1975, **56**, 79.
[594] S. Mulhern, E. Eisenberg, and W. W. Kielly, *Biochemistry*, 1975, **14**, 3863.
[595] J. J. Leger and F. Marotte, *F.E.B.S. Letters*, 1975, **52**, 17.
[596] P. D. Wagner and R. G. Yount, *Biochemistry*, 1975, **14**, 5156.
[597] P. D. Wagner and R. G. Yount, *Biochemistry*, 1975, **14**, 1907.
[598] D. D. Thomas, J. C. Seidel, J. S. Hyde, and J. Gergely, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1729.
[599] J. C. Seidel, *J. Biol. Chem.*, 1975, **250**, 5681.
[600] A. Muhlrad, R. Lamed, and A. Oplatka, *J. Biol. Chem.*, 1975, **250**, 175.
[601] A. D'Albis, *F.E.B.S. Letters*, 1975, **58**, 241.
[602] S. P. Banerjee, P. Cuatrecasas, and S. H. Synder, *J. Biol. Chem.*, 1975, **250**, 1427.
[603] M. Lazdunski, *Biochemistry*, 1975, **14**, 2081.
[604] F. Castillo, F. F. De La Rosa, and E. Palacian, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 546.
[605] D. H. Boxer and R. A. Clegg, *F.E.B.S. Letters*, 1975, **60**, 54.
[606] M. Loffler and F. Schneider, *F.E.B.S. Letters*, 1975, **56**, 66.
[607] C. Di Bello and J. H. Griffin, *J. Biol. Chem.*, 1975, **250**, 1445.
[608] E. J. Simon and J. Groth, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2404.
[609] A. A. Ansari, S. A. Kidwai, and A. Salahuddin, *J. Biol. Chem.*, 1975, **250**, 1625.
[610] H. G. Offler and F. R. Schneider, *Biochim. Biophys. Acta*, 1975, **386**, 221.
[611] R. W. Cowgill, *Biochemistry*, 1975, **14**, 4277.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Paramyosin	*Mercenaria*	CNBr	Met	seven bands on SDS gels	612
Paramyosin	*Pectin maximus*	[^{14}C]iodoacetic acid	Cys	chains are heterogeneous	613
Pepsin	chicken	thiol reagents	Cys	enhanced catalytic activity	614
Pepsin	chicken	dimethyl-(2-hydroxy-5-nitro-benzyl)sulphonium bromide, diazoacetylnorleucine methyl ester, 2-diazo-4-bromo-acetophenone, 1,2-epoxy-3-(*p*-nitrophenoxy)propane		stoicheiometries with enzyme determined	615
Pepsin	pig, ox	hydrogen peroxide	Met	affects activity	616
Penicillinase	*Staphylococcus aureus*	3-carboxy-2-quinoxalinyl penicillanic acid		attached by lactam carboxy-group	617
Pepsin		carbodi-imide coupled to amino-substituted hydroxyalkyl methacrylate gels		active	618
Phenol hydroxylase	*Trichosporon cutaneum*	*p*-hydroxymercuribenzoate, DTNB	Cys	16 thiols per 150 000	619
Phenylalanyl tRNA synthetase	yeast	photo-crossed linked to $tRNA^{Phe}$		attached fragments of RNA identified	502
Phenylalanyl tRNA synthetase	yeast	PCMB	Cys	separation of modified α and β subunits	620
Phenylalanyl tRNA synthetase	yeast	PCMB, *p*-aminophenyl-mercuriacetate	Cys	rapid inactivation of aminoacylation but not of pyrophosphate exchange	621
Phenylalanyl tRNA synthetase	*E. coli*	*p*-azidoanilidate ATP		competitive inhibitor of ATP; covalent bond formed	622
Phenylalanyl tRNA synthetase	*E. coli*	PCMB	Cys?	inactivation and dissociation of structure	623
Phenylalanyl tRNA synthetase	*E. coli*	[^{14}C]*N*-chlorambucilyl $tRNA^{Phe}$		kinetics of labelling	624
Phosphate transport	rat liver mitochondria	[^{3}H]-NEM	Cys	five labelled species on polyacrylamide gels	625

Phosphofructokinase	rabbit muscle	dimethyl suberimidate	Lys	electron microscope study of oligomers	626
Phosphoglycerate kinase	yeast	*O*-methylisourea 2-methoxy-5-nitrotropone pyridoxal 5′-phosphate	Lys	three essential Lys	627
Phosphoglycerate kinase	yeast		Tyr	mainly monoiodo Tyr	628
Phosphoglycerate kinase	ox liver	PCMB, DTNB	Cys	inactivated	
		sodium borohydride–DTNB	S—S	one disulphide bridge	629
Phosphoribosylpyrophosphate synthetase	*Salmonella typhimurium*	potassium permanganate	Cys	one per mol subunit oxidized; $KMnO_4$ is phosphate analogue	630
Phosphorylase *b*	rabbit muscle	DTNB	Cys	three classes of thiol groups	631
Polygalacturonase	*Botrytis cinerea*	PCMB, TNM			632
Polynucleotide phosphorylase	*E. coli*	dimethyl suberimidate	Lys	trimer	633

[612] J. W. Weisel and A. G. Szent-Gyorgyi, *J. Mol. Biol.*, 1975, **98**, 665.
[613] I. D. Walker and M. Stewart, *F.E.B.S. Letters*, 1975, **58**, 16.
[614] Y. Shechter, M. Rubenstein, R. Becker, and Z. Bohak, *European J. Biochem.*, 1975, **58**, 123.
[615] J. M. Llewellin and M. L. Green, *Biochem. J.*, 1975, **151**, 319.
[616] K. Kidd and B. Kassell, *Biochemistry*, 1975, **14**, 631.
[617] R. Virden, A. F. Bristow, and R. H. Pain, *Biochem. J.*, 1975, **149**, 397.
[618] O. Valentova, J. Turkova, R. Lapka, J. Zima, and J. Coupek, *Biochim. Biophys. Acta*, 1975, **403**, 192.
[619] H. Y. Neujhar and A. Gaal, *European J. Biochem.*, 1975, **58**, 351.
[620] F. Fasiolo and Y. Boulanger, *F.E.B.S. Letters*, 1975, **60**, 156.
[621] A. Murayama, J. P. Raffin, P. Remy, and J. P. Ebel, *F.E.B.S. Letters*, 1975, **53**, 15.
[622] V. N. Ankilova, D. G. Knorre, V. V. Kravchenko, O. I. Lovrik, and G. A. Nevinsky, *F.E.B.S. Letters*, 1975, **60**, 172.
[623] T. Hanke, P. Bartman, and E. Holler, *European J. Biochem.*, 1975, **56**, 605.
[624] I. I. Gorshkova and O. I. Lovrik, *F.E.B.S. Letters*, 1975, **52**, 135.
[625] W. A. Coty and P. L. Pederson, *J. Biol. Chem.*, 1975, **250**, 3515.
[626] J. N. Telford, P. M. Lad, and G. G. Hammes, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3054.
[627] F. S. Markland, A. D. E. Bacharach, B. H. Weber, T. C. O. Grady, G. C. Saunders, and N. Umemura, *J. Biol. Chem.*, 1975, **250**, 1301.
[628] A. Fattoum, C. Rouston, L. Pradel, and N. Van Thoai, *F.E.B.S. Letters*, 1975, **51**, 18.
[629] K. D. Kulbe, M. Bojanovski, and W. Lamprecht, *European J. Biochem.*, 1975, **52**, 239.
[630] M. F. Roberts, R. L. Switzer, and K. R. Schubert, *J. Biol. Chem.*, 1975, **250**, 5364.
[631] M. H. Buc-Caron and H. C. Buc, *European J. Biochem.*, 1975, **52**, 575.
[632] H. Urbanek and J. Zalewska-Sokczak, *Biochim. Biophys. Acta*, 1975, **377**, 402.
[633] C. Porter, *F.E.B.S. Letters*, 1975, **50**, 79.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Purine nucleoside phosphorylase	*E. coli, Salmonella typhimurium*	dimethyl suberimidate	Lys		634
Protamine Y-I	herring sperm	methyl 4-mercaptobutyrimidate, followed by mercuric chloride	*N*-terminal	labelled at amino-terminus	635
Protein kinase (in membrane)	human erythrocytes	[^{32}P]-8-azidoadenosine 3,5-cyclic monophosphate (u.v.)		activates cAMP dependent protein kinase; photolysis gives label in 49 000 and 55 000 components	636
Protein kinase	ox brain	[^{32}P]-8-azidoadenosine 3,5-monophosphate (u.v.)		incorporation abolished by excess cAMP	637
Protein kinase	rat muscle	CNBr-activated Sepharose	Lys	phosphorylation of proteins *in vitro*	638
Pyruvate kinase	ox	trinitrobenzenesulphonic acid		inactivation; kinetics of hybrids	639
Pyruvate kinase	rabbit muscle	2-hydroxy-5-nitrobenzyl bromide	Trp	one per mol subunit modified; no effect on activity	640
Pyruvate kinase	yeast	phosphoenol-3-bromopyruvate		irreversible inactivation	641
Pyruvate carboxylase	sheep liver	bromopyruvate	Cys	inactivates; pyruvate and oxaloacetate protect	642
Quinone receptor	*Periplanata americana*	NEM, iodoacetic acid	Cys	iodoacetic reaction competitively inhibited by quinones	643
Renin	mouse sub-maxillary gland	α-bromoisocaproyl peptides, β-bromopropionyl peptides		active-site-directed inhibitors	644
Replicase	Qβ-phage	dimethyl suberimidate, methyl 4-mercaptobutyrimidate		cross-link I+II, III+IV, I+II+III+IV	645
Retinol binding protein	human plasma	*N*-acetylimidazole	Lys, Tyr	affects RBP/pre-albumin binding	646
Reverse transcriptase	RNA virus	butanedione, borate	Arg	inactivated, protection by poly-rA	647
Rhodanese	ox kidney	pyridoxal 5′-phosphate	Lys	inactivated, two Lys per mol subunit	648

Rhodanese	ox kidney	selenosulphate			649
Rhodopsin	ox	NEM, PCMB, *p*-chloro-mercuribenzene sulphonate	Cys	six thiol groups divide into three pairs	650
Ribose 5-phosphate isomerase	*E. coli*	iodoacetate	Cys	inducible type inactivated; constitutive type unaffected	651
30S Ribosomes	*E. coli*	methyl 4-mercaptobutyrimidate, followed by oxidation	Lys	cross-linked pairs: S2–S3, S4–S6, S4–S8, S4–S9, S4–S12, S5–S8, S5–S9, S7–S8, S7–S9	652
30S Ribosomes	*E. coli*	tartryl diazide, tartryl di(glycyl azide), tartryl di(ε-amino-caproyl azide)	Lys	cross-linked pairs: S5–S8, S7–S9, S13–S19, S2–S3, S2–S5, S2–S8, S3–S10, S6–S18, S4–S5	653
30S Ribosomes	*E. coli*	dimethyl suberimidate	Lys	cross-linked complexes: S4–S5, S5–S8, S8–S15?, S4–S5–S8, S5–S8–S15?, S7–S9, S7–S13–S19	654

634 K. F. Jensen and P. Nygaard, *European J. Biochem.*, 1975, **51**, 253.
635 F. P. Ottensmeyer, R. F. Whiting, and A. P. Korn, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4953.
636 B. E. Haley, *Biochemistry*, 1975, **14**, 3852.
637 A. H. Pomerantz, S. A. Rudolph, B. E. Haley, and P. Greengaro, *Biochemistry*, 1975, **14**, 3858.
638 V. Kinzel, A. Alonso, and D. Kubler, *European J. Biochem.*, 1975, **55**, 361.
639 D. R. Hubbard and J. M. Cardenas, *J. Biol. Chem.*, 1975, **250**, 4931.
640 A. Brevet, C. Roustan, L. A. Pradel, and N. Van Thoai, *European J. Biochem.*, 1975, **52**, 345.
641 K. Blumberg and J. Stubbe, *Biochim. Biophys. Acta*, 1975, **384**, 120.
642 P. J. Hudson, D. B. Keech, and J. C. Wallace, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 213.
643 G. Singer, J. M. Rozental, and D. M. Norris, *Nature*, 1975, **256**, 222.
644 Y. Suketa and T. Inagami, *Biochemistry*, 1975, **14**, 3188.
645 R. A. Young and T. Blumenthal, *J. Biol. Chem.*, 1975, **250**, 1829.
646 J. Heller and J. Horowitz, *J. Biol. Chem.*, 1975, **250**, 3019.
647 C. L. Borders, J. F. Riordan, and D. S. Auld, *Biochim. Biophys. Res. Comm.*, 1975, **66**, 490.
648 C. Cannella, L. Pecci, M. Costa, B. Pensa, and D. Cavallini, *European J. Biochem.*, 1975, **56**, 283.
649 C. Cannella, L. Pecci, A. Finazzi Agro, G. Federici, B. Pensa, and D. Cavallini, *European J. Biochem.*, 1975, **55**, 285.
650 W. J. De Grip, S. L. Bonting, and F. J. M. Daemen, *Biochim. Biophys. Acta*, 1975, **396**, 104.
651 M. K. Essenberg and R. A. Cooper, *European J. Biochem.*, 1975, **55**, 323.
652 A. Sommer and R. R. Traut, *J. Mol. Biol.*, 1975, **97**, 471.
653 L. C. Lutter, C. G. Kurland, and G. Stoffler, *F.E.B.S. Letters*, 1975, **54**, 144.
654 D. Barritault, A. Expert-Bezancon, M. Milet, and D. H. Hayes, *F.E.B.S. Letters*, 1975, **50**, 114.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
50S Ribosomes	*E. coli*	dimethyl suberimidate	Lys	L2–L9, L10–L11, L17–L32	655
30S Ribosomes	*E. coli*	tartryl diazide	Lys	IF3–S7	656
30S Ribosomes	*E. coli*	dimethyl suberimidate	Lys	IF2 in links with: S1, S2, S11, S12, S13, S14, S19	657
30S Ribosomes	*E. coli*	periodate–sodium borohydride	Lys?	S1 and S21 bound to 3′ end of 16S RNA	658
70S Ribosomes	*E. coli*	[^{3}H]*N*-(ethyl-2-diazomalonyl)-puromycin (u.v.)		mainly L23 labelled (aminoacyl-tRNA acceptor site)	659
70S Ribosomes	*E. coli*	*S*-(*p*-azidophenacyl)valyl-tRNA (u.v.)		bound to both 50S and 30S proteins	660
70S Ribosomes	*Bacillus stearothermophilus*	[^{3}H]-1-(4-azidophenyl)-2-(5-guanyl)-pyrophosphate (u.v.)		BL2, BL10, BL22	661
70S Ribosomes	*E. coli*	5-(*N*-bromoacetyl-*p*-aminophenyl)-AUG		S4 and S18 labelled; decoding site for fMet-tRNA$_f^{Met}$	662
70S Ribosomes	*E. coli*	*p*-nitrophenoxycarbonyl tRNA^{Phe}		labels at both donor and acceptor sites	663
50S, 30S Ribosomes	*E. coli*	photo-irradiation		cross-links of rRNA and protein in two dose-dependent steps	664 665
70S Ribosomes	*E. coli*	photo-irradiation, [^{3}H]-poly-U		covalent attachment to S1	666
70S Ribosomes	*E. coli*	photo-irradiation, poly(4-thio-U)		covalent attachment to S1	667
16S rRNA S4 complex	*E. coli*	photo-irradiation		covalent linkage-tryptic digestion of S4 shows two or three basic clusters which have formed covalent bonds	668
30S, 50S Ribosomes	*E. coli*	[^{131}I]iodide, lactoperoxidase	Tyr	[^{3}H]-ribosomes; ^{131}I : ^{3}H ratio shows change on association of 50S with 30S	669

50S Ribosomes	*E. coli*	[^{131}I]iodide, lactoperoxidase	Tyr	effect of removing L7, L12 on iodination pattern	670
40S, 60S Ribosomes	rat liver	[^{125}I]iodide, lactoperoxidase	Tyr	most proteins accessible	671
50S A protein	*Bacillus stearothermophilus*	[^{3}H, ^{14}C]acetic anhydride	*N*-terminal Met and Lys-3	competitive labelling; p*K* of Met-1 and Lys-3	672
30S Ribosomes		[^{14}C]-NEM	Cys	labelling pattern of 30S different in 70S and in 70S, aminoacyl tRNA complex	673
80S Ribosomes	human tonsils	[^{14}C]-NEM	Cys	characterization of reactive subunits	674
70S Ribosomes	*E. coli*	[^{3}H]-3-ethoxy-1,1-dihydroxybutan-2-one	Arg, (single strand G)	changes in modification by prebinding with streptomycin	675

[655] A. Expert-Bezancon, D. Barritault, J. C. S. Clegg, M. Milet, Y. Khouvine, and D. H. Hayes, *F.E.B.S. Letters*, 1975, **59**, 64.
[656] J. Vanduin, C. G. Kurland, J. Dondon, and M. Grynberg-Manago, *F.E.B.S. Letters*, 1975, **59**, 287.
[657] A. Bollen, R. L. Heimark, A. Cozzone, R. R. Traut, and J. W. B. Hershey, *J. Biol. Chem.*, 1975, **250**, 4310.
[658] A. P. Czernilofsky, C. G. Kurland, and G. Stoffler, *F.E.B.S. Letters*, 1975, **58**, 281.
[659] B. S. Cooperman, E. N. Jaynes, C. J. Brunswick, and M. A. Luddy, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2974.
[660] I. Schwartz, E. Gordon, and J. Ofengand, *Biochemistry*, 1975, **14**, 2907.
[661] J. A. Maassen and W. Moller, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 1175.
[662] O. Pongs and E. Lanka, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1505.
[663] K. Bauer, A. P. Czernilofsky, and E. Kuechler, *Biochim. Biophys. Acta*, 1975, **395**, 146.
[664] L. Gorelic, *Biochim. Biophys. Acta*, 1975, **390**, 209.
[665] L. Gorelic, *Biochemistry*, 1975, **14**, 4627.
[666] I. Fiser, P. Margaritella, and E. Kuechler, *F.E.B.S. Letters*, 1975, **52**, 281.
[667] I. Fisher, K. H. Scheit, G. Stoffler, and E. Kuechler, *F.E.B.S. Letters*, 1975, **56**, 226.
[668] B. Ehresmann, J. Reinbolt, and J. P. Ebell, *F.E.B.S. Letters*, 1975, **58**, 106.
[669] C. J. Michalski and B. H. Sells, *European J. Biochem.*, 1975, **52**, 385.
[670] C. J. Michalski and B. H. Sells, *F.E.B.S. Letters*, 1975, **58**, 85.
[671] D. P. Leader, *Biochem. J.*, 1075, **152**, 373.
[672] L. P. Visentin and H. Kaplan, *Biochemistry*, 1975, **14**, 463.
[673] I. Ginsburg and A. Zamir, *J. Mol. Biol.*, 1975, **93**, 465.
[674] U. Ucer, M. Munkemeyer, and E. Bermek, *Biochim. Biophys. Acta*, 1975, **402**, 206.
[675] N. Delihas, E. Topol, and I. Larrinua, *F.E.B.S. Letters*, 1975, **53**, 170.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
30S Ribosomes	*E. coli*	*N*-(3-pyrene)-maleimide		quenches intrinsic fluorescence	676
30S Ribosomes	*E. coli*	7-chloro-4-nitrobenzo-2-oxa-1,3-diazole	Cys, Lys	to label 30S proteins and follow assembly process; critical role of S10 in positioning some others	677
30S, 50S Ribosomes	*E. coli*	7-chloro-4-nitrobenzo-2-oxa-1,3-diazole	Cys, Lys	six per 30S; < 1 per 50S	678
30S Ribosomes	*E. coli*	fluorescein isothiocyanate, and *N*-iodoacetyl-*N'*-(5-sulphonic-1-naphthyl)ethylene diamine	probably Lys	singlet–singlet energy transfer between labelled pairs to estimate their distance apart	679
50S Ribosomes	*E. coli*	photochemical oxidation		loss of peptidyl transferase activity	680
30S Ribosomes	*E. coli*	photochemical oxidation	His?	S2, S3 restore tRNA binding and poly-U binding	681
Ribulose 1,5-bis-phosphate carboxylase	*Chromatium*	PCMB, DTNB, iodoacetic	Cys	64 thiols per mol	682
Ribulose 1,5-bis-phosphate carboxylase	spinach	3-bromo-1,4-dihydroxybutan-2-one 1,4-bisphosphate	Lys	essential Lys	683
RNAse		formaldehyde–sodium borohydride	*N*-terminal and Lys	^{1}H n.m.r.; pK of *N*-terminal and Lys-41 determined	684
RNAse		TNM	Tyr	changes in pK of nitro-tyrosine in refolding of nitro-RNAse	685
RNAse	ox pancreas	2(3)-*O*-bromoacetyluridine	His	*N*-3-carboxymethyl-His-12 alkylation inhibited competitively by uridine	686
RNAse	ox	methyl iodide–guanidine hydrochloride	Met	methylation at Met-30 or -13 prevents refolding	687
RNAse	ox	rabbit liver extracts/UDP-*N*-acetylglucosamine	Asn-34	*N*-acetylglucosamine-Asn linked	688
RNAse	ox semen	DTT–iodoacetic acid	S—S	preparation of catalytically active monomers	689

RNA polymerase	bacteriophage T7	DTNB, [^{14}C]iodoacetamide	Cys	one mol per mol enzyme inactivates; modification of four surface Tyr	690
		TNM	Tyr	Tyr prevents binding to T7 DNA	
RNA polymerase	*E. coli*	pyridoxal 5-phosphate–sodium borohydride	Lys	inactivated; one low pK Lys found	691
RNA polymerase	*E. coli*	3-(2-bromo-acetamidoethyl)-thiorifamycin		core, β-labelled; holo, β, σ-labelled; holo + *p*-hydroxymercuri-benzenesulphonic acid, mainly β'-labelled	692
RNA polymerase I + II RNA polymerase I	rat liver yeast	pyridoxal 5′-phosphate–sodium borohydride	Lys	inactivated, *N*-pyridoxyl-lysine found in acidic hydrolysis	693
Succinate thiokinase	*E. coli*	dimethyl suberimidate	Lys	cross-links: $\alpha\beta$, $\alpha_2\,\beta$, $\alpha_2\,\beta_2$	695
		NN-phenylenedimaleimide		cross-links: $\alpha\beta$	

[676] N. Schechter, D. Elson, and P. Spitnik-Elson, *F.E.B.S. Letters*, 1975, **57**, 149.
[677] K. H. Huang and C. R. Cantor, *J. Mol. Biol.*, 1975, **97**, 423.
[678] M. Perrin and F. Pochon, *European J. Biochem.*, 1975, **57**, 319.
[679] K. Huang, R. H. Fairclough, and C. R. Cantor, *J. Mol. Biol.*, 1975, **97**, 443.
[680] K. K. Wan, N. D. Zahid, and R. M. Baxter, *European J. Biochem.*, 1975, **58**, 397.
[681] G. Thomas, R. Sweeney, C. Chang, and H. F. Noller, *J. Mol. Biol.*, 1975, **95**, 91.
[682] T. Takabe and T. Akazawa, *Arch. Biochem. Biophys.*, 1975, **169**, 686.
[683] I. L. Norton, M. H. Welch, and F. C. Hartman, *J. Biol. Chem.*, 1975, **250**, 8062.
[684] L. R. Brown and J. M. Bradbury, *European J. Biochem.*, 1975, **54**, 219.
[685] J. R. Garel and R. L. Baldwin, *J. Mol. Biol.*, 1975, **94**, 621.
[686] M. Pincus, L. L. Thi, and R. P. Carty, *Biochemistry*, 1975, **14**, 3653.
[687] G. R. Stark and T. P. Link, *Biochemistry*, 1975, **14**, 3476.
[688] Z. Khalkhali and R. D. Marshall, *Biochem. J.*, 1975, **146**, 229.
[689] G. D'Alessio, M. C. Malorni, and A. Parente, *Biochemistry*, 1975, **14**, 1116.
[690] J. L. Oakley, J. A. Pascale, and J. E. Coleman, *Biochemistry*, 1975, **14**, 4684.
[691] P. Bull, J. Zaldivar, A. Venegas, J. Martial, and P. Valenzuela, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 1152.
[692] W. Stender, A. A. Stutz, and K. H. Scheit, *European J. Biochem.*, 1975, **56**, 129.
[693] J. Martial, J. Zaldivar, P. Bull, A. Venegas, and P. Valenzuela, *Biochemistry*, 1975, **14**, 4907.
[694] P. D. J. Weitzman, *Biochem. J.*, 1975, **149**, 281.
[695] J. A. Teherani and J. S. Nishimura, *J. Biol. Chem.*, 1975, **250**, 3883.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Succinate dehydrogenase	ox heart mitochondria	NEM	Cys	essential thiols	696
Taurocyamine kinase	*Arenicola marina*	[^{14}C]-NEM	Cys	essential Cys peptide sequenced	697
Tetanus toxin	*Clostridium tetani*	formaldehyde–sodium borohydride	Lys	c.d. study, toxicity affected	698
Thermolysin		*N*-hydroxysuccinimide esters of amnio-acids	Tyr?		699
Thrombin		cyclohexanedione	Arg	heparin sensitivity affected	700
(Pro)thrombin	ox, rat	[^{3}H]diborane		identification of G1a as 5,5-dihydroxyleucine	701
Thymidylate synthetase	*Lactobacillus casei*	[^{14}C]iodoacetamide	Cys	inactivates enzyme; 0.5 mol Cys per mol subunit	702
Thyrotropin	ox	[^{125}I]iodide chloramine T	Tyr	active	703
Transaldolase	*Candida utilis*	CNBr, maleic anhydride		active centre, subunit structure	704
Transcobalamine	human plasma	*N*-succinimidyl-3-(4-hydroxy-phenyl) propionate		plasma clearance study	705
Transglutaminase	guinea-pig	alkyl isocyanates	Cys	one per mol	706
Tropomyosin	rabbit muscle	*N*-(1-oxyl-2,2,6,6-tetramethyl-piperidinyl)maleimide	Cys	spin-label denaturation studied	707
Tropomyosin	rabbit muscle	DTNB	Cys	cross-links formed; two chains in register	708
Troponin	rabbit muscle	tartryl diazide, tartryl di(glycyl-hydrazide)	Lys	cross-links: T-I, T-C, I-C, T-I-C cleavable links	709
Troponin	rabbit muscle	dimethyl imido-esters	Lys	T and I close together	710
Troponin C	rabbit muscle	TNM	Tyr-10, Tyr-108	c.d. study. Tyr not involved in calcium interaction	711
β-Trypsin		methyl acetamidate	Lys	active and stable at pH 8.0	712
Trypsin inhibitor	ox pancreas	(mercaptoethanol, glutathione; DTT) followed by [^{14}C]iodoacetic acid	S—S	intermediates in disulphide bond formation during refolding identified	713, 714

Trypsin inhibitor	ox pancreas	tetranitromethane	Tyr	n.m.r.; Tyr assignments	715
Trypsin inhibitor chymotrypsin complex	ox	maleic anhydride	Lys	Lys-15 (inhibitor) shielded in complex	716
Tryptophanase	*E. coli*	pyridoxal 5′-phosphate–Sepharose, sodium borohydride	Lys	active solid-state enzyme	717
Tryptophan synthase	*E. coli*	α-cyanoglycine		inactivates; substrate analogue	718
Tryptophan synthetase	ox pancreas	[^{14}C]-NEM	Cys	loss of activity; dissociates into subunits	719
Tryptophan tRNA synthetase	ox pancreas	[^{14}C]-NEM	Cys	peptide map of Cys peptides; reactivity of each	720

[696] W. C. Kenney, *J. Biol. Chem.*, 1975, **250**, 3089.
[697] A. Brevet, Y. Zeitoun, and L. A. Pradel, *Biochim. Biophys. Acta*, 1975, **393**, 1.
[698] J. P. Robinson, J. B. Picklesimer, and D. Puett, *J. Biol. Chem.*, 1975, **250**, 7435.
[699] S. Blumberg and B. L. Vallee, *Biochemistry*, 1975, **14**, 2410.
[700] R. Machovich, *Biochim. Biophys. Acta*, 1975, **412**, 13.
[701] T. H. Zytkovicz and G. L. Nelsestuen, *J. Biol. Chem.*, 1975, **250**, 2968.
[702] R. P. Leary, N. Beaudette, and R. L. Kisliuk, *J. Biol. Chem.*, 1975, **250**, 4864.
[703] R. L. Tate, H. I. Schwartz, J. M. Holmes, L. D. Kohn, and R. J. Winand, *J. Biol. Chem.*, 1975, **250**, 6509.
[704] H. Schutt and K. Brand, *Arch. Biochem. Biophys.*, 1975, **169**, 287.
[705] R. L. Burger, R. J. Schneider, C. S. Mehlman, and R. H. Allen, *J. Biol. Chem.*, 1975, **250**, 7707.
[706] M. Gross, N. K. Whetzel, and J. E. Folk, *J. Biol. Chem.*, 1975, **250**, 7693.
[707] Y. H. Chao and A. Holtzer, *Biochemistry*, 1975, **14**, 2164.
[708] S. S. Lehrer, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3377.
[709] S. E. Hitchcock and L. C. Lutter, *F.E.B.S. Letters*, 1975, **57**, 172.
[710] S. E. Hitchcock, *Biochemistry*, 1975, **14**, 5162.
[711] W. D. McCubbin and C. M. Kay, *F.E.B.S. Letters*, 1975, **55**, 183.
[712] A. Nureddin and T. Inagami, *Biochem. J.*, 1975, **147**, 71.
[713] T. E. Creighton, *J. Mol. Biol.*, 1975, **95**, 167.
[714] T. E. Creighton, *J. Mol. Biol.*, 1975, **96**, 767.
[715] G. H. Snyder, R. Rowan, S. Karplus, and B. D. Sykes, *Biochemistry*, 1975, **14**, 3765.
[716] J. Chauvet and R. Archer, *European J. Biochem.*, 1975, **54**, 31.
[717] S. Fukui, S. Ikeda, M. Fujimura, H. Yamada, and H. Kumagai, *European J. Biochem.*, 1975, **51**, 155.
[718] E. W. Miles, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 248.
[719] F. Iborra, B. Labouesse, and J. Labouesse, *J. Biol. Chem.*, 1975, **250**, 6659.
[720] F. Iborra, C. Gros, B. Labouesse, and J. Labouesse, *J. Biol. Chem.*, 1975, **250**, 6666.

Table 2 (*cont.*)

Protein	*Source*	*Reagent*	*Residue*	*Comments*	*Ref.*
Tubulin	rat brain	fluorescein isothiocyanate, rhodamine isothiocyanate	Lys	follow polymerization process, and tubulin membrane interaction	721
Tyrosine phenol lyase	*E. intermedia*	diethylpyrocarbonate	His	inactivates; two His per mol	722
Uridine diphosphoglucose dehydrogenase		DTNB	Cys	reaction mechanism; essential thiol	723
Uridine nucleosidase	yeast	DTNB	Cys	one Cys per mol	724
Valyl tRNA synthetase	yeast	u.v. irradiation; $tRNA^{Ile}$, (*E. coli*)		cross-links to tRNA; regions of tRNA identified	502
β-D-Xylosidase	*Bacillus pumilis*	glutaraldehyde	Lys	dimer, tetramer	725

[721] J. S. Becker, J. M. Oliver, and R. D. Berlin, *Nature*, 1975, **254**, 152.
[722] H. Kumagai, T. Utagawa, and H. Yamada, *J. Biol. Chem.*, 1975, **250**, 1661.
[723] W. P. Ridley, J. P. Houchins, and S. Kirkwood, *J. Biol. Chem.*, 1975, **250**, 8761.
[724] G. Magni, E. Fioretti, P. L. Ipata, and P. Natalini, *J. Biol. Chem.*, 1975, **250**, 9.
[725] M. Claeyssens, E. Saman, H. Hilderson, and C. K. De Bruyne, *Biochim. Biophys. Acta*, 1975, **405**, 475.

3 Chemical Modification

A comprehensive list of proteins modified appears in Table 2 (p. 55). The column headed *Residue* describes those residues which the authors specifically assert are modified: in the absence of such comment from the authors, the residues listed are those which the Reporter believes are *probably* modified.

The comments in the *Comments* column are necessarily terse, attempting merely to give either the main result of the modification or the type of experiments conducted. Membrane proteins of no proved function appear under 'membrane proteins' and ribosomes and all components thereof appear under 'ribosomes'.

New Reagents and Techniques.—Several new reagents, or new uses of known reagents have been reported: those more extensively investigated appear in Table 3. Of these, the resurrection of cyclohexane-1,2-dione for reversible modification of arginine could prove important for protein sequence work by restricting the action of trypsin to lysine residues. The product is N^7,N^8-(1,2-dihydroxycyclohex-1,2-ylene)-L-arginine. Other arginine reagents require harsh conditions, or have significant side reactions with α- or ε-amino group, resulting in multiple products, or are irreversible or all too easily reversible.[726] Cyclohexane-1,2-dione is alleged to suffer none of these drawbacks, and has the additional merit of allowing modified peptides to be identified either by a specific colour reaction, or by a simple electrophoretic technique which utilizes the observation that the complex of dihydroxycyclohexylene-arginine with borate differs in electrophoretic mobility from the borate-free derivative. Diagonal electrophoresis is run at pH 6.5 or 4.7 for the first dimension; and at the same pH in the presence of borate for the second dimension. Only those peptides containing the arginine derivative move off the diagonal.[727]

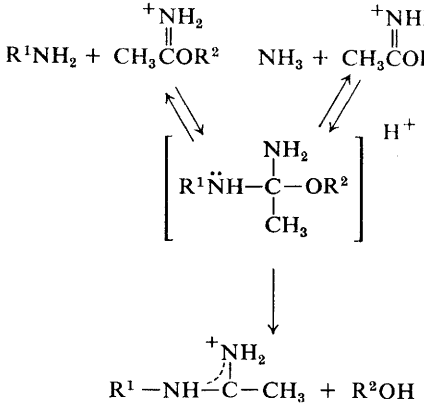
The selective oxidation of methionine to methionine sulphoxide by chloramine T or *N*-chlorosuccinimide could also prove useful in protein sequencing by allowing the generation of overlapping cyanogen bromide (CNBr) fragments. The mild, near-neutral conditions contrast with the acidic conditions usual for selective labelling at methionine and should aid the study of the role of methionine in specific proteins.[731] The detection and quantitation of methionine sulphoxide was achieved by the use of CNBr followed by acidic hydrolysis in the presence of dithiothreitol (DTT). The CNBr converts methionine into homoserine lactone, leaving the methionine sulphoxide unchanged. Subsequent acidic hydrolysis in the presence of DTT converts the methionine sulphoxide into methionine, leaving the homoserine lactone unchanged. Hence, on amino-acid analysis, the ratio of homoserine species to methionine reflects the original ratio of methionine to methionine sulphoxide.

Reinvestigation of Known Reagents and Techniques.—The specificity, reaction mechanisms, and drawbacks of some common methods have recently come under scrutiny. Halogenoacetates are known to react with methionine, cysteine,

[726] L. Patthy and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 557.
[727] L. Patthy and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 565.
[728] M. Z. Atassi and W. Zablocki, *Biochim. Biophys. Acta*, 1975, **386**, 233.
[729] R. B. Homer, S. R. Allsopp, and J. E. Arrieta, *F.E.B.S. Letters*, 1975, **59**, 173.
[730] D. J. Smith, E. T. Maggio, and G. L. Kenyon, *Biochemistry*, 1975, **14**, 766.
[731] Y. Shechter, Y. Burstein, and A. Patchornik, *Biochemistry*, 1975, **14**, 4497.

lysine, and histidine. Each of these derivatives can be recognized by amino-acid analysis after acidic hydrolysis of the protein. N.m.r. studies now show that [^{13}C]bromoacetic acid can give significant amounts of the glycollate esters of aspartic acid and glutamic acid. These are destroyed on acidic hydrolysis and thus cannot be detected by amino-acid analysis.[408]

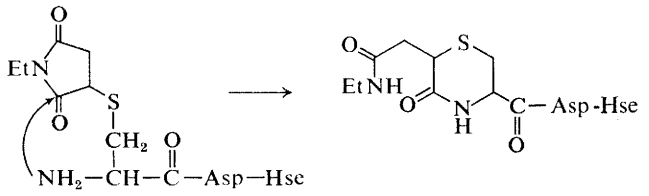
Imido-esters were believed to react with primary amine groups, and hence ε-amino-groups of lysine, to give *N*-alkylamidine derivatives. This seems to be true at pH 10.0 where amidine products are formed directly, but at pH 8.0, the initial products of reaction have been shown by ^{1}H n.m.r. studies to be *N*-alkylimidates. These undergo slower reactions to regenerate the amine or form the *N*-alkylamidine (Scheme 3).[732]



Scheme 3

Should it be necessary to use imido-esters at pH 8.0 only repeated addition of imido-esters can give quantitative conversion into the amidine derivative.[312] One serious implication of these results is that monofunctional imido-esters could cross-link adjacent lysine residues as *N,N'*-disubstituted amidines. That this possibility is more than academic is illustrated by the unexplained observation [710] that methyl acetimidate apparently cross-links components of troponin.

A possibly important limitation in the sequencing of *N*-ethylmaleimide (NEM) labelled peptides has emerged. The tritiated peptide was isolated from a tryptic digest of elongation factor Tu labelled with [^{3}H]NEM at the cysteine essential for GTP binding. It had a blocked *N*-terminus, and this was established to be a modified cysteine residue rather than the usual culprit, pyroglutamic acid. A



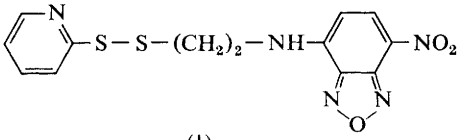
Scheme 4

[732] D. T. Browne and S. B. H. Kent, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 126.

mechanism for the blocking reaction was proposed (Scheme 4). This reaction would also presumably operate during Edman sequencing when the NEM labelled cysteine became *N*-terminal in the degradation. Thus a drop in the cleavage yield could be expected at this point.[420]

It had been proposed (Robyt *et al.*, *Arch. Biochem. Biophys.*, 1971, **147**, 262) that DTNB attacked and cleaved disulphide bonds. The attacking species was alleged to be mercaptonitrobenzoate formed initially by reactions of DTNB with thiol groups. This now seems not to be the case: if the thiol is prepared by electrolytic reduction of the disulphide, it does not attack disulphide-containing proteins.[694]

Reporter Groups.—A new reporter group delivery system has been evaluated for the 7-nitrobenzo-2-oxa-1,3-diazole (NBD) fluorophore.[733] The NBD group was combined with the 2-aminoethyl-2′-pyridyl disulphide delivery system as in (1).



(1)

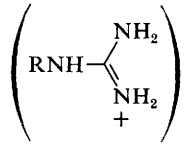
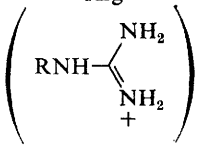
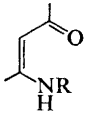
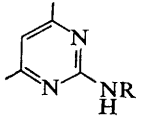
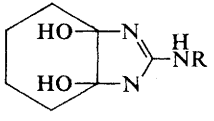
The advantages of this method over use of NBD-chloride include an increased specificity toward thiol, a greater reactivity, a more highly fluorescent product, and easier regeneration of native proteins from labelled protein.

Singlet–singlet energy-transfer measurements between donor (fluorescein isothiocyanate) and acceptor [*N*-iodoacetyl-*N*′-(1-naphthyl-5-sulphonic acid)-ethylene diamine] fluorescent dyes on different proteins have been used to estimate their separation. Active 30S ribosomes were prepared containing pairs of proteins carrying different dyes, one a donor, the other an acceptor. Two extreme models were used in the estimation: the centre-to-centre model, and the point-to-point model. The former assumed that for each protein the dyes were located at random on the surface of a sphere and the results derived represent the distance between centres of proteins. The latter assumed that for each protein, the dyes had reacted at a unique point on the protein surface and the results derived represent the point distance between labelling sites on proteins. There was agreement between the results obtained from each of two models, and qualitative agreement with cross-linker studies. The advantage of this technique over cross-linker studies is that distant protein pairs as well as proximate ones can be identified. A model for the arrangement of ribosomal proteins was constructed (Figure 1).[679] Such models, in which proteins are represented as spheres, are misleading, however, since the conformations of some proteins within the ribosome are known to be highly extended. This technique was able to assess this possibility from the variations in energy transfer efficiency as a function of the degree of labelling. Thus, S20 protein appears to be elongated.

Qualitatively, the technique has been applied to following the polymerization of tubulin (by labelling one population of subunits with donor, and another

[733] T. Stuchbury, M. Shipton, R. Norris, J. P. G. Malthouse, K. Brocklehurst, J. A. L. Herbert, and H. Suschitzky, *Biochem. J.*, 1975, **151**, 417.

Table 3

Reagent	pentane-2,4-dione		cyclohexane-1,2-dione
Group(s) modified	Lys (RNH_2)	Arg 	Arg 
Products formed	 unstable pH 7.0	 stable	 stable in acid or in borate buffers
Detecting the extent of modification	λ_{max}. 310 nm	(i) λ_{max}. 310 nm (ii) acid hydrolysis, amino-acid analysis showed increase in ornithine	acid hydrolysis in presence of mercaptoacetic acid, amino-acid analysis gives product eluting after aromatic amino-acids
Reaction conditions	0.2M pentane-2,4-dione, 0.5M potassium bicarbonate, pH 7.0 or pH 9.0, 21 °C		0.15M cyclohexane-1,2-dione, 0.25M sodium borate, pH 9.0, 35 °C
Time for reaction	7 h	50—100 h	2—20 h
Reversal of modification?	hydroxylamine	—	hydroxylamine
Tested out on	lysozyme, n-butylamine, guanidine		lysozyme, RNase, arginine
Comments	(i) selective modification of Lys at pH 7.0, short reaction times: (ii) selective modification of Arg at pH 9.0, long reaction times, followed by hydroxylamine		sequence applications: (i) restricts tryptic cleavage to Lys; (ii) colour reaction of modified peptide; (iii) diagonal electrophoresis for modified peptides
Reference	543		726, 727

Reagent	2,3-dioxo-5-indoline-sulphonic acid	potassium nitro-disulphonate (Fremy's salt)	alkyl alkanethiol sulphonates ($R^1SSO_2R^2$) alkoxycarbonylalkyl disulphides ($R^2OCOSSR^1$)	chloramine T	*N*-chloro-succinimide
Group(s) modified	Trp	Tyr	Cys (XSH)	Met Cys	Met Trp
Products formed	unknown	unknown, probably many	XSSR	Met sulphoxide	
Detecting the extent of modification	(i) 367 nm (yellow colour) (ii) amino-acid analysis after hydrolysis with *p*-toluene sulphonic acid shows Trp decrease	(i) changes in fluorescence and probably absorption spectrum (ii) acid hydrolysis–amino-acid analysis showed loss of Tyr	use of radio-active reagent	CNBr followed by hydrolysis in 6M-HCl, DTT and amino-acid analysis	
Reaction conditions	0.1M acetic acid	10mM Fremy's salt. 0.1M bicarbonate pH 8.5, 25 °C	0.2mM reagent, 0.01M glycine, sodium hydroxide, pH 7.8, 4 °C	10mM oxidant, 0.1M Tris-HCl, pH 8.5, 20 °C	
Time for reaction	7 h	24 h	30 min	20 min	
Reversal of modification	—	—	DTT or 2-mercapto-ethanol	—	
Tested out on	albumin, amino-acids, synthetic peptide	L-asparaginase, RNase A	papain, glyceraldehyde, phosphate dehydro-genase, creatine kinase	lactalbumin, chymotrypsin, RNase, lysozyme	
Comments	highly soluble in water, high (alleged) specificity	different pattern in Tyr products and accessibilities from those of tyrosinase	mild conditions, mixed disulphides are not charged (as with DTNB)	(i) surface Met differentiated from buried (ii) location of surface Met (iii) Met overlapping fragments for sequencer analysis	
Reference	728	729	730	731	

population with acceptor) and to demonstrating considerable interaction between tubulin and cell membrane.[721]

E.s.r. probes have been used to extend the range of approaches to the elucidation of the topography and conformation of proteins in solution. The location of the most reactive thiol group in bovine serum albumin was explored by a molecular dipstick, a series of spin labels consisting of the nitroxide free radical and a maleimide or iodoacetamide attaching group separated by various chain lengths. From the change in e.s.r. spectra as a function of chain length, the thiol group was found to be located in a crevice 9.5 Å deep.[301]

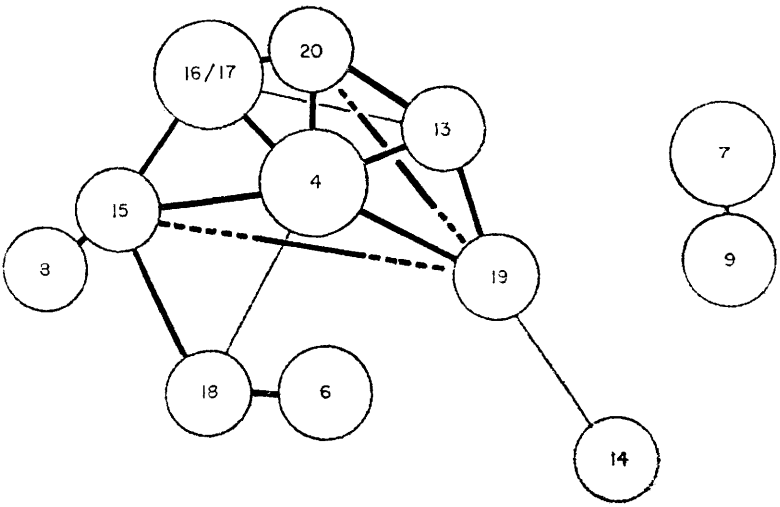


Figure 1 *Schematic summary of all the average distances between ribosomal proteins. Protein radii and distances are drawn roughly to scale. Thick solid lines indicate distances, thin lines are lower limits. To display the data in two dimensions, distances* S15—S19 *and* S19—S20 *had to be increased artificially (dashed lines)*
(Reproduced by permission from *J. Mol. Biol.*, 1975, **97**, 443)

Dipolar interactions between paramagnetic centres can be quantitated by e.s.r., and it had been suggested that this could be used to calculate the distance between the paramagnetic centres. There are, however, experimental and theoretical difficulties in doing this. An attempt has been made with glyceraldehyde phosphate dehydrogenase which may be labelled at two sites with 4-(2-iodoacetamido)-2,2,6,6-tetramethylpiperidinio-oxyl to give one highly immobile label and one which can tumble. The addition of the paramagnetic gadolinium ion reduces the intensity of both spin labels, indicating that the metal ion site is close to both types of label. Quenching is inversely related to separation of the paramagnetic centres on the enzyme. The distance of the immobile label from the gadolinium ion was therefore calculated from the degree of quenching. However, the calculation could not be applied to the tumbling label since a main assumption in this calculation is that dipoles are fixed rigidly with respect to each other. This result will be tested by the appearance in the near future of the crystal structure of human glyceraldehyde phosphate dehydrogenase.[464]

A new technique, saturation transfer e.p.r., which is sensitive to rotation in the range 10^{-7}—10^{-3} s (conventional e.s.r. is limited to 10^{-7} s or shorter) has been applied to myosin. This shows, after spin labelling of the thiol groups, a considerable flexibility of the subfragment I segment relative to the rest of the myosin molecule.[598]

A possible misinterpretation of composite e.s.r. spectra has surfaced, in the claim that a weakly immobilized spectrum may be due to a probe free in solution, rather than on a mobile binding site.[734]

Chemical Cross-linking.—Bifunctional reagents have been used to probe the structure of homo- and hetero-oligomers. The symmetry of quaternary structure of polypeptide chains in homo-oligomers has been investigated by cross-linking with several di-imidates and quantitating the species formed (monomer, dimer, trimer, tetramer) on SDS gels.[735] Comparison with a theoretical species quantitation for the cross-linking of tetrameric polypeptide chains arranged as a tetrahedron, as a square, or as two pairs may allow the type of symmetry to be deduced. The major premise is that any preferences for particular species must reflect the symmetry of the quaternary structure. This method assumes that there is no association or dissociation of tetramers during the cross-linking reaction, and that there is no change in symmetry induced by the cross-linking reaction.

The neighbourhood patterns of hetero-oligomers such as histones, ribosomes, and troponin have been deduced by identifying cross-linked products. Means of identification have included molecular weight, ^{35}S-composition,[654] and ^{125}I-iodinated peptide maps.[496] However, the most promising approach is that of the cleavable cross-linker, with its advantage of the 2-D gel diagonal. Cross-linked species running in the first dimension are broken at the cross-link before running in the second. Those proteins cross-linked together will fall off the diagonal on the same straight vertical line.

The product of imido-ester and amino-groups is cleavable by ammonolysis and yields of 70% are alleged.[654] Other cross-linkers which cleave not at the functional group, but within the spacer arm have been used. Two such species have evolved: the tartryl diazide [653, 709] which is cleaved by mild periodate, and the mercaptoalkylimidates. The latter react under reducing conditions with the ε-amino-groups of lysines in the protein. Mild oxidation promotes the formation of intermolecular S—S bonds only between neighbouring thiol groups of the reagent. At a later stage, cross-links can be broken under reducing conditions.[503, 652] A potential danger of using disulphide cross-linkers is that traces of free thiol might catalyse disulphide interchange. In histone oligomers, it has been shown that if interchange occurs, it does not generate new histone–histone associations.[503]

The results of cross-linker studies have had immediate impact in determination of histone arrangements in chromatin. Not only has a repeating histone octamer $(H4)_2(H3)_2(H2A)_2(H2B)_2$ been identified with the use of dimethyl suberimidate and dithiobis(succimidylpropionate), but the proximity relationships within the octamer have been partly determined [495, 503] with methyl 3-mercaptopropionimidate (Figure 2).

[734] G. Benga and S. J. Strach, *Biochim. Biophys. Acta*, 1975, **400**, 69.
[735] F. Hucho, H. Mullner, and H. Sund, *European J. Biochem.*, 1975, **59**, 79,

A novel method of cross-linking has been reported. Transglutaminase enzymes catalyse the formation of ε-(γ-glutamyl)lysyl cross-links between glutamine and lysine side-chains of proteins. By this means some of the membrane proteins of mouse erythrocyte membranes have been cross-linked. Alternatively transglutaminase may be used to attach a variety of primary amines to glutamine side-chains. The advantages of this technique over chemical methods include mild reaction conditions, high specificity, and for membrane studies, a distinct membrane sidedness.[344]

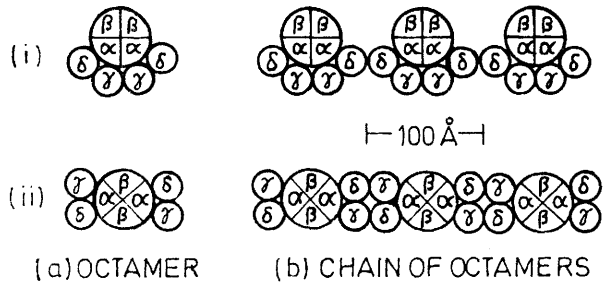
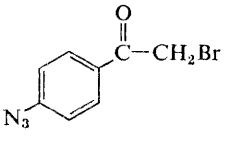
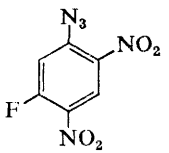


Figure 2 *Possible arrangements of histones* (a) *within the octamer and* (b) *in chains of octamers*; α, β, γ, *and* δ *represent* H4, H3, H2A, *and* H2B, *respectively* (Reproduced by permission from *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2626)

Photochemical Cross-linking.—Photolabile azides have been used as affinity reagents for the labelling of active sites, and successful uses have included labelling of antibody binding sites (see last year's Report). However, photo-affinity reagents have also been found to give random labelling of proteins, even when bound tightly, though non-covalently to the active site. This may be due to the long lifetime of arylnitrenes (up to 10^{-4} s), which can therefore diffuse out of the binding site and label anywhere on the protein surface. These observations have prompted a somewhat different use of photolabile reagents – as bifunctional cross-linking reagents with a conventional warhead at one end, and the photolabile group at the other. For example *p*-azidophenacyl bromide (2)[460] and



(2)



(3)

2,4-dinitro-5-fluorophenyl azide (3)[736] have been prepared. These are anchored to the protein through the conventional warhead, and then irradiated. Any portion of the protein chain labelled after photolysis must be close to the first site, and the residues involved will be linked to the first site through the spacer arm of the reagent. The advantage of these bifunctional reagents over con-

[736] D. F. Wilson, Y. Miyata, M. Erecinska, and J. M. Vanderkooi, *Arch. Biochem. Biophys.*, 1975, **711**, 104.

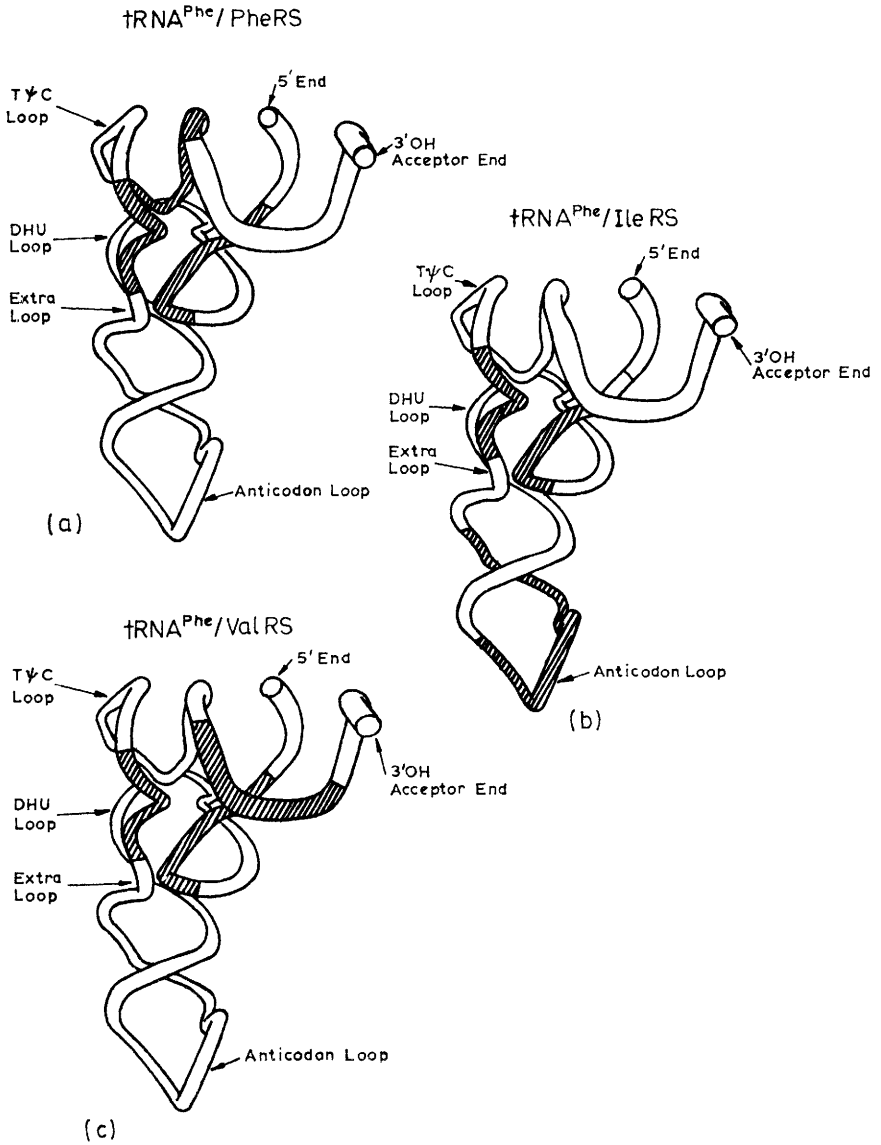


Figure 3 (a) *Three-dimensional model of tRNA*^{Phe} *with regions cross-linked to phenylalanyl-tRNA synthetase indicated by shading.* (b) *Three-dimensional model of tRNA*^{Phe} *with regions cross-linked to isoleucyl-tRNA synthetase indicated by shading.* (c) *Three-dimensional model of tRNA*^{Phe} *with regions cross-linked to valyl-tRNA synthetase indicated by shading*

(Reproduced by permission from *J. Biol. Chem.*, 1975, **250**, 4440)

ventional bifunctional reagents is that there is no requirement for a specific chemical group at the second locus of reaction. Nitrenes will even link to the peptide backbone. This technique could be used to map proximity relationships within a protein or to cross-link proteins which specifically interact (large molecules have slower exchange rates than the lifetime of the nitrene). Thus, cytochrome *c* was labelled[736] with (3) to give a 2,4-dinitro-5-phenylamine derivative. Irradiation of cytochrome *c* depleted mitochondrial membranes supplemented by an excess of photo-affinity labelled cytochrome *c* resulted in covalent binding of the derivative to cytochrome *c* oxidase in a 1 : 1 complex active in electron transport.

Photochemical cross-linking of protein and nucleic acid in ribosomal proteins/rRNA,[664, 665, 668] ribosomal protein/mRNA analogues,[666, 667] and aminoacyl-tRNA synthetases/tRNA,[501] without the intervention of specific cross-linking reagents, has been reported. Though the precise chemistry of the reaction remains unclear (see refs. 737 and 737*a* for references), it is assumed to start with the photoactivation of the nucleotide base, and may involve free radical intermediates. A reasonable *a priori* objection to the technique must be that if the average lifetimes of the free radicals are comparable to the rate of movement of the nucleic acid at the protein binding site, there is a chance that a cross-link unrepresentative of the usual binding contacts will occur. Results, however, of cross-linking $tRNA^{Phe}$ to its cognate synthetase, or to two non-cognate synthetases which nevertheless show a strong binding interaction, are that in each case three regions of the tRNA are cross-linked. Only two of these regions are in common with all the three synthetases. Hence differences in physiological tRNA/synthetase interactions are at least mirrored in differences in cross-linking pattern, indicating that in this case, the tRNA probably does not cross-link in a physiologically irrelevant mode (Figure 3).[501] The cross-linked regions both on proteins and on nucleic acid have been identified but usually indirectly. Thus a tryptic digest of the photocross-linked 16S RNA–S4 protein complex was compared on thin layer sheets with a tryptic digest of protein S4. Those peptides missing or staining weakly in the former digest were assumed to be bound to the 16S RNA. Comparison of these spots with the known sequence of S4 protein indicated a minimum of two or three clusters of basic regions of S4 that can form covalent bonds with 16S RNA (Figure 4).[668]

In a similarly indirect experiment, radioactively labelled tRNA was used to make the photocross-linked tRNA/aminoacyl tRNA synthetase complex which was then digested with T1 RNAse. The phenol-insoluble oligonucleotide fragments (and hence those *not* cross-linked to the proteins) were compared on a 2-D map with a digest of 'cold' native tRNA. The specific activity of fragments which bind to the synthetase was reduced, and so the binding regions of tRNA to the synthetase could be recognized. The synthetase spans the distance from anticodon to 3′-terminus, making extensive contact with the region in which the two helical arms of the tRNA structure come together.[501, 502]

Labelling Methods.—Four general labelling methods have been used to probe the topography of protein surfaces. They are 'kinetic', competitive, affinity, and

[737] M. D. Shetlar, H. N. Schott, H. G. Martinson, and E. T. Lin, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 88.

[737a] E. I. Budowsky, N. A. Simukova, M. F. Turchinsky, I. V. Boni, and Y. M. Skoblov, *Nucleic Acid Res.*, 1976, **3**, 261.

difference labelling. Intrinsic reactivity differences of side-chains are exploited in 'kinetic' and competitive labelling, whereas in affinity and difference labelling the specific binding of reagent or of protecting molecule, respectively, directs the course of the modification.



Figure 4 *Sequence of protein* S4. *The tryptic peptides which are cross-linked to the* 16S *rRNA in the complex are underlined*
(Reproduced by permission from *F.E.B.S. Letters*, 1975, **58**, 106)

'Kinetic' Labelling. An excess of reagent is used in this method, and the reactivity of each reacting group is assessed from the specific activity of label or group at a series of times. Peptide maps of digests are prepared after labelling to identify the labelled sequences. In tryptophan-tRNA synthetase, the eight cysteines fall into four groups on the basis of their reactivity towards NEM in different conditions. Surface, buried, and tryptophan binding-site cysteines were identified.[720] Similar methods, using iodoacetic acid, demonstrate the order of reactivity of cysteines in glutamate dehydrogenase.[452]

The method, as usually practised, has several disadvantages: (i) in the absence of a device for rapid mixing of reagent and protein, followed by rapid quenching

of the reaction after a carefully timed interval, the method cannot be applied to fast reactions; (ii) the excess of reagent can be expected to label several sites on the same protein molecule. However, the reactivity of one group on the protein may have been altered by the previous modification of another group. Reactivities of groups other than the first labelled on any protein molecule may therefore bear little relationship to reactivities on the native enzyme; (iii) it is often not obvious whether protein groups are reacting faster or slower than comparable groups free in solution. The inclusion of an internal standard, which also became labelled, would immediately answer this point.

Competitive Labelling. This technique avoids the above disadvantages bar the requirement for rapid mixing. A trace amount of reagent, radioactively labelled, is treated with the protein and internal standard such that complete trace labelling would give an average of only one modified group per protein. The groups on the protein react in nucleophilic competition with each other for the limited amount of reagent. Their degree of labelling will depend on their pK_a and accessibility of reagent. To complete the labelling an excess of cold [380] or radioactivity labelled [544, 672] reagent of a different isotope is used under denaturing conditions. After protease digestion, the extent of labelling of each group is determined experimentally from the specific activity or isotope ratio of the label found in peptides containing the group. The ratio of the specific activity of each group to that of the internal standard is collected at different pH values, enabling the pK_a of each competing group to be determined. This can be used to place the groups on the Brønsted plot (a straight line plot, relating reactivities to pK_a of simple compounds of the same chemical type). Groups lying on the line correspond to those in free solution, the ones above are super-reactive, and ones below are presumably shielded (Figure 5).

Competitive labelling with [^{14}C,^{3}H]acetic anhydride has been applied to *Bacillus stearothermophilus* ribosomal A protein, and it was shown that the amino-terminus has a pK_a of 7.5 and is super-reactive.[672] Applied to a chymotrypsin with [^{3}H]fluorodinitrobenzene (FDNB), it has shown that His-40 and His-57 are super-reactive, but that the binding of indole to the specificity site causes both histidines to become less reactive. This suggests that the charge-relay system is only set up when the substrate, or suitable ligand, is bound to the specificity site (Figure 6).[380]

However, the use of bulky reagents such as FDNB is open to the criticism that the reagent could bind in the vicinity of certain groups, so artificially altering their rate of reaction. Thus a group of normal reactivity might appear to be super-reactive. In addition, if the binding of the bulky reagent distorted the structure of the proteins, it might shift the pK_a of the group it was about to modify.

Affinity Labelling. This method has been used extensively: for example, bromoacetyl analogues of NAD show that two cysteines are present near the active site of alcohol dehydrogenase.[309] A new criterion for affinity labelling (for a list of other criteria see ref. 622), 'catalytic competence', has been prompted by the reaction of estradiol-17β dehydrogenase with bromoacetoxyoesterone.[423] The covalently bound steroid is oxidized and reduced by the modified enzyme, clearly direct evidence for binding of the label at the active centre of the enzyme. Equally

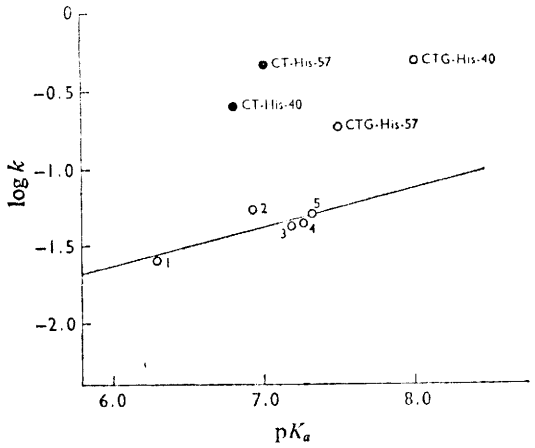


Figure 5 *Brønsted plot for the reaction of imidazole compounds with* 1-*fluoro*-2,4-*dinitrobenzene*: (1) 4-*hydroxymethylimidazole*; (2) *imidazole*; (3) α-N-*acetyl*-L-*histidine*; (4) L-β-*imidazolyl-lactic acid*; (5) *imidazolylacetic acid. The reactivities of the histidine residues of native chymotrypsin* (CT) *and chymotrypsinogen* (CTG) *are also indicated*
(Reproduced by permission from *Biochem. J.*, 1975, **147**, 411)

clearly, the method is applicable only to those enzymes in which the label reacts with a group not essential to catalysis and is covalently bound in the right orientation for enzymic catalysis. This may not be such a rare event, as illustrated by the diphosphonate GTP analogues, which appear to capture the GTP regulatory site of adenyl cyclase in a covalent bond, leaving the cyclase permanently switched on.[298]

Difference Labelling. One of the few examples of this technique has been the identification of the NAD binding-site lysine in alcohol dehydrogenase. This

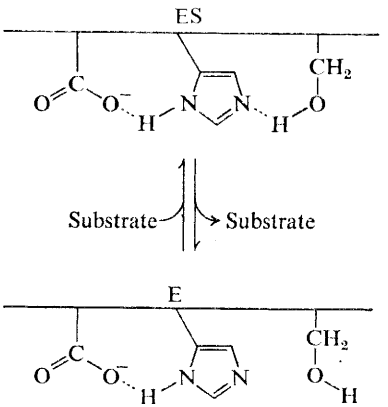
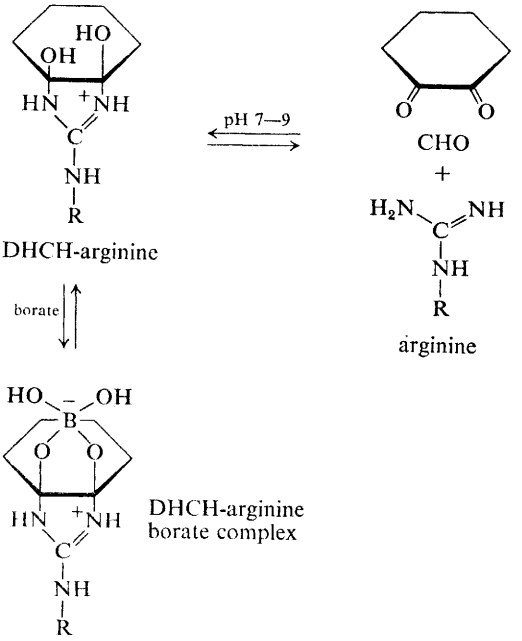


Figure 6 *Proposed hydrogen bonding of serine*-195 *in the presence and absence of a substrate or ligand at the specificity site of chymotrypsin*
(Reproduced by permission from *Biochem. J.*, 1975, **147**, 411)

can be protected from acetimidation by NAD^+–pyrazole complex. Further acetimidation with [^{14}C]ethyl acetimidate, after removal of NAD^+–pyrazole, radioactively labels only Lys-228.[313]

4 Protein Sequence Methods

Chain Cleavage and Separation of Peptides. Blocking of Arginine Residues.— The availability of large peptide fragments suitable for sequence analysis by automatic methods is becoming crucially important to the speed and efficiency of sequence determination. Reversible blocking of lysine residues as a means of restricting the specificity of trypsin has been very useful in generating such fragments, and recent detailed reports describing a reversible blocking reaction for arginine residues may extend the usefulness of trypsin even further.[738, 739] Arginyl residues can be specifically and quantitatively converted into N^7,N^8-(1,2-dihydroxycyclohex-1,2-ylene) adducts (DHCH-arginine) by treatment with cyclohexane-1,2-dione providing borate buffers (pH 8—9) are used (Scheme 5).



Scheme 5

The importance of borate is that it forms a chemically stable complex with DHCH-arginine thereby preventing side reactions which accompany the use of buffers other than borate. DHCH-arginyl residues are completely resistant to trypsin which cleaves exclusively at lysine residues. The blocking groups can be easily and quantitatively removed after tryptic cleavage by mild treatment with

[738] L. Patthy and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 557.
[739] L. Patthy and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 565.

hydroxylamine which results in the quantitative conversion of released cyclohexane-1,2-dione into its inactive dioxime. One possible drawback in the method is that 1,2-diones such as cyclohexane-1,2-diones react with urea and guanidine, precluding their use as protein denaturants. Nonetheless the method is superior to previous methods for chemically modifying arginine since both blocking and unblocking reactions are efficient and highly specific.

Detection of Peptides.—Separation of peptides is becoming routine, but their detection is still a subject of interest, particularly when performed non-destructively and at high sensitivity. Preparative SDS-polyacrylamide gels have been previously used for high-resolution separation of protein chains, and a recent report has suggested the use of highly fluorescent dansyl tracers to monitor preparative gel separation.[740] A small sample of the mixture to be separated is dansylated and, after removal of excess dansyl chloride, is remixed with the remainder which is then separated by gel electrophoresis. Since the tracer dansylated proteins have mobilities identical with those of their unlabelled counterparts on SDS-gels, subsequent inspection of the gel under u.v. light allows the protein zone of interest to be excised. In this paper a simple means of eluting and concentrating the purified protein by electrodialysis is also described and the method should be useful for protein separations at the milligram level or lower. Polyacrylamide gels have also been suggested for the separation of very small peptides ('prelabelled' with fluorescamine).[741] Separations of peptides from 3 to 215 residues long are reported in this paper. Although the authors suggest that the main use of the method may be in testing the purity of peptide samples, it appears to have no advantage, for small peptides at least, over the more common techniques of paper electrophoresis or end-group analysis by dansylation.

A rapid, although relatively insensitive, method for identifying peptides containing cysteine cross-links in column eluents has been described.[742] It involves alkaline hydrolysis of samples, subsequent neutralization, and finally addition of 5,5′-dithiobis-(2-nitrobenzoic acid). The product of this reaction with cysteine is chromogenic and may be measured by its absorbance at 420 nm. Approximately 100 nmol of peptides must be used per determination, however, and this may limit the usefulness of the method.

Another method for the location of carboxy-terminal fragments after paper electrophoresis of digests has been reported.[743] The intact protein chain is treated with carbodi-imide and ethanolamine so as to block all free carboxyls and then digested with a suitable enzyme. The resulting fragments can be separated by paper electrophoresis at pH 2.1 and then at pH 4.4 in the perpendicular direction. The only peptide which contains no free carboxy-groups corresponds to the carboxy-terminal peptide of the protein, and such peptides can be recognized by their position relative to internal standards added to the mixture before electrophoresis. From peptic digests of α-chymotrypsin the two expected carboxy-terminal peptides were clearly identified, and the apparent ease and sensitivity of this method recommend it.

[740] R. E. Stevens, *Analyt. Biochem.*, 1975, **65**, 369.
[741] M. S. Rosemblatt, M. N. Margolies, L. E. Cannon, and E. Haber, *Analyt. Biochem.*, 1975, **65**, 321.
[742] W. L. Anderson and D. B. Wetlaufer, *Analyt. Biochem.*, 1975, **67**, 493.
[743] R. G. Duggleby and H. Kaplan, *Analyt. Biochem.*, 1975, **65**, 346.

The usefulness of fluorescamine for detecting peptides in both column eluents and after separation on paper has been further extended this year. In one approach [744] column eluant is mixed with buffer and fluorescamine and peptide material is detected by fluorimetry. The salient variation, however, is that *all* the column eluent is automatically sampled for short time intervals. The resulting trace, although discontinuous, is nonetheless adequate for detection of peptides. The sensitivity of this method is impressive: 700 pmol of a ribonuclease digest, of which one seventh was sampled for detection, produced a useful elution profile. The detection of peptides with amino-terminal proline or hydroxyproline with fluorescamine after their separation on paper has been problematical. However, it can be accomplished either by heating at 110 °C or by pretreatment of papers with *N*-chlorosuccinimide to convert secondary amines into primary amines.[745]

Amino-acid Analysis.—*Fluorescamine.* Although excellent systems are now available for amino-acid analysis of peptide or protein hydrolysates, there is a growing need to increase the sensitivity to the subnanomole level. The use of fluorescamine for this purpose has been detailed in previous Reports, but a serious drawback is that proline, a secondary amine, cannot be detected. It has recently been reported [746] that the aminoenone of proline, its fluorescamine adduct, although non-fluorescent, can be detected by absorbance at 380 nm and it is suggested that a combination of fluorimetry (for primary amino-acids) and absorbance measurements for proline will circumvent the problem. There is a flaw, however. The proline aminoenone is a weak chromophore and at least 10 nmol is needed for accurate quantitation, thus precluding its determination at the subnanomole level, the range for which fluorescamine analysis was designed.

o-Phthalaldehyde. *o*-Phthalaldehyde also forms fluorescent adducts with amino-acids and peptides, and a report of its use in amino-acid analysis has appeared.[747] In this work, the sensitivity of detection of amino-acids using ninhydrin, fluorescamine, and *o*-phthalaldehyde after their separation on the same column were compared. Use of exactly comparable separation conditions for the three separations, the impressive feature of this work, was absent in previous reports in which fluorescamine was compared to ninhydrin. The authors found that whereas ninhydrin and fluorescamine were equally sensitive, *o*-phthalaldehyde was more sensitive by approximately an order of magnitude. Full scale deflection on the recorder is produced by 0.1 nmol. Only proline was not detectable although the cysteine responses was anomalously low. Another advantage of *o*-phthalaldehyde over fluorescamine is its water solubility which largely overcomes problems with organic solvents inherent in the use of fluorescamine.

Dansylation. An even more sensitive means of amino-acid analysis depends upon the quantitation of radioactive dansyl amino-acids prepared by reaction of aminopeptidase M hydrolysates of peptides with ^{3}H-dansyl chloride.[748] Dansyl

[744] P. Böhlen, S. Stein, J. Stone, and S. Udenfriend, *Analyt. Biochem.*, 1975, **67**, 438.
[745] E. Mendez and C. Y. Lai, *Analyt. Biochem.*, 1974, **65**, 281.
[746] A. M. Felix, V. Toome, S. DeBernardo, and M. Weigele, *Arch. Biochem. Biophys.*, 1975, **168**, 601.
[747] J. R. Benson and P. E. Hare, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 619.
[748] S. R. Burzynski, *Analyt. Biochem.*, 1975, **65**, 93.

amino-acids are then separated on polyamide layers, detected by fluorography and quantified by densitometry of the resulting autoradiograph. The authors claim detection of 2.5×10^{-5} nmol. The usefulness of techniques capable of quantitation of amino-acids at the subnanomole level is limited by the difficulty in detecting this amount of peptide, if not protein, and by the background levels of amino-acids present in buffers and electrophoresis papers commonly used for separations.

Tryptophan Determination. The determination of tryptophan, due to its lability in acid, remains a subject for investigation. One group [749] suggest that absorbance measurements on protein after its complete enzymic hydrolysis to amino-acids are accurate enough since conformational quenching effects are eliminated. Another group has reported on the use of 2-hydroxyl-5-nitrobenzyl bromide, which reacts specifically with tryptophan to form a chromophoric product.[750] It is doubtful whether either method has a sensitivity comparable to that of most amino-acid analysers and their use will be limited to proteins available in large amounts.

Gas–Liquid Chromatography (g.l.c.). Gas–liquid chromatography as a means of separating amino-acids and their derivatives (including phenylthiohydantoins and methylthiohydantoins) has been comprehensively reviewed this year.[751] In a more recent report [752] g.l.c. has been used for the separation of lysine and hydroxylysine and of proline and hydroxyproline from collagen digests, and in another report the *N*(*O*)-perfluorobutyryl isoamyl esters have been used for amino-acid analysis by g.l.c.[753]

Manual Sequence Methods. Three reports have appeared which describe modifications to commonly used methods for sequencing peptides by manual means. Two of these are mainly concerned with maintaining a nitrogen atmosphere in the reaction tube during coupling and cleavage steps, by use either of a closed nitrogen chamber,[754] or of a reaction tube fitted with a teflon cap through which reagents can be introduced.[755] A nitrogen line and a vacuum line ensure that the contents of the tube are evacuated or under nitrogen continuously. Using the latter method 39 residues were sequenced of a total of 44 in the peptide examined. In a third report,[756] diborane reduction of aspartic and glutamic acid residues in peptides prior to sequence analysis by the dansyl-Edman method is suggested to distinguish these residues from asparagine and glutamine, respectively. Reduction of aspartic acid and glutamic acid with diborane produces homoserine and α-amino-β-hydroxyvaleric acid, respectively, both of which are well separated as dansyl derivatives from dansyl-aspartic and dansyl-glutamic acids. Asparagine and glutamine are unaffected by diborane reduction and, after dansylation and hydrolysis, appear as their respective acid forms. The additional steps involved in the generation of diborane and reduction of peptides

[749] T. Sasaki, B. Abrams, and B. L. Horecker, *Analyt. Biochem.*, 1975, **65**, 396.
[750] Y. D. Karkhanis, D. J. Carlo, and J. Zeltner, *Analyt. Biochem.*, 1975, **69**, 55.
[751] P. Husěk and K. Macek, *J. Chromatogr.*, 1975, **113**, 139.
[752] S. L. MacKenzie and D. Tenaschuck, *J. Chromatogr.*, 1975, **104**, 176.
[753] P. Felker and R. S. Bandurski, *Analyt. Biochem.*, 1975, **67**, 245.
[754] R. B. Meagher, *Analyt. Biochem.*, 1975, **67**, 404.
[755] G. E. Tarr, *Analyt. Biochem.*, 1975, **63**, 361.
[756] L. P. D. Airoldi and S. Doonan, *F.E.B.S. Letters*, 1975, **50**, 155.

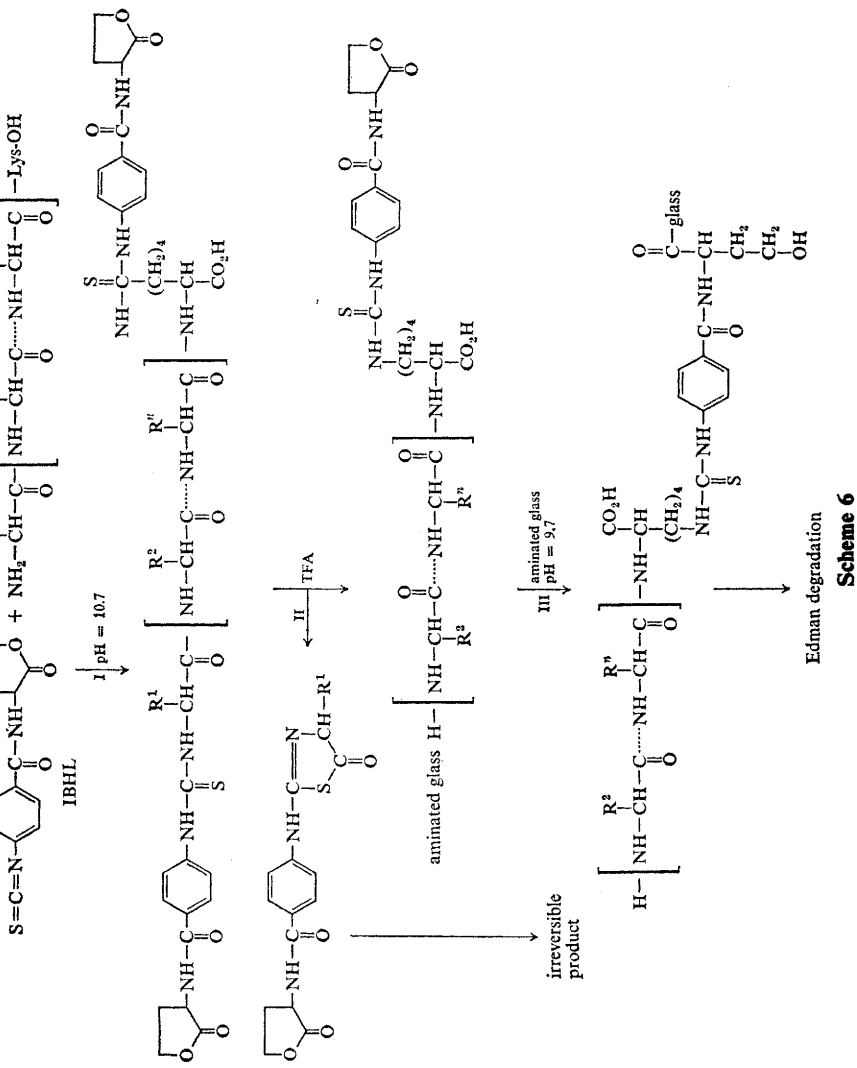
detract from the usefulness of the method, and identification of PTH–amino-acids by established t.l.c. techniques circumvents the need to distinguish the acid amide pairs by such an indirect means.

Automatic Sequence Methods. Liquid-phase sequences. A program which minimizes solvent extraction of small peptides from the cup of the liquid-phase sequencer has been reported.[757] The program seems to work even with hydrophobic tripeptides and relies on the ability of hydrated quadrol, deliberately left in the cup after coupling, to retain selectively the phenylthiocarbamyl peptide which would normally be removed during solvent washes. No comparisons of this method and the other commonly used peptide programs were given in this report. Other applications of automatic liquid-phase sequencing are reported in the Microsequencing section and elsewhere later in this chapter.

Solid-phase sequences. When peptides are covalently attached to solid supports, the problems of losses due to solvent washes during sequencing become irrelevant. A major factor which limits the applicability of the technique is the lack of methods for covalent attachment of peptides to resins. Perhaps the most useful general method involves the attachment of peptides *via* lysine amino-groups to aminated glass by *p*-phenylene di-isothiocyanate (DITC) (see previous Reports). A variation on this method has recently appeared.[758] Instead of DITC a new compound *N*-(*p*-isothiocyanatobenzoyl)-DL-homoserine lactone (IBHL), is used. As indicated in Scheme 6 the α-amino-group and *C*-amino-groups of lysine residues in the peptide react with the isothiocyanate moiety of IBHL in a manner analogous to the coupling step of an Edman degradation. Treatment with trifluoroacetic acid results in the removal of the amino-terminal residue of the peptide which may then be coupled through modified lysine residues to a suitably aminated support *via* the lactone function which is susceptible to nucleophilic attack by the amino-groups of the support. Sequential degradation may then be carried out in the usual manner. Both covalent attachment and sequence analysis have been shown to work satisfactorily on the peptides tried, but the method suffers from the same limitations as that using DITC, namely that it is applicable only to peptides containing lysine and that sequence analysis cannot continue past the carboxyl proximal lysine in the peptide. Furthermore, the extra effort involved in chemically synthesizing IBHL for use is likely to ensure that the commercially available DITC remains the reagent of choice for lysine-containing peptides.

Whereas the solid-phase sequencing methods currently in use rely on the Edman chemistry for sequential degradation of peptides from the amino-terminus, a report has appeared [759] which advocates coupling *via* the amino-terminus and sequential degradation from the carboxy-terminus. Lysine residues present are firstly blocked by a cycle of Edman degradation which also removes the amino-terminal residue. The blocked peptide may then be coupled to hydroxysuccinimide–porous glass by its sole remaining α-amino-group and degraded by the Stark method which sequentially removes amino-acids from the carboxy-terminus. The major drawback of the work is that certain residues,

[757] W. G. Crewther and A. S. Inglis, *Analyt. Biochem.*, 1975, **68**, 572.
[758] P. Herbrink, G. I. Tesser, and J. J. M. Lamberts, *F.E.B.S. Letters*, 1975, **60**, 313.
[759] M. J. Williams and B. Kassel, *F.E.B.S. Letters*, 1975, **54**, 353.


IBHL
I pH = 10.7
II TFA
III aminated glass pH = 9.7
irreversible product
aminated glass
Edman degradation

Scheme 6

e.g. aspartic acid and proline, cannot be removed by this method and this will preclude its use for extended degradations without considerable improvement.

Schiltz and Reinbolt [760] have reported a potentially useful variation of existing methods for coupling peptides devoid of lysine and homoserine to resins for conventional sequence analysis. Normally such peptides are coupled to aminated supports by activation of carboxylate groups by carbodi-imides or carbonyl-di-imidazole. Reversible blocking of peptide α-amino-groups present is necessary, however, to prevent intermolecular cross-linking which would otherwise occur. The initial blocking reaction can be circumvented by coupling *via* the amino-terminal residue with DITC in the usual way and then activating peptide carboxylate groups which can then react with excess amino-groups on the support. The sequencing program begins with a TFA cleavage which exposes the free penultimate amino-terminal residue, and subsequent sequence determination is straightforward. These authors reported that coupling yields are better than with previous methods, and the speed and simplicity of the method are appealing.

An automatic system suitable for a solid-phase sequencer has been described [761] whereby thiazolinones released at each cycle of Edman degradation can be converted into PTH–amino-acids for immediate identification. The importance of installing such a system is questionable since manual conversion is routine and fairly rapid. Laboratories in which technical assistance is lacking, however, may find it useful.

Protein chemists interested in techniques in solid-phase sequencing should find two books [762, 763] which have appeared this year to be useful. Both treat this topic critically and comprehensively, and discuss the advantages and limitations of various methods in far more detail than is possible here. Further applications of solid-phase sequencing are noted below in the Microsequencing section.

PTH–Amino-acids. The initial products of Edman degradation of peptides or proteins are the 2-anilino-5-thiazolinones. These cannot be directly identified and must be converted into PTH–amino-acids which can. Two methods have appeared which claim to minimize the time and effort involved in this conversion. In one method,[764] heating anilinothiazolinones at 80 °C for 30 min is claimed to be sufficient for conversion, and in the other method [765] anilinothiazolinones are applied to silica gel plates with 1 μl of heptafluorobutyric acid and heated to 140 °C before t.l.c. An advantage of both methods is that the conventional ethyl acetate extraction step is omitted, allowing PTH–arginine and PTH–histidine to be identified on the same t.l.c. plates as the other amino-acids.

Inman and Appella (in ref. 762) describe the conversion of anilinothiazolinones obtained from a solid-phase sequencer to PTC–amino-acid methylamides rather than PTH–amino-acids. Conversion of anilinothiazolinones into methyl-

[760] E. Schiltz and J. Reinbolt, *European J. Biochem.*, 1975, **56**, 467.
[761] C. Birr and R. Frank, *F.E.B.S. Letters*, 1975, **55**, 61.
[762] 'Solid Phase Methods in Protein Sequence Analysis', ed. R. A. Laursen, Pierce Chemical Company, Rockford, Illinois, 1975.
[763] 'Instrumentation in Amino Acid Sequence Analysis', ed. R. N. Perham, Academic, London, 1975.
[764] R. L. Guyer and C. W. Todd, *Analyt. Biochem.*, 1975, **66**, 400.
[765] A. S. Inglis, P. W. Nicholls, and P. M. Strike, *J. Chromatogr.*, 1975, **107**, 73.

amides is accomplished by heating in 0.3 ml of 2.4M methylamine in propan-2-ol for 2 min and conversion for most amino-acids seems to be complete in this time. Serine and threonine are problematical due to acid-catalysed breakdown of their anilinothiazolinones before conversion. However, if conversion is prompt, the problem is largely circumvented. PTC–amino-acid methylamides can be prepared for 19 of the 20 amino-acids (cysteine derivatives were not examined) and excellent t.l.c. systems for their separation have been developed; high-pressure liquid chromatography (h.p.l.c.) also looks very promising for the purpose of identification and quantitation. Their only drawback is that their application is at present confined to solid-phase sequencers since impurities present in fractions obtained from spinning-cup sequences interfere with their identification.

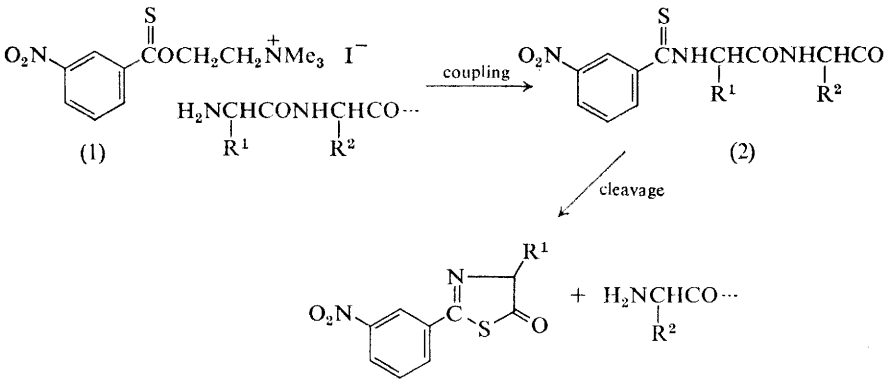
New t.l.c. solvents for the identification of PTH–aminoethyl cysteine, carboxymethyl cysteine, cysteic acid, and homoserine on polyamide layers have been reported,[766] and methods for quantifying PTH–amino-acids after t.l.c. have also appeared.[767]

Further progress has been made in the use of high-pressure liquid chromatography (h.p.l.c.) for the analysis of PTH–amino-acids.[768–770] The advantages of h.p.l.c. are its sensitivity and the fact that arginine, histidine, and tryptophan derivatives do not present the same problems as in g.l.c. To date no single h.p.l.c. system capable of resolving all PTH–amino-acids has been reported. However, two systems are now available,[770] one which separates all PTH–amino-acids except histidine, arginine, and cysteic acid, and another which can adequately separate these three. The sensitivity of these h.p.l.c. methods enables quantitation at a useful level, and the cost of instrumentation is comparable to that of a gas-chromatograph thus making h.p.l.c. a viable alternative to g.l.c. for analysis of PTH–amino-acids.

Where hydrolysis of Edman degradation products to amino-acids is necessary for identification, either anilinothiazolinones or PTH–amino-acids can be used. A paper has appeared in which conditions of conversion of thiazolinones into amino-acids have been investigated.[771] Recommended conditions involve hydrolysis in 1M-HCl for 4 h in the presence of tin(II) chloride. The thiohydantoins produced by sequential degradation from the carboxy-terminus by the Stark procedure can also be identified as amino-acids after acidic hydrolysis, according to another report.[772]

Miscellaneous. Although the Edman degradation is almost universally used for sequence studies, alternatives are available to the alkyl or aryl isothiocyanates used. One such reagent which was reported this year[773] was *m*-nitrobenzoylthionocholine iodide [(4), in Scheme 7]. Coupling to the α-amino-group of the peptide results in the formation of the *N*-(*m*-nitrothiobenzoyl) peptide (5) which

[766] K. D. Kulbe and Y. M. Noguerra-Hattesohl, *Analyt. Biochem.*, 1975, **63**, 624.
[767] M. Kubota, N. Takahashi, K. Goto, and T. Murachi, *Analyt. Biochem.*, 1975, **64**, 494.
[768] E. W. Matthews, P. G. H. Byfield, and I. MacIntyre, *J. Chromatogr.*, 1975, **110**, 369.
[769] J. X. deVries, R. Frank, and C. Birr, *F.E.B.S. Letters*, 1975, **55**, 65.
[770] J. Bridgen, A. P. Graffeo, B. L. Kager, and M. D. Waterfield, in ref. 762.
[771] E. Mendez and C. Y. Lai, *Analyt. Biochem.*, 1975, **68**, 47.
[772] M. Rangarajan and A. Darbre, *Biochem. J.*, 1975, **147**, 435.
[773] G. C. Barret and P. H. Leigh, *F.E.B.S. Letters*, 1975, **57**, 19.



(1) (2) (3)

Scheme 7

can be cleaved by TFA to expose the second amino-acid residue with concomitant release of a thiazolone (6). The advantage of this alternative reaction is speed; coupling takes 10 min and cleavage 2—3 min. The efficiency of coupling and cleavage seems to be comparable with the Edman degradation, but no data were given in this paper on the identification of thiazolone derivatives, a crucial factor in determining the practical usefulness of the chemistry presented in sequence analysis.

An interesting and potentially useful enzyme has been purified from the pronase mixture of *Streptomyces griseus*.[774] It is an aminopeptidase whose action terminates solely at residues penultimate to proline in a sequence (at X-Pro bonds). The authors have outlined a potential use for the aminopeptidase in sequence analysis (Figure 7). Successive rounds of aminopeptidase digestion and single cycles of Edman degradation should produce a series of fragments progressively shortened from the amino-terminus, each containing penultimate proline residues and suitable for extended automatic sequence analysis. Unfortunately, no attempt was made to digest an intact protein chain with the aminopeptidase whose specificity and efficiency were examined only on model peptides.

Microsequencing. The importance of determining sequences of proteins available in tiny amounts is discussed in later sections on hormones and histocompatibility antigens. The purpose of the present section is to delineate recent approaches to the general problem of microsequencing. Two general approaches have been used.

(*i*) *Biological incorporation of radioisotope.* In this approach radioactive amino-acids are incorporated either by incubation of cells or tissue slices in a suitable medium, or by cell-free translation of m-RNA. The radioactively labelled proteins of interest are purified and their amino-terminal sequences determined by the identification of radioactive PTH–amino-acids.

[774] K. D. Vosbeck, B. D. Greenberg, and W. M. Awad, jun., *J. Biol. Chem.*, 1975, **250**, 3981.

Huang *et al.*[775] labelled human parathyroid tissue individually with six different ^{3}H-amino-acids, purified labelled proparathyroid hormone in each case, and submitted samples to automatic degradation using non-radioactive apomyoglobin as carrier to minimize solvent extraction of protein from the cup. Radioactive PTH–amino-acids were checked by t.l.c. to exclude *in vivo* conversion of one amino-acid into another. Habener *et al.*[776] added [^{35}S]methionine

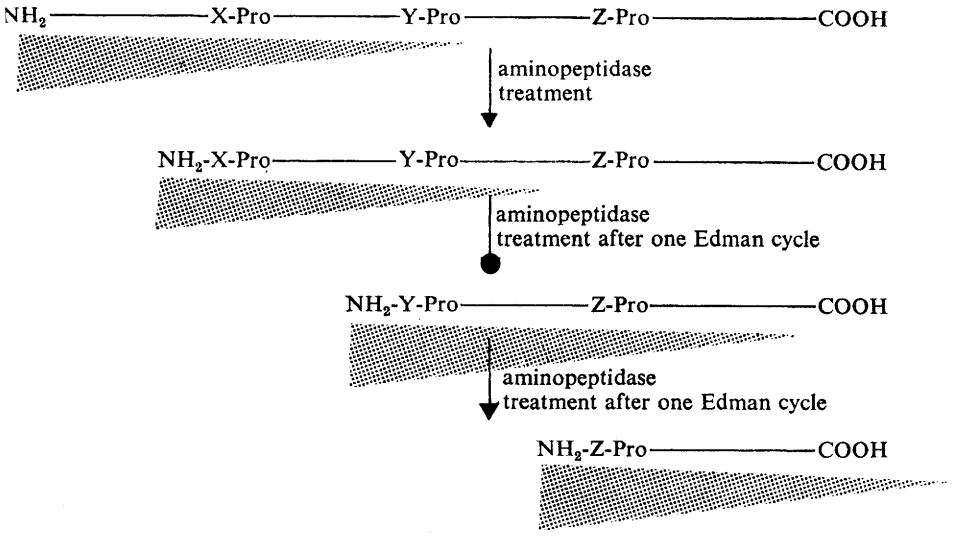


Figure 7 *Proposed scheme for generating a series of peptides from a parent polypeptide chain by sequential use of Pronase aminopeptide and phenylisothiocyanate (Edman) cleavages. The stippled areas represent the diminishing confidence in values for residues derived by sequence analysis*
(Reproduced by permission from *J. Biol. Chem.*, 1975, **250**, 3981)

and m-RNA from bovine parathyroid tissue to a protein-synthesizing cell-free system, purified the newly synthesized pre-proparathyroid hormone (see Hormone section) by immunoprecipitation and gel electrophoresis, and sequenced the labelled product also in the presence of carrier apomyoglobin. Despite the greater technical difficulty of the second method, its main advantage is that much less dilution of radioactive amino-acid by endogenous amino-acids takes place in a cell-free system facilitating the use of smaller amounts of radioactivity and greater sensitivity of detection. Both methods suffer from the obvious drawbacks that biological labelling is only practicable with a relatively small number of proteins and all 20 amino-acids must be incorporated individually to obtain extended sequences.

[775] W. Huang, L. L. H. Chu, J. W. Hamilton, D. H. McGregor, and D. V. Cohn, *Arch. Biochem. Biophys.*, 1975, **166**, 67.
[776] J. F. Habener, B. Kemper, J. T. Potts, jun., and A. Rich, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1114.

(*ii*) *Chemical approaches.* Brauer *et al.*[777] have developed a new program for the spinning-cup sequencer which minimizes the considerable amount of protein loss which takes place during solvent washing steps. The major problem with conventional programs is that small amounts of heptafluorobutyric acid (HFBA) remain in the lines after cleavage and dissolve in the chlorobutane during the subsequent wash. This results in the acidification of the chlorobutane making it a solvent not only for the desired thiazolinones but also for residual protein. This problem has been circumvented by cleaving the HFBA after its introduction by the subsequent introduction of nitrogen through the same line. Otherwise this program resembles previous peptide programs, utilizing a diluted quadrol coupling buffer and an abbreviated solvent wash after coupling. These authors report 20 residues from only 3 nmol of apomyoglobin.

Jacobs and Niall[778] have reported the use of phenyl [^{35}S]isothiocyanate (PITC) to chemically introduce radioactivity into PTH–amino-acids during the coupling step. They have developed a program whereby protein in the spinning cup is treated with a trace of very highly radioactive PITC and subsequently with excess 'cold' PITC to complete the coupling. Cleavage and extraction follow and after their conversion [^{35}S]PTH–amino-acids are identified by t.l.c. Quantitation is also possible by determining radioactivity in spots excised from t.l.c. plates. The authors reported 15 residues from 4.6 nmol of bovine proparathyroid hormone. Another report[779] has also appeared describing a similar method although with less impressive results. A solid-phase application using [^{35}S]PITC of much lower specific activity has been reported[780] and, here too, the results are impressive – an amino-terminal sequence of seven residues obtained with 5 nmol of insulin B chain. The design and operation of the solid-phase sequencer make it particularly well suited to incorporate an initial pulse of highly radioactive PITC before the conventional 'cold' coupling, and the instrument is likely to find extensive application in sequencing at the sub-nanomole level.

5 Primary Structures

Table 4 presents a summary of the amino-acid sequence studies reported during 1975.

Enzymes.—*Alcohol Dehydrogenase.* Reaction of an NAD analogue with either horse liver or yeast alcohol dehydrogenases (ADH) has been shown to result in the covalent attachment of the active site reagent to a single cysteine residue in each case.[781] The respective sequences surrounding the modified cysteines in each enzyme are unrelated, and in yeast ADH cysteine-43 is modified whereas in horse ADH it is cysteine-174. The authors show that although cysteine-43 is unreactive to the NAD analogue in horse ADH, it is present in the sequence and likewise that cysteine-174 is present but inactive in the yeast enzyme. They suggest that both cysteines are present in close proximity to the active sites of

[777] A. W. Brauer, M. N. Margolies, and E. Haber, *Biochemistry*, 1975, **14**, 3029.
[778] J. W. Jacobs and H. D. Niall, *J. Biol. Chem.*, 1975, **250**, 3629.
[779] J. Silver and L. Hood, *Analyt. Biochem.*, 1975, **67**, 392.
[780] J. Bridgen, *F.E.B.S. Letters*, 1975, **50**, 159.
[781] H. Jornvall, C. Woenckhaus, and G. Johnscher, *European J. Biochem.*, 1975, **53**, 71.

Table 4 *Amino-acid sequences reported in 1975*

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
Enzymes				
Aldolase	rabbit muscle	361	complete sequence	782—785
	rabbit brain	27	active centre peptide	786
	bovine muscle	28	active centre peptides, all highly homologous to rabbit muscle counterpart	787
	whale muscle	28		
	Loligo vulgaris	28		
Aspartate aminotransferase	pig heart (cytoplasmic)	412	complete sequence	788
	pig heart (mitochondrial)	37	pyridoxal phosphate binding peptide, highly homologous to cytoplasmic counterpart	789
	sheep liver		pyridoxal phosphate binding peptides from mitochondrial and cytoplasmic forms shown to be similar	790
Carboxypeptidase B	bovine pancreas	305	complete sequence	791
6S Carboxypeptidase A subunit III	bovine pancreas	9	amino-terminal sequence – homologous to residues 17—26 in chymotrypsinogen	792
Cytochrome oxidase	bovine heart	36	cysteine-containing peptide	793
Dehydrofolate reductase	*Streptococcus faecium*	167	complete sequence of mutant enzyme resistant to methotrexate	794, 795

[782] C. Y. Lai, *Arch. Biochem. Biophys.*, 1975, **166**, 330.
[783] N. Nakai, D. Chang, and C. Y. Lai, *Arch. Biochem. Biophys.*, 1975, **166**, 339.
[784] N. Nakai, D. Chang, and C. Y. Lai, *Arch. Biochem. Biophys.*, 1975, **166**, 347.
[785] C. Y. Lai, *Arch. Biochem. Biophys.*, 1975, **166**, 358.
[786] Y. Lee, R. Felicioli, and B. L. Horecker, *Arch. Biochem. Biophys.*, 1975, **166**, 592.
[787] J. Mareschal, E. Schonne, R. R. Crichton, and D. A. Strosberg, *F.E.B.S. Letters*, 1975, **54**, 97.
[788] S. Doonan, H. J. Doonan, R. Hanford, C. A. Vernon, J. M. Walker, L. P. D. Airoldi, F. Bossa, D. Barra, M. Carloni, P. Fasella and F. Riva, *Biochem. J.*, 1975, **149**, 497.
[789] H. Kagamiyama, K. Teranishi, and H. Wada, *Biochem. Biophys. Res. Comm.*, 1975, **72**, 993.
[790] M. Campos-Cavieres and C. P. Milstein, *Biochem. J.*, 1975, **147**, 275.
[791] K. Titani, L. H. Ericsson, K. A. Walsh, and H. Neurath, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1666.
[792] A. Puigserver and P. Desnuelle, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2442.
[793] M. Tanaka, M. Haniu, S. Zeitlin, K. T. Yasunobu, C. A. Vu, L. Yu, and T. E. King, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 357.
[794] D. L. Peterson, J. M. Gleisner, and R. L. Blakley, *J. Biol. Chem.*, 1975, **250**, 4949.
[795] J. M. Gleisner, D. L. Peterson, and R. L. Blakley, *J. Biol. Chem.*, 1975, **250**, 4937.

Table 4 (*cont.*)

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
	bovine liver	27	amino-terminal sequence, homologous to *S. faecium* and *E. coli* enzymes	796
N,O-Diacetylmuraminidase	*Chalaropis*	211	complete sequence, 198 residues by automatic methods	797, 798
Glutamate dehydrogenase (NAD specific)	*Neurospora crassa*	886	partial sequence	799
Glutamate dehydrogenase (NADP specific)	*Neurospora crassa*	452	complete sequence – high conservation of structure in coenzyme and substrate-binding portions between *Neurospora* enzyme and other species	800—803
Glutathione reductase	yeast	35	cystine-containing sequence and three cysteine-containing sequences reported	804
Glycogen phosphorylase	yeast	9	phospho-peptide	805
		18	pyridoxal phosphate-containing peptide	
Glycogen synthetase	rabbit muscle	8	amino-terminal sequence	806
Histidine decarboxylase	*Micrococcus* sp.n.	43	from light chain	807
		67	from heavy chain	
β-Lactamase I	*Bacillus cereus*		80% of sequence elucidated; peptides aligned by homology	808
Lactate dehydrogenase (M4)	*Squalus acanthus*		37 of 40 tryptic peptides sequenced	809
Lysozyme	*Asteria rubens*	25	amino-terminal sequence	810
Malate dehydrogenase	pig heart	6	pyridoxal phosphate-containing peptide	811
Ornithine decarboxylase	*E. coli*	8	pyridoxal phosphate-containing peptide	812
Penicillinase	*Staphylococcus aureus*	257	complete sequence	813
Pepsin	pig	327	complete sequence, three genetic variants identified by sequence analysis	814—816
	human	34	amino-terminal sequence, shows pronounced homology to human gastricin and *R. chinensis* acid protease sequences	817
	bovine	65	amino-terminal sequence	818
Phosphoglycerate mutase	chicken muscle	10	active-site peptide, homologous to substrate binding loop in lactate dehydrogenase	819
Phosphorylase	rabbit muscle	4	amino-terminal sequence	820

Phosphorylase kinase	rabbit muscle	12(α) 9(β)	phospho-peptides from α and β chains	821
Prochymosin	—	42	amino-terminal sequence, overlapped into chymosin sequence; activation sequence very similar to that of pepsinogen	823
Proline racemase	*Clostridium sticklandii*	5	active centre peptide	824
Prophospholipase	bovine pancreas	7	both bovine prophospholipase variants have identical activation (*N*-terminal) sequences but sheep variants have different activation sequences	825
	sheep pancreas	7		

[796] D. L. Peterson, J. M. Gleisner, and R. L. Blakley, *Biochemistry*, 1975, **14**, 5261.
[797] N. Wahba, J. W. Felch, W. Shih, and J. H. Hash, *J. Biol. Chem.*, 1975, **250**, 3709.
[798] J. W. Felch, T. Inagami, and J. H. Hash, *J. Biol. Chem.*, 1975, **250**, 3713.
[799] B. M. Austen, J. F. Nyc, Y. Degani, and E. L. Smith, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4891.
[800] J. C. Wooton, J. G. Taylor, A. A. Jackson, G. K. Chambers, and J. R. S. Fincham, *Biochem. J.*, 1975, **149**, 739.
[801] A. A. Holder, J. C. Wooton, A. J. Baron, G. K. Chambers, and J. R. S. Fincham, *Biochem. J.*, 1975, **149**, 757.
[802] J. C. Wooton, A. J. Baron, and J. R. S. Fincham, *Biochem. J.*, 1975, **149**, 749.
[803] K. M. Blumenthal, K. Moon, and E. L. Smith, *J. Biol. Chem.*, 1975, **250**, 3644.
[804] E. T. Jones and C. H. Williams, jun., *J. Biol. Chem.*, 1975, **250**, 3779.
[805] K. Lerch and E. H. Fischer, *Biochemisty*, 1975, **14**, 2009.
[806] Y. Takeda, H. B. Brewer, jun., and J. Larner, *J. Biol. Chem.*, 1975, **250**, 8943.
[807] V. Prozorovski and H. Jörnvall, *European J. Biochem.*, 1975, **53**, 169.
[808] D. S. Thatcher, *Biochem. J.*, 1975, **147**, 313.
[809] S. S. Taylor, W. S. Allison, and N. D. Kaplan, *J. Biol. Chem.*, 1975, **250**, 8740.
[810] J. Jolles and P. Jolles, *European J. Biochem.*, 1975, **54**, 19.
[811] M. J. Wimmer and J. H. Harrison, *J. Biol Chem.*, 1975, **250**, 8768.
[812] D. Applebaum, D. L. Sabo, E. H. Fischer, and D. L. Morris, *Biochemistry*, 1975, **14**, 3675.
[813] R. P. Ambler, *Biochem. J.*, 1975, **151**, 197.
[814] K. C. S. Chen, N. Tao, and J. Tang, *J. Biol. Chem.*, 1975, **250**, 5068.
[815] J. Marciniszyn, jun., P. Sepulveda, W. Huang, J. P. Lainer, and J. Tang, *J. Biol. Chem.*, 1975, **250**, 5076.
[816] P. Sepulveda, J. Marcinszyn, jun., D. Liu, and J. Tang, *J. Biol. Chem.*, 1975, **250**, 5082.
[817] P. Sepulveda, K. W. Jackson, and J. Tang, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 1106.
[818] M. K. Harboe and B. Foltmann, *F.E.B.S. Letters*, 1975, **60**, 133.
[819] Z. B. Rose, N. Hamasaki, and S. Dube, *J. Biol. Chem.*, 1975, **250**, 7939.
[820] K. Titani, P. Cohen, K. A. Walsh, and H. Neurath, *F.E.B.S. Letters*, 1974, **55**, 120.
[821] P. Cohen, D. C. Watson, and G. H. Dixon, *European J. Biochem.*, 1975, **51**, 79.
[822] S. J. Yeaman and P. Cohen, *European J. Biochem.*, 1975, **51**, 93.
[823] V. B. Pedersen and B. Foltman, *European J. Biochem.*, 1975, **55**, 95.
[824] G. Rudnick and R. H. Abeles, *Biochemistry*, 1975, **14**, 4515.
[825] C. E. Dutilth, P. J. van Doren, F. E. A. M. Verhuel, and G. H. de Haas, *European J. Biochem.*, 1975, **53**, 91.

Table 4 *(cont.)*

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
Protease	*Staphylococcus aureus*	15	amino-terminal sequence	778
Neutral protease	*Bacillus subtilis*	171	54% of sequence elucidated; convincing homology to thermolysin (49%) – even greater at active-site region	826
Inorganic pyrophosphatase	yeast	100	amino-terminal sequence	827
Pyruvate kinase	sturgeon	7	sturgeon and rabbit muscle peptides closely homologous	828
	rabbit muscle	8		
	rat liver	20	phospho-peptide sequence, very similar to counterpart in pig	829
Pancreatic ribonuclease	guinea-pig A	125	complete sequences	830
	guinea-pig B	128		830
	coypu	128		830
	chinchilla	124		830
	Camelius drometrius	123		831
	reindeer moose	124		832
	fallow deer	124		832
	Connochaetes taurinus	124		833
Ribonuclease U2	*Ustilago sphaerogena*	113	complete sequence	834
RNA polymerase	*E. coli*		four amino-terminal residues of all four subunits (α, β, β', and γ) reported, Met is amino-terminal residue in all	835
Taurocyamine kinase	*Arenicola marina*	16	active centre peptide	836
Transaldolase III	*Candida utilis*	9	active centre peptide	837
Triose phosphate isomerase	rabbit muscle	248	complete sequence	838
Trypsin	*Streptomyces griseus*	221	complete sequence	839
	Squalus acanthus	222	complete sequence	840
	Astacus leptodactylus	20	amino-terminal sequence	841
Trypsinogen	*Balaenoptera acutostrata*	8	amino-terminal sequence	842
Tryptophan tRNA synthetase	*E. coli*	9	sequences found to be highly homologous between two species	843
	human placenta	10		
UDP galactose-4-epimerase	*E. coli*	30	amino-terminal sequence	844

Hormones				
Follicle-stimulating hormone α-chain	human pituitary	92	complete (α-chain) sequence; identical with α-chain of human chorionic gonadotropin	846
Chorionic gonadotropin (α and β chains)	human	92(α) 146(β)	complete sequences; four corrections made to previously published sequence	847
Growth hormone	bovine	38	revised sequence of isolated peptide	848
Insulin	hagfish		23 of the 24 'invariant' residues conserved in hagfish	849

[826] P. L. Levy, M. K. Pangburn, Y. Burstein, L. H. Ericsson, H. Neurath, and K. A. Walsh, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4341.
[827] R. Sterner and R. L. Heinrikson, *Arch. Biochem. Biophys.*, 1975, **168**, 693.
[828] P. J. Anderson and R. F. Randall, *Biochem. J.*, 1975, **145**, 575.
[829] B. Edlund, J. Andersson, V. Titanji, V. Dahlquist, P. Ekman, O. Zetterquist, and L. Engström, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1517.
[830] A. van den Berg and J. J. Beintema, *Nature*, 1975, **253**, 207.
[831] G. W. Welling, G. Groen, and J. J. Beintema, *Biochem. J.*, 1975, **147**, 505.
[832] G. Leijenaar, A. van der Berg, and J. J. Beintema, *F.E.B.S. Letters*, 1975, **56**, 101.
[833] G. Groen, G. W. Welling, and J. J. Beintema, *F.E.B.S. Letters*, 1975, **60**, 300.
[834] S. Sato and T. Uchida, *Biochem. J.*, 1975, **145**, 353.
[835] H. Fujiki and G. Zurek, *F.E.B.S. Letters*, 1975, **55**, 242.
[836] A. Brevet, Y. Zeitoun, and L. Pradel, *Biochim. Biophys. Acta*, 1975, **393**, 1.
[837] D. Tsolas and S. C. Sun, *Arch. Biochem. Biophys.*, 1975, **167**, 525.
[838] P. H. Corran and S. G. Waley, *Biochem. J.*, 1975, **145**, 335.
[839] R. W. Olafson, L. Jurásěk, M. R. Carpenter, and L. B. Smillie, *Biochemistry*, 1975, **14**, 1168.
[840] K. Titani, L. H. Ericsson, H. Neurath, and K. A. Walsh, *Biochemistry*, 1975, **14**, 1358.
[841] R. Zwilling, H. Neurath, L. H. Ericsson, and D. L. Enfield, *F.E.B.S. Letters*, 1975, **60**, 247.
[842] S. Bricteux-Grégoire, R. Schyns, M. Florkin, M. Emmens, G. W. Welling, and J. J. Beintema, *Biochim. Biophys. Acta*, 1975, **386**, 244.
[843] K. H. Muench, M. S. Lipscomb, M. Lee, and G. V. Kuehl, *Science*, 1975, **187**, 1089.
[844] R. Deeley, P. Blackburn, W. Ferdinand, and J. Bridgen, *European J. Biochem.*, 1975, **60**, 371.
[845] A. Devilliers-Thiery, T. Kindt, G. Scheele, and G. Blobel, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 5016.
[846] P. Rathman and B. B. Saxena, *J. Biol. Chem.*, 1975, **250**, 6735.
[847] F. J. Morgan, S. Birken, and R. E. Canfield, *J. Biol. Chem.*, 1975, **250**, 5247.
[848] N. Yamasaki, J. Shimanaka, and M. Sonenberg, *J. Biol. Chem.*, 1975, **250**, 2510.
[849] J. D. Peterson, D. F. Steiner, S. O. Emdin, and S. Falkmer, *J. Biol. Chem.*, 1975, **250**, 5183.
[850] C. R. Snell and D. G. Smyth, *J. Biol. Chem.*, 1975, **250**, 6291.

Table 4 (*cont.*)

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
Lutropin (β chain)	human pituitary	115	complete sequence	851
Melanotropin (α and β chains)	camel	13(α) 18(β)	amino-terminal sequences	852
Neurophysin	sheep	95	complete sequence	853—855
Neurotensin	bovine hypothalamus	13	amino-terminal sequence	856
Pancreatic hormone	chicken	36	complete sequence, function of hormone unknown	857
Proinsulin	guinea-pig	29	C-peptide region	861
PS-1	*Drosophila funebris*	27	complete sequence, male hormone which reduces receptivity of female after mating	862
Vasoactive peptide	chick intestine	28	complete sequence	863
Ubiquitin	human thymus	74	complete sequences; ubiquitins from human and bovine species identical in sequence – can induce T and B lymphocyte differentiation	864 865
	bovine thymus	74		
Urogastrone	human urine	53	complete sequence; 37 residues found to be identical with mouse epidermal growth factor	866
Immunoglobulins				
IgA (α-chain)	human	130	peptides from H-chain	871
IgG (γ_3-chain)	human	15	sequence from Omm, a shortened disease γ_3-chain; covers the hinge region	872
IgG	human		amino-terminal sequences of γ_1-, γ_2-, γ_3-, μ-, and α-chain switch regions; found that six amino-acids were invariant regardless of group or subgroup	873
λ Mutant	human	135	complete sequence; 81 residues deleted from V region immediately preceding C region	874
μ Disease chain	human	15	amino-terminal sequence; closely resembles residues 338—353 in normal μ-chain sequence, represents only Cμ3 domain	875
A.I.T. (heavy chain disease protein)	human	26	extent of deletion elucidated	876

Secretory component	human	10	sequences of intrachain disulphide bond	877
Secretory component	human	10	amino-terminal sequence	878
MOPC 173 κ-chain	mouse	214	complete sequence	879
Myeloma δ-chain	mouse	53	sequence of Fc fragment; similar to corresponding regions of ε- and γ-chains	880
Myeloma γ-chain	mouse	138	carboxyl-terminal sequence; mouse γ_1 more similar to human γ_1 than mouse γ_2	881
Myeloma γ_{2a}-chain	mouse	173	completes 447 residue sequence	882

[851] M. R. Sairam and C. H. Li, *Biochim. Biophys. Acta*, 1975, **412**, 70.
[852] C. H. Li, W. D. Danho, D. Chung, and A. J. Rao, *Biochemistry*, 1975, **14**, 947.
[853] M. Chauvet, J. Chauvet, and R. Acher, *F.E.B.S. Letters*, 1975, **58**, 234.
[854] M. Chauvet, J. Chauvet, and R. Acher, *F.E.B.S. Letters*, 1975, **52**, 212.
[855] D. H. Schlesinger, M. Ernst, A. Nicholas, W. B. Watkins, and R. Walter, *F.E.B.S. Letters*, 1975, **57**, 55.
[856] R. Carraway and S. E. Leeman, *J. Biol. Chem.*, 1975, **250**, 1907.
[857] J. R. Kimmel, L. J. Hayden, and H. G. Pollock, *J. Biol. Chem.*, 1975, **250**, 9369.
[858] H. T. Keutmann, H. D. Niall, L. J. H. O'Riordan, and J. T. Potts, jun., *Biochemistry*, 1975, **14**, 1842.
[859] L. L. H. Chu, W. Huang, E. Littledike, J. W. Hamilton, and D. V. Cohn, *Biochemistry*, 1975, **14**, 3631.
[860] G. Suchanek, I. Kindås-Mügge, G. Kreil, and M. H. Schreier, *European J. Biochem.*, 1975, **60**, 309.
[861] D. E. Massey and D. G. Smyth, *J. Biol. Chem.*, 1975, **250**, 6288.
[862] H. Baumann, K. J. Wilson, P. S. Chen, and R. E. Humbel, *European J. Biochem.*, 1975, **52**, 521.
[863] A. Nilsson, *F.E.B.S. Letters*, 1975, **60**, 322.
[864] D. H. Schlesinger and G. Goldstein, *Nature*, 1975, **255**, 423.
[865] D. H. Schlesinger, G. Goldstein, and H. D. Niall, *Biochemistry*, 1975, **14**, 2214.
[866] H. Gregory, *Nature*, 1975, **257**, 326.
[867] D. M. Pratt and L. E. Mole, *Biochem. J.*, 1975, **151**, 337.
[868] L. E. Mole, *Biochem. J.*, 1975, **151**, 351.
[869] G. A. Gutman, E. Lohn, and L. Hood, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 5046.
[870] J. W. Fett and H. F. Deutsch, *Immunochemistry*, 1975, **12**, 643.
[871] C. Wolfenstein-Todel, B. Frangione, and E. C. Franklin, *Biochim. Biophys. Acta*, 1975, **379**, 627.
[872] J. B. Aldersberg, E. C. Franklin, and B. Frangione, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 723.
[873] A. Wang and H. H. Fudenberg, *Arch. Biochem. Biophys.*, 1975, **168**, 657.
[874] F. A. Garver, L. Chang, J. Medicino, T. Isobe, and E. F. Osserman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4559.
[875] J. P. Lebreton, C. Ropartz, J. Rouseaux, P. Roussel, M. Dautrevaux, and G. Biserte, *European J. Immunol.*, 1975, **5**, 179.
[876] C. Wolfenstein-Todel, E. Mihaesco, and B. Frangione, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 47.
[877] C. Cunningham-Rundles and M. E. Lamm, *J. Biol. Chem.*, 1975, **250**, 1987.
[878] K. Sletten, T. B. Christensen, and P. Brantzaeg, *Immunochemistry*, 1975, **12**, 783.
[879] C. Schiff and M. Fougereau, *European J. Biochem.*, 1975, **59**, 525.
[880] H. L. Spiegelberg, *Nature*, 1975, **254**, 723.
[881] K. Adetugbo, E. Poskus, J. Svasti, and C. Milstein, *European J. Biochem.*, 1975, **56**, 503.
[882] J. Rocca-Serra, M. Milli, and M. Fougereau, *European J. Biochem.*, 1975, **59**, 511.

Table 4 (*cont.*)

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
MOPC 104E μ-chain	mouse	32	sequences from four isolated peptides	883
L-chain	rabbit	210	complete L-chain sequence of homogeneous antibody to streptococcal CHO	884, 885
Monoclonal antibody	rabbit V(L)	145	amino-terminal sequences covering V regions of both L and H chains of antibody to type III pneumococci	886
	V(H)	116		
γ-Chain	sheep	18	carboxyl-terminal 18 residues of γ_1- and γ_2-chains identical	887
Pooled λ	guinea-pig	102	carboxyl-terminal (C-region) sequence	
Bence-Jones protein (λ)	guinea-pig	17	amino-terminal sequence – similar to λIV subgroup of human	889
γ-Chain	pig	30	amino-terminal sequence of pooled subgroup III γ-chains	890
Anti-Ar antibodies	mouse	38	H-chain amino-terminal sequences of antibodies of shared specificity and idiotype; all sequences identical	891
Anti-pneumococcal polysaccharide antibodies	rabbit	40	L-chain amino-terminal sequences of antibodies of shared specificity	892
Anti-streptococcal group A polysaccharide antibodies	rabbit	42	L-chain amino-terminal sequences of antibodies of shared specificity; all sequences identical	893
Blood clotting proteins				
Factor X1	bovine	307	complete sequence; homologous to trypsin, chymotrypsin, elastase, and thrombin	894
Fibrinogen	human	9	carboxyl-terminal sequence of β chain	904
Fibrinogen	human	38	γ-chain peptide containing four plasmin cleavage points	905
Fibrin	human		amino-terminal sequences of separated α-, β-, and γ-chains	906
Plasmin (H-chain)	human	86	evidence for internal sequence repeats	907
Plasminogen	human	38	peptide fragment encompassing cleavage site for activating protease; homologous to 'pro' part of prothrombin	908

Plasminogen	human	122	peptide fragment contains plasmin A and B chain cross-linking site	909
Ribosomal proteins				
S4	*E. coli*	203	complete sequence	763
S6	*E. coli*	131	complete sequence	910
S9	*E. coli*	128	complete sequence	911
S18	*E. coli*	74	complete sequence	912

[883] C. P. Milstein, N. E. Richardson, E. V. Deverson, and A. Feinstein, *Biochem. J.*, 1975, **151**, 615.
[884] K. C. S. Chen, T. J. Kindt, and R. M. Krause, *J. Biol. Chem.*, 1975, **250**, 3280.
[885] K. C. S. Chen, T. J. Kindt, and R. M. Krause, *J. Biol. Chem.*, 1975, **250**, 3289.
[886] J. Jaton, *Biochem. J.*, 1975, **147**, 235.
[887] F. P. Conde, E. V. Deverson, and C. P. Milstein, *European J. Immunol.*, 1975, **5**, 291.
[888] J. Novotny and F. Franěk, *F.E.B.S. Letters*, 1975, **58**, 25.
[889] F. K. Stevenson, L. E. Mole, C. M. Raymont, and G. T. Stevenson, *Biochem. J.*, 1975, **151**, 751.
[890] F. Franěk, R. L. Wasserman, J. Novotny, and J. M. Kehoe, *European J. Immunol.*, 1975, **5**, 427.
[891] B. Friedenson, A. S. Tung, and A. Nisonoff, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3676.
[892] M. Freedman, T. Hofmann, S. Rhee, H. Yeger, M. Slaughter, and J. Milandre, *Immunochemistry*, 1975, **12**, 263.
[893] D. G. Braun, H. Huser, and J. Jaton, *Nature*, 1975, **258**, 363.
[894] K. Titani, K. Fujikawa, D. L. Enfield, L. H. Ericsson, K. A. Walsh, and H. Neurath, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3082.
[895] J. B. Howard and G. L. Nelsestuen, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1281.
[896] T. H. Zytkovicz and G. L. Nelsestuen, *J. Biol. Chem.*, 1975, **250**, 2968.
[897] J. Jesty, A. K. Spencer, Y. Nakashima, Y. Nemerson, and W. Konigsberg, *J. Biol. Chem.*, 1975, **250**, 4497.
[898] J. Stenflo, P. Fernlund, W. Egan, and P. Roepstorff, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2730.
[899] P. Fernlund, J. Stenflo, P. Roepstorff, and J. Thomsen, *J. Biol. Chem.*, 1975, **250**, 6125.
[900] J. B. Howard, M. D. Fausch, and G. L. Nelsestuen, *J. Biol. Chem.*, 1975, **250**, 6178.
[901] P. V. Hauschka, J. B. Lian, and P. M. Gallop, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3925.
[902] M. R. Downing, R. J. Butkowski, M. M. Clark, and K. G. Mann, *J. Biol. Chem.*, 1975, **250**, 8897.
[903] K. Fujikawa, K. Titani, and E. W. Davie, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3359.
[904] T. Takagi and R. F. Doolittle, *Biochim. Biophys. Acta*, 1975, **386**, 617.
[905] T. Takagi and R. F. Doolittle, *Biochemistry*, 1975, **14**, 5149.
[906] D. Collen, B. Kudryk, B. Hessel, and B. Blombäck, *J. Biol. Chem.*, 1975, **250**, 5808.
[907] K. A. Laursen and H. Lee, *F.E.B.S. Letters*, 1975, **56**, 70.
[908] L. Sottrup-Jensen, M. Zajdel, H. Claeys, T. E. Petersen, and S. Magnusson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2577.
[909] B. Wiman and P. Wallen, *European J. Biochem.*, 1975, **58**, 539.
[910] H. Hitz, D. Schafer, and B. Wittmann-Liebold, *F.E.B.S. Letters*, 1975, **56**, 259.
[911] R. Chen and B. Wittmann-Liebold, *F.E.B.S. Letters*, 1975, **52**, 139.
[912] M. Yaguchi, *F.E.B.S. Letters*, 1975, **59**, 217.

Table 4 (*cont.*)

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
L18	*E. coli*	117	complete sequence	913
L25	*E. coli*	94	complete sequence	914
L27	*E. coli*	84	complete sequence	915
L30	*E. coli*	58	complete sequence	916
Tu	*E. coli*	42	peptide important in GTP binding	917
Structural proteins				
Collagen	chick skin	693	CNBr peptides of α_1-chain; known structure entirely of (Gly XY) type	919—922
	calf skin (α1)	120	sequences of three CNBr peptides	923
	calf skin (α2)	321	sequence of CNBr peptide	924, 925
	calf bone	4	sequence represents cross-link between α_1- and α_2-chains	926
	rabbit	43	sequences of CNBr peptides of α_1- and α_2-chains	927
	guinea-pig	61	sequences of CNBr peptides of α_2-chain	928
Crystallin α-chain	human	173	complete sequence: partial comparative sequences of pig, horse, dog, cat, rabbit, rat, and rhesus monkey	929, 930
Keratin	angora sheep	97	high sulphur protein SCMKB-M1.2; complete sequence	931
Tropoelastin	chick	42	amino-terminal sequence	932
Globins				
α-Chain mutants				
Arya	human		α47, Asp → Asn	934
Titusville	human		α94, Asp → Asn	935
Hirosaki	human		α43, Phe → Leu	936
Antharaj	human		α11, Lys → Glu	937
Strumica (Serbia)	human		α112, His → Arg	938, 939
J. Paris I	human		α12, Ala → Asp	938
Montgomery	human		α48, Leu → Arg	946
Norfolk	human		α85, Asp → Asn	940
β-Chain mutants				
Djelfa	human		β98, Val → Ala	941

Wood	human	β97, His → Leu	942
Fort Gordon	human	β145, Tyr → Asp	943
C. Ziguinchor	human	β6, Glu → Val, β58, Pro → Arg	944

[913] J. Brosius, E. Schiltz, and R. Chen, *F.E.B.S. Letters*, 1975, **56**, 359.
[914] N. V. Douglas, L. F. Markova, T. A. Mednikova, L. M. Vinokurov, Y. B. Alakhov, and Y. B. Ovchinnikov, *F.E.B.S. Letters*, 1975, **53**, 351.
[915] R. Chen, L. Mende, and U. Arfsten, *F.E.B.S. Letters*, 1975, **59**, 96.
[916] E. Ritter and B. Wittmann-Liebold, *F.E.B.S. Letters*, 1975, **60**, 153.
[917] M. Wade, R. A. Laursen, and D. L. Miller, *F.E.B.S. Letters*, 1975, **53**, 37.
[918] T. Housley, M. L. Tanzer, E. Henson, and P. M. Gallop, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 824.
[919] S. N. Dixit, A. H. Kang, and J. Gross, *Biochemistry*, 1975, **14**, 1929.
[920] S. N. Dixit, J. M. Seyer, A. O. Oronsky, C. Corbett, A. H. Kang, and J. Gross, *Biochemistry*, 1975, **14**, 1933.
[921] J. H. Highberger, C. Corbett, A. H. Kang, and J. Gross, *Biochemistry*, 1975, **14**, 2872.
[922] A. H. Kang, S. N. Dixit, C. Corbett, and J. Gross, *J. Biol. Chem.*, 1975, **250**, 7428.
[923] P. P. Fietzek and K. Kuhn, *European J. Biochem.*, 1975, **52**, 77.
[924] F. W. Rexrodt, P. P. Fietzek, and K. Kuhn, *European J. Biochem.*, 1975, **59**, 105.
[925] P. P. Fietzek and F. W. Rexrodt, *European J. Biochem.*, 1975, **59**, 113.
[926] K. Fujii, D. Corcoran, and M. L. Tanzer, *Biochemistry*, 1975, **14**, 4409.
[927] U. Becker, P. P. Fietzek, H. Furthmayr, and R. Timpl, *European J. Biochem.*, 1975, **54**, 359.
[928] C. C. Clark, P. P. Fietzek, and P. Bornstein, *European J. Biochem.*, 1975, **56**, 327.
[929] W. W. de Jong, E. C. Terwindt, and H. Blomendal, *F.E.B.S. Letters*, 1975, **58**, 311.
[930] W. W. de Jong, F. J. van der Ouderaa, M. Versteeg, G. Groenwoud, J. M. van Amelsvoort, and H. Bloemendal, *European J. Biochem.*, 1975, **53**, 237.
[931] D. Parris and K. S. Swart, *Biochem. J.*, 1975, **145**, 459.
[932] J. A. Foster, R. Shapiro, P. Voynow, G. Crombie, G. Faris, and C. Franzblau, *Biochemistry*, 1975, **14**, 5343.
[933] H. F. Bunn, G. J. Schmidt, D. N. Haney, and R. G. Dluhy, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3609.
[934] S. Rahbar, N. Mahdavi, G. Nowzari, and I. Mostafavi, *Biochim. Biophys. Acta*, 1975, **386**, 525.
[935] R. G. Schneider, R. J. Atkins, T. S. Hosty, G. Tomlin, R. Casey, H. Lehmann, P. A. Lorkin, and K. Nagai, *Biochim. Biophys. Acta*, 1975, **400**, 365.
[936] Y. Ohba, T. Muyaji, M. Matsuoka, M. Yokoyama, H. Numakura, K. Nagata, Y. Takebe, Y. Izumi, and S. Shibata, *Biochim. Biophys. Acta*, 1975, **405**, 155.
[937] S. Pootrakul, B. Kematorn, S. Na-Nakorn, and S. Suanpan, *Biochim. Biophys. Acta*, 1975, **405**, 161.
[938] G. A. Niazi, G. D. Emfremov, N. Nicolov, E. Hunter, jun., and T. H. J. Huisman, *Biochim. Biophys. Acta*, 1975, **412**, 181.
[939] D. Beksedić, T. Rajevska, P. A. Lorkin, and H. Lehmann, *F.E.B.S. Letters*, 1975, **58**, 228.
[940] P. A. Lorkin, R. G. Huntsman, J. A. M. Ager, H. Lehmann, F Vella, and P. D. Darbre, *Biochim. Biophys. Acta*, 1975, **379**, 22.
[941] G. Gacon, H. Wajcman, D. Labie, and A. Cosson, *F.E.B.S. Letters*, 1975, **58**, 238.
[942] F. Taketa, Y. P. Huang, J. A. Libnoch, and B. H. Dessel, *Biochim. Biophys. Acta*, 1975, **400**, 348.
[943] H. B. Kleckner, J. B. Wilson, J. G. Lindeman, P. D. Stevens, G. Niazi, E. Hunter, C. J. Chen, and T. H. J. Huisman, *Biochim. Biophys. Acta*, 1975, **400**, 343.
[944] M. Goossens, M. C. Garel, J. Auvinet, P. Basset, P. F. Gomes, and J. Rosa, *F.E.B.S. Letters*, 1975, **58**, 149.

Table 4 (*cont.*)

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
Castilla	human		β32, Leu → Arg	945
Newcastle	human		β92, His → Pro	946
Alabama	human		β39, Glu → Lys	947
Hamadan	human		β56, Gly → Arg	948
Olmstead	human		β141, Leu → Arg	949
Atlanta	human		β75, Leu → Pro	950
Saki	human		β14, Leu → Pro	951
Camperdown	human		β104, Arg → Ser	952
F. Victoria Jubilee	human		$^{A}\gamma$80, Asp → Tyr	953
Coburg	human		δ116, Arg → His	954
α-chain	rat		peptides aligned by homology to mouse and rabbit α-chains	955, 956
β-chain	*Rana catesbuana* (tadpole)	35	amino-terminal sequence; tadpole chains six residues longer than frog chains	957
Myoglobin	*Inia geoffrensis*	153	complete sequence	958
	Didelphis marsupialis	153	complete sequence	959
	Erinaceus europaeus	153	complete sequence	960
	chick	153	complete sequence	961
	gorilla	153	one difference from human Ser-22 → Pro	962
Muscle proteins				
Actin	rabbit muscle	374	complete sequence	964—967
Myosin light chain	bovine heart	80	partial sequence peptides aligned by homology	968
Paramyosin	*Pectin maximus*	16	cysteine containing peptides	963
Parvalbumin	rabbit muscle	109	complete sequence	969
	frog	108	complete sequence	970
Troponin C	bovine heart	161	complete sequence three Ca^{2+} binding sites	971
Troponin I	rabbit muscle	179	complete sequence	972
Troponin T	rabbit muscle	40	partial sequence of CNBr peptides	973
Miscellaneous				
Albumin	human serum	585	different groups report different sequences	975—977
Calcium-binding protein	bovine intestine	85	complete sequence: contains calcium-binding sequence	978
Complement protein C1s	human	8	amino-terminal sequence of C1s and activated $C1\bar{s}$	979

945 M. C. Garel, Y. Blouquit, J. Rosa, and C. R. Garcia, *F.E.B.S. Letters*, 1975, **58**, 145.
946 R. Finney, R. Casey, H. Lehmann, and W. Walker, *F.E.B.S. Letters*, 1975, **60**, 435.
947 B. Brimhall, R. T. Jones, R. G. Schneider, T. S. Hosty, G. Tomlin, and R. Atkins, *Biochim. Biophys. Acta*, 1975, **379**, 28.
948 S. Rahbar, G. Nowzari, H. Haydari, and P. Daneshmand, *Biochim. Biophys. Acta*, 1975, **379**, 645.
949 P. A. Lorkin, H. Lehmann, and V. F. Fairbanks, *Biochim. Biophys. Acta*, 1975, **386**, 256.
950 M. Hubbard, E. Winton, J. G. Lindeman, P. L. Dessauer, J. B. Wilson, R. N. Wrightsone, and T. H. J. Huisman, *Biochim. Biophys. Acta*, 1975, **386**, 538.
951 Y. Beuzard, P. Basset, F. Braconnier, H. E. Gammal, L. Martin, J. L. Oudard, and J. Thillet, *Biochim. Biophys. Acta*, 1975, **393**, 182.
952 T. Wilkinson, C. G. Chua, R. W. Carrel, H. Robin, T. Exner, K. M. Lee, and H. Kronenberg, *Biochim. Biophys. Acta*, 1975, **393**, 195.
953 E. Ahern, W. Holder, V. Ahern, G. R. Serjeant, B. E. Serjeant, M. Forbes, B. Brimhall, and R. T. Jones, *Biochim. Biophys. Acta*, 1975, **393**, 188.
954 R. S. Sharma, L. Williams, J. B. Wilson, and T. H. J. Huisman, *Biochim. Biophys. Acta*, 1975, **393**, 379.
955 C. G. Chua, R. W. Carrell, and B. H. Howard, *Biochem. J.*, 1975, **149**, 259.
956 L. M. Garrick, V. S. Sharma, N. J. McDonald, and H. M. Ranney, *Biochem. J.*, 1975, **149**, 245.
957 K. W. K. Watt and A. Riggs, *J. Biol. Chem.*, 1975, **250**, 5934.
958 F. E. Dwulet, R. A. Bogardt, B. N. Jones, L. D. Lehman, and F. R. N. Gurd, *Biochemistry*, 1975, **24**, 5336.
959 A. E. Romero-Herrera and H. Lehmann, *Biochim. Biophys. Acta*, 1975, **400**, 387.
960 A. E. Romero-Herrera, H. Lehmann, and W. Fakes, *Biochim. Biophys. Acta*, 1975, **379**, 13.
961 M. Deconinck, S. Peiffer, C. Paul, A. G. Schnek, and J. Leonis, *Biochim. Biophys. Acta*, 1975, **386**, 567.
962 A. E. Romero-Herrera, H. Lehmann and D. Fossey,, *Biochim. Biophys. Acta*, 1975, **393**, 383.
963 I. D. Walker and M. Stewart, *F.E.B.S. Letters*, 1975, **58**, 16.
964 W. M. Kuehl, M. A. Conti, and R. S. Adelstein, *J. Biol. Chem.*, 1975, **250**, 5890.
965 J. H. Collins and M. Elzinga, *J. Biol. Chem.*, 1975, **250**, 5906.
966 M. Elzinga and J. H. Collins, *J. Biol. Chem.*, 1975, **250**, 5897.
967 J. H. Collins and M. Elzinga, *J. Biol. Chem.*, 1975, **250**, 5915.
968 A. G. Weeds, *F.E.B.S. Letters*, 1975, **59**, 203.
969 D. L. Enfield, L. H. Ericsson, H. E. Blum, E. H. Fischer, and H. Neurath, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1309.
970 J. Capony, J. Demaille, C. Pina, and J. Pechère, *European J. Biochem.*, 1975, **56**, 215.
971 J. van Eerd and K. Takahashi, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 122.
972 J. M. Wilkinson and R. J. A. Grand, *Biochem. J.*, 1975, **149**, 493.
973 P. Jackson, G. W. Amphlett, and S. V. Perry, *Biochem. J.*, 1975, **151**, 85.
974 J. H. Russel and D. M. Geller, *J. Biol. Chem.*, 1975, **250**, 3409.
975 B. Meloun, M. A. Saber, and J. Kušnír, *Biochim. Biophys. Acta*, 1975, **393**, 505.
976 B. Meloun, L. Moravek, and V. Kostka, *F.E.B.S. Letters*, 1975, **58**, 134.
977 K. K. Gambhir, R. H. McMenamy, and F. Watson, *J. Biol. Chem.*, 1975, **250**, 6711.
978 W. Huang, D. V. Cohn, J. W. Hamilton, C. Fullmer, and R. H. Wasserman, *J. Biol. Chem.*, 1975, **250**, 7647.
979 K. Takahashi, S. Nagasawa, and J. Koyama, *F.E.B.S. Letters*, 1975, **50**, 330.

Table 4 (*cont.*)

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
C3a (anaphylatoxin)	human	77	pig and human amino-terminal sequences 80% homologous	980, 981
Electron transport proteins				
Cytochrome b^2	yeast	14	amino-terminal sequence	982
Cytochrome *c*	*Euglena gracilis*	98	complete sequence	983
	Crithidia oncopelti	111	complete sequence	983
	Rhodospirillum rubrum	126	complete sequence	984
	mouse testes		thirteen differences from somatic form	985
	Crithidia fasciculata	103	complete sequence	986
Cytochrome *f*	*Spirulina maxima*	89	complete sequence	987
	Alaria esculenta	86	complete sequence	988
Ferredoxin	*Spirulina maxima*	98	complete sequence	989, 991
	Spirulina platensis	98	complete sequence	990
	Chlorobium limicola	61	complete sequence	992
	Equisetum	31	amino-terminal sequence (18) and cysteine-containing peptide (13)	993
Plastocyanin	*Spinacia oleracea*	99	complete sequence	994
	Anabaena variabilis	105	complete sequence	995
Glycophorin	human erythrocyte	131	complete sequence	996
Lipoprotein C-1	human serum	57	complete sequence	1013
Apolipoprotein (Apo A-I)	human serum	245	complete sequence	1014, 1015
Bromelain inhibitor	pineapple stem	52	complete sequence	1016
Carboxypeptidase inhibitor	potato	39	complete sequence	1017
Trypsin inhibitor	*Phaseolus vulgaris*	71	partial sequence	1018
Protease inhibitor	*Helix pomatia*	58	complete sequence	1019
β2-microglobulin	rabbit	35	amino-terminal sequence 30/35 positions identical with human	998
Allergen Ra5	ragweed pollen	45	sensitivity of allergen associated with HL-A phenotype A-7	999
Histone H5	chick	91	complete sequence	1000, 1001
Basic protein	rat testis	54	complete sequence	1002
Lac repressor	*E. coli*	347	complete sequence	1013
Leghaemoglobin I	*Vicia faba* L.	143	complete sequence: polymorphism at 14 positions	1007
Leghaemoglobin component a	*Phaseolus vulgaris*	145	complete sequence	1008

[980] T. E. Hugli, *J. Biol. Chem.*, 1975, **250**, 8293.
[981] T. E. Hugli, E. H. Vallota, and H. J. Muller-Eberhard, *J. Biol. Chem.*, 1975, **250**, 1472.
[982] B. Guiard, F. Lederer, and C. Jacq, *Nature*, 1975, **255**, 423.
[983] G. W. Pettigrew, J. L. Leaver, T. E. Meyer, and A. P. Ryle, *Biochem. J.*, 1975, **147**, 291.
[984] T. E. Meyer, R. P. Ambler, R. G. Bartsch, and M. D. Kamen, *J. Biol. Chem.*, 1975, **250**, 8416.
[985] B. Hennig, *European J. Biochem.*, 1975, **55**, 167.
[986] G. C. Hill and G. W. Pettigrew, *European J. Biochem.*, 1975, **57**, 265.
[987] R. P. Ambler and R. G. Bartsch, *Nature*, 1975, **253**, 285.
[988] M. V. Laycock, *Biochem. J.*, 1975, **149**, 271.
[989] M. Tanaka, M. Haniu, K. T. Yasunobu, K. K. Rao, and D. O. Hall, *Biochemistry*, 1975, **14**, 5535.
[990] K. Wada, T. Hase, H. Tokunaga, and H. Matsubara, *F.E.B.S. Letters*, 1975, **55**, 102.
[991] M. Tanaka, M. Haniu, S. Zeitlin, K. T. Yasunobu, M. C. W. Evans, K. K. Rao, and D. O. Hall, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 399.
[992] M. Tanaka, M. Haniu, K. T. Yasunobu, M. C. W. Evans, and K. K. Rao, *Biochemistry*, 1975, **14**, 1938.
[993] H. Kagamiyama, K. K. Rao, D. O. Hall, R. Cammack, and H. Matsubara, *Biochem. J.*, 1975, **145**, 121.
[994] M. D. Scawen, J. A. M. Ramshaw, and D. Boulter, *Biochem. J.*, 1975, **147**, 343.
[995] A. Aitken, *Biochem. J.*, 1975, **149**, 675.
[996] M. Tomita and V. T. Marchesi, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2964.
[997] J. Silver and L. Hood, *Nature*, 1975, **256**, 63.
[998] B. A. Cunningham and I. Berggard, *Science*, 1975, **187**, 1079.
[999] L. E. Mole, L. Goodfriend, C. B. Lapkoff, J. M. Kehoe, and J. D. Capra, *Biochemistry*, 1975, **14**, 1216.
[1000] P. Sauiter, D. Kmiecik, O. Loy, G. Briand, G. Biserte, A. Garel, and M. Champagne, *F.E.B.S. Letters*, 1975, **50**, 200.
[1001] A. Garel, A. Mazen, H. Champagne, P. Sautiere, D. Kmiecik, O. Loy, and G. Biserte, *F.E.B.S. Letters*, 1975, **50**, 195.
[1002] W. S. Kistler, C. Noyes, R. Hsu, and R. L. Heinrikson, *J. Biol. Chem.*, 1975, **250**, 1847.
[1003] K. Beyreuther, K. Adler, E. Fanning, C. Murray, A. Klemm, and N. Geisler, *European J. Biochem.*, 1975, **59**, 491.
[1004] K. E. Langley, A. V. Fowler, and I. Zabin, *J. Biol. Chem.*, 1975, **250**, 2587.
[1005] K. E. Langley, M. R. Villarejo, A. V. Fowler, P. J. Zamenhof, and I. Zabin, *Proc. Nat. Acad. Sci.*, 1975, **72**, 1254.
[1006] J. G. Files, K. Weber, C. Coulondre, and J. H. Miller, *J. Mol. Biol.*, 1975, **95**, 327.
[1007] M. Richardson, M. J. Dilworth, and M. D. Scawen, *F.E.B.S. Letters*, 1975, **51**, 33.
[1008] P. Lehtovaara and N. Ellfolk, *European J. Biochem.*, 1975, **54**, 577.
[1009] J. L. Wang, B. A. Cunningham, M. J. Waxdal, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1490.
[1010] B. A. Cunningham, J. L. Wang, M. J. Waxdal, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1503.
[1011] R. Lotan, R. Cacan, M. Cacan, H. Debray, W. G. Carter, and N. Sharon, *F.E.B.S. Letters*, 1975, **57**, 100.
[1012] J. B. Miller, R. Hsu, R. Henrikson, and S. Yachnin, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1388.
[1013] R. S. Shulman, P. N. Herbert, K. Wearly, and D. S. Fredrickson, *J. Biol. Chem.*, 1975, **250**, 182.
[1014] T. Delahunty, H. N. Baker, A. M. Gotto, jun., and R. L. Jackson, *J. Biol. Chem.*, 1975, **250**, 2718.
[1015] H. N. Baker, A. M. Gotto, jun., and R. L. Jackson, *J. Biol. Chem.*, 1975, **250**, 2725.
[1016] N. N. Reddy, P. S. Keim, R. L. Heinrikson, and F. J. Kezdy, *J. Biol. Chem.*, 1975, **250**, 1741.
[1017] G. M. Hass, H. Nau, K. Biemann, D. T. Grahn, L. H. Ericsson, and H. Neurath, *Bio- chemistry*, 1975, **14**, 1334.
[1018] K. A. Wilson and M. Laskowski, *J. Biol. Chem.*, 1975, **250**, 4261.
[1019] H. Tschesche and T. Dietl, *European J. Biochem.*, 1975, **58**, 439.

Table 4 (*cont.*)

Protein	*Source*	*No. of residues*	*Notes*	*Ref.*
Concanavalin A	*Canavalia ensiformis*	237	complete sequence	1009, 1010
Ricin	*Ricinus communis*		amino-terminal sequence of A(28 residues) and B(14) chains	1020
Soyabean agglutinin		10	amino-terminal sequence	1011
Phytohaemagglutinin	*Phaseolus vulgaris*		amino-terminal sequences of L and R chains examined; first eight residues differ between chains but residues 9—24 are identical	1012
Phycocyanin	*Cyanidium caldarum*		amino-terminal sequences of both α and β phycocyanin chains; little homology between first 10 residues but residues 11—27 in α and β chains identical	1021
Toxins				
Tuberculin-active protein	*Myobacterium tuberculosis*	89	complete sequence	1022
Venom protein	*Naja melanoleuca*	61	complete sequence	1023
Toxin component S4 C11	*Naja melanoleuca* venom	65	complete sequence	1024
Toxin III	*Naja haje* venom	72	complete sequence	1025
Cardiotoxin	*Naja naja* venom	60	complete sequence	1026
Cardiotoxin (CTx) – analogue I		60	complete sequence	1027
Cardiotoxin	*Naja nigricollis* venom	60	complete sequence	1028
Neurotoxin I	*Centruroides sculpturatus*	64	complete sequence	1029
Cholera toxin	*Vibrio cholera*	53	sequences of cysteine-containing peptides of A1, A2, and B chains	1030
Melittin	*Apis dorsata*	26	complete sequence	1031
Presynaptic neurotoxin	*Notechus scutatus scutatus*	119	complete sequence – homologous to phospholipase	1032
Phospholipase (DE III)	*Naja melanoleuca*	119	complete sequence	1033
Phospholipase DE I and DE II	*Naja melanoleuca*	118 (DE I) 119 (DE II)	complete sequences	1034
Phospholipase A2-α	*Crotalus adamanteus*	54	amino-terminal sequence	1035
Phospholipase A	*Hemachatus hemachatus*	119	complete sequence	1036
Transferrin	human serum		partial sequence	1037, 1038

Transferrin			sequences of glycopeptides from human, bovine, porcine, and duck reported	1039
Ovotransferrin	chick	94	glycopeptide	1040
Retinol-binding protein			amino-terminal 50 residues of human and dog sequences reported	1041
Viral proteins				
Group specific antigen (p30)			amino-terminal sequences of C-type virus antigens from cat, mouse, rat, gibbon, and baboon	1042, 1043
TMV coat protein	cowpea strain	161	complete sequence	1044
PFL coat protein	Pf1 virus	46	complete sequence	1045
Qβ host factor	Qβ	5	amino-terminal sequence	1047

[1020] S. S. Li, C. H. Wei, J. Lin, and T. Tung, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 1191.
[1021] R. F. Troxler, J. A. Foster, A. S. Brown, and C. Franzblau, *Biochemistry*, 1974, **14**, 268.
[1022] S. Kuwabara, *J. Biol. Chem.*, 1975, **250**, 2563.
[1023] R. A. Shipolini, M. Kissonerghis, and B. E. C. Banks, *European J. Biochem.*, 1975, **56**, 449.
[1024] F. H. H. Carlsson, *Biochim. Biophys. Acta*, 1975, **400**, 310.
[1025] C. Kopeyan, F. Miranda, and H. Rochat, *European J. Biochem.*, 1975, **58**, 117.
[1026] L. Fryklund and D. Eaker, *Biochemistry*, 1975, **14**, 2860.
[1027] K. Hayashi, M. Takechi, T. Sasaki, and C. Y. Lee, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 360.
[1028] L. Fryklund and D. Eaker, *Biochemistry*, 1975, **14**, 2065.
[1029] D. R. Babin, D. D. Watt, S. M. Goos, and R. V. Mlejnek, *Arch. Biochem. Biophys.*, 1975, **166**, 67.
[1030] E. Mendez, C. Y. Lai, and A. Wondar-Flipowicz, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1435.
[1031] G. Kreil, *F.E.B.S. Letters*, 1975, **54**, 101.
[1032] J. Halpert and D. Eaker, *J. Biol. Chem.*, 1975, **250**, 6990.
[1033] F. J. Joubert, *Biochim. Biophys. Acta*, 1975, **379**, 329.
[1034] F. J. Joubert, *Biochim. Biophys. Acta*, 1975, **379**, 345.
[1035] F. H. C. Tsao, P. S. Keim, and R. L. Heinrikson, *Arch. Biochem. Biophys.*, 1975, **167**, 706.
[1036] F. J. Joubert, *European J. Biochem.*, 1975, **52**, 539.
[1037] R. T. A. MacGillivray and K. Brew, *Science*, 1975, **190**, 1306.
[1038] M. R. Sutton, R. T. A. MacGillivray, and K. Brew, *European J. Biochem.*, 1975, **51**, 43.
[1039] I. Graham and J. Williams, *Biochem. J.*, 1975, **145**, 263.
[1040] I. B. Kingston and J. Williams, *Biochem. J.*, 1975, **147**, 463.
[1041] M. D. Poulik, D. Farrah, G. H. Malek, C. J. Shinnick, and O. Smithies, *Biochim. Biophys. Acta*, 1975, **412**, 326.
[1042] S. Oroszlan, T. Copeland, M. R. Summers, G. Smythers, and R. V. Gilden, *J. Biol. Chem.*, 1975, **250**, 6232.
[1043] S. Oroszlan, M. R. Summers, and R. V. Gilden, *Virology*, 1975, **64**, 581.
[1044] M. W. Rees and M. N. Short, *Biochim. Biophys. Acta*, 1975, **393**, 15.
[1045] Y. Nakashima, R. L. Wiseman, W. Konigsberg, and D. A. Marvin, *Nature*, 1975, **253**, 68.
[1046] J. J. Skehel and M. D. Waterfield, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 93.
[1047] G. G. Carmichael, K. Weber, A. Niveleau, and A. J. Wahba, *J. Biol. Chem.*, 1975, **250**, 3607.

both enzymes but that small differences in three-dimensional structure are sufficient to totally alter the preference of the NAD analogue towards cysteine-174 in horse and cysteine-43 in yeast.

Carboxypeptidase B. The complete sequence of bovine carboxypeptidase B was recently reported [791] and not surprisingly shows extensive homology to carboxypeptidase A. Examination of the sequence of carboxypeptidase B with reference to the known three-dimensional structure of carboxypeptidase A indicated that an isoleucine residue (Ile-255), which in carboxypeptidase A is located at the pit of the side-chain binding pocket, is substituted for an aspartic acid residue in carboxypeptidase B. The authors suggest that positively charged lysine or arginine side-chains could form a salt bridge with the carboxylate group of Asp-255, thereby stabilizing the interaction of basic side-chains with the enzyme and altering its specificity in the desired manner. The residues believed to bind to zinc ions in carboxypeptidase A are preserved in unaltered positions in carboxypeptidase B, and the only residue known to be part of the active site which differs between the A and B enzymes is Ile/Asp at position 255.

Phosphorylase Kinase. Glycogenolysis in muscle tissue is stimulated by the hormone adrenalin which produces a cascade of events resulting finally in rapid glycogen breakdown. One of the intermediate events is the activation of phosphorylase kinase, the enzyme responsible for the conversion of inactive glycogen phosphorylase into its active form. Phosphorylase kinase is composed of α- and β-subunits, both of which can be phosphorylated either *in vivo* or *in vitro*. Cohen *et al.*[821] have isolated the peptides phosphorylated *in vitro* by cyclic AMP (c-AMP) dependent protein kinase, and found that only one site was phosphorylated on each type of subunit. No obvious sequence similarities were apparent between the phosphorylated α- and β-chain sequences which were:

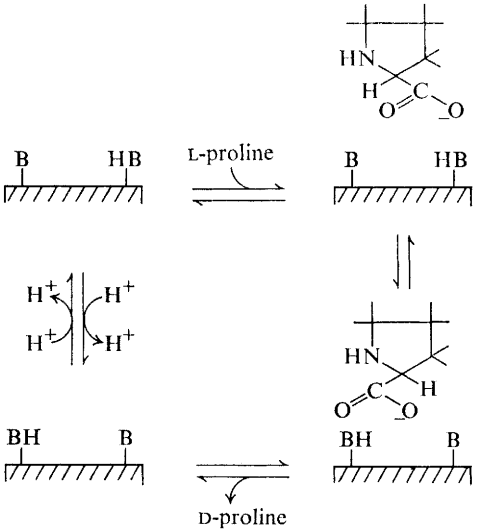
Arg-Leu-Ser(P)-Ile-Ser-Thr-Glu-Ser-Glx-Pro-Asx-Gly (α)

Glu-Ser-Gly-Ser(P)$\left\{\begin{matrix}\text{Val}\\\text{Ile}\end{matrix}\right\}$Tyr-Pro-Leu-Lys ($\beta$)

The fact that only two out of a total of 200 serine residues in phosphorylase kinase were phosphorylated indicated a very high degree of specificity in the c-AMP dependent protein kinase catalysed reaction, and the authors point out that since the α- and β-chain phosphopeptides are so different in sequence, the three-dimensional structure must be of paramount importance in native enzyme for determining the susceptibility of only two serine residues to phosphorylation.

Adrenalin administered to living rabbits was shown [822] to result in the phosphorylation of the same two serine residues in α- and β-chains of phosphorylase kinase as does the c-AMP dependent protein kinase *in vitro*, establishing that the protein kinase is the enzyme responsible for *in vivo* phosphorylation. These experiments remove any doubts as to whether c-AMP dependent protein kinase has an important physiological role and add important molecular details necessary for the understanding of the adrenalin-induced stimulation of glycogenolysis.

Proline Racemase. Interconversion of the D- and L-forms of proline by the proton abstraction mechanism shown in Scheme 8 is catalysed by the enzyme



Scheme 8

proline racemase. Two bases are important in catalysis, one to remove the α-proton of proline and one, as its conjugate acid, to supply a proton from the other side of the α-carbon. Rudnick and Abeles [824] have demonstrated that although proline racemase is a dimeric enzyme of identical subunits, it contains only one active site. Two moles of iodoacetate per mole of dimer were shown to be rapidly incorporated resulting in a complete loss of activity, which could be prevented by the competitive inhibitor pyrrole-2-carboxylate indicating the importance and proximity of the residues modified to the active site of the enzyme. Only one radioactive peptide was present in digests of proline racemase, previously labelled with [^{14}C]iodoacetate, indicating that the same residue was modified in both subunits of the dimer. The sequence of this peptide was

Ser-Pro(Cm)-Cys-Gly-Thr

indicating that an equivalent cysteine residue was modified in both subunits. The authors conclude that the active site of proline racemase is a composite from two identical subunits each providing an equivalent cysteine residue capable of abstracting or supplying protons at the α-carbon of either D- or L-proline, resulting in racemization.

Pancreatic Enzyme Precursors. Trypsin and chymotrypsin are secreted from the pancreas as zymogens which are subsequently activated by proteolysis. Recently, a precursor of trypsin, even longer than trypsinogen, has been discovered [845] and it appears that the additional residues of the precursor may be important in the process of secretion from the cells which synthesize it. mRNA from pancreatic microsomes was translated in a heterologous cell-free protein synthesizing system and the radioactively labelled proteins were separated by SDS-gel electrophoresis. Five major protein bands were found to be radioactive and the proteins of each were eluted and their amino-terminal sequences determined

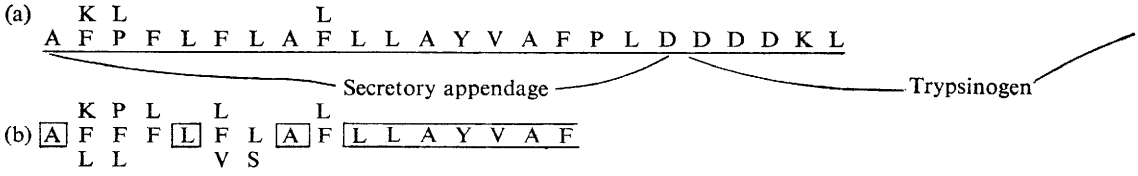


Figure 8 *Amino-terminal sequences of pancreatic precursor proteins.* (a) *Trypsinogen precursor sequence.* (b) *Composite sequence showing alternate residues in different proteins. Residues common to all proteins examined are boxed*
(Reproduced by permission from *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 5016)

(*cf.* Microsequencing section). The sequence of one protein band indicated that it was a lengthened form of trypsinogen containing 18 amino-terminal residues appending the normal trypsinogen sequence (Figure 8). The authors suggest that the function of the appendage is to direct newly synthesized proteins, destined for secretion, to the cell membrane which they must traverse and the sequence of the trypsinogen precursor is certainly hydrophobic enough to bind it to the membrane. The other labelled protein bands have amino-terminal sequences which are all very similar to the trypsinogen appendage (Figure 8). Indeed, nine of the first 16 positions contain invariant residues in all the proteins examined, at least some of which correspond to other known pancreatic secretory proteins. This high conservation of sequence suggests an important and general role in secretion for the amino-terminal appendages.

Hormones.—*Proinsulin.* In proinsulin, the A and B chains of insulin are connected by a C-peptide which bridges amino-terminal B chain with carboxy-terminal A chain. Whereas A and B chain sequences are highly conserved between different species the C-peptide sequences are not. Snell and Smyth [850] have attempted to build models of proinsulin based on amino-acid sequences from 10 different species (Figure 9). They knew that in proinsulin, the C-peptide had to be on the surface, since hexamer formation occurs equally well in both insulin and proinsulin, indicating that the points of intersubunit contact in the interior of the hexamer were not changed by the presence of C-peptide. Using this information, knowledge of the three-dimensional structure of insulin, and empirical rules for predicting secondary structure, an ingenious model was built in which all (but one) of the disparate C-peptide regions could be accommodated into a common structure. One striking feature of the model is that the few highly conserved residues in the C-peptide are spatially close enough to conserved residues in the A or B chains for stable interactions to occur in accord with the view that the C-peptide sequence is important in directing correct folding of proinsulin before its excision. The C-peptide in the proinsulin model also shields residues which are normally accessible in insulin and which are known to be important for biological activity. The authors suggest that, since the conserved residues in A and B chains which interact with the C-peptide are those important in biological activity, the same interactions may occur between insulin and its specific receptor on target cells as occur between insulin and C-peptide.

Parathyroid Hormone and its Biosynthetic Precursors. Parathyroid hormones from different species have recently been the subject of extensive investigations at the level of amino-acid sequence. Two sequences of the human hormone, differing at three positions, have been published, and Keutmann *et al.*[858] have used a novel approach to validate their original work. In the original sequence from Niall's laboratory the three residues in dispute were Glu-22, Leu-28, and Asp-30, whereas the other sequence from Brewer's group had Gln-22, Lys-28, and Leu-30. The Glu, Gln ambiguity in particular is difficult to resolve definitively, since partial deamidation of glutamine is common during Edman degradation. To circumvent the problem, tissue slices were incubated with single radioactive amino-acids and labelled parathyroid hormone purified and sequenced. Glutamine-labelled hormone contained little or no radioactivity at position 22, whereas hormone labelled with glutamic acid contained prominent

	BC1	BC2	C1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Human (14, 15)	Arg	Arg	Glu	Ala	Glu	Asp	Leu	Gln	Val	Gly	Gln	Val	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Ser	Leu	Gln	Pro
Monkey (16)			Glu	Ala	Glu	Asp	Pro	Gln	Val	Gly	Gln	Val	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Ser	Leu	Gln	Pro
Guinea-pig (17)			Glu	Leu	Glu	Asp	Pro	Gln	Val	Glu	Gln	Thr	Glu	Leu	Gly	Met	Gly	Leu	Gly	Ala	Gly	Gly	Leu	Gln	Pro
Chinchilla			Glu	Leu	Glu	Asp	Pro	Gln	Val	Gly	Gln	Ala	Asp	Pro	Gly	Val	Val	Pro	Glu	Ala	Gly	Arg	Leu	Gln	Pro
Rat I (18, 19)			Glu	Val	Glu	Asp	Pro	Gln	Val	Pro	Gln	Leu	Glu	Leu	Gly	Gly	Gly	Pro	Glu	Ala	Gly	Asp	Leu	Gln	Thr
Rat II (18, 19)			Glu	Val	Glu	Asp	Pro	Gln	Val	Ala	Gln	Leu	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Ala	Gly	Asp	Leu	Gln	Thr
Horse (19)			Glu	Ala	Glu	Asp	Pro	Gln	Val	Gly	Glu	Val	Glu	Leu	Gly	Gly	Gly	Pro	Gly	Leu	Gly	Gly	Leu	Gln	Pro
Pig (6)			Glu	Ala	Glu	Asn	Pro	Gln	Ala	Gly	Ala	Val	Glu	Leu	Gly	Gly	Gly	Leu	-	-	Gly	Gly	Leu	Gln	Ala
Ox (20, 21), sheep (16)			Glu	Val	Glu	Gly	Pro	Gln	Val	Gly	Ala	Leu	Glu	Leu	Ala	Gly	Gly	Pro	Gly	Ala	Gly	Gly	Leu	-	-
Dog (16)											Asp	Val	Glu	Leu	Ala	Gly	Ala	Pro	Gly	Glu	Gly	Gly	Leu	Gln	Pro

	24	25	26	27	28	29	30	C31	CA1	CA2
Human (14, 15)	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln	Lys	Arg
Monkey (16)	Leu	Ala	Leu	Glu	Gly	Ser	Leu	Gln		
Guinea-pig (17)	Leu	-	-	Gln	Gly	Ala	Leu	Gln		
Chinchilla	Leu	Ala	Leu	Glu	Met	Thr	Leu	Gln		
Rat I (18, 19)	Leu	Ala	Leu	Glu	Val	Ala	Arg	Gln		
Rat II (18, 19)	Leu	Ala	Leu	Glu	Val	Ala	Arg	Gln		
Horse (19)	Leu	Ala	Leu	Ala	Gly	Pro	Gln	Gln		
Pig (6)	Leu	Ala	Leu	Glu	Gly	Pro	Pro	Gln		
Ox (20, 21), sheep (16)	-	-	-	Glu	Gly	Pro	Pro	Gln		
Dog (16)	Leu	Ala	Leu	Glu	Gly	Ala	Leu	Gln		

Figure 9 C-*peptide sequences from* 10 *mammalian species of proinsulin*
(Reproduced by permission from *J. Biol. Chem.*, 1975, **250**, 6291)

amounts at this position. This excludes the initial incorporation of glutamine and its subsequent deamidation; the validity of the approach depends upon the fidelity of the translational apparatus and is not subject to post-synthetic or preparative artefacts. By the same approach leucine was identified at position 28 as predicted by Niall's original work, but not at position 30.

Proparathyroid hormone is a lengthened precursor which contains additional sequences at both the amino and carboxy ends of the normal sequence. The

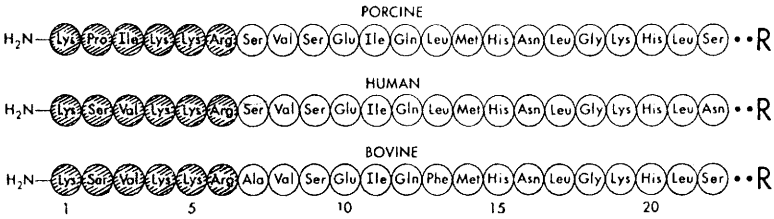


Figure 10 *Partial amino-acid sequences of porcine, human, and bovine proparathyroid hormone. The unshaded residues represent the PTH sequences of the respective species* (Reproduced by permission from *Biochemistry*, 1975, **14**, 3631)

human and bovine 'pro' sequences are identical (ref. 775 and last year's Report, p. 125), and the porcine 'pro' sequence which appeared recently [859] is very similar (Figure 10). The authors suggest that in all three species similar enzymes must exist which are capable of recognizing and cleaving the 'pro' sequence.

An even longer form of the protein, pre-proparathyroid hormone, has been isolated [776] and sequence studies carried out (see Microsequencing section, p. 000). The presence of methionine residues at positions 1, 2, 7, 11, and 14 was established in bovine pre-proparathyroid hormone, which must contain at least 14 more residues at its amino-terminus than proparathyroid hormone.

A protein containing the entire promellitin sequence but elongated at both ends has also been identified recently [860] by sequencing the product of cell-free translation of bee mRNA. The authors suggest that the additional sequences present in elongated promellitin (protomellitin) may be important in the secretion process, and the amino-acid sequence of the peptide appendages may be useful in testing this idea (*cf.* Enzyme section, p. 120).

Immunoglobulins.—*V-Region allotypes.* The rabbit heavy-chain allotypic specificities a1, a2, and a3 were originally identified serologically and were shown to behave as three alleles at a single genetic locus. Sequence analysis of rabbit immunoglobulin heavy chains showed that there were many differences between the variable (V) regions of all three allotypes but it was not clear which of the sequence differences were uniquely associated with allotype differences. It has recently been established that the constant (C) regions of three allotypes are identical in sequence,[867] indicating that the V-region sequence alone must be responsible for the differences between allotypes. The use of alloantisera to detect allotypes can be criticized, since evidence is available to suggest that they recognize not only one sequence but a family of related sequences. To circumvent this problem, Mole [868] devised a peptide mapping system capable of separating

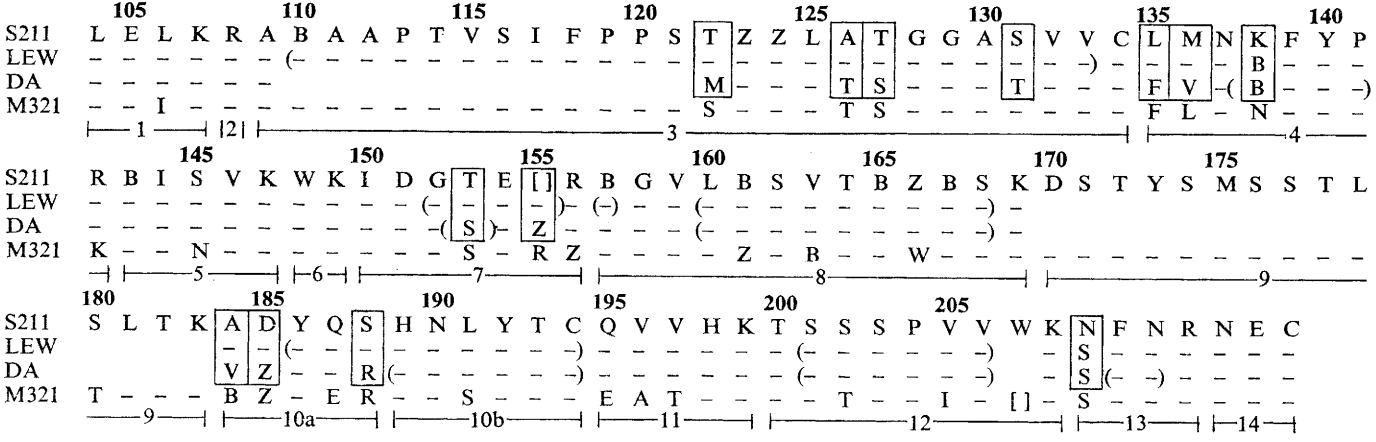


Figure 11 *Amino-acid sequences of rodent κ chain constant regions.* S211 *is a myeloma κ chain from the* LOU *rat,* LEW *and* DA *are sequences from pooled light chains, and* M321 *is a mouse myeloma κ chain. The numbering of peptides is given below the sequences. Differences between* DA *and* LEW *are boxed. The two differences between pooled* LEW *and* S211C_κ *regions are boxed and shaded. Dashes indicate an amino-acid identical with the* S211 *sequence. Parentheses indicate that the sequence of the corresponding residues has not been determined. The one-letter code of Dayhoff is used for the amino-acids*

(Reproduced by permission from *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 5046)

two V-region peptides each associated specifically with one allotype but not with the other. By performing genetic crosses with rabbits of known allotype, it could be established that all rabbits of the a1 allotype possessed a V-region sequence containing glutamic acid at position 85 where the corresponding residue in the a3 allotype was alanine. The author suggests that the use of peptide mapping to detect specific sequences corresponding to allelic variants is a more rigorous means of defining genetic markers than the use of antisera.

C-Region Allotypes. Until recently, different C-region allotypes were explained by allelic genes which each encoded a different but closely related protein sequence. Gutman *et al.*[869] have questioned this view and, on the basis of sequence studies on rat C_κ region allotypes, proposed that allelic control genes exist, each capable of promoting the expression of only one of a number of structural genes for the different C-region allotypes present in every genome. The sequence of three rat C_κ allotypes is compared with that of the mouse C_κ region in Figure 11. The sequences of LEW and DA are of C_κ regions from two different inbred strains of rat homozygous for b and a specificities, respectively, whereas the S211 (LOU) sequence is of C_κ chains of a rat tumour which serologically is also of the b specificity. The LEW and DA chains differ by at least 10 residues whereas LEW and LOU differ by only two residues. Three other models can account for the sequence variations of Figure 11.

In (a) of Figure 12 an ancestral C_κ gene is considered to have accumulated mutations resulting in the divergence of two alleles encoding sequences similar to LEW and DA. A recent gene duplication of the LEW allele is then postulated to have taken place resulting, after further divergence, in LEW and LOU genes. The authors consider this model unlikely, and point out that the degree of divergence between LEW and DA sequences of at least 10 residues is far greater than would be expected for allelic genes. Different abnormal human haemoglobins differ by no more than three residues in 145.

The second model [Figure 12(b)] involves initial gene duplication of an ancestral C_κ region, independent divergence of the two genes into LEW and DA types, and subsequent elimination of one of the genes after divergence to produce a single locus with two much altered alleles. Gene duplication of the LEW allele and divergence into LEW and LOU genes completes the picture. This scheme has the advantage that during the period when LEW and DA were present as non-allelic genes, rapid divergence could take place without the conservation apparently imposed upon different haemoglobin alleles.

The third model postulates that all rats have structural genes encoding each of the allotypes but that expression of a given allotype depends upon the presence of a control gene whose different alleles determine whether DA or LEW (and LOU) will be expressed [Figure 12(c)]. This model, although involving more assumptions than the first two, can explain why, under abnormal circumstances, more than two allotypes can be expressed in a diploid organism.

V—C Joining in Human λ-chains. The Mcg λ-chain is a human Bence-Jones protein whose C-region sequence differs from other known human C-regions by at least three amino-acid substitutions. Two of these substitutions, Asn-116 and Thr-118, occur within a single peptide which can be distinguished by a combination of peptide mapping and sequence analysis from 'normal' peptides

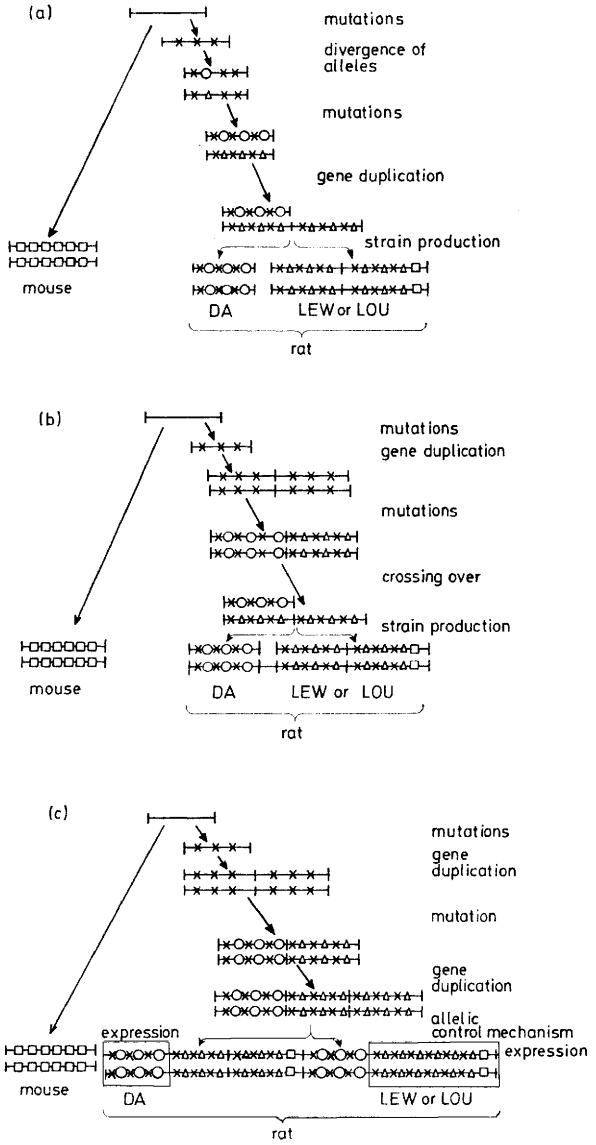


Figure 12 *Genetic models for the expression and evolution of the rat* C_K *allotypes.* (a) *Classical alleles.* (b) *Alleles by crossing over.* (c) *Alleles by gene duplication and a control mechanism*
(Reproduced by permission from *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 5046)

containing Ala-116 and Ser-118. The third substitution (Lys-167 in Mcg and Thr-167 in normal λ-chains) can easily be recognized by peptide mapping alone. Fett and Deutsch [870] have reported experiments in which λ-chains carrying the three characteristic Mcg substitutions have been identified in the serum of four normal individuals studied. Rough measurements suggest that Mcg type chains

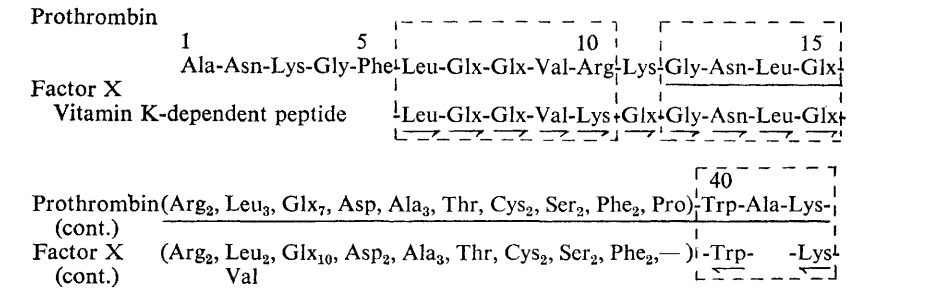


Figure 13 *Partial amino-acid sequence of the vitamin* K*-dependent,* Ca^{2+}*-binding region of factor* X. *The amino-terminal sequence was determined by sequential dansyl-Edman degradation* (→) *and the carboxy-terminal region was determined by carboxypeptidase* A + B *digestion* (←). *Shown for comparison is the amino-terminal region of prothrombin. The underlined portion of the prothrombin sequence designates the barium citrate adsorbing peptide* (*vitamin* K*-dependent peptide*). *The sequence for this peptide in prothrombin is -Gly-Asn-Leu-Glx-Arg-Glx-Glx-Leu-Glx-Glx-Pro-Cys-Cys-Arg-Glx-Glx-Ala-Phe-Leu-Glx-Ser-Leu-Ser-Ala-Asp-Thr-Ala-Phe-Trp-Ala-Lys-. All of the Glx in this portion of prothrombin and most or all of the Glx in this portion of factor* X *are γ-carboxyglutamic acid residues*
(Reproduced by permission from *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1281)

account for *ca.* 25% of λ-chains in normal human serum and indicate that Mcg type λ-chains are normal constituents of human serum and represent a hitherto undetected λ C-region subclass. When Mcg type chains from monoclonal sources were examined, an important observation was made concerning the variable region sequences of this new subclass. Chains with the characteristic Mcg C-region sequence possessed V-region sequences having threonine at position 103 and not glycine as occurs in all other V_λ regions studied to date. The authors suggest that the presence of Thr-103 indicates the presence of a new type of V_λ gene which joins preferentially or exclusively with C_λ regions of the Mcg type. This implies that V_λ and C_λ joining is, to some degree at least, selective and not completely random.

Blood Clotting Proteins.—*Factor X.* A general scheme describing the cascade process involved in blood clotting was described in last year's Report (p. 114). Factor X, a component of this cascade, has previously been shown to be similar to prothrombin in amino-terminal sequence and in its requirement for calcium ions for activity. The isolation of a peptide from factor X, whose sequence resembles that of the calcium-binding peptide of prothrombin, further emphasizes the homology between the two proteins (Figure 13).[895] The authors report that all glutamic residues are present in the peptide as γ-carboxyglutamic acid, as in the prothrombin case. γ-Carboxyglutamic acid was determined by amino-acid analysis after reduction to 5,5′-dihydroxyleucine by treatment with borohydride,

a method which prevents the decarboxylation of γ-carboxyglutamic acid after acidic hydrolysis.[896] The activation of factor X by factor VII and tissue factor has been shown to proceed *via* an initial cleavage close to the amino-terminus and a subsequent autoproteolytic cleavage close to the carboxy-terminus.[897, 903]

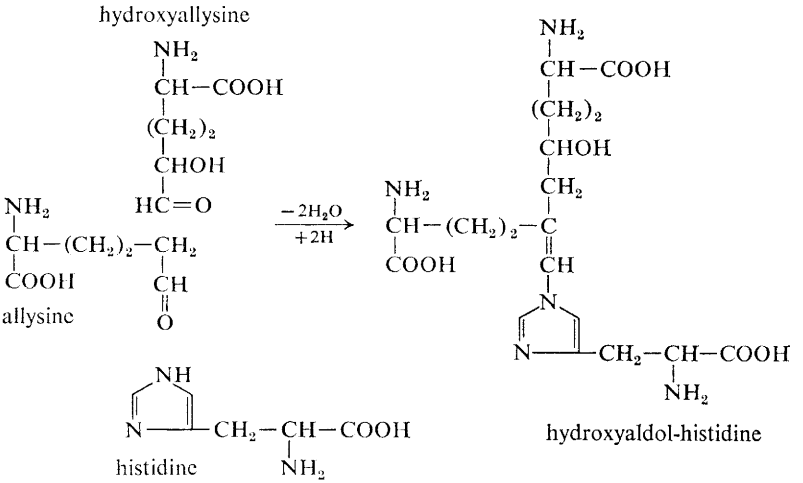


Figure 14 *Postulated origin of hydroxyaldol-histidine by condensation of three amino-acids present in collagen*
(Reproduced by permission from *Biochem. Biophys. Res. Comm.*, 1975, **67**, 824)

Prothrombin. Vitamin K is crucial in the blood clotting process and its role has recently been investigated by the use of dicoumarol, a structural analogue and antagonist of vitamin K. Prothrombin isolated from dicoumarol-treated animals has been shown to lack the 14γ-carboxyglutamic acid residues found in the amino-terminal sequence of normal prothrombin.[898, 899] Otherwise the two prothrombins are indistinguishable and in particular the thrombin parts of both are identical. It therefore appears that the role of vitamin K is to mediate the carboxylation of 14 specific glutamic acid residues in prothrombin which can then be activated in a process which depends upon the binding of calcium ions to some or all of the γ-carboxyglutamic acid residues. The sequence containing the 14 glutamic acid residues which are carboxylated in normal prothrombin has been reported by two groups,[899, 900] but the two sequences differ by four residues although both are from the same species. The activation of human and bovine prothrombin has been compared by sequence analysis of activation intermediates and the sites of cleavage are reported [902] to be very similar in both cases.

γ-Carboxyglutamic acid residues have also been detected [901] in a major protein of bone which may be important in the process of calcification in this tissue.

Ribosomal Proteins.—Wittmann's group and others are systematically studying the proteins of both large and small subunits of *E. coli* ribosomes. Sequences which have recently appeared are listed in Table 4 (p. 129).

Structural Proteins.—*Collagen.* A new cross-link, hydroxyaldol histidine, has recently been isolated [918] from acid hydrolysates of calf skin collagen (Figure 14).

It results from the condensation of two molecules of allysine and one of histidine and could possibly cross-link all three chains of the collagen triple helix. Recent reports dealing with collagen sequences are listed in Table 4 (p. 130).

Globins. Procedures for identifying and characterizing mutant haemoglobins are well established, and new variants of α-, β-, γ-, and δ-chains of human haemoglobin have been reported recently (Table 4). Most of these mutants result from single base changes in the DNA but a new frameshift mutant, haemoglobin Cranston, has also been reported.[933] The carboxy-terminal sequences of normal chains and of Cranston β-chains are

Lys	Tyr	His	Term			normal
AAG	UAU	CAC	UAA			
Lys	Ser	Ile	Thr	Lys	Leu	Cranston
AAG	[AG]U	AUC	ACU	AAG		

The insertion of AG as shown can account for the sequence differences between normal and Cranston β-chains, and the terminator codon UAA is the only one compatible with both amino-acid sequences.

Muscle Proteins.—*Paramyosin.* Paramyosin is a major constituent of mollusc muscle where it forms the backbone of the thick filaments. In native muscle or in paracrystals, paramyosin exists as aggregates of dimers of chains indistinguishable in size by SDS-gel electrophoresis. Recently, sequence heterogeneity has been demonstrated in paramyosin chains from *Pectin maximus* by the isolation and sequence analysis of cysteine-containing peptides.[963] Although all paramyosin chains contain only two cysteine residues, three cysteine-containing peptides A, B, and C were violated from a thermolytic digest in amounts which suggested that peptide C represented a sequence present on all chains, whereas peptides A and B were each present only on 50% of chains. Whether all paramyosin dimers are composed of one chain of each type, or whether two entirely different types of dimer exist was not established, but the two chain types appeared to be present in about equimolar amounts, suggesting that their respective genes were co-ordinately expressed in muscle cells.

Actin. The complete amino-acid sequence of muscle actin has recently appeared,[962–964] and with partial sequence data on cardiac actin no more than one or a few amino-acid substitutions are indicated between the two tissue-specific forms. The authors explain this very high conservation of sequence as a consequence of the need to preserve not only the interior residues of native actin but also those on outside surfaces which must interact with other highly conservative muscle proteins.

Miscellaneous.—*Proalbumin.* Serum albumin, like other proteins destined for export, is synthesized as a precursor which is subsequently converted into the form normally present in blood. The amino-terminal sequence of rat proalbumin has been determined [974] and contains the hexapeptide sequence Arg-Gly-Val-Phe-Arg-Arg, appending the normal amino-terminal sequence of albumin. The authors remark upon the similarity of this sequence to the hexapeptide appendage of proparathyroid hormone (see Hormone section, p. 141). The

distribution of basic amino-acids is certainly similar in both cases and may underlie a related role for both amino-terminal appendages.

Histocompatibility Antigens. Failure of a tissue graft between two individuals is usually due to the ability of the recipient's immune system to recognize and mount a response to the histocompatibility antigens of donor tissue. To understand the factors which underlie the process of graft rejection, histocompatibility antigens of man and mouse have been purified, and recently a report has appeared [997] in which amino-acid sequences of both heavy and light chains of purified H-2 (mouse histocompatibility antigens) have been investigated.

Individual radioactive amino-acids were incorporated into H-2 chains (see Microsequencing, p. 118) which were purified by immunoprecipitation with alloantisera followed by SDS-gel electrophoresis. The light chain was shown to have lysine, valine, and tyrosine residues at positions 3, 9, and 10, respectively, and these are the residues expected for mouse β-microglobulin, a urinary protein previously believed, on the basis of immunological data, to be part of the H-2 complex. The heavy chain was shown to have leucine and tyrosine at positions 5 and 7, respectively. Although far more work is required before those sequences which differ between different allogenic specificities are known, the present work establishes that H-2 antigens at least are unblocked and amenable to automatic sequencing methods.

α-Complementation. Certain mutants of *E. coli* galactosidase possess no enzyme activity but can be complemented by other mutants having an intact amino-terminal region which can restore some activity to the otherwise inactive enzyme. The sequence of a cyanogen bromide peptide of β-galactosidase which can complement mutant enzyme has been shown [1004] to represent residues 3—92 of the normal sequence, thereby implicating at least part of this region in α-complementation. Sequence analysis of the M15 mutant enzyme (inactive but can be complemented) has indicated that residues 11—42 are deleted from the normal sequence.[1005] The importance of the region deleted in the M15 mutant in normal enzyme is apparently not in substrate binding since the mutant enzyme can be purified by affinity chromatography for which an intact substrate binding site is essential. The mutant enzyme cannot form tetramers, however, and the deleted region may be important in establishing stable intersubunit contacts which are probably important for enzyme activity.

Translational Restarts. *N*-Formyl methionine is normally the first residue inserted into most or all newly initiated protein chains in *E. coli.* In certain mutants where translation is prematurely terminated, residues other than methionine are found at the amino-terminus of chains initiated at translational restart sites which are never normally used. Lac operon repressor mutants carrying premature termination codons have previously been shown to reinitiate at a leucine codon corresponding to position 62. The codon corresponding to position 62 in the lac repressor sequence has recently been determined [1006] by sequence analysis of an amber mutant synthesized in a suppressor host in which tyrosine residues are inserted in response to UAG, a normal terminator codon. Analysis of the tryptic peptides of the suppressed amber mutant indicated that tyrosine was present at position 62, indicating an UAG codon at this position in the mutant.

The only codon for leucine, the normal residue at position 62, which can be mutated to UAG in a single step is UUG, which must therefore be the normal codon present and which must be the codon recognized as a translational restart in premature termination mutants.

Influenza Virus Precursor Protein. Cells infected by influenza virus synthesize a virus-specific haemagglutinin which, when isolated from the cell membrane, consists of two subunits, HA1 (mol. wt. 58 000) and HA2 (26 000). Abortive infection of cells by influenza virus results in the accumulation of a chain of mol. wt. 80 000, whose cleavage to the normal smaller subunits is inhibited. The amino-terminal sequences of the precursor, HA1, and HA2 have recently been reported [1046] and they establish that the HA1 sequence occurs at the amino-terminus of the precursor. The amino-terminal sequence of HA2 is

Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly-Phe-Ile

The sequence is a palindrome centred at Ile-6, and the authors point out that it may represent a recognition sequence for the protease which cleaves the precursor chain.

PART II: *X*-Ray Studies *by H. Muirhead*

1 Introduction

Although the major effort in protein crystallography has been concentrated on the structure and function of enzymes, the structures of many molecules of a wider biological interest are being investigated. These include glucagon, the immunoglobulins, concanavalin A, and the purple membrane protein. Both electron microscopy and *X*-ray diffraction have revealed a wealth of information about biological structures. The combination of the two techniques has been particularly important in the study of muscle and viruses. One important advance has been the use of the electron microscope to determine the structure of an unstained biological specimen, the purple membrane protein, at a resolution of 7 Å by combining the information present in electron micrographs and the electron diffraction pattern. With so much material available the choice of emphasis in this review is bound to be somewhat arbitrary.

Crystallographic studies of amino-acids and peptides have given information about the conformation of amino-acid residues and hydrogen-bonding. Complexes of amino-acids with nucleic acids, carbohydrates, and metals can give useful information about the possible interactions of proteins with such substances. Structures which have been determined are listed in Table 1.

Preliminary *X*-ray data have been published for many proteins and these are listed in Table 2, while Table 3 lists those protein structures which have been determined at low resolution.

2 Methods and Techniques

The success of *X*-ray analysis is dependent upon the growth of suitable crystals and this can still be an intractable problem. Koeppe *et al.*[1] describe a pulsed diffusion technique which was used to grow crystals of glutamate–aspartate

[1] R. E. Koeppe, R. M. Stroud, V. A. Pena, and D. V. Santi, *J. Mol. Biol.*, 1975, **98**, 155.

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

Compound	*Space group*	*a*/Å	*b*/Å	*c*/Å	α	β	γ	*Z*	*R*	*Ref.*
L-Phe hydrochloride [a]	$P2_12_12_1$	27.76	7.06	5.38					0.084	*b*
DL-Trp ethyl ester hydrochloride	$P\bar{1}$	15.85	8.73	5.35	82.73	88.51	106.33	2	0.089	*c*
DL-Leu	$P\bar{1}$	14.12	5.39	5.19	111.1	97.0	86.4	2	0.058	*d*
Cyclosarcosyl-L-Val	$P2_12_12_1$	6.52	9.91	14.58				4	0.057	*e*
L-Cysteine [a, f]	$P2_12_12_1$	8.12	12.19	5.43				4	0.046	*g*
L-Cystine dihydrobromide dihydrate [h]	$P2_1$	9.51	13.46	6.02		91.13		2	0.05	*i*
3,3,3′,3′-tetramethyl-D-cystine (D-penicillamine disulphide) dihydrochloride [f]	$P2_12_12_1$	10.51	26.42	6.10				4	0.043	*j*
L-Cystine dimethyl ester dihydrochloride monohydrate	$P2_1$	14.80	9.34	5.85		91.47		2	0.103	*k*
S-Benzyl-L-Cys-*S*-benzyl-L-Cys	$P2_12_12_1$	27.70	15.00	4.86				4	0.061	*l*
Benzoyl-DL-Leu-Gly ethyl ester [m]	$P2_1/n$	15.10	16.63	14.70		91.28		8	0.039	*n*
L-Met-L-Met [o]	$P2_12_12_1$	16.18	16.43	5.07				4	0.051	*p*
L-Ala-L-Ala-L-Ala [q]	$C2$	18.51	5.33	24.78		98.64		8	0.031	*r*
Gly-DL-Thr monohydrate	$Pca2_1$	16.26	4.87	23.24				8	0.108	*s*
Cyclo-L-Pro-Gly [t]	$P2_12_12_1$	9.67	5.78	13.07				4	0.049	*u*
S-Benzyl-L-Cys-L-Pro-L-Leu-Gly-NH_2 [v]	$P2_1$	13.65	8.97	20.27		93.33		2	0.085	*w*
Cyclo-L-Pro-L-Pro-L-Hypro [x]	$P2_12_12_1$	16.28	9.45	9.26				4	0.062	*y*
L-Ser(OBu^t)-β-Ala-Gly-L-Asp-(OMe) [z]	$P2_1$	5.62	10.30	17.86		99.07		2	0.057	*z*
Diglycine selenate [aa]	*Pbca*	18.16	11.15	10.00				8	0.041	*bb*

Bis-(L-Asn)-Cu^{II} [aa]	$P2_1$	5.03	18.93	6.17	96.4		2	0.107	*cc*
Bis-(L-Asp)-Co^{III} [aa]	$P6_3$	15.97		21.16			3	0.054	*dd*
Gly-Gly-Cu^{II} [aa]	$C2/c$	12.49	9.25	13.36	97.68		8	0.038	*ee*
L-Leu-L-Tyr-Cu^{II} [aa]	$P2_12_12_1$	15.55	16.12	8.68			4	0.035	*ff*
Ferrichrysin [x, aa]								0.095	*gg*
Beauvericin–Ba^{2+} [x]	$P2_12_12_1$	28.05	15.79	16.99					*hh*
O-(β-D-xylopyranosyl)-L-Ser [ii]	$P2_1$	6.05	9.53	9.43		94.5		0.058	*jj*
O-(β-D-xylopyranosyl)-L-Ser-Cu^{II} [aa, ii]	$P4_32_12$	9.82		21.28				0.093	*jj*
(Gly-Gly)(cytosine)-Cu^{II} [kk]	$P2_1/n$	10.70	8.08	14.76	92.38		4	0.084	*ll*
1 : 1 Cytidine-*N*-benzyloxycarbonyl-Glu dihydrate [mm]	$P2_1$	29.63	8.43	5.14	91.2		2	0.10	*nn*

(*a*) Neutron diffraction. (*b*) A. R. Al-Karaghouli and T. F. Koetzle, *Acta Cryst.*, 1975, **B31**, 2461. (*c*) B. K. Vijayalakshmi and R. Srinivasan, *Acta Cryst.*, 1975, **B31**, 999. (*d*) B. Di Blasio, C. Pedone, and A. Sirigu, *Acta Cryst.*, 1975, **B31**, 601. (*e*) P. A. Timmins, *Acta Cryst.*, 1975, **B31**, 2561. (*f*) Sulphur involved in short contacts. (*g*) K. A. Kerr, J. P. Ashmore, and T. F. Koetzle, *Acta Cryst.*, 1975, **B31**, 2022. (*h*) Cystine does not possess two-fold symmetry. (*i*) R. E. Rosenfield and R. Parthasarathy, *Acta Cryst.*, 1975, **B31**, 816. (*j*) R. E. Rosenfield and R. Parthasarathy, *Acta Cryst.*, 1975, **B31**, 462. (*k*) B. K. Vijayalakshmi and R. Srinivasan, *Acta Cryst.*, 1975, **B31**, 993. (*l*) S. Capasso, C. Mattia, A. Zagari, and R. Puliti, *Acta Cryst.*, 1975, **B31**, 2466. (*m*) Twisted parallel pleated sheet. (*n*) P. A. Timmins, *Acta Cryst.*, 1975, **B31**, 2240. (*o*) Planar peptide. (*p*) R. E. Stenkamp and L. H. Jensen, *Acta Cryst.*, 1975, **B31**, 857. (*q*) Antiparallel pleated sheet. (*r*) J. K. Fawcett, N. Camerman, and A. Camerman, *Acta Cryst.*, 1975, **B31**, 658. (*s*) P. Swaminathan, *Acta Cryst.*, 1975, **B31**, 1608. (*t*) Refinement of high-angle diffraction data. (*u*) R. B. Von Dreele, *Acta Cryst.*, 1975, **B31**, 966. (*v*) Protected *C*-terminal fragment of oxytocin. (*w*) A. D. Rudko and B. W. Low, *Acta Cryst.*, 1975, **B31**, 713. (*x*) Cyclic peptide. (*y*) G. Kartha and G. Ambady, *Acta Cryst.*, 1975, **B31**, 2035. (*z*) I. L. Karle, B. K. Handa, and C. H. Hassall, *Acta Cryst.*, 1975, **B31**, 555. (*aa*) Amino-acid–metal complex. (*bb*) S. Olejnik, K. Lukaszewicz, and T. Lis, *Acta Cryst.*, 1975, **B31**, 1785. (*cc*) F. S. Stephens, R. S. Vagg, and P. A. Williams, *Acta Cryst.*, 1975, **B31**, 841. (*dd*) I. Oonishi, S. Sato, and Y. Saito, *Acta Cryst.*, 1975, **B31**, 1318. (*ee*) T. J. Kistenmacher and D. J. Szaldo, *Acta Cryst.*, 1975, **B31**, 1659. (*ff*) D. van der Helm, S. E. Ealick, and J. E. Burks, *Acta Cryst.*, 1975, **B31**, 1013. (*gg*) R. Norrestam, B. Stensland, and C.-I. Brändén, *J. Mol. Biol.*, 1975, **99**, 501. (*hh*) J. A. Hamilton, L. K. Steinrauf, and B. Braden, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 151. (*ii*) Carbohydrate–protein interaction. (*jj*) L. T. J. Delbaere, B. Kamenar, and K. Prout, *Acta Cryst.*, 1975, **B31**, 862. (*kk*) Protein–metal–nucleic acid ternary complex. (*ll*) T. J. Kistenmacher, D. J. Szalda, and L. G. Marzilli, *Acta Cryst.*, 1975, **B31**, 2416. (*mm*) Protein–nucleic acid complex. (*nn*) T. Hata, M. Yoshikawa, S. Sato, and C. Tamura, *Acta Cryst.*, 1975, **B31**, 312.

Table 2 *Preliminary X-ray data on proteins*

Protein – source	*Space group*	*a*/Å	*b*/Å	*c*/Å	*β*	*Mol. wt. of asymmetric unit*	*Ref.*
Nerve growth factor – mouse	$P6_122$	56.1		181.4		13 250 ($\frac{1}{2}$ molecule)	*a*
Nontoxin lectin – *Abrus precatorius*	$P4_12_12$	140		210		250 000 (2 molecules)	*b*
Acetylcholinesterase – electric eel	$P6_222$ or $P6_422$	187		292		260 000 (1 molecule)	*c*
Adenylate kinase – carp muscle	$P4_122$ or $P4_322$	86		128		44 000 (2 molecules)	*d*
Venom phospholipase – *C. adamanteus*	$C222_1$	108.1	79.4	63.9		30 000 (1 dimer)	*e*
Venom phospholipase – *C. atrox*	$P2_12_12_1$	53.8	99.0	49.5		30 000 (1 dimer)	*e*
Monellin – serendipity berry (sweet)	$P2_12_12$	54.4	113.0	40.8		21 000 (2 molecules)	*f*
Thaumatin – sweet-tasting protein	$P4_12_12$ or	58.5		151.8		21 000 (1 molecule)	*g*
	$P2_12_12_1$	73.04	52.89	52.32		21 000 (1 molecule)	*g*
Calcium-binding protein – bovine	$P2_12_12$	56.3	43.0	29.4		9600 (1 molecule)	*h*
D-Ribulose-1,5-diphosphate carboxylase – tobacco leaves	$I4_132$	383				140 000 ($\frac{1}{4}$ molecule)	*i*
L-Asparaginase – *Proteus vulgaris*	$P2_1$	69.3	125.6	101.4	97.33	120 000 (1 tetramer)	*j*
L-Glutaminase-asparaginase	$P6_2$ or	145.0		63.5		70 000 ($\frac{1}{2}$ tetramer)	*k*
	$C222_1$	149.7	70.5	132.8		70 000 ($\frac{1}{2}$ tetramer)	*k*
ATP:L-Arginine phosphotransferase – lobster	$P2_1$	75.9	88.3	58.2	102.0	76 000 (2 molecules)	*l*
Lipoprotein – phosphoprotein complex in oocytes from *Xenopus laevis*	orthorhombic	88.5	174.8	198.9		960 000 (2 molecules) or 4 molecules/unit cell	*m*
Glutamine synthetase – *E. coli*	hexagonal	140		148		100 000 (4 subunits)	*n*
Proteinase K – fungus	$P4_12_12$	68.3		108.5		37 000 (2 molecules)	*o*
β-Amylase – soybean	$P3_121$	86.1		144.4		60 000 (1 molecule)	*p*
p-Hydroxybenzoate hydroxylase – *Pseudomonas fluorescens*	$C222_1$	71.9	164.4	88.3		55 000 (1 molecule)	*q*

Glutathione reductase – human erythrocytes	$C2$	120	63	85	58.2	50 000 ($\frac{1}{2}$ molecule)	*r*
Flavodoxin – blue–green alga	$P2_12_12_1$	57.08	69.24	45.55		20 000 (1 molecule)	*s*
Met-myoglobin – gasteropod	$P2_12_12_1$	52.9	70.4	32.5		18 000 (1 molecule)	*t*
Catalase – bovine liver	$P3_121$	173.3		237.4		250 000 (1 molecule)	*u*
Catalase – bovine liver	$P2_12_12_1$	69	173.5	206		250 000 (1 molecule)	*v*
Haemocyanin – spiny lobster	$P2_1$	119.8	193.1	122.2	118.1	450 000 (1 molecule)	*w*
Haemerythrin – sipunculid	$P4$	86.5		80.6		50 400 (4 subunits)	*x*
Thymidylate synthetase – *Lactobacillus casei*	$P6_122$ or $P6_522$	152 or 76		298 298		280 000 (4 dimers) 70 000 (1 dimer)	*y* *y*
Adenovirus-induced protein crystal in KB cells	$P2_1$	630	1200	900	95.5	130×10^6 (1 tubule)	*z*

(*a*) A. Wlodawer, K. O. Hodgson, and E. M. Shooter, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 777. (*b*) C. H. Wei, C. Koh, P. Pfuderer, and J. R. Einstein, *J. Biol. Chem.*, 1975, **250**, 4790. (*c*) C. Chothia and W. Leuzinger, *J. Mol. Biol.*, 1975, **97**, 55. (*d*) L. Noda, G. E. Schulz, and I. Von Zabern, *European J. Biochem.*, 1975, **51**, 229. (*e*) M. Pasek, C. Keith, D. Feldman, and P. B. Sigler, *J. Mol. Biol.*, 1975, **97**, 395. (*f*) A. Wlodawer and K. O. Hodgson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 398. (*g*) H. van der Wel, T. C. V. Soest, and E. C. Rogers, *F.E.B.S. Letters*, 1975, **56**, 316. (*h*) K. Moffatt, C. H. Fullmer, and R. H. Wasserman, *J. Mol. Biol.*, 1975, **97**, 661. (*i*) T. S. Baker, D. Eisenberg, F. A. Eiserling, and L. Weissmann, *J. Mol. Biol.*, 1975, **91**, 391. (*j*) B. Lee, H. J. Yang, G. M. Henry, J. P. Seymour, and I. Chibata, *J. Biol. Chem.*, 1975, **250**, 6228. (*k*) A. Wlodawer, K. O. Hodgson, and K. Bensch, *J. Mol. Biol.*, 1975, **99**, 295. (*l*) J. Berthou, C. Rérat, B. Rérat, A. Gadet, R. Fourme, M. Renaud, C. Dubord, L. A. Pradel, C. Roustan, and N. van Thoai, *J. Mol. Biol.*, 1975, **95**, 331. (*m*) D. H. Ohlendorf, M. L. Collins, E. O. Puronen, L. J. Banaszak, and S. C. Harrison, *J. Mol. Biol.*, 1975, **99**, 153. (*n*) R. P. Bywater, C. H. Carlisle, R. B. Jackson, A. L. Mackay, and P. A. Timmins, *J. Mol. Biol.*, 1975, **91**, 293. (*o*) J. K. Dattagupta, T. Fujiwara, E. V. Grishin, K. Lindner, P. C. Manon, N. J. Pieniazek, W. Saenger, and D. Suck, *J. Mol. Biol.*, 1975, **97**, 267. (*p*) Y. Morita, S. Aibara, H. Yamashita, F. Yagi, T. Suganuma, and K. Hiromi, *J. Biochem.*, 1975, **77**, 343. (*q*) J. Drenth, W. G. J. Hol, and R. K. Wierenga, *J. Biol. Chem.*, 1975, **250**, 5268. (*r*) G. E. Schulz, H. Zappe, D. J. Worthington, and M. A. Rosemeyer, *F.E.B.S. Letters*, 1975, **54**, 86. (*s*) W. W. Smith, H. L. Crespi, B. Entsch, M. L. Ludwig, and C. E. Nordman, *J. Mol. Biol.*, 1975, **94**, 123. (*t*) T. L. Blundell, M. Brunori, B. Curti, M. Bolognesi, A. Coda, M. Fumagalli, and L. Ungaretti, *J. Mol. Biol.*, 1975, **97**, 665. (*u*) W. Eventoff and G. V. Gurskaya, *J. Mol. Biol.*, 1975, **93**, 55. (*v*) P. N. T. Unwin, *J. Mol. Biol.*, 1975, **98**, 235. (*w*) H. A. Kuiper, W. Gaastra, J. J. Beintema, E. F. J. van Bruggen, A. M. H. Schepman, and J. Drenth, *J. Mol. Biol.*, 1975, **99**, 619. (*x*) J. S. Loehr, K. N. Meyerhoff, L. C. Sieker, and L. H. Jensen, *J. Mol. Biol.*, 1975, **91**, 521. (*y*) R. E. Koeppe, R. M. Stroud, V. A. Pena, and D. V. Santi, *J. Mol. Biol.*, 1975, **98**, 155. (*z*) A. Lifchitz, A. Rimsky, G. Torpier, and P. A. Boulanger, *Acta Cryst.*, 1975, **B31**, 441.

Table 3 *Low-resolution structures*

Protein	*Resolution in* Å	*Comments*	*Ref.*
Acid proteinase from *Endothia parasitica*	5.2	Two carboxylate groups in the active-site region	*a*
Monoclinic ribonuclease-S	6	Two crystallographically independent molecules which differ in the conformation of the *N*-terminal tail	*b*
Porcine heart lactate dehydrogenase	6	H_4 Isoenzyme	*c*
Coenzyme deficient LDH:NAD-pyruvate	5	Loss of coenzyme substitution on two of four subunits	*d*
Cat muscle pyruvate kinase	6	Binding of Mn^{2+}, Mn-ATP, and phosphoenolpyruvate	*e*
Leghaemoglobin from lupin root nodules	5	Similar structure to other haemoglobins	*f*
Sickle-cell deoxyhaemoglobin	5	Molecules arranged in pairs of interlocking strands; intermolecular contacts involve Val-6β	*g*
Myohaemerythrin – sipunculan worm	5.5	Monomeric non-haem iron protein	*h*
Octameric haemerythrin	5.5	Structure solved by molecular search techniques using structure of monomeric myohaemerythrin	*i*
Horse-spleen apoferritin	6	24 Subunit structure	*j*
Purple membrane protein	7	Membrane protein contains α-helices running perpendicular to the plane of the membrane	*k, l, m*
Human erythrocyte membrane	9	Phases assigned by minimum-wavelength principle	*n*
Herring sperm protamine	5	E.m. study showing helical structure	*o*

(*a*) J. A. Jenkins, T. L. Blundell, I. J. Tickle, and L. Ungaretti, *J. Mol. Biol.*, 1975, **99**, 583. (*b*) Y. Mitsui and H. W. Wyckoff, *J. Mol. Biol.*, 1975, **94**, 17. (*c*) W. Eventoff, M. L. Hackert, and M. G. Rossmann, *J. Mol. Biol.*, 1975, **98**, 249. (*d*) J. White, M. G. Rossmann, and G. C. Ford, *J. Mol. Biol.*, 1975, **98**, 259. (*e*) D. K. Stammers and H. Muirhead, *J. Mol. Biol.*, 1975, **95**, 213. (*f*) B. K. Vainshtein, E. H. Harutyunyan, I. P. Kuranova, V. V. Borisov, N. I. Sosfenov, A. G. Pavlovsky, A. I. Grebenko, and N. V. Konareva, *Nature*, 1975, **254**, 163. (*g*) B. C. Wishner, K. B. Ward, E. E. Lattman, and W. E. Love, *J. Mol. Biol.*, 1975, **98**, 179. (*h*) W. A. Hendrickson, G. L. Klippenstein, and K. B. Ward, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2160. (*i*) K. B. Ward, W. A. Hendrickson, and G. L. Klippenstein, *Nature*, 1975, **257**, 818. (*j*) R. J. Hoare, P. M. Harrison, and T. G. Hoy, *Nature*, 1975, **255**, 653. (*k*) R. Henderson and P. N. T. Unwin, *Nature*, 1975, **257**, 28. (*l*) R. Henderson, *J. Mol. Biol.*, 1975, **93**, 123. (*m*) A. E. Blaurock, *J. Mol. Biol.*, 1975, **93**, 139. (*n*) J. B. Stamatoff, S. Krimm, and N. R. Harvie, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 531. (*o*) F. P. Ottensmeyer, R. F. Whiting, and A. P. Korn, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4953.

transaminase and of thymidylate synthetase. In this technique a concentration of salt or organic solvent which will produce precipitation is alternated with one which will dissolve precipitated protein. The method is useful when only small quantities of protein are available. It eliminates unwanted amorphous precipitate and reduces the number of crystal nuclei which form. Many crystals, including those of intact immunoglobulins, are highly radiation sensitive. DOB immunoglobulin crystals grown in the presence of monomers of styrene last much longer in the *X*-ray beam and diffract to a higher resolution.[2] This treatment does not cause any major conformational change in the protein and it is possible to prepare heavy-atom derivatives.

Photographic techniques are becoming more and more important for the collection of diffraction data. When using screenless film methods the setting angles of the crystal can be derived directly from the cut-off reflexions on the films.[3] Setting photographs are only necessary for the initial approximate alignment of the crystal and normally the accuracy of the calculated setting angles is better than 3′. Cork *et al.*[4] describe a xenon-filled multiwire area detector for measuring intensities. This system was used to collect high-resolution data which gave good agreement with diffractometer data. It was possible to measure 1400 intensities per hour and 40 000 per crystal (space group $P6_1$, $a = 93$ Å, $c = 74$ Å).

Tickle[5] describes a method for the calculation of integrated peak intensities of reflexions measured with a computer-controlled four-circle diffractometer. This is an alternative to the ordinate analysis method when the computer has limited store and no external storage devices. Unlike ordinate analysis this method does not systematically overestimate weak reflexions. Systematic errors between sets of isomorphous-replacement and anomalous-scattering data may sometimes be reduced by a technique of local scaling.[6] A proposed method may be used to analyse a data set for significant systematic errors and to predict the optimum number of reflexions to be included in the determination of local scale factors. This technique may be useful when forced to be content with poor data.

One of the prime objectives of the protein crystallographer is to relate the function of a biological molecule to its three-dimensional structure. The structure obtained from a crystallographic study of a protein must be one which is stable for about a day, since this is the time-scale over which measurements must be made. It is impossible to study transients which are present during a reaction unless they can be trapped in a reasonably stable form. Douzou *et al.*[7] have studied the feasibility of an *X*-ray analysis of a productive enzyme–substrate complex. The enzymic hydrolysis of bacterial cell walls and oligosaccharides appears to proceed by the same mechanism in mixed aqueous/organic solvents as in aqueous solution. At low temperatures the reaction is slowed progressively and at -25 °C the enzyme–substrate complex in mixed solvents is stable indefinitely. The conformation of the enzyme is not significantly altered and an

[2] R. Sarma and G. Zaloga, *J. Mol. Biol.*, 1975, **98**, 479.
[3] P. Schwager, K. Bartels, and A. Jones, *J. Appl. Cryst.*, 1975, **8**, 275.
[4] C. Cork, R. Hamlin, W. Vernon, and Ng. H. Xuong, *Acta Cryst.*, 1975, **A31**, 702.
[5] I. J. Tickle, *Acta Cryst.*, 1975, **B31**, 329.
[6] B. W. Matthews and E. W. Czerwinski, *Acta Cryst.*, 1975, **A31**, 480.
[7] P. Douzou, G. Hui Bon Hoa, and G. A. Petsko, *J. Mol. Biol.*, 1975, **96**, 367.

enzyme–substrate complex can be formed by the direct addition of substrate to enzyme at sub-zero temperatures. A study of the pH profile allows the conditions of optimum binding to be selected. This shows the possibility of determining the three-dimensional structure of an enzyme–substrate complex directly. Petsko [8] has developed a procedure for maintaining the integrity of protein crystals at sub-zero temperatures. Crystals will diffract to a high resolution after repeated cooling to below -70 °C. Radiation damage is negligible, there are no changes in unit cell dimensions, and no special equipment or cross-linking procedures are necessary.

Until recently all detailed knowledge of the tertiary structure of proteins came from crystallographic studies using the method of isomorphous replacement with anomalous scattering. Molecular replacement techniques for direct structure determination by analogy with another protein of known structure have been used in the determination of the structure of trypsin by Bode, Fehlhammer, and Schwager.[9, 10] These workers have shown that detailed information about the new structure can be obtained if one works at a sufficiently high resolution. The trypsin structure obtained from the complex of pancreatic trypsin inhibitor with trypsin was used as a starting point. This structure was used to give calculated phase angles and the structure was refined using measured intensities and calculated phase angles. Working at the lower resolution of 3.5 Å and using these rotational and translational search procedures, Ward *et al.* have determined the structure of human deoxyhaemoglobin A crystallized from polyethylene glycol solutions.[11] At this resolution no refinement was possible but the results should be of interest in determining the structure of deoxyhaemoglobin S which can be crystallized from similar solutions. This structure has been determined at 5 Å resolution using the same procedures.[12] The parts of the structure near the Val-6β residues are not significantly different from the corresponding parts of the haemoglobin A structure. The molecules are arranged in pairs of interlocking strands and the two strands in each pair are related by a two-fold screw axis running between them longitudinally. Intermolecular contacts involve Val-6β. Such double strands may be incorporated into a fibre model which is consistent with other information about the structure of deoxyhaemoglobin S fibres *in vivo*. Further structural information at a higher resolution may throw light on the mechanism by which sickle cell haemoglobin aggregates into fibres. Similar techniques have been used to study the quaternary and tertiary structures of an octameric haemerythrin using the known structure of a monomeric haemerythrin.[13, 14]

Argos *et al.* have studied the application of molecular replacement techniques to the known structure of glyceraldehyde-3-phosphate dehydrogenase.[15] Their

[8] G. A. Petsko, *J. Mol. Biol.*, 1975, **96**, 381.
[9] H. Fehlhammer and W. Bode, *J. Mol. Biol.*, 1975, **98**, 683.
[10] W. Bode and P. Schwager, *J. Mol. Biol.*, 1975, **98**, 693.
[11] K. B. Ward, B. C. Wishner, E. E. Lattman, and W. E. Love, *J. Mol. Biol.*, 1975, **98**, 161.
[12] B. C. Wishner, K. B. Ward, E. E. Lattman, and W. E. Love, *J. Mol. Biol.*, 1975, **98**, 179.
[13] W. A. Hendrickson, G. L. Klippenstein, and K. B. Ward, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2160.
[14] K. B. Ward, W. A. Hendrickson, and G. L. Klippenstein, *Nature*, 1975, **257**, 818.
[15] P. Argos, G. C. Ford, and M. G. Rossmann, *Acta Cryst.*, 1975, **A31**, 499.

method involved successive cycles of electron-density calculations, averaging among the non-crystallographically equivalent subunits, and structure factor calculations based upon the averaged map. This was used to extend the phases from a known 6 Å isomorphous replacement set to at least 4.9 Å, to determine a reasonable structure at low resolution given the molecular envelope and non-crystallographic symmetry, and to use the resulting low-resolution phases in the location of heavy atoms. Their results indicated that such techniques should be useful when applied to the determination of virus structures. Litvin has developed a mathematical method to predict all the peaks of a rotation function corresponding to a given molecular crystal.[16] The method is used to determine possible models of bovine liver catalase and the peaks corresponding to the model of satellite tobacco necrosis virus are determined and tabulated. This method should be useful for the analysis of the rotation function when determining non-crystallographic symmetry for use in the molecular replacement technique.

Several groups have refined protein structures at high resolution and extended phases to a higher resolution. High-order probability laws have been applied to the phase refinement and extension of protein structures.[17] Using structure factors calculated from the atomic model of insulin an order-400 covariance matrix leads to phases with an average error of 15°. The method has also been applied to the actual experimental data for insulin for phase refinement and phase extension from 2.8 to 2.0 Å. The results showed that high-resolution data were important. Deisenhofer and Steigemann[18] have carried out a refinement of the bovine pancreatic trypsin inhibitor (58 residues) at 1.5 Å resolution. The model structure was fitted to the electron-density map at 1.9 Å by application of Diamond's real-space refinement procedure. Further refinement was done at 1.5 Å by cyclic real-space refinement against electron-density maps computed with observed structure factors and phases calculated from the preceding model. Difference Fourier maps led to the correction of errors in the model and the location of solvent molecules. After 15 cycles the crystallographic *R*-factor was 0.225.

For larger molecules such procedures can be very time consuming and expensive. Collins has shown that Fourier phase refinement for large molecules can be accomplished using electron-density maps sampled at intervals of half the minimum interplanar spacing for which diffraction data have been measured.[19] A modification function can be used to increase the resolution and sharpness of low density levels while leaving the highest levels unchanged. This function can be used both to improve and to extend phases. Computationally this is a very efficient method. This method of phase refinement and extension coupled with the development of a computer-driven display to fit molecular models to electron-density sections has been used to study the structures of rubredoxin and staphylococcal nuclease.[20] It proved possible to extend the resolution by collecting native data only between 2.0 and 1.5 Å.

[16] D. B. Litvin, *Acta Cryst.*, 1975, **A31**, 407.
[17] C. De Rango, Y. Mauguen, and G. Tsoucaris, *Acta Cryst.*, 1975, **A31**, 227.
[18] J. Deisenhofer and W. Steigemann, *Acta Cryst.*, 1975, **B31**, 238.
[19] D. M. Collins, *Acta Cryst.*, 1975, **A31**, 388.
[20] D. M. Collins, F. A. Cotton, E. E. Hazen, E. F. Mayer, and C. N. Morimoto, *Science*, 1975, **190**, 1047.

These refinement procedures can only be used when a reasonable starting model has been obtained. A method which had been used to automate the interpretation of an electron-density map was unable to represent α-helical regions correctly. The problem was due in part to the density produced by strong hydrogen bonds and has been overcome by using a 0.5 Å instead of a 1 Å grid.[21] Sometimes the initial model contains systematic errors as well as random errors. The random errors can be treated by Diamond's real-space refinement and systematic errors by a simple unconstrained steepest-descent method.[22] In the case described the major shift was a translation perpendicular to the map sections.

Many criteria are available for the initial evaluation of a trial structure. The volumes occupied by atoms or groups of atoms in ribonuclease S have been calculated considering buried and exposed atoms separately.[23] The volumes occupied by particular atom types fall within a narrow range and the summation of these volumes over main and side-chain groups leads to an even narrower distribution. These volume distributions could be used as stringent criteria against which to test trial tertiary structures. Scanlon and Eisenberg describe a theory which relates the mass of solvent bound to a crystalline protein to its molecular weight and easily measurable densities and crystallographic parameters.[24] The theory is applicable when the density of the protein crystal is a linear function of the density of the mother liquor.

There have been further developments in the use of the electron microscope for determining crystal structures. Langer *et al.* describe experiments on the preparation of thin film sections of embedded protein crystals.[25] The best embedding media is aquon and electron diffraction patterns and micrographs can be observed with a resolution of 10 Å. Unwin and Henderson determined the projected structures of two unstained periodic biological specimens, the purple membrane and catalase, to resolutions of 7 and 9 Å respectively.[26] Glucose was used to facilitate their *in vacuo* preservation and extremely low electron doses were applied to avoid their destruction. The information was extracted from defocused bright-field micrographs and electron diffraction patterns. Fourier analysis of the micrograph data gave the phases of the Fourier components of the structures; measurement of the electron diffraction pattern gave the amplitudes. Large regions (3330—10 000 unit cells) were required because of the intrinsically low image contrast and the statistical noise due to the low electron dose. The methods are limited in resolution only by the performance of the microscope at the unusually low magnifications which were necessary, and a resolution close to 3 Å should be possible. The advantage of the method is that it determines an undamaged structure, but a highly ordered array is required since the redundancy of information present in the image is used.

The purple membrane is a specialized part of the cell membrane of *Halobacterium halobium*. It functions *in vivo* as a light-driven proton pump involved in photosynthesis. It contains identical protein molecules of molecular weight

[21] J. Greer, *J. Mol. Biol.*, 1975, **98**, 649.
[22] R. J. Fletterick and H. W. Wyckoff, *Acta Cryst.*, 1975, **A31**, 698.
[23] J. L. Finney, *J. Mol. Biol.*, 1975, **96**, 721.
[24] W. J. Scanlon and D. Eisenberg, *J. Mol. Biol.*, 1975, **98**, 485.
[25] R. Langer, Ch. Poppe, H. J. Schramm, and W. Hoppe, *J. Mol. Biol.*, 1975, **93**, 159.
[26] P. N. T. Unwin and R. Henderson, *J. Mol. Biol.*, 1975, **94**, 425.

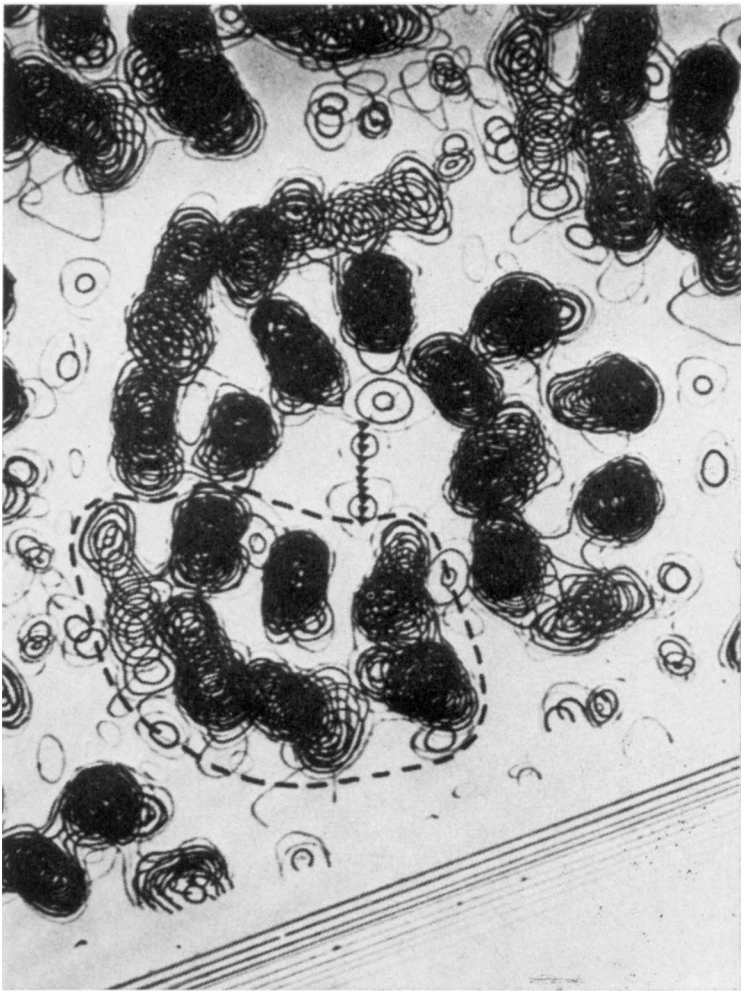


Figure 1 *Part of the* 7 Å *resolution map of the purple membrane showing a region* 50 Å *thick spanning the membrane. The view shows protein molecules grouped about one of the three-fold axes. The probable boundary of one of them is indicated by the broken line. The angle of view is such that the* α*-helices furthest from the three-fold axis partly overlap in the protein molecule outlined, but are seen almost end-on in the one to the right*

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26 000, which make up 75% of the total mass, and lipid which makes up the remaining 25%. Retinal, covalently linked to each protein molecule in a 1 : 1 ratio is responsible for the characteristic purple. These components together form an extremely regular two-dimensional array or crystal. An *X*-ray diffraction analysis showed that the protein and lipid components are packed in a *P*3 hexagonal lattice, with one protein molecule per asymmetric unit. The structure is made up of a single layer of protein molecules, oriented vectorially in the same direction across the membrane.[27] A three-dimensional map at a resolution of 7 Å was obtained by collecting data from 15 diffraction patterns and 18 micrographs of membranes tilted at angles from 0 to 57° to the incident electron beam.[28] These data were combined using the Fourier method of three-dimensional image reconstruction. The protein in the membrane contains seven, closely packed α-helical segments which extend roughly perpendicular to the plane of the membrane for most of its width (Figure 1). Lipid bilayer regions fill the spaces between the protein molecules. This is the first time that the tertiary structure of a membrane protein has been obtained and that significant detail at a resolution higher than about 20 Å has been revealed in biological structures by electron microscopy. In an analysis of the *X*-ray diffraction pattern Blaurock has reached similar conclusions.[29] He presents model calculations based on the bilayer profile calculated for the extracted lipids and on two simple profiles for the protein. The calculations favour a structure having the lipid molecules in two layers as in a bilayer, although there may be more of the lipid on one side of the membrane than on the other. The diffraction pattern suggests that the lipid molecules may be located mainly in a few discrete regions approximately 20 Å across between the protein molecules.

Low-angle *X*-ray scattering in solution can be used to give information about surface organization. Experimental data for wild cucumber mosaic virus and polyoma virus have been analysed and it has been shown that the differences between the observed scattering curve and that expected from a solid sphere of uniform density may be accounted for by deviations from spherical symmetry. An analysis in terms of icosahedral harmonics shows how the clustering pattern of the coat protein may be deduced.[30] In order to study the intramolecular packing in proteins by the method of *X*-ray scattering in solution a cylindrical model of a helical region of the polypeptide chain has been proposed.[31] This model is characterized by five parameters – helix length, electron density per unit length, and external diameter, plus two depending on the radial distributions of the electron density. The parameters are calculated from the amino-acid content. This is a way of gaining information about structure as well as overall dimensions and shape.

Stubbs and Diamond [32] give a procedure for separating and phasing the terms originating from the different Bessel function orders in cylindrically averaged fibre diffraction patterns using isomorphous heavy-atom derivatives. They give model calculations and discuss the application to tobacco mosaic virus.

[27] R. Henderson, *J. Mol. Biol.*, 1975, **93**, 123.
[28] R. Henderson and P. N. T. Unwin, *Nature*, 1975, **257**, 28.
[29] A. E. Blaurock, *J. Mol. Biol.*, 1975, **93**, 139.
[30] A. Jack and S. C. Harrison, *J. Mol. Biol.*, 1975, **99**, 15.
[31] B. A. Federov, *J. Mol. Biol.*, 1975, **98**, 341.
[32] G. J. Stubbs and R. Diamond, *Acta Cryst.*, 1975, **A31**, 709.

3 Hormones

Insulin.—Insulin is a protein hormone produced and stored in the β-cells of the islets of Langerhans of the pancreas. It is synthesized as a single-chain precursor, proinsulin. Within the membrane-bound storage granules it is converted into the active hormone by enzymatic removal of a 30-residue peptide. The insulin molecules are stored within the granules as zinc-containing hexamers. They are released into circulation in response to raised blood glucose levels and facilitate the removal of glucose and other metabolites to the tissues. The hormone binds to specific receptor sites on the target cell surfaces but the details of how a biological response is initiated are not understood.

Research workers in China have published papers on the structure of rhombohedral 2-zinc insulin at 1.8 Å resolution.[33, 34] They have calculated phases to this resolution using the method of multiple isomorphous replacement and their results agree well with those obtained by Hodgkin's group at Oxford. Interactions of insulin and insulin analogues with receptor proteins in liver and fat cells are discussed. Wood *et al.* have studied the structures and association properties of insulins of different sequences and receptor-binding affinities in order to define the residues involved in the hormone–receptor interaction.[35] They show that decreased receptor binding does not necessarily implicate the changed residues in receptor binding. It may be due to a change in the three-dimensional shape of the insulin molecule. The receptor-binding region comprises residues which are widely separated in the primary structure but are held closely together by the tertiary structure. This suggests that for insulin conformational integrity is a prerequisite for full binding. Blundell and Wood present a model for the evolution of insulin which depends on the effect of changes of sequence on the tertiary structure and the role of various parts of this structure in the conversion of proinsulin into the active form, the storage of insulin, its transport to the site of action, and its interaction with a receptor.[36]

Glucagon.—Glucagon is a polypeptide hormone of 29 amino-acids which is synthesized and stored in the α-cells of the islets of Langerhans of the pancreas. Its action is antagonistic to that of insulin since its release from the pancreas is stimulated by low circulating glucose levels and its effect is to release glucose from liver cells. The hormone acts by binding to a plasma membrane receptor site on the regulatory component of adenylate cyclase which leads to an increase in intracellular levels of cyclic AMP. In order to understand the hormone–receptor interaction it is necessary to know the conformation of the hormone when bound to the receptor. Large crystals can be obtained at pH 9.2 whereas the physiological pH is in the range 5.6—7.5. If the pH of the mother liquor is changed large conformational changes take place although the crystals still diffract to a resolution of about 3 Å. This implies that glucagon is a flexible molecule.

These crystals were used to determine the structure of glucagon at physiological pH to a resolution of 3 Å using the method of multiple isomorphous replace-

[33] D. C. Hodgkin, *Nature*, 1975, **255**, 103.
[34] *Scientia Sinica*, 1974, **17**, 752, 779.
[35] S. P. Wood, T. L. Blundell, A. Wollmer, N. R. Lazarus, and R. W. J. Neville, *European J. Biochem.*, 1975, **55**, 531.
[36] T. L. Blundell and S. P. Wood, *Nature*, 1975, **257**, 197.

ment.[37] The electron-density map showed a well defined helical region containing 16 residues (10—25) extended at either end by a few residues of less regular right-handed helix. Residues 1—5 form a flexible region. In the crystal the molecules aggregate about crystallographic three-fold axes. These trimers are

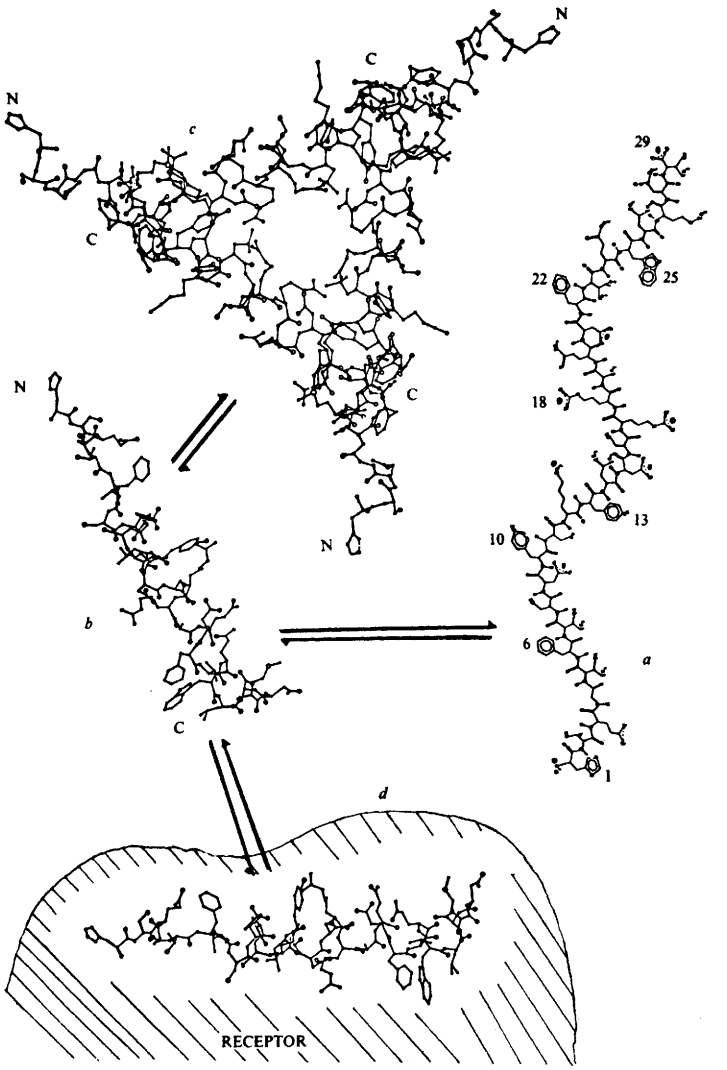


Figure 2 *A schematic representation of the equilibrium between conformers of glucagon. (a) Hypothetical random coil structure in equilibrium with a helical form (b). The helical form is stabilized either as trimers (c) or by association with a receptor (d) by hydrophobic interactions*
(Reproduced by permission from *Nature*, 1975, **257**, 751)

[37] K. Sasaki, S. Dockerill, D. A. Adamiak, I. J. Tickle, and T. Blundell, *Nature*, 1975, **257**, 751.

formed by interactions between patches of hydrophobic side-chains in two regions of each molecule (Figure 2). It is probable that in solution glucagon has a flexible structure. At high concentrations the α-helical conformation is induced and is accompanied by an association of molecules to form trimers. It is suggested that at physiological concentrations glucagon exists as a monomer and that it is unlikely that the trimers play a role in binding to the receptor. There is evidence that the glucagon–receptor complex is stabilized by hydrophobic interactions which suggests that the helical conformation could be stabilized by the interaction of glucagon with two hydrophobic regions on the receptor. This model implies that the glucagon molecule exists in solution as an equilibrium population of conformers with little retention of structure and that the helical conformation is stabilized by hydrophobic interaction either as an oligomer or as a complex with the receptor (Figure 2). An alternative model would involve an induced fit for the interaction of the glucagon molecule with the receptor. The length of the helical molecule is over 40 Å and the centres of the two hydrophobic regions are about 20 Å apart. This is a very long structure to be bound to one regulatory unit of adenylate kinase and it is possible that glucagon binds to two regulatory subunits. This would tie in with the approximate two-fold symmetry of the central part of the molecular structure.

The structures of insulin and glucagon contrast strongly in that glucagon is a flexible structure whereas insulin has a well defined and relatively inflexible globular structure. In insulin, residues widely separated in the primary sequence are involved in a relatively small receptor-binding region. There are similarities in that both associate in concentrated solution through mainly hydrophobic interactions. In both hormones the hydrophobic residues involved in association are also involved in receptor binding.

4 Immunoglobulins

Antibodies have a common basic structure in which the antigen-binding function is carried out by the variable domain whose hypervariable regions are involved in the binding process. This variable domain is composed of the amino-terminal half of one light chain and the amino-terminal quarter of one heavy chain. Repetitive amino-acid sequences along the heavy and light chains were suggestive of a number of very similar tertiary structural domains along the polypeptide chains and this was confirmed by the three-dimensional structures. Most of the detailed structural information about antibodies has come from crystallographic analysis of fragments of myeloma proteins.

The Fc fragment consists of the *C*-terminal half of the heavy chains and an Fab fragment is an antigen-binding fragment of the immunoglobulin and consists of one light chain and the *N*-terminal half of a heavy chain. The structure of the variable portion of the χ-type Bence-Jones protein REI has been determined at 2.0 Å resolution.[38] The Bence-Jones dimer consists of two identical light chains and resembles an Fab fragment, in which one light chain plays the structural role of the heavy chain. This fragments represents the variable half of an Fab fragment. The structure has been refined using a constrained crystallographic

[38] O. Epp, E. E. Lattman, M. Schiffer, R. Huber, and W. Palm, *Biochemistry*, 1975, **14**, 4943.

refinement procedure to give a crystallographic *R*-factor of 0.24. A detailed analysis of the structure is presented in terms of hydrogen bonds and conformational angles and the elements of secondary structure are described. The precise atomic arrangement of the amino-acid residues forming the contact region between the two chains and the hapten binding site are given. The amino-acid residues which form the binding cavity in a λ-type Bence-Jones dimer are almost identical to those found in this χ-type dimer. Two *cis*-prolines were found and the intrachain disulphide bridge occurs statistically in two alternative conformations. The knowledge of the refined molecular structure of this fragment enables analysis of related molecules by Patterson search techniques. In addition the calculated phases based on the refined structure are more accurate than isomorphous phases and will be used to analyse the effects of hapten binding on the molecular structure. Since this will improve the quality of the difference Fourier maps it may be possible to observe subtle structural changes which might occur upon binding. The structure of a second χ-type Bence-Jones protein variable fragment Au has been determined by molecular replacement methods using the known structure of the REI fragment.[39] This structure was very similar and gave a crystallographic *R*-factor of 0.31.

Other groups have been investigating a complete Fab fragment. The Fab fragment of the mouse myeloma immunoglobulin McPC603 crystallizes in both a hexagonal and a cubic crystal lattice. Models of the structure showing the approximate molecular packing were deduced from electron micrographs and *X*-ray analysis.[40] Using low-angle *X*-ray scattering Pilz *et al.*[41] have studied the shape and volume of bivalent and univalent Fab fragments of anti-poly-(D-alanyl) antibodies in the presence and absence of tetra-D-alanine. Under conditions in which more than 90% of the binding sites were occupied by hapten there were no significant changes in volume or radius of gyration. This contrasts with a 10% decrease in volume and a 7.7% decrease in radius of gyration found upon reaction of hapten with intact anti-poly-(D-alanyl) antibodies. This indicates that a conformational change occurs within the intact antibody molecule but not in the Fab fragment upon reaction with the tetra-alanine hapten. The changes in the intact molecule suggest a conformational change affecting large portions of the molecule and involving a role for either the Fc fragment or the hinge region which connects the Fab and Fc fragments or possibly for both. Recently the structure of an intact human antibody molecule Kol has been determined at low resolution using three heavy-atom derivatives.[42] The density describing the Fab fragment has been interpreted in terms of the known structures of the variable half of the Bence-Jones protein REI and the mouse McPC603 Fab fragment. It shows a quaternary structure different from those of previously reported Fab fragments and provides evidence for flexibility in the switch peptides which occur in the centre of the Fab fragment between the variable and constant domains of the light and heavy chains. The Fc fragment appears to be disordered in the

[39] H. Fehlhammer, M. Schiffer, O. Epp, P. M. Colman, E. E. Lattman, P. Schwager, and W. Steigemann, *Biophys. Struct. Mech.*, 1975, **1**, 139.
[40] L. W. Labaw, E. A. Padlan, D. M. Segal, and D. R. Davies, *J. Ultrastruct. Res.*, 1975, **51**, 326.
[41] I. Pilz, O. Kratky, A. Licht, and M. Sela, *Biochemistry*, 1975, **14**, 1326.
[42] P. M. Colman, J. Deisenhofer, R. Huber, and W. Palm, *J. Mol. Biol.*, 1976, **100**, 257.

crystal lattice, implying that it is flexible. The differences in structure between the Fab fragment and the same region of the intact antibody involve bending in the switch region. This structural transition could influence the complement binding properties localized in the Fc fragment and explain why conformation changes upon hapten binding are not seen in the Fab fragment although they occur in the intact molecule.

These *X*-ray determinations of the structures of immunoglobulin Fab fragments and Bence-Jones protein have enabled crystallographers working in the field to correlate the structure with existing data and also to gain new information. In a review Poljak [43] has shown how the mass of chemical and immunological information about the immunoglobulins can be unified in terms of the three-dimensional structure. Padlan and Davies [44] have compared the various immunoglobulin domains and have shown that the similarity of the structural framework is in good agreement with the evolutionary relationships of the chains as derived from sequence analysis. There are three hypervariable regions in the light chains and four in the heavy chains. The polypeptide segments in hypervariable regions are quite dissimilar in folding. These regions, which together form the antigen-binding surface, are in the form of loops; some loops are longer or shorter, depending on insertions or deletions, some have extended configurations, while some are helical. The non-hypervariable segments, on the other hand, provide the rigid framework necessary for maintaining the structural integrity of the molecule. Padlan and Davies used the diagonal plot method to demonstrate the similarity of folding and used a difference diagonal plot to assess the difference between two similar structures. These calculations showed that the hypervariability of sequence is paralleled by an equivalent structural variability. Kabat *et al.*[45] have compared five constant region sequences of human and mouse and λ-immunoglobulin chains in order to reveal sequence homologies and evolutionary relationships. A comparison with the tertiary structure of one mouse χ-chain has suggested structural reasons why many of the residues are invariant or conserved along χ *versus* λ lines. Edmundson *et al.*[46] have used the known structures to investigate what changes could have led to the functional differentiation of the constant and variable domains. The structure of the human (Mcg) λ-type light-chain (Bence-Jones) dimer has been correlated with the amino-acid sequence. Each light chain is composed of amino (variable) and carboxyl (constant) regions or domains which are cylinders of β-pleated sheets enclosing hydrophobic interiors. The structures of the variable and constant domains support the hypothesis of a common ancestral gene. The comparison of the sequences of the variable and constant domains with the tertiary structure as a basis for alignment confirms earlier views that sequence homologies have been obscured by divergent evolution. The types of change are indicated by the domain structure. For example variable and constant domains are rotational allomers, being related by a rotation of approximately 165° about an axis passing through homologous half-cystinyl residues of the intrachain disulphide bonds.

[43] R. J. Poljak, *Nature*, 1975, **256**, 373.
[44] E. A. Padlan and D. R. Davies, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 819.
[45] E. A. Kabat, E. A. Padlan, and D. R. Davies, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2785.
[46] A. B. Edmundson, K. R. Ely, E. E. Abola, M. Schiffer, and N. Panagiotopoulos, *Biochemistry*, 1975, **14**, 3953.

The functions of the three- and four-chain layers of antiparallel β-pleated sheet constituting the basic immunoglobulin fold have changed during evolution. In the light-chain dimer the three-chain layers of the variable domains face each other across a solvent channel in which hapten-like molecules can be bound while the corresponding layers in the constant domains supply most of the external surfaces of the dimeric molecule. Conversely the four-chain layers furnish the external surface of the variable domain but interact across a solvent-free zone to help maintain the compact arrangement of the constant domains. Patterns of amino-acid sequence in parts of the pleated sheets can be correlated with these differences. In the variable domains an alternating pattern of polar and apolar residues is broken in the three-chain layers by the substitution of aromatic for aliphatic polar residues in positions important for the maintenance of the general architecture of the hapten-binding sites. Similarly the alternating sequence pattern in the four-chain layers of the constant domains is interrupted by substitution of hydrophobic for polar residues at sites of interaction in the domain interface. The polar constituents in the four-chain layers of the variable domains are mainly Ser and Thr, which form most of the dimer surface. The polar residues on the constant surface are more diversified. These differences in surface properties may help to explain why the variable domains have been implicated in the formation of amyloid fibrils and in the characteristic thermal behaviour of Bence-Jones proteins. This functional differentiation of the constant and variable domains is an aspect of immunoglobulin structure that would have been difficult to foresee.

5 Concanavalin A

Concanavalin A is one of a group of plant proteins, termed lectins, that bind carbohydrates and agglutinate a variety of animal cells. Structural studies on such non-enzyme proteins which are capable of these types of interactions with cell surfaces should give information of wide biological importance. Since its first isolation from jack bean concanavalin A has been shown to possess haemagglutinating activity and to precipitate various polysaccharides. It is mitogenic for lymphocytes; that is it is able to stimulate the transformation of lymphocytes from resting cells to actively growing cells which are capable of undergoing mitotic division, and is also capable of specifically agglutinating malignant cells. Such a lectin can be used as a molecular probe of the number, distribution, and mobility of the receptors in the plasma membrane of various cells and concanavalin A has been used to study the proliferation of lymphoid cells and the interaction among their surface receptors. Such experiments have suggested that the binding of the lectin to the cell surface results in the restriction of mobility of the concanavalin A receptors as well as those for a variety of other ligands. The specific inhibition of the various biological activities of lectins by simple sugars has indicated that the initial step in such activities involves the binding of the lectin to saccharide chains on the cell surface. The study of the structure and activity of concanavalin A could lead to an understanding of the molecular basis of its recognition of specific sugars and the mechanism of its action on cells, which should in turn throw light on the structure and behaviour of the cells themselves.

The concanavalin A molecule is composed of identical polypeptide chains each with a molecular weight of 26 000; below pH 6 the molecule exists as a dimer, and above pH 6.5 as a tetramer. Each subunit contains 237 amino-acid residues and there is no covalently bound carbohydrate moiety or other detectable prosthetic group. The protein binds to mono-, oligo-, and poly-saccharides with terminal non-reducing α-D-mannopyranosyl and α-D-glucopyranosyl residues and to certain non-terminal mannose residues. Other stereoisomers of these saccharides do not inhibit its biological activity. Like other lectins concanavalin A requires the ordered binding of manganese and calcium ions before it is capable of binding sugars. During this sequential binding there are conformational changes affecting aromatic residues although apparently the gross secondary structure of the protein is not affected. Crystallographic results from several groups have shown that the metal-free protein is generally similar in structure to the native protein although the geometrical relationships among the subunits are different. A series of four papers from Edelman's group at the Rockefeller University gives the complete amino-acid sequence of the protein and a detailed description of its structure.[47–50] This includes a list of the co-ordinates of non-hydrogen atoms and an analysis of all the intra and inter subunit interactions in the molecule. The *X*-ray analysis to a resolution of 2.0 Å was carried out on the tetrameric protein with both types of metal ion bound. The overall shape of the monomer is a flat based dome of dimensions 42 × 40 × 39 Å. The structure is dominated by two large, anti-parallel β-sheets. One of these sheets, containing six strands, forms the base of the dome and it is slightly bowed, almost completely lacking the twist normally seen in β-sheets. The second sheet, which forms the centre of the subunit, lies at an angle to the first sheet with its seven strands twisted through 90°. The top of the dome is formed by a number of loops, mainly derived from the *N*-terminal and *C*-terminal parts of the polypeptide chain. There are two types of interaction between subunits, both largely involving the flat β-sheet. One type of interaction causes the monomers to associate in pairs with equivalent strands at the edge of the flat β-sheet forming antiparallel hydrogen bonds to give dimers that contain a 12-stranded antiparallel sheet. The second type of interaction involves the 12-stranded sheets from two dimers being placed face to face so that contacts between dimers involve both hydrophobic and hydrophilic side-chains that point outwards from the sheets. There are marked similarities between the structure of concanavalin A and the structures of the immunoglobulins and prealbumin. The domains of the immunoglobulins have a similar β-structure and associate through interactions very like these dimer–dimer interactions, and the thyroxine-binding protein, prealbumin, has the same type of tertiary structure and subunit interactions.

There are two distinct regions in the amino-acid sequence; the distribution of charged residues is more dense in the *N*-terminal half of the polypeptide chain

[47] J. L. Wang, B. A. Cunningham, M. J. Waxdal, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1490.
[48] B. A. Cunningham, J. L. Wang, M. J. Waxdal, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1503.
[49] J. W. Becker, G. N. Reeke, J. L. Wang, B. A. Cunningham, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1513.
[50] G. N. Reeke, J. W. Becker, and G. M. Edelman, *J. Biol. Chem.*, 1975, **250**, 1525.

than in the *C*-terminal portion and in the latter region there is a linear stretch composed of many hydrophobic residues. This hydrophobic region is located in the interior of the molecule. Residues 1—42 contain all the protein ligands for binding Mn^{2+} and Ca^{2+} while residues 43—129 provide many of the groups involved in interactions between subunits. Three clusters of charged groups stand out, a group of six negatively charged residues between Asp-2 and Asp-28, a closely spaced group of positive charges between Lys-30 and Arg-60, and another cluster of negative charges between Asp-71 and Glu-87. The majority of these charged clusters are exposed on the surface of the monomer with the exception of the first negatively charged cluster in which three residues are involved in metal-ion binding. In the *C*-terminal portion of the chain there are fewer charged residues and these charged residues are usually counterbalanced by the presence of oppositely charged residues in their vicinity. In each subunit the metal ions are located in a surface cavity near the top of the dome; they are about 4.6 Å apart. Each ion is in an octahedral environment composed of four protein ligands, two of which are shared, and two water molecules. The side-chains involved in the binding of Mn^{2+} are those of Glu-8, Asp-10, Asp-19, and His-24 while Ca^{2+} binds to Asp-10, Asn-14, Asp-19, and the carbonyl oxygen of Tyr-12. The acidic nature of these residues accounts for the pH dependence of metal binding and the presence of joint ligands explains the fact that transition-ion binding is a necessary precursor of Ca^{2+} binding. The geometry of the binding site suggests that in the metal-free protein a precursor transition-metal binding site exists consisting of residues 8, 10, and 24. The binding of Mn^{2+} to this site might induce a conformational change, hence creating the Ca^{2+} binding site. Calcium binding would stabilize the native conformation of the *C*-terminal portion of the chain, and possibly the saccharide binding site.

β-(*o*-Iodophenyl)-D-glucopyranoside is an inhibitor of concanavalin A-mediated agglutination of erythrocytes and precipitation of polysaccharides. In the crystal it binds about 20 Å from the metal positions in a deep cavity between the two large β-sheets in the monomer. This cavity contains distinct hydrophobic and hydrophilic subsites. There is some evidence that the binding behaviour of the protein differs in the crystalline state and in solution and it was thought possible that such saccharides labelled with heavy atoms were bound by their hydrophobic aglycones, rather than by their saccharide moieties. Other simple sugars that specifically inhibit the protein cause the crystals to dissolve or to lose their diffraction pattern. This behaviour indicates some sort of conformational change and precludes the direct observation of protein–saccharide interactions. A recent paper by Becker *et al.* describes the results of using crystals of concanavalin A which had been cross-linked with 1% glutaraldehyde.[51] In order to avoid potential non-specific binding to a hydrophobic aglycone, 2-deoxy-2-iodo-methyl-α-D-mannopyranoside, an inhibitor labelled with heavy atom was used to form a protein–saccharide complex. The changes were too large for difference Fourier techniques to be used so new phases were calculated and a new electron-density map plotted. The positions of chain segments 95—105 and 195—207 relative to the rest of the molecule are altered resulting in a disruption

[51] J. W. Becker, G. N. Reeke, B. A. Cunningham, and G. M. Edelman, *Nature*, 1976, **259**, 406.

of the β-structure in this region. The entire metal-binding region seemed to have moved 4—6 Å with respect to the rest of the molecule. To locate the bound sugar molecule a difference map between the complexes with the iodo-sugar and its chloro-analogue was calculated. The iodine peak was about 13 Å from the Mn^{2+} ion near a shallow pocket at the top of the molecule (Figure 3). Several

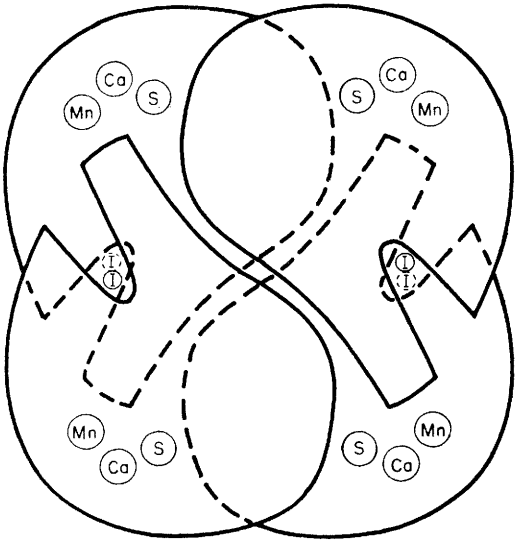


Figure 3 *Schematic representation of the concanavalin A tetramer. Each subunit is approximately* 42 × 40 × 39 Å, *and the tetramer complex possesses exact* 222 *symmetry. The manganese and calcium sites are indicated by* Mn *and* Ca *respectively. The saccharide-binding site near the metals is indicated by* S *and the* o-*iodophenyl-*β-D-*glucopyranoside-binding site in the cavity by* I
(Reproduced by permission from *Nature*, 1976, **259**, 406)

residues in this region are involved in intermolecular interactions in the crystal. Definitive proof of the location of the saccharide-binding site and its association with the metal-binding region of concanavalin A must await the determination at high resolution of the structure of the protein–saccharide complex. Hardman has also obtained crystals of a concanavalin A – carbohydrate complex.[52]

6 Bacteriochlorophyll Protein

Green photosynthetic bacteria contain small amounts of a water-soluble bacteriochlorophyll protein which can be extracted in aqueous salt solutions without the use of detergent. This complex has been shown to participate in the transfer of excitation energy from the antenna pigment chlorobium chlorophyll to the photochemical reaction centres of the organism. The three-dimensional structure of this bacteriochlorophyll protein from *Chlorobium limicola* has been

[52] K. D. Hardman, Collected Abstracts, Tenth International Congress of Crystallography, Amsterdam, 1975, 03.1—8, printed by P. J. Schmidt Tryk, Vojens.

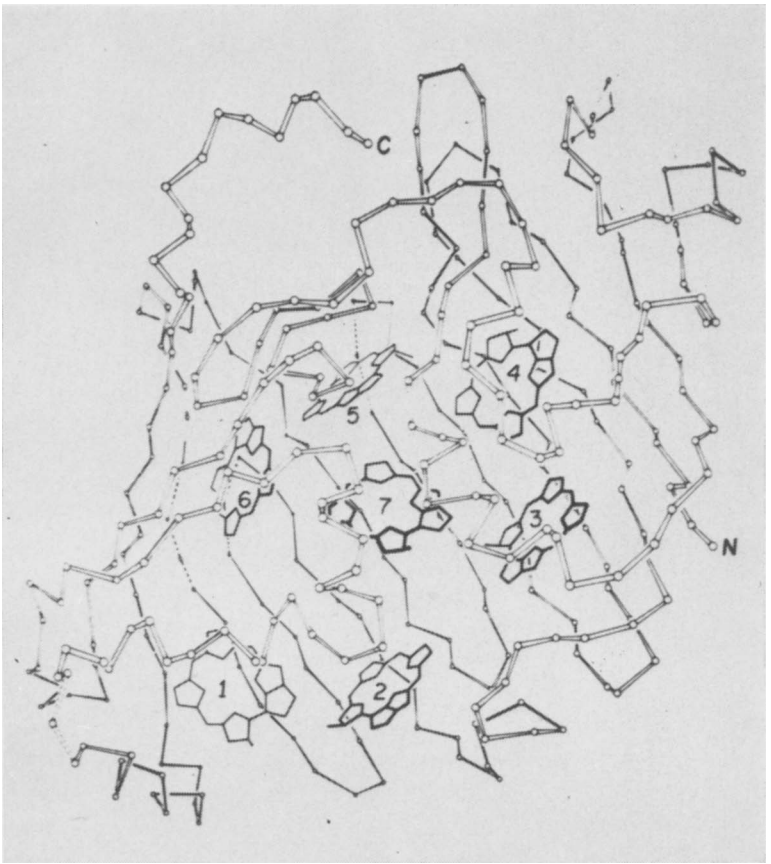


Figure 4 *Schematic diagram showing the arrangement of the polypeptide backbone and chlorophyll core of one subunit of the bacteriochlorophyll protein. Presumed α-carbon positions are indicated by circles. Ambiguous regions of the polypeptide chain are indicated by broken lines. For clarity the magnesium atoms, the chlorophyll ring substituents, and the phytyl chains, except for the first bond, are omitted. The direction of view is from the three-fold axis, which is horizontal, towards the exterior of the molecule*

(Reproduced by permission from *Nature*, 1975, **258**, 573)

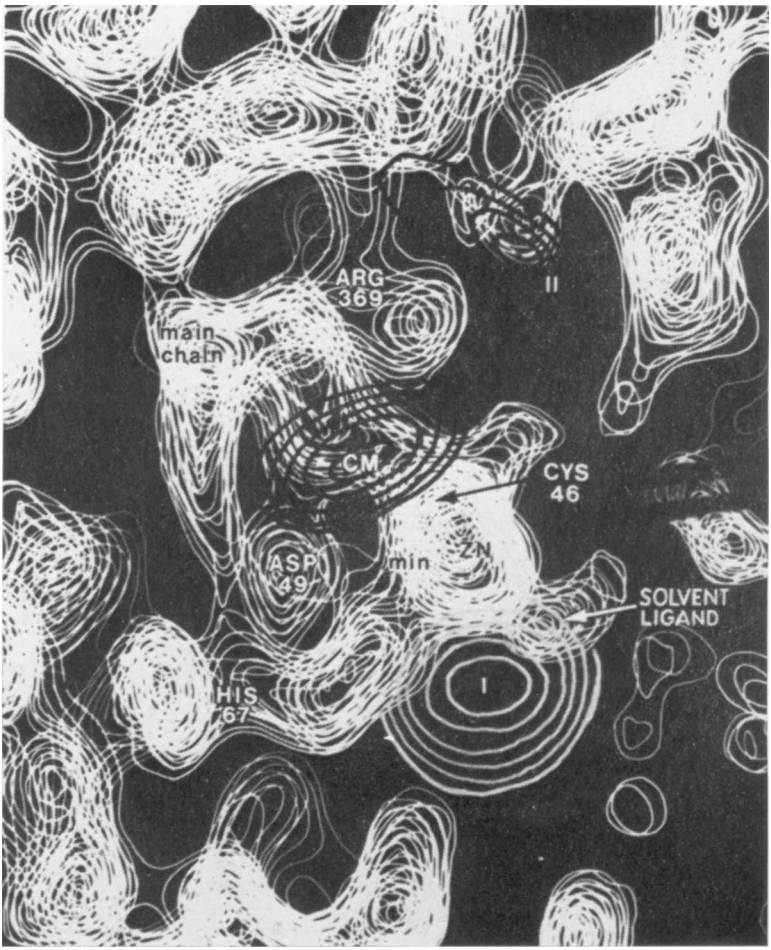


Figure 5 *Sections from the electron-density map to* 2.4 Å *resolution of the active-site zinc region in native liver alcohol dedydrogenase. Superimposed are the difference electron densities of carboxymethylated enzyme*; CM = *carboxymethyl group on* Cys-46, I = *iodine*, II = *new position of* Arg-369
(Reproduced by permission from *European J. Biochem.*, 1975, **58**, 95)

determined at 2.8 Å resolution.[53] It is the first chlorophyll-containing protein for which the structure is known and provides information on the arrangement of chlorophyll *in vivo*.

The molecule has a molecular weight of 150 000 and contains 21 molecules of bacteriochlorophyll *a*, tightly, although not covalently, associated with the molecule. It crystallizes in the space group $P6_4$ with $a = b = 112.4$ Å and $c = 98.4$ Å. It is a trimer with one subunit of molecular weight 50 000 per asymmetric unit. The amino-acid sequence is not known. Each of the three identical subunits contains seven bacteriochlorophyll molecules arranged in an apparently irregular fashion. The conformation of the polypeptide chain forms a distorted hollow cylinder. One end could be open but is blocked by the *C*-terminal helix of an adjacent subunit of the trimer which could possibly form part of the same subunit. The hollow cavity within the cylinder contains the bacteriochlorophyll aggregate (Figure 4). The area of the cylindrical wall which is exposed to the solvent in the trimer is composed almost entirely of 15 strands of β-sheet which seem to be main antiparallel. The side in contact with an adjacent subunit is more complex, consisting of four short lengths of α-helix interspersed with regions of irregular conformation.

The seven chlorophyll molecules are within the core of each subunit. They are confined within an ellipsoid of axial dimensions 45 × 35 × 15 Å and the average centre-to-centre nearest-neighbour distance between porphine rings is 12 Å. This means that although there is no direct contact between porphine rings efficient energy transfer is possible within each subunit. The orientations of the porphine rings do not conform to any obvious pattern. Although their planes do, to a first approximation, lie parallel with one another the azimuthal orientation of the nearly coplanar rings varies considerably. The normal to the average plane makes an angle of 62° with the three-fold axis of the trimer and the centre of the aggregate in each subunit is about 20 Å from this axis. In each subunit the phytyl chains lie close together and the hydrophobic core is completely buried in the protein, being shielded from the aqueous surroundings by the β-sheet. The binding of the aggregate to the protein is partly due to interactions between the phytyl chains and hydrophobic residues on the interior of the β-sheet. There are also extensive interactions involving the magnesium ions of the porphine rings, either through hydrogen-bonding to protein *via* a water molecule or through direct interaction with a suitable side-chain.

Thus in common with other globular proteins this chlorophyll-containing protein possesses the usual types of secondary structure. Unlike any other protein of known tertiary structure it contains a large non-protein core. It is also unusual in being trimeric and this may be related to some underlying symmetry in the membrane. In contrast to current models for chlorophyll arrangement *in vivo* the chlorophyll packing is dominated by interactions between chlorophyll and protein rather than between chlorophyll and lipid or between adjacent chlorophyll molecules. Furthermore the chlorophylls are arranged in an irregular array rather than in strictly ordered one-dimensional or two-dimensional arrays. Although this is an atypical chlorophyll protein the authors suggest that the arrangement of chlorophyll molecules seen here, in close association with protein, typifies the usual arrangement of chlorophyll *in vivo*.

[53] R. E. Fenna and B. W. Matthews, *Nature*, 1975, **258**, 573–577.

7 Glycolytic Enzymes

The structures of many of the enzymes involved in glycolysis are being studied by *X*-ray analysis. Complete amino-acid sequence information, which is essential for the full interpretation of the *X*-ray results, is available for only three enzymes of known structure.

Hexokinase.—The B isoenzyme of yeast hexokinase, which has a molecular weight of 51 000, has been crystallized in three crystal forms, I, II, and III. In forms I and II the subunits associate to form dimers. The quaternary structures in the two forms are different although they both show heterologous interactions between the two subunits. In form III hexokinase does not associate into dimers. The structure of the BIII monomer is known at 2.7 Å resolution.[54] The BI form does not bind substrates but the enzyme–substrate complexes of the BII dimer have been studied at low resolution.[55] The binding of sugar and nucleotide substrates to dimeric yeast hexokinase (BII) exhibits both negative and positive co-operativity. Sugar substrates and inhibitors bind in the deep cleft that divides each subunit into two lobes and nucleotide substrates bind nearby to one site per dimer which lies between the subunits and on the molecular symmetry axis. Although several inhibitors bind equally to both subunits the degree of substitution of glucose or xylose is very different for the two subunits. This negative co-operativity in substrate binding may result from the heterologous or non-equivalent environments for the two chemically identical subunits. There is a positive allosteric interaction between the sugar- and nucleotide-binding sites. Sugar binding is required for nucleotide binding at the inter-subunit site and the binding of nucleotides modifies the binding of sugars. These positive heterotropic interactions appear to be mediated by extensive substrate-induced structural changes in the enzyme.

Triosephosphate Isomerase.—This enzyme has a molecular weight of 53 000 and is a dimer with two identical subunits. The two subunits are independently active and the reaction mechanism is well understood. The structure of the enzyme from chicken muscle has been determined at 2.5 Å resolution using amino-acid sequence data.[56] Each subunit is roughly spherical with diameter about 35 Å. Within each subunit the polypeptide chain is folded in a surprisingly regular manner to form an inner cylinder or barrel in which the staves are represented by eight strands of parallel β-pleated sheet. Following the normal twist these strands are inclined to the axis of the barrel. Adjacent strands follow one another consecutively in the sequence and are linked by mainly helical segments of chain which form the outer surface of each subunit. About 55% of the residues are involved in α-helices and about 22% in β-structure. The general distribution of the side-chains is as expected. The two subunits are related by a non-crystallographic two-fold axis. A loop of chain forms a hydrophobic pocket around a Met from the adjacent subunit. The substrate dihydroxyacetone phosphate binds

[54] R. J. Fletterick, D. J. Bates, and T. A. Steitz, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 38.
[55] W. F. Anderson and T. A. Steitz, *J. Mol. Biol.*, 1975, **92**, 279.
[56] D. W. Banner, A. C. Bloomer, G. A. Petsko, D. C. Phillips, C. I. Pogson, I. A. Wilson, P. H. Corran, A. J. Furth, J. D. Milman, R. E. Offord, J. D. Priddle, and S. G. Waley, *Nature*, 1975, **255**, 609.

at the carboxyl end of the β-barrel. A few residues of the adjacent subunit are involved.

Glyceraldehyde-3-phosphate Dehydrogenase.—This enzyme is a tetramer of molecular weight 140 000. The structure of the lobster muscle enzyme has been calculated at 2.9 Å resolution.[57] Two heavy-atom derivatives were used and the electron-density map, in which the asymmetric unit is the complete tetramer, was averaged over the molecular dyad axis which is known to be exact in the human holoenzyme. The map shows possible asymmetry between subunits in which the active centres are closely related. The major differences occur around the active site. The coenzyme is more tightly bound in one pair of active sites than in the other pair and it is suggested that the conformation of the adenine about the glycosidic bond could be *anti* with a C-2′ *endo* conformation for the ribose ring in one pair of subunits but probably *syn* with C-3′ *endo* in the other. Detailed mechanistic proposals are given. The amino-acid sequences of the pig muscle and yeast enzymes have been compared with the tertiary structure of the lobster enzyme.[58]

Lactate Dehydrogenase.—A 5 Å map of a coenzyme-deficient LDH:NAD–pyruvate ternary complex shows loss of coenzyme substitution on two of the four subunits.[59] Thus in this enzyme as well there is a reduction of molecular symmetry when coenzyme is removed. A low-resolution study of pig heart lactate dehydrogenase showed that the only observable difference between this H_4 isoenzyme and the M_4 dogfish structure was a displacement in the vicinity of the essential His-195.[60]

Pyruvate Kinase.—Pyruvate kinase catalyses the phosphorylation of ADP by phosphoenolpyruvate. Both a bivalent and a univalent cation are required. The muscle enzyme, which unlike that present in liver does not show allosteric activation, has a molecular weight of 240 000 and is composed of four identical subunits. The low-resolution electron-density map shows that each subunit is organized into two very unequal domains, divided by a cleft.[61] Phosphoenolpyruvate, Mn^{2+}, and Mn–ATP bind closely together on the side of this cleft which appears to be the active site. There is a second nucleotide-binding site some 25 Å away.

8 Electron Transport and Oxygen Reactions

Thioredoxin-S2.—This is a low molecular weight protein which transports electrons *via* an oxidation–reduction active disulphide. The structure of the *E. coli* protein, which contains 108 residues of known sequence, has been determined at 2.8 Å resolution.[62] The molecule is built up of a central core of three parallel and two antiparallel strands of pleated sheet surrounded by four helices.

[57] D. Moras, K. W. Olsen, M. N. Sabesan, M. Buehner, G. C. Ford, and M. G. Rossmann, *J. Biol. Chem.*, 1975, **250**, 9137.
[58] K. W. Olsen, D. Moras, M. G. Rossmann, and J. I. Harris, *J. Biol. Chem.*, 1975, **250**, 9313.
[59] J. White, M. G. Rossmann, and G. C. Ford, *J. Mol. Biol.*, 1975, **98**, 259.
[60] W. Eventoff, M. L. Hackert, and M. G. Rossmann, *J. Mol. Biol.*, 1975, **98**, 249.
[61] D. K. Stammers and H. Muirhead, *J. Mol. Biol.*, 1975, **95**, 213.
[62] A. Holmgren, B.-O. Söderberg, H. Eklund, and C.-I. Brändén, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2305.

The residues involved in the active-centre 14-membered disulphide ring form a protrusion between one of the helices and the middle strand of the sheet. This region of the molecule, comprising two parallel strands joined by the protrusion and a helix, is structurally very similar to the corresponding functionally important regions in the nucleotide-binding domains of flavodoxin and the dehydrogenases. Thioredoxin is a substrate for two enzymes and presumably these enzymes will have active-site clefts designed to fit this active-site protrusion.

High-potential Iron Protein.—The structure of *Chromatium* high-potential iron protein has been refined to give a standard crystallographic *R*-factor of 0.25.[63] The refined iron–sulphur cluster is more regular in that geometrically equivalent bond lengths and angles are more nearly equal, and the geometry of the refined cluster more closely resembles that of a small model analogue.

Cytochromes.—Crystals of cytochrome b_5 reduced by sodium dithionite are isomorphous with the oxidized form. The difference Fourier at 2.8 Å resolution shows that there is no change in the conformation of the main chain or in the orientation of internal side-chains upon reduction.[64] However, an ion becomes attached at the entrance of the haem crevice, causing displacement of a surface Lys side-chain in an adjacent molecule. This cation appears to neutralize one of the haem propionate groups, which is partially buried. It is proposed that the negatively charged propionate neutralizes the positive charge on the haem ion in the oxidized cytochrome and that the neutralization of the haem iron upon reduction leads to the binding of a cation to the propionate. The reduction mechanism *in vivo* probably involves the direct transfer of an electron to the porphyrin ring.

The structure of bonito ferrocytochrome *c* at 2.3 Å is very similar to that observed for other cytochrome *c*'s.[65] Phe-82 is in close contact with the iron atom.

Superoxide Dismutase.—The structure of bovine Cu,Zn superoxide dismutase at 3 Å resolution has been described.[66] 50% of the backbone forms an eight-stranded barrel of antiparallel β-pleated sheet. The interior of the barrel is packed with hydrophobic side-chains. There is one short helical section and two long loops. The Cu and Zn ions are bound between the loops and one side of the barrel and are about 6 Å apart with a common His ligand. The Cu has four His ligands in a somewhat distorted square plane and the Zn is liganded to three His and an Asp in an approximately tetrahedral arrangement. The two Cu's of a dimer are about 34 Å apart. The overall folding pattern of the polypeptide chain is very similar to that of an immunoglobulin domain. The Cα co-ordinates have been published.[67]

Myoglobin and Haemoglobin.—The crystal structures of acid metmyoglobin and deoxy-cobalt(II)-mesoporphyrin IX myoglobin were compared by a difference

[63] S. T. Freer, R. A. Alden, C. W. Carter, and J. Kraut, *J. Biol. Chem.*, 1975, **250**, 46.
[64] P. Argos and F. S. Mathews, *J. Biol. Chem.*, 1975, **250**, 747.
[65] N. Tanaka, T. Yamane, T. Tsukihara, T. Ashida, and M. Kakudo, *J. Biochem.*, 1975, **77**, 147.
[66] J. S. Richardson, K. A. Thomas, B. H. Rubin, and D. C. Richardson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1349.
[67] J. S. Richardson, K. A. Thomas, and D. C. Richardson, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 986.

Fourier analysis at 2.5 Å resolution.[68] The structural changes in the porphyrin ring were clearly observed but it was not clear whether there was any change of the metal position relative to the porphyrin plane or proximal His.

The structure of human deoxyhaemoglobin at 2.5 Å resolution has been refined.[69] The distance of the iron atom from the porphyrin plane is 0.60 Å in the α-haem and 0.63 Å in the β-haem; the distance of the N_ε of the iron-linked His from the porphyrin plane is 2.6 Å in the α-haem and 2.8 Å in the β-haem. The structures of deoxy- and carbonmonoxy-haemoglobin Kansas (Asn-102β → Thr) in the deoxy quaternary conformation have been compared with that of normal human deoxyhaemoglobin.[70] Hb Kansas has four functional haems with a lower than normal oxygen affinity. A hydrogen bond between Asp-99α and Asn-102β stabilizes the oxy quaternary structure in normal human haemoglobin. Human deoxyhaemoglobin crystals grown from polyethylene glycol solution had an almost identical structure to that of normal haemoglobin.[11] There were small conformational changes, many of which were suggestive of the structural changes that accompany the $T \rightarrow R$ transition. A comparison of the haem binding pockets in globins and cytochrome b_5 shows that they are structurally and topologically equivalent.[71] The only major difference between the two structures is a rotation of the haem group about its normal by 53°.

A stereochemical basis of heat stability in bacterial ferredoxins and in haemoglobin A2 appears to lie in the presence of external salt bridges and hydrogen bonds.[72]

9 Proteolytic Enzymes

The refined crystal structure of bovine β-trypsin at 1.8 Å resolution has been determined.[9, 10] At pH 7 the small inhibitor benzamidine is used for protection against autodigestion. The structure has been compared with that of trypsin in the trypsin–trypsin inhibitor complex. Any differences are very small. In particular Ser-195 O_γ does not change its dihedral angle significantly. This indicates that on binding the inhibitor adapts to the trypsin which in the main remains rigid. The structure shows some discrepancies with that obtained earlier by other workers. There is one calcium-binding site in the trypsin molecule. The calcium ion is complexed by six different ligands positioned at the edges of an almost regular octahedron.[73]

Although primary structural homology between bacterial serine proteinases and those from the mammalian pancreas is slight, two-thirds of the residues in the bacterial enzyme SGPB adopt a similar polypeptide chain conformation to that of the trypsin family.[74] Boronic acid adducts with subtilisin BPN′ contain a covalent bond between O_γ of the catalytic Ser-221 and the inhibitor boron atom.[75]

[68] E. A. Padlan, W. A. Eaton, and T. Yonetani, *J. Biol. Chem.*, 1975, **250**, 7069.
[69] G. Fermi, *J. Mol. Biol.*, 1975, **97**, 237.
[70] L. Anderson, *J. Mol. Biol.*, 1975, **94**, 33.
[71] M. G. Rossmann and P. Argos, *J. Biol. Chem.*, 1975, **250**, 7525.
[72] M. F. Perutz and H. Raidt, *Nature*, 1975, **255**, 256.
[73] W. Bode and P. Schwager, *F.E.B.S. Letters*, 1975, **56**, 139.
[74] L. T. J. Delbaere, W. L. B. Hutcheon, M. N. G. James, and W. E. Thiessen, *Nature*, 1975, **257**, 758.
[75] D. A. Matthews, R. A. Alden, J. J. Birktoft, S. T. Freer, and J. Kraut, *J. Biol. Chem.*, 1975, **250**, 7120.

The boron atom is co-ordinated tetrahedrally and this complex appears to be a good model for the postulated tetrahedral intermediate.

Various metal ions have been bound to triclinic lysozyme.[76] These bind near the oxygens of the side-chains of Glu-35 and Asp-52 and act as inhibitors. An *X*-ray study has been made of the binding properties of iodine-inactivated lysozyme.[77] This work provides support for the proposal that distortion of the sugar residue in subsite D is important in the catalytic mechanism.

10 Other Globular Proteins

Rhodanese is a sulphur transferase of molecular weight 32 000. The 3 Å electron density map of bovine liver rhodanese shows that it consists of a single polypeptide chain.[78] There is a very clear double domain structure and the two domains are of equal size with very similar conformations. The single chain appears to have evolved from a monomer of half the size after gene duplication. Each domain contains a four-stranded β-structure which is similar to the nucleotide-binding fold found in other proteins.

The tertiary structure of human erythrocyte carbonic anhydrase B is very similar to the structure of the C isozyme.[79] The essential zinc is firmly bound to the enzyme through three His ligands and is located at the bottom of a conical cavity. The topography of the active site in the immediate vicinity of the zinc is slightly different in B and C.

A study of the conformation of coenzyme analogues when bound to liver alcohol dehydrogenase showed that the steric requirements of the hydrophobic adenosine-binding pocket are such that for proper nucleotide binding the adenine moiety must be oriented in a specific way.[80] The electron-density map of the active-site region in native LADH shows the zinc ligands (Cys-46, Cys-174, His-67, and solvent molecule) very clearly (see Figure 5 facing p. 171).[81] The enzyme was alkylated with iodoacetate under conditions resulting in the single substitution of Cys-46. Under these conditions the carboxymethylated sulphur atom of Cys-46 is still liganded to the zinc and the iodide ion liberated during alkylation is bound as the fourth zinc ligand, displacing imidazole (Figure 5).

11 Viruses

Tobacco Mosaic Virus.—Three-dimensional image reconstruction techniques have been applied to three related structures; the *dahlemense* strain of TMV, the RNA-free protein helix of the same strain, and the protein helix of the *vulgare* strain. In all three cases the arrangement of subunits is regularly perturbed in such a way that adjacent turns of the basic helix are paired but the strength and nature of the pairing interaction varies from one structure to another.[82] This

[76] K. Kurachi, L. C. Sieker, and L. H. Jensen, *J. Biol. Chem.*, 1975, **250**, 7663.
[77] C. R. Beddell, C. C. F. Blake, and S. J. Oatley, *J. Mol. Biol.*, 1975, **97**, 643.
[78] J. Bergsma, W. G. J. Hol, J. N. Jansonius, K. H. Kalk, J. H. Ploegman, and J. D. G. Smit, *J. Mol. Biol.*, 1975, **98**, 637.
[79] K. K. Kannan, B. Notstrand, K. Fridborg, S. Lövgren, A. Ohlsson, and M. Petef, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 51.
[80] M. A. Abdallah, J.-F. Biellmann, B. Nordström, and C.-I. Brändén, *European J. Biochem.* 1975, **50**, 475.
[81] E. Zeppezauer, H. Jörnvall, and I. Ohlsson, *European J. Biochem.*, 1975, **58**, 95.
[82] R. Sperling, L. A. Amos, and A. Klug, *J. Mol. Biol.*, 1975, **92**, 541.

pairing interaction appears to play an essential role in the self-assembly of the virus and represents a non-equivalent interaction between chemically identical subunits. The electron-density distribution of the intact virus has been calculated at a resolution of 6.7 Å by analysis of the *X*-ray diffraction pattern given by oriented gels.[83] This was achieved by separation of overlapping Bessel function terms by a technique analogous to isomorphous replacement. The course of the polypeptide chain of the coat protein was traced for much of its length although there were several ambiguous regions (Figure 6). In addition an electron-density map of the TMV disc at 5 Å resolution has been obtained using isomorphous replacement and non-crystallographic symmetry.[84] The axial contacts between protein subunits are unlike those in the virus, the disc being a more open structure apparently designed for rapid interaction with the RNA. The polypeptide chain was traced with little ambiguity. The map reveals reasonable agreement in gross features with that obtained for the intact virus. However, it is not surprising to find that the connections between helical regions do not agree with those derived from the lower-resolution map of the helical virus (Figures 6 and 7).

Spherical Viruses.—A three-dimensional reconstruction from electron micrographs of tomato bushy stunt virus has been used to determine the *X*-ray phases to 28 Å resolution by analogy with the single isomorphous replacement method of protein crystallography.[85] The electron-density map computed from *X*-ray amplitudes and these phases differs in two important respects from the reconstruction. The exclusion of stain from the five-fold vertices, previously attributed to the presence of a minor protein component, is shown to be an artefact of staining. In addition there is positive staining of the RNA at the quasi three-fold positions. An electron-density map has been calculated at 16 Å resolution using multiple isomorphous replacement and non-crystallographic symmetry.[86] The relative orientation of the three quasi-equivalent protein subunits is clearly defined. The geometry of the dimer contacts is very similar at the strict and local dyads. The contacts around the five-fold axis are similar to those in the $T = 1$ structure of turnip crinkle virus, and the larger $T = 3$ structure involves a flattening across the true dyad. The nucleic acid chain is intertwined among the protein subunits with extensive regions of protein–RNA interaction.

Southern bean mosaic virus is a small $T = 3$ plant virus. Rotation function studies at 22 Å resolution have been used to find the orientation of the virus in the rhombohedral unit cell.[87] The results differ from an earlier prediction based on apparent spikes in an *hk*0 photograph. A new crystal form has been found which should be suitable for high-resolution structural studies.[87a]

Filamentous Bacterial Viruses.—Marvin and Wachtel have reported the first model of the structure of a filamentous bacterial virus.[88] The protein coat of these viruses is constructed from segments of α-helix that extend in an axial direction and overlap each other to form a hollow cylindrical shell which contains

[83] K. C. Holmes, G. J. Stubbs, E. Mandelkow, and U. Gallwitz, *Nature*, 1975, **254**, 192.
[84] J. N. Champness, A. C. Bloomer, G. Bricogne, P. J. G. Butler, and A. Klug, *Nature*, 1976, **259**, 20.
[85] A. Jack, S. C. Harrison, and R. A. Crowther, *J. Mol. Biol.*, 1975, **97**, 163.
[86] S. C. Harrison and A. Jack, *J. Mol. Biol.*, 1975, **97**, 173.
[87] J. E. Johnson, P. Argos, and M. G. Rossmann, *Acta Cryst.*, 1975, **B31**, 2577.
[87a] T. Akimoto, M. A. Wagner, J. E. Johnson, and M. G. Rossmann, *J. Ultrastruct. Res.*, 1975, **53**, 306.
[88] D. A. Marvin and E. J. Wachtel, *Nature*, 1975, **253**, 19.

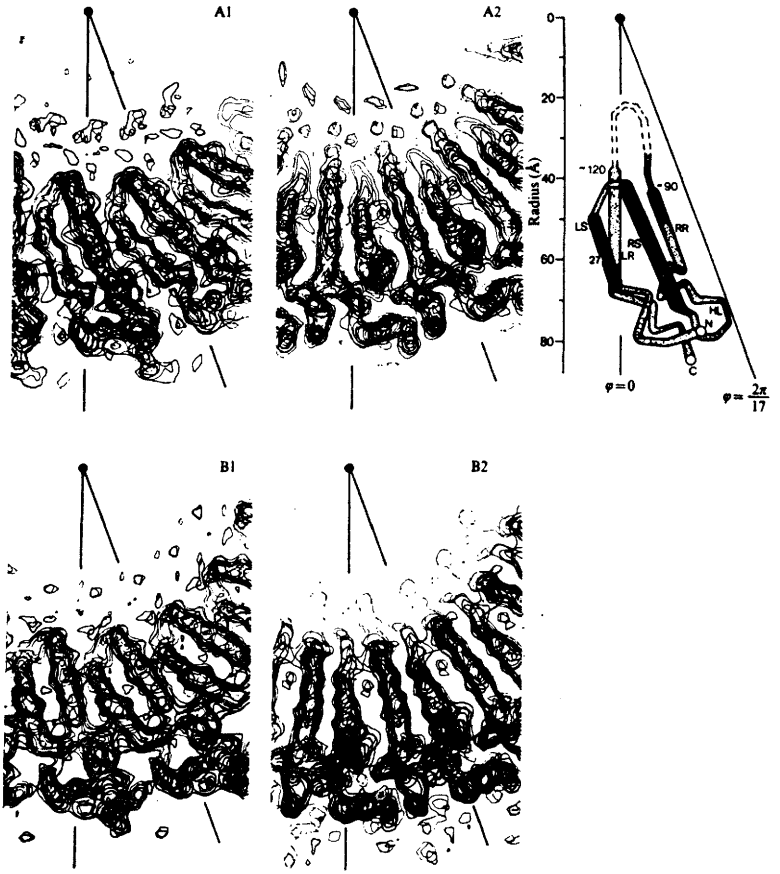


Figure 7 *Averaged electron-density map of a sector through the TMV protein disc. The sector comprises two subunits per layer and is shown in four composite slices.* A *and* B *represent the two rings which compose the disc and* 1 *and* 2 *represent the upper and lower halves of the corresponding rings. The schematic diagram indicates the course proposed for the polypeptide chain in one subunit. The slewed rods* LS *and* RS *are in the upper half-layer* 1 *and the radial rods* LR *and* RR *are in half-layer* 2. *The 'hanging loop' connecting rods* RS *and* RR *is denoted* HL
(Reproduced by permission from *Nature*, 1976, **259**, 20)

the viral DNA. The low-resolution *X*-ray data were used to define the general arrangement of α-helices and molecular model building was used to define atomic positions (Figure 8). This linear assembly of coat protein subunits encapsulating single-stranded circular DNA is a valuable model for studying the molecular structure of fibrous proteins and nucleoproteins.

Bacteriophages.—Optical reconstruction has been used to study the structures of T4, R17, and f2 bacteriophages.[89–91]

[89] T. Ishii and M. Yanagida, *J. Mol. Biol.*, 1975, **97**, 655.
[90] L. A. Amos and A. Klug, *J. Mol. Biol.*, 1975, **99**, 51.
[91] R. A. Crowther, L. A. Amos, and J. T. Finch, *J. Mol. Biol.*, 1975, **98**, 631.

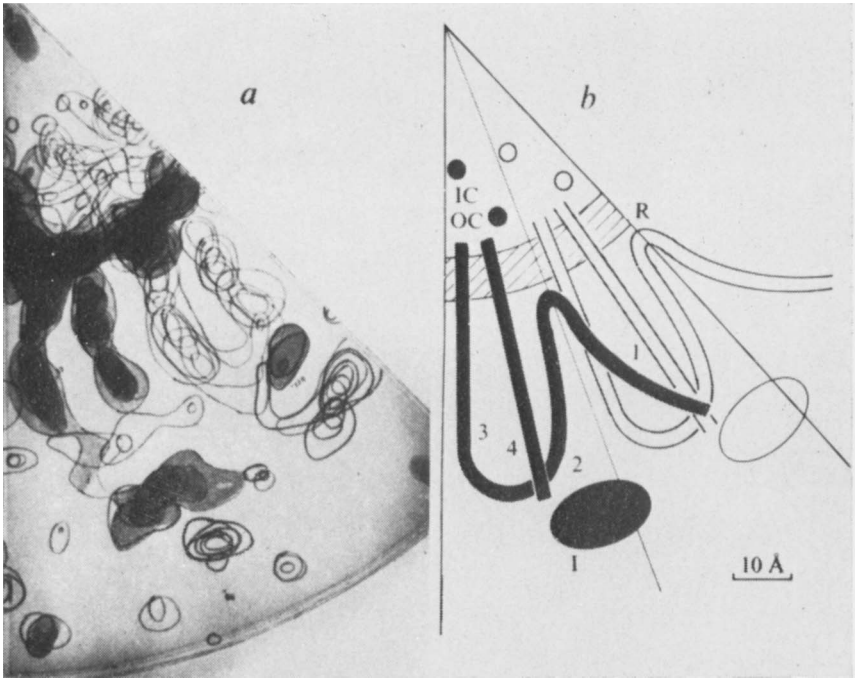


Figure 6 (*a*) *Sections through the electron-density map of tobacco mosaic virus. The sections contain parts of two adjacent subunits. One subunit and the RNA strand are shaded. The RNA strand, some ribbons of density, and an island of density are clearly visible.* (*b*) *Schematic diagram of the main features in the electron-density map. The diagram represents two subunits, one shown by open symbols.* IC *and* OC *represent columns of density,* I *an island of density, and* 1, 2, 3, 4 *ribbons of density*
(Reproduced by permission from *Nature*, 1975, **254**, 192)

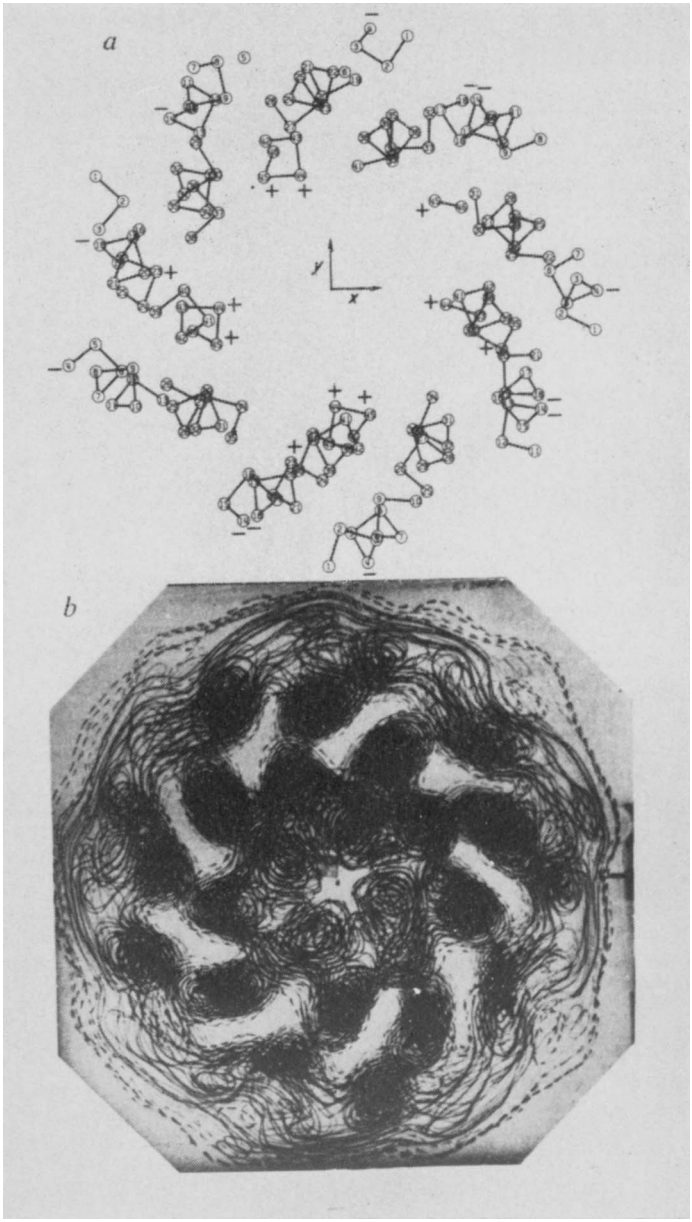


Figure 8 *Axial section of the model for class II filamentous bacterial virus. A* 14 Å *slab through one virion, equivalent to four helix asymmetric units, is shown. (a) Ball-and-stick representation of the α-carbon atoms, projected on to a plane normal to the axis of the virion. Numbers indicate residues. The basic and acidic residues are indicated by + and − respectively. The box measures* 30 Å *on a side. (b) Electron density in a corresponding slab, calculated using the observed amplitudes and the phases calculated from the protein model. The DNA is in the central region*
(Reproduced by permission from *Nature*, 1975, **253**, 19)

12 Muscle

Considerable efforts are being made to crystallize the various components of muscle. The troponin complex, which controls the onset of muscular contraction, contains three different subunits T, I, and C. This complex is bound at regular intervals along the thin filaments which are composed of a double helix of actin monomers plus tropomyosin which runs along the groove of the actin helix. Each tropomyosin molecule spans seven actin monomers and one molecule of the troponin complex is bound to each tropomyosin molecule through subunit T. Subunit I binds to actin and C binds to both T and I. In the absence of calcium, I and T inhibit contraction by inhibiting the interaction of actin and myosin. In the presence of calcium, the inhibition is reversed by calcium binding to C and contraction occurs. Subunit C of rabbit troponin has a molecular weight of about 17 000. Crystals have been obtained [92] which are about 0.1 mm long and diffract to a resolution of 8—10 Å. The lattice is tetragonal with $a = b = 127$ Å, $c = 156$ Å.

The tropomysin molecule consists of two α-helices, which form a coiled-coil a little over 400 Å long. From the optical diffraction pattern the pitch of the double helix is 114 Å so that the repeat distance in the molecule is 57 Å. In the actin helix the distance along the helix between the actin subunits at the radius at which tropomyosin lies is close to 57 Å. Thus each actin subunit would be in a position to interact with one half-turn of the tropomyosin double helix.[93]

When subunit C of troponin is removed from the actin–tropomyosin–troponin complex the thin filament is not calcium sensitive and remains permanently 'switched off' as in a resting muscle. Without any of the troponin components present the actin–tropomyosin complex is also insensitive to calcium, but in this case it stimulates the splitting of ATP by myosin, *i.e.* it is active or 'switched on'. Wakabayashi *et al.*[94] formed paracrystals of both these complexes by the addition of Mg^{2+}. Using three-dimensional image reconstruction from electron micrographs they showed that in the actin–tropomyosin complex the tropomyosin may be clearly distinguished running near the centre of each of the grooves between actin strands. However, in the inhibited complex produced by the addition of subunits T and I of troponin the tropomyosin is displaced through about 10 Å and the contact between actin and tropomyosin becomes very close. This supports the proposal that inhibition is caused by the blocking by tropomyosin of the sites of actin that interact with myosin. O'Brien, Gillis, and Couch [95, 96] have studied the effect of calcium ions in the range 10^{-6}—10^{-5} mol l^{-1} on the structure of thin filaments reconstituted from actin, tropomyosin, and troponin using electron microscopy and *X*-ray diffraction. Their results show that the tropomyosin shift can occur reversibly in thin filaments and is controlled by the level of calcium.

X-Ray evidence has been obtained for conformational changes in the myosin filaments of vertebrate striated muscle.[97] An analysis of the axial *X*-ray diffrac-

[92] D. Mercola, B. Bullard, and J. Priest, *Nature*, 1975, **254**, 634.
[93] W. Longley, *Nature*, 1975, **253**, 126.
[94] T. Wakabayashi, H. E. Huxley, L. A. Amos, and A. Klug, *J. Mol. Biol.*, 1975, **93**, 477.
[95] J. M. Gillis and E. J. O'Brien, *J. Mol. Biol.*, 1975, **99**, 445.
[96] E. J. O'Brien, J. M. Gillis, and J. Couch, *J. Mol. Biol.*, 1975, **99**, 461.
[97] J. C. Haselgrove, *J. Mol. Biol.*, 1975, **92**, 113.

tion patterns from relaxed, contracted, and rigor muslces at different sarcomere lengths has shown that the initial movement of cross-bridges from their positions in resting muscle does not depend on the interaction of each cross-bridge with actin, but on a conformational change in the backbone of the myosin filament. It is possible that the myosin filament, like the actin filament, has an activation control mechanism and can exist in either of two possible states.

Lymn has studied the effect of a non-hydrolysed analogue of ATP on the low-angle *X*-ray patterns from skeletal muscle.[98] The patterns obtained were different from the patterns characteristic of either relaxed or rigor muscle. The ATP analogue changes the actin–myosin bonds but does not restore the thick filament to the configuration typical of relaxed muscle. Using this analogue it may be possible to observe a transitory state. An analysis of the equatorial *X*-ray reflexions from skeletal muscle[99] has shown that models with the myosin heads or cross-bridges projecting to a radius of 180—200 Å fit the *X*-ray data best. It is possible that the change in the ratio of intensities of the two principal equatorial reflexions arises mainly from azimuthal variations in the configuration of the cross-bridges within the equatorial projection with little radial movement outwards from the thick filament. *X*-Ray diffraction patterns from relaxed invertebrate muscles have been used to show the thick filament symmetries and cross-bridge configurations.[100] The cross-bridges are markedly tilted to the filament axes in the resting state and take up diverse configurations and positions. Measurements of the low-angle diffraction pattern of a calcium-activated insect flight muscle, which was sinusoidally oscillated in length to produce mechanical work, were interpreted in terms of a movement of the heads of the myosin molecules into contact with the actin filaments.[101] Between 10 and 20% of myosin heads were attached to actin during this work-producing oscillation of the muscle. Calculations suggested that not all the myosin molecules attached to actin at any one moment were generating tension.

Carp muscle calcium-binding parvalbumin is a low molecular weight, water-soluble protein. Its function is unknown but a role in calcium ion control and muscle contraction has been suggested. The structure has been refined to give an *R*-factor of 0.25.[102] Two calcium ions are bound.[103] One is completely surrounded by protein ligands; the second has one water molecule in the primary co-ordination sphere.

13 Fibrous Proteins

X-Ray studies have been carried out on the structures of poly-(γ-ethyl)-L-glutamate[104] and poly-γ-benzyl-DL-glutamate,[105] which form helical structures, and poly-L-alanine, poly-(L-alanylglycine) and poly-L-homoarginine hydro-

[98] R. W. Lymn, *J. Mol. Biol.*, 1975, **99**, 567.
[99] R. W. Lymn, *Nature*, 1975, **258**, 770.
[100] J. S. Wray, P. J. Vibert, and C. Cohen, *Nature*, 1975, **257**, 561.
[101] P. M. Armitage, R. T. Tregear, and A. Miller, *J. Mol. Biol.*, 1975, **92**, 39.
[102] P. C. Moews and R. H. Kretsinger, *J. Mol. Biol.*, 1975, **91**, 201.
[103] P. C. Moews and R. H. Kretsinger, *J. Mol. Biol.*, 1975, **91**, 229.
[104] G. Valle, A. Del Pra, P. Spadon, and M. Mammi, *Biopolymers*, 1975, **14**, 437.
[105] F. Heitz, B. Lotz, and G. Spach, *J. Mol. Biol.*, 1975, **92**, 1.

chloride, which form β-pleated sheet structures.[106, 107] Two models for the structure of the collagen fibril have been presented.[108, 109]

14 Low-angle X-Ray Scattering

Low-angle *X*-ray scattering has been used to study cattle and rabbit optic nerves swollen in glycerol solution,[110] the interaction between lysine transfer RNA ligase from yeast and transfer RNA,[111] deoxyribonucleoprotein,[112] and whole and broken *Bacillus subtilis* bacteria.[113]

Part III: Conformation and Interaction of Peptides and Proteins in Solution *edited by R. H. Pain, with contributions by J. M. Asher, R. J. Burgess, D. P. E. Dickson, D. Osguthorpe, H. W. E. Rattle, B. Robson, P. J. Seeley, R. M. Stephens, and E. J. Wood*

1 Theoretical Aspects of Protein Conformation

Contributed by J. M. Asher, D. Osguthorpe, and B. Robson

Introduction.—The past year has been particularly interesting. Although there have been no radical revisions in basic concepts, workers are attending to the practical problems concerned with simulating the folding of globular proteins and predicting their native structure. Since hydrophobic interactions are largely responsible for the conformation and stability of globular proteins, it is appropriate that a great deal of work is being carried out on solvent structure. We therefore continue to give considerable attention to relevant work on water structure and solvent–polypeptide interactions.

Conformational Energy Calculations.—Work continues on the improvement of semi-empirical energy functions by which the conformational energy of a molecule can be estimated.[1] An interesting new approach is the EPEN method (*i.e.* Empirical Potential using Electrons and Nuclei) in which properties are assigned to points inside bonds as well as to the nuclei.[2, 3]

Quantum mechanical methods continue to be of value in investigating hydrogen bonding, and the conclusions are likely to be of value in defining the function form for semi-empirical potentials.[4–6]

[106] F. Colonna-Cesari, S. Premilat, and B. Lotz, *J. Mol. Biol.*, 1975, **95**, 71.
[107] M. Suwalsky, M. Bunster, and K. G. Wagner, *Biopolymers*, 1975, **14**, 1197.
[108] A. Veis and L. Yuan, *Biopolymers*, 1975, **14**, 895.
[109] J. Woodhead-Galloway, D. W. L. Hukins, and J. S. Wray, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 1237.
[110] S. Lalitha and C. R. Worthington, *J. Mol. Biol.*, 1975, **96**, 625.
[111] R. Österberg, B. Sjöberg, L. Rymo, and U. Lagerkvist, *J. Mol. Biol.*, 1975, **99**, 383.
[112] V. I. Aleksanyan, V. I. Vorobev, and B. A. Fedorov, *Biopolymers*, 1975, **14**, 1133.
[113] M. K. Gee, D. L. Parker, L. C. Ford, and W. E. Dibble, *J. Ulstrastruct. Res.*, 1975, **52**, 300.
[1] F. A. Momany, R. F. McGuire, A. W. Burgess, and H. A. Scheraga, *J. Phys. Chem.*, 1975, **79**, 2361.
[2] L. L. Shipman, A. W. Burgess, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 543.
[3] A. W. Burgess, L. L. Shipman, and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 854.
[4] J. E. Del Bene, *J. Chem. Phys.*, 1975, **62**, 1961.
[5] J. D. Dill, L. C. Allen, and W. C. Topp, *J. Amer. Chem. Soc.*, 1975, **97**, 7220.
[6] A. Støgard, A. Strich, J. Almhof, and B. Roos, *Chem. Phys.*, 1925, **8**, 405.

Energy calculations by semi-empirical methods are still being carried out for short oligopeptides such as diproline,[7] the tripeptide thyrotropin-releasing factor,[8] and tetrapeptides which occur in reverse turns of chymotrypsin homologues,[9] and for synthetic polypeptide crystal systems.[10, 11] An interesting new application is to the study of amino-acid replacements by mutation in cytochrome *c*.[12]

Energy calculations of this type should be checked by predicting conformational properties of systems for which experimental conformational data are available. In this connection, a critical discussion of the accuracy and significance of the experimental techniques is of interest.[13]

Water Structure.—An understanding of water structure is essential for understanding polypeptide–water interactions in general and interactions between hydrophobic side-chains in particular. Stillinger[14] has reviewed a variety of models and Anderson[15] has discussed models of liquid in general. Three models have been receiving particular attention in the past two years: the cluster model, the interstitial model, and the random network model. Lentz *et al.*[16–18] have considered the implications of a cluster model. The cluster model has been contested by Symons[19] largely on the grounds that the predicted degree of hydrogen bonding is less than that observed by i.r. and n.m.r. spectroscopy. A new formulation of the interstitial model has been proposed by Bell and Sallouta.[20] A rather more novel description of water is as a random network of fully hydrogen-bonded molecules, and Rice[21] has shown that this is an experimentally testable model. Although the co-ordination number for water molecules proposed by this theory is somewhat less than that observed by small-angle *X*-ray scattering, Kell[22] has overcome this difficulty by proposing an interpenetrating network for water at low temperatures.

A number of papers have treated details of the interaction between water molecules in a dimer or small clusters.[23–25] In the case of larger clusters or bulk water, Monte Carlo or Molecular Dynamic approaches are best. Monte Carlo calculations have recently been carried out by Lie and Clementi,[26] using a modified Hartree–Fock potential, and by Sarkisov *et al.*,[27] who used a 6-exp

[7] C. M. Venkatachalam, B. J. Price, and S. Krimm, *Biopolymers*, 1975, **14**, 1121.
[8] A. W. Burgess, F. A. Momany, and H. A. Scheraga, *Biopolymers*, 1975, **14**, 2645.
[9] J. C. Howard, A. Ali, H. A. Scheraga, and F. A. Momany, *Macromolecules*, 1975, **8**, 607.
[10] F. Colonna-Cesari, S. Premilat, and B. Lotz, *J. Mol. Biol.*, 1975, **95**, 71.
[11] M. D. Alagni, E. Giglio, and N. V. Pavel, *Polymer*, 1976, **17**, 257.
[12] P. K. Warme, *Biochemistry*, 1975, **14**, 3518.
[13] L. C. Craig, D. Cowburn, and H. Bleich, *Ann. Rev. Biochem.*, 1975, **4**, 477.
[14] F. H. Stillinger, *Adv. Chem. Phys.*, 1975, **31**, 1.
[15] H. C. Anderson, *Ann. Rev. Phys. Chem.*, 1975, **26**, 145.
[16] B. R. Lentz, A. T. Hagler, and H. A. Scheraga, *J. Phys. Chem.*, 1974, **78**, 1531.
[17] B. R. Lentz, A. T. Hagler, and H. A. Scheraga, *J. Phys. Chem.*, 1974, **78**, 1844.
[18] J. C. Owicki, B. R. Lentz, A. T. Hagler, and H. A. Scheraga, *J. Phys. Chem.*, 1975, **79**, 2352.
[19] M. C. R. Symons, *Phil. Trans. Roy. Soc.*, 1975, **B272**, 13.
[20] G. M. Bell and H. Sallouta, *Mol. Phys.*, 1975, **29**, 1621.
[21] S. A. Rice, *Topics Current Chem.*, 1975, **60**, 109.
[22] C. G. S. Kell, *Chem. Phys. Letters*, 1975, **30**, 223.
[23] F. M. Stillinger and H. L. Lemberg, *J. Chem. Phys.*, 1975, **62**, 1340.
[24] L. L. Shipman, J. C. Owicki, and H. A. Scheraga, *J. Phys. Chem.*, 1974, **78**, 2055.
[25] J. C. Owicki, H. A. Sheraga, and L. L. Shipman, *J. Phys. Chem.*, 1975, **79**, 1794.
[26] G. C. Lie and E. Clementi, *J. Chem. Phys.*, 1975, **62**, 2195.
[27] G. N. Sarkisov, V. G. Dashevski, and G. G. Malenkov, *J. Mol. Phys.*, 1974, **27**, 1249.

potential. Molecular Dynamics continues to be a popular technique, and Briant and Burton [28] have applied it to water clusters. Stillinger and colleagues [29, 30] have applied a central force model to facilitate Molecular Dynamic simulations.

Hydrophobic Interactions.—Both analytical treatment through models[31, 32] and more exact treatment through Molecular Dynamic and Monte Carlo [33] approaches may be applied to the problem of hydrophobic bonding.

Most of the current studies in hydrophobic bonding are, however, experimental. The solvophobic effect in general,[34] and the way in which the hydrophobic effect depends on solute [35] has been investigated. Howarth [36] has reassessed hydrophobic bonding in the light of n.m.r. measurements and has proposed that the hydrophobic effect possibly originates in the restricted motion of the solute itself. Malcolm [37] has determined experimental parameters for hydrophobic side-chain interactions in proteins and synthetic polypeptides at the air–water interface.

General Polypeptide–Solvent Interactions.—A simplified model of solvent effects on conformational stability in general rather than hydrophobic bonding in particular has been presented.[38] Tanaka and Scheraga [39] have also discussed the phenomenon of solute–solvent interactions on the *cis–trans* isomerization of polyproline and the change in the sense of the polyproline helix. In a second paper,[40] the solvent–solute effects for the polyproline helix coil are broken down into a bulk dielectric contribution and an interaction which is dependent on the types of atom in the polyproline. In a third paper,[41] the detailed effects of solvent are considered. Studies of the above kind often use the water 'supermolecule' approach (in particular, see ref. 38) in which the water is placed in specific positions relative to solute groups and treated as an extension of the solute molecule. Pullman and Berthod have used this approach in PCILO calculations of the effect of solvent on the conformation of γ-amino-butyric acid.[42]

Solvation of Globular Proteins.—Recent studies by Finney [43] confirm that the interior of a protein is well packed and excludes solvent molecules. The exposure of groups at the protein surface is also considered in this study. Such analyses are based on the *X*-ray structure of proteins in crystals. A theory for determining the amount of bound water in protein crystals has been discussed by Scanlan and Eisenberg,[44] and the amount of strongly bound water in proteins has been

[28] C. L. Briant and J. J. Burton, *J. Chem. Phys.*, 1975, **63**, 3327.
[29] H. L. Lemberg and F. H. Stillinger, *J. Chem. Phys.*, 1975, **62**, 1677.
[30] A. Rahman, F. M. Stillinger, and H. L. Lemberg, *J. Chem. Phys.*, 1975, **63**, 5223.
[31] A. Ben Naim, *Biopolymers*, 1975, **14**, 1337.
[32] R. B. Herman, *J. Phys. Chem.*, 1975, **79**, 163.
[33] V. G. Dashevsky and G. N. Sarkisov, *Mol. Phys.*, 1974, **27**, 1271.
[34] M. Yaacobi and A. Ben Naim, *J. Phys. Chem.*, 1974, **78**, 175.
[35] A. Ben Naim and M. Yaacobi, *J. Phys. Chem.*, 1975, **78**, 170.
[36] O. W. Howarth, *J.C.S. Faraday I*, 1975, **71**, 2303.
[37] B. R. Malcolm, *Adv. Chem. Soc.*, 1975, **145**, 338.
[38] D. L. Beveridge and G. W. Schnuelle, *J. Phys. Chem.*, 1975, **78**, 2064,
[39] S. Tanaka and H. A. Scheraga, *Macromolecules*, 1975, **8**, 494.
[40] S. Tanaka and H. A. Scheraga, *Macromolecules*, 1975, **8**, 504.
[41] S. Tanaka and H. A. Scheraga, *Macromolecules*, 1975, **8**, 516.
[42] B. Pullman and H. Berthod, *Theor. Chim. Acta*, 1975, **36**, 317–328.
[43] J. L. Finney, *J. Mol. Biol.*, 1975, **96**, 4.
[44] W. J. Scanlan and D. Eisenberg, *J. Mol. Biol.*, 1975, **98**, 485.

measured experimentally.[45] Of related interest is a study of the water sorption isotherm of polymers and proteins.[46]

In solution rather than in crystals, well-packed globular proteins are in dynamic equilibrium with a variety of more open conformations in which interaction between the interior of the protein and the solvent is possible. Woodward and colleagues have carried out an extensive study on tritium exchange in globular proteins, and this throws considerable light on the time-averaged degrees of exposure of regions of structure classically regarded as buried inside the protein.[47–50]

Conformational Information from Synthetic Polypeptides.—Experiments on synthetic polypeptides continue to provide valuable parameters and information about the conformational behaviour of amino-acid residues in proteins. As well as supplementing energy calculations they also yield experimental properties against which predictions using energy parameters may be tested.

The search for helix–coil stability parameters of various amino-acids in homo- and hetero-polypeptides continues,[51–53] while the β-pleated sheet to α-helix transition of polylysine has been studied as a function of temperature and pH.[54]

Conformational transitions of oligo-γ-methyl glutamate in solution and at the air–water interface have been investigated[55] for different chain lengths. The effect of the ordering of Gly, Leu, and Pro in poly(Gly-Leu-Pro) and the production of collagen-like conformations in the crystal and in solution have been examined.[56]

Secondary Structure of Globular Proteins.—It has been argued that an accurate prediction of secondary structure (including α-helix, β-pleated sheet, reverse turns, and details of the remaining coil conformations) may be a necessary prerequisite for successful computer simulation of the folding of globular proteins.[57] Comparison of predicted secondary structure with that observed by *X*-ray crystallography continues to be the major way of assessing the accuracy of predictions, although other kinds of experimental data have recently been used.[58, 59] In 1975 there has been a reduction in interest in predicting secondary structure. This appears to be for two principal reasons. First, most algorithms are predicting about 80% of residues correctly as helix or non-helix, and as pleated-sheet and non-pleated sheet (or as extended chain, non-extended chain). This is likely to be a theoretical limit imposed by the nature of the code relating sequence

[45] P. Bhaskara Rao and W. P. Bryon, *J. Mol. Biol.*, 1975, **97**, 119–122.
[46] H. W. Starkweather, *Macromolecules*, 1975, **8**, 476–479.
[47] C. K. Woodward, L. M. Ellis, and A. Rosenberg, *J. Biol. Chem.*, 1975, **250**, 432.
[48] C. K. Woodward, L. M. Ellis, and A. Rosenberg, *J. Biol. Chem.*, 1975, **250**, 440.
[49] L. M. Ellis, V. A. Bloomfield, and C. K. Woodward, *Biochemistry*, 1975, **14**, 3413.
[50] L. M. Ellis and C. K. Woodward, *Biochemistry*, 1975, **14**, 3419.
[51] J. E. Alter, G. T. Taylor, and H. A. Scheraga, *Macromolecules*, 1975, **8**, 479.
[52] T. Iio and S. Takahashi, *Bull. Chem. Soc. Japan*, 1975, **48**, 1240.
[53] K. J. Wenn and R. W. Woody, *Biopolymers*, 1975, **14**, 1827.
[54] I. Satake and J. T. Yang, *Biopolymers*, 1975, **14**, 1841.
[55] J. Caspan and W. Hecq, *Biopolymers*, 1975, **14**, 2263.
[56] A. Scatturin, A. N. Tomburro, A. Del Pra, and E. Bordignon, *Internat. J. Peptide Protein Res.*, 1975, **7**, 425.
[57] A. W. Burgess and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1221.
[58] B. M. Dunn and I. M. Chaiken, *J. Mol. Biol.*, 1975, **95**, 497.
[59] P. Y. Chou, A. J. Adler, and G. D. Fasman, *J. Mol. Biol.*, 1975, **96**, 29.

to conformation, so that higher degrees of accuracy cannot be obtained without considering 'long-range' interactions, *i.e.* interactions between residues far apart in the amino-acid sequence. Secondly, interaction between regions of organized secondary structure is of increasing interest in its own right as the likely next step in the folding process, and is occupying the attention of most workers interested in predictions. For example, Nagano has extended his earlier studies [60, 61] to take into account the 'long range' interactions.[62] A few recent papers, however, direct attention to other approaches to the prediction problem. The work of Lim is, in this respect, particularly worthy of note. Most predictions of secondary structure, like those of Nagano, are based on parameters obtained by statistical analysis of proteins of known sequence and conformation. The studies of Lim are, however, partly based on stereochemical 'rules-of-thumb' concerning side-chain interactions [63] from which good predictions can be made.[64] Another somewhat different approach makes use of pattern recognition to analyse proteins of known sequence and conformation and so establish prediction criteria.[65]

Analysis of interactions involving charged side-chains may be of value in enhancing the predictive power of current algorithms. Whether glutamic acid is protonated or charged is likely to affect its behaviour in the formation of α-helical regions in proteins,[66] and this behaviour is in part determined by the charges of other side-chains which are close to the glutamate on the surface of the helix.[67]

Other kinds of analyses which may also be of value in understanding secondary structure are an investigation of non-helix and helix length distributions,[68] and an analysis of the correlation of amino-acid residue properties along the primary sequence.[69] In the latter case, the significance of some of the properties examined seems to us rather obscure, for example, the pK of the backbone carboxy-group of the free amino-acids.

Tertiary Structure.—Considerable efforts are still being made to find ways of summarizing or representing in two dimensions the complex, three-dimensional tertiary structure of a protein.[70–72] Using techniques of this type, tertiary structure homologies of related proteins have been explored.[73] The topology of disulphide bond arrangements has recently been examined in the light of knot theory.[74]

Thermodynamic models for tertiary interactions can be found in the aggregating or micelle-forming behaviour of partly hydrophobic artificial polypeptides, and studies in this area continue.[75]

[60] K. Nagano, *J. Mol. Biol.*, 1973, **75**, 401.
[61] K. Nagano, *J. Mol. Biol.*, 1974, **84**, 337.
[62] K. Nagano and K. Hasegawa, *J. Mol. Biol.*, 1975, **94**, 257.
[63] V. J. Lim, *J. Mol. Biol.*, 1974, **88**, 857.
[64] V. J. Lim, *J. Mol. Biol.*, 1974, **88**, 873.
[65] D. A. Denisov, *J. Theoret. Biol.*, 1975, **55**, 107.
[66] P. J. Belobrov, *Biophysics*, 1975, **20**, 18.
[67] F. R. Maxfield and H. A. Scheraga, *Macromolecules*, 1975, **8**, 491.
[68] D. A. Denisov, *J. Theoret. Biol.*, 1975, **54**, 383.
[69] D. D. Jones, *J. Theoret. Biol.*, 1975, **50**, 167.
[70] R. Srinivasan, R. Balasubramanian, and S. S. Rajan, *J. Mol. Biol.*, 1975, **98**, 739.
[71] J. B. R. Dunn and I. M. Klotz, *Arch. Biochem. Biophys.*, 1975, **167**, 615.
[72] I. D. Kuntz, *J. Amer. Chem. Soc.*, 1975, **97**, 4362.
[73] K. Nishikawa and T. Ooi, *J. Theoret. Biol.*, 1975, **43**, 351.
[74] G. M. Crippen, *J. Theoret. Biol.*, 1975, **43**, 327.
[75] E. V. Amifrieva, U. E. Bychkova, M. G. Krakovyak, U. D. Pautor, and O. B. Ptitsyn, *F.E.B.S. Letters*, 1975, **55**, 46.

The relationship between tertiary structure and biological activity is highlighted in the interesting studies of Gutte.[76] In this work, a 70-residue chain was synthesized which resembled the bovine ribonuclease sequence, but with extensive deletions where experimental data (*e.g.* concerning residue mutations) suggest the region is not vital to biological activity. Under various conditions, such as dimerization, a significant amount of biological activity persists in the 70-residue molecule.

Quaternary Structure.—Quaternary structure is not only of interest in its own right, but may provide a model for intramolecular interactions between subdomain regions of a protein.[77] The stability of protein–protein interactions is a motivation for recent studies on the trypsin–trypsin inhibitor complex,[78] while the general concept of protein–protein recognition has been surveyed.[79]

The Unfolding of Globular Proteins.—The unfolding of globular proteins is currently of interest with respect to understanding the folding process, since the two processes are thought usually to involve negotiating the same pathway in opposite directions. Since this pathway implies the existence of intermediates, two recent theoretical models of denaturation may help us to understand the conditions in which intermediates may or may not be observed.[80, 81] Based on experimental data, a detailed model for the unfolding of bovine ribonuclease has been proposed.[82]

The Folding of Globular Proteins.—Both experimental and theoretical aspects of protein folding up to 1974 have been reviewed by Anfinsen and Scheraga.[83]

Experimental data support the theoretically important concept of a constrained pathway of folding. In the case of trypsin inhibitor protein, the pathway seems to be almost obligatory with few opportunities for generating transient misfolded states (see also p. 196).[84] In other cases,[85] there is evidence for the generation of misfolded states, which then must be unfolded so that the productive pathway may be taken.[86–89] Our current knowledge of the intermediates on the productive pathway has been reviewed by Baldwin.[90] Structures experimentally observed early in the folding process of several proteins are likely to involve α-helix or β-pleated sheet.[91–94]

[76] B. Gutte, *J. Biol. Chem.*, 1975, **250**, 889.
[77] D. B. Wettlaufer, *Proc. Nat. Acad. Sci. U.S.A.*, 1973, **70**, 697.
[78] J. W. Donovan and R. A. Beardslee, *J. Biol. Chem.*, 1975, **280**, 1966.
[79] C. Chothia and J. Janin, *Nature*, 1975, **256**, 705.
[80] A. J. Hopfinger, *J. Macromol. Sci.*, 1974, **B9**, 484.
[81] N. Gō, *Internat. J. Peptide Protein Res.*, 1975, **7**, 313.
[82] A. W. Burgess and H. A. Scheraga, *J. Theoret. Biol.*, 1975, **53**, 403.
[83] C. B. Anfinsen and H. A. Scheraga, *Adv. Protein Chem.*, 1975, **29**, 205.
[84] T. E. Creighton, *J. Mol. Biol.*, 1975, **95**, 167.
[85] H. C. Hawkins and R. B. Freedman, *F.E.B.S. Letters*, 1975, **58**, 7.
[86] A. Ikai, W. W. Fish, and C. Tanford, *J. Mol. Biol.*, 1973, **73**, 165.
[87] V. P. Saxena and D. B. Wetlaufer, *Biochemistry*, 1970, **9**, 5015.
[88] R. R. Hantgan, G. G. Hammes, and H. A. Scheraga, *Biochemistry*, 1974, **13**, 3421.
[89] C. B. Anfinsen, *Harvey Lect.*, 1962, **61**, 95.
[90] R. L. Baldwin, *Ann. Rev. Biochem.*, 1975, **44**, 453.
[91] D. H. Sachs, A. N. Schechter, A. Eastlake, and C. B. Anfinsen, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3790.
[92] D. H. Sachs, A. N. Schechter, A. Eastlake, and C. B. Anfinsen, *Biochemistry*, 1972, **11**, 4268.
[93] J. R. Gavel and R. L. Baldwin, *J. Med. Biol.*, in press.
[94] T. E. Creighton, *J. Mol. Biol.*, 1974, **87**, 603.

In the case of the folding of the exopenicillinase of *Staphylococcus aureus*, the presence of α-helical structure in an early intermediate can actually be detected.[95] A fact of particular interest is that the α-helical intermediate is stable at 20—30 °C in 0.8 M-GuCl. Such an intermediate can therefore be studied at leisure for the first time. Difference spectroscopy and hydrodynamic measurements, in conjunction with thermodynamic considerations, suggest that interactions between the helical regions occur and so stabilize them. Such an intermediate may well be involved in the folding of other proteins, but the authors show that it cannot be observed by similar techniques applied to proteins more stable than *ca.* -10 kcal mol^{-1}. Since experimental studies have previously been carried out only on globular proteins with this degree of stability, such an intermediate has not been previously seen as a stable state (see also p. 191).

The coming together of ordered secondary structure regions to form the native structure has been recently considered.[96, 97]

Theoretical studies on the association of α-helical regions in myoglobin is likely to be a major topic in the near future. The earlier work of Ptitsyn and Rashin [98, 99] has now been published in English. These authors start with the known helical regions and manipulate a model system by hand so as to simulate the folding process. An approximation to the native structure was one of two most favourable structures. The regions between helices were treated as freely jointed, but Nakata and Suzuki [100] have recently carried out energy calculations of the flexibility of the single residues (aspartate and serine, respectively) separating the A and B, and D and E helices of myoglobin. ϕ, ψ Energy maps were produced for these residues, the *N*- and *C*-termini being blocked by five-residue helices having side-chains in the experimentally observed conformation in myoglobin. In many respects these maps resemble those for alanine blocked by five-residue polyalanine helices, also produced by these workers. The important result is that for single residues separating helices only three or four narrowly constrained conformations are allowed.

Because of its small size (58 residues), trypsin inhibitor protein continues to be a popular choice for computer simulations of the folding process. Tanaka and Scheraga [101] carried out a Monte Carlo simulation of the folding, constraining right-hand α-helical and extended chain conformations to regions around their observed conformation on the ϕ, ψ map. This simulation therefore largely represents a coming together of established ordered secondary structure regions. It therefore differs somewhat from the computer simulation of Levitt and Warshel,[102] in which no such constraints were applied except that the observed α-helical region had to be started in the α-helical conformation. In order to save computing time, Levitt and Warshel used a particularly simplified representation of the protein chain. An attempt to simulate the folding of a globular protein has also been carried out by Taketomi, Ueda, and Gō.[103]

[95] B. Robson and R. H. Pain, *Biochem. J.*, 1976, **155**, 331.
[96] K. Nagano and K. Hasegawa, *J. Mol. Biol.*, 1975, **94**, 257.
[97] J. D. Kuntz, *J. Amer. Chem. Soc.*, 1975, **97**, 4362.
[98] O. B. Ptitsyn and A. A. Rashin, *Doklady Akad. Nauk S.S.S.R.*, 1973, **213**, 473.
[99] O. B. Ptitsyn and A. A. Rashin, *Biophys. Chem.*, 1975, **3**, 1.
[100] Y. Nakata and K. Suzuki, *Polymer. J.*, 1975, **7**, 444.
[101] S. Tanaka and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3802.
[102] M. Levitt and A. Warshel, *Nature*, 1975, **253**, 694.
[103] H. Taketomi, Y. Ueda, and N. Gō, *Internat. J. Peptide Protein Res.*, 1975, **7**, 445.

Protein-bound Ions and Cofactors.—The concept of metal cation recognition by polypeptides and proteins has recently been discussed.[104]

In some proteins, the presence of a strongly bound ion or cofactor suggests the possibility that the amino-acid sequence alone may not be sufficient to allow correct simulation of the folding process and a concurrent prediction of tertiary structure. Nevertheless, proteins of this type can be experimentally refolded in the absence of the associated ion or cofactor. For example, carbonic anhydrase is less stable in the absence of Ca^{2+} ions and refolds less rapidly, but the biologically active structure is still recovered.[105] More intermediate states appear in the denaturation profile in the absence of Ca^{2+}, and the authors discuss the possibility that the refolding pathways in the presence and absence of the cation may differ. This does not necessarily follow, since it may be possible to raise the free energy of intermediates (*e.g.* by removing an ion or cofactor) so that they cannot be detected experimentally but are still involved in the folding (see, for example, the above discussion on nucleating helical regions in penicillinase and other proteins). At least in some cases, therefore, it may still be possible to simulate folding without consideration of the associated ion or cofactor. Also of considerable interest in this respect is apomyoglobin (*i.e.* myoglobin without the haem group) which by o.r.d. measurements has *ca.* 20% less α-helix than myoglobin. The stability of apomyoglobin in various aqueous organic solvents has also been investigated.[106]

2 Mechanisms of Folding in Globular Proteins

Contributed by R. J. Burgess

The field of protein folding has been extensively reviewed,[83] as have intermediates in folding and their importance in the folding mechanism.[107]

The Two-state Transition.—The validity of the two-state hypothesis for the unfolding of globular proteins continues to be a subject of interest, with thermodynamic evidence generally in favour while kinetic evidence is frequently cited as being contradictory. The two-state unfolding of protein molecules requires that in the transition region both native and denatured molecules exist in equilibrium. Such a situation has been shown for bovine glutamate dehydrogenase.[108] Electrophoresis of the equilibrium mixture of chymotrypsin denatured at high pressure allows the two states to be visualized, but does not rule out the existence of low levels of intermediates.[109] Fluorimetric titration of the SDS denaturation of barnase, an extra-cellular ribonuclease from *Bacillus amyloliquefaciens*, provides evidence in accord with a two-state transition.[110]

The biphasic kinetics of denaturation have been quoted as evidence for the presence of significant intermediate states. Earlier Volumes covered the work of Ikai and Tanford on this topic.[111, 112] However, a model proposed by Brandts

[104] M. R. Truter, *Phil. Trans. Roy. Soc.*, 1975, **B272**, 29.
[105] K.-P. Wong and L. M. Hamlin, *Arch. Biochem. Biophys.*, 1975, **170**, 12.
[106] T. T. Herskovits and N. J. Solli, *Biopolymers*, 1975, **14**, 319.
[107] R. L. Baldwin, *Ann. Rev. Biochem.*, 1975, **44**, 453.
[108] K. S. Rogers, *Proc. Soc. Exp. Biol. Med.*, 1975, **149**, 1023.
[109] S. A. Hawley and R. M. Mitchell, *Biochemistry*, 1975, **14**, 3257.
[110] R. W. Hartley, *Biochemistry*, 1975, **14**, 2367.
[111] This series, 1973, Vol. 5, p. 188.
[112] This series, 1974, Vol. 6, pp. 216, 218.

and co-workers suggests that only one phase, the fast one, represents the unfolding reaction and that the slow phase is due to the *cis–trans* isomerism of proline residues in the denatured state. This model accounts for the experimentally observed kinetic phenomena associated with unfolding and refolding, and shows that biphasic kinetics need not be inconsistent with a two-state model for unfolding.[113]

Although much thermodynamic evidence points to a simple two-state situation, numerous studies have shown that intermediates may occur on the folding pathway even if they are only transient and in low concentration. Multi-state structural transitions have recently been shown for many proteins. Thus the denaturation of α-amylase is triphasic,[114] each phase having its own characteristic pH range and involving the ionization of specific tyrosine residues. Only the first phase is reversible, however. The catalytic 'activity' of bovine serum albumin has been used to probe its refolding.[115] Activity is regained before other structural features, implying that the domain containing the active site has a greater stability than other regions of the molecule and hence an intermediate may exist with the 'active domain' folded and other parts of the molecule unfolded. An intermediate is also found in the magnesium chloride dissociation of rabbit muscle aldolase, exhibiting a changed local environment of six tyrosine and two tryptophan residues and a loss of 20% helix.[116] Renaturation of this enzyme is affected by the substrate fructose-1,6-diphosphate.[117] If denaturant is diluted out with fructose-1,6-diphosphate present from the start, the enzyme cannot refold. But if it is added after an initial pause, fructose-1,6-diphosphate enhances refolding. Three species are shown up by this method: native active monomer, unfolded inactive monomer, and a monomer having similar c.d. and u.v. absorption spectra to the native monomer but lacking in activity. Since both intermediate and final conditions are important in the folding process, kinetic considerations must be involved in the folding process.

A model proposed to account for the stabilization of thermomycolase [118] by Ca^{2+} requires a critically activated intermediate on the folding pathway that binds Ca^{2+} at a lower affinity. The integrity of the Ca^{2+} binding site and the initial stages of unfolding must be closely linked since only one Ca^{2+} ion is needed for stabilization. By lowering the free energy of its binding site[119] the Ca^{2+} raises the local activation energy needed for unfolding and hence blocks it. Exposure of lactate dehydrogenase to pH 2—3 for increasing periods of time reveals denatured states differing according to their ability to renature and their kinetics of activity recovery. It is suggested that state B may represent an intermediate on the pathway from native to dissociated and unfolded lactate dehydrogenase.[120]

Multi-state systems that do not exhibit transient intermediates do exist. Thus lysozyme, which denatures between 60 and 70 °C, undergoes a localized conformational transition between 20 and 30 °C,[121] which is reflected in a change of

[113] J. F. Brandts, H. R. Halvorson, and M. Brennan, *Biochemistry*, 1975, **14**, 4953.
[114] K. Hiromi, M. Ohnishi, K. Kanaya, and T. Matsumoto, *J. Biochem.*, 1975, **77**, 957.
[115] R. P. Taylor, S. Berga, V. Chau, and C. Bryner, *J. Amer. Chem. Soc.*, 1975, **97**, 1943.
[116] L.-S. Hsu and K. E. Neet, *J. Molec. Biol.*, 1975, **97**, 351.
[117] C. Vimard, G. Orsini, and M. E. Goldberg, *European J. Biochem.*, 1975, **51**, 521.
[118] G. Voordouw and R. S. Roche, *Biochemistry*, 1975, **14**, 4659.
[119] G. Voordouw and R. S. Roche, *Biochemistry*, 1975, **14**, 4667.
[120] R. B. Vallee and R. C. Williams, jun., *Biochemistry*, 1975, **14**, 2574.
[121] P. J. Cozzone, S. J. Opella, O. Jardetzky, J. Berthou, and P. Jolles, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2095.

crystal structure. *In vivo* the protein probably folds to form the B conformational state. The biphasic unfolding of ribonuclease A may be explained by the existence of an equilibrium mixture of fast and slow renaturing species (U_1 and U_2) in the denatured material.[122] The folding–unfolding reaction may be represented in two possible ways:

$$U_1 \underset{}{\overset{\text{slow}}{\rightleftharpoons}} U_2 \overset{\text{fast}}{\rightleftharpoons} N \quad \text{or} \quad U_1 \overset{\text{slow}}{\rightleftharpoons} N \overset{\text{fast}}{\rightleftharpoons} U_2$$

U_2 may contain nucleated regions which may refold rapidly or U_1 may refold slowly as a consequence of its containing incorrectly folded structure. Measurement of the pK's of the three surface tyrosines reveals a physical difference between the two species[123] in that the pK changes only with the slow refolding mechanism.

Theory of Nucleation and Folding.—Robson has discussed some proposed pathways of folding.[124]

A theoretical treatment relating protein denaturation to the conformation of native proteins and the forces stabilizing it has been developed.[125] If amino-acid residues of different kinds behave as having homogeneous averaged properties denaturation is shown to follow a two-state path. However, if averaging over different residues does not give a good approximation, *i.e.* melting takes place in large blocks of predominantly polar residues and predominantly non-polar residues, significant intermediate states will exist.

A simplified representation of protein conformation employing a concept of virtual bonds, similar to that suggested by Ptitsyn,[126] has been used, together with the normal mode thermalization method of energy minimization, to simulate the pathway of folding of trypsin inhibitor.[127] A model is proposed where long-range forces direct folding to a compact shape at which short-range forces become important. A remarkably successful approximation to the native conformation of pancreatic trypsin inhibitor was achieved, assuming only the location of the helical region in the protein. This paper is discussed by Robson and a reply given by the authors.[128]

Long-range interactions are also favoured as directors of nucleation by a study of the energetics of folding of a lysozyme bend. The destabilizing influence of the β-bend must first be overcome by long-range interactions and then localized by short-range forces.[129] Prediction of strong long-range interactions has been examined by Nagano and Hasegawa[130] and a function derived from sequence and conformation data. This was used to predict the structure of T_4 phage lysozyme and other proteins. Helix to β-candidate interactions are found to be almost as important as β to β-candidate interactions in folding.

[122] J. R. Garel and R. L. Baldwin, *J. Mol. Biol.*, 1975, **94**, 611.
[123] J. R. Garel and R. L. Baldwin, *J. Mol. Biol.*, 1975, **94**, 621.
[124] B. Robson, *Nature*, 1975, **256**, 89.
[125] N. Gō, *Internat. J. Peptide Protein Res.*, 1975, **7**, 313.
[126] O. B. Ptitsyn and A. A. Rashin, *Doklady Akad. Nauk S.S.S.R.*, 1973, **213**, 473.
[127] M. Levitt and A. Warshel, *Nature*, 1975, **253**, 694.
[128] B. Robson, *Nature*, 1975, **254**, 386.
[129] W. A. Hiltner and A. G. Walton, *J. Mol. Biol.*, 1975, **92**, 567.
[130] K. Nagano and K. Hasegawa, *J. Mol. Biol.*, 1975, **94**, 247.

Theoretical treatments of protein folding require the structural properties of proteins to be represented by a set of functions. Relatively simple equations have been derived for the accessible surface area of a protein, the number of intermolecular H bonds, and the close packing of amino-acids in a protein.[131]

Nucleating Regions. A plot of distance between α carbon atoms against residue numbers enables visualization of the folding domains of proteins and the secondary structures involved in making them. In a modified version, idealized contour lines have been used to represent set structures such as hairpin turns.[132] Myoglobin, trypsin inhibitor, and cytochrome b_5 are given as examples.

The glutathione renaturation of ribonuclease A has been explained in terms of folding theory.[133] The interaction of hydrophobic side-chains of localized helical or β structures leads to nucleation and rapid formation of disulphide bonds in the nucleated region. Thus, three or four disulphide bonds form prior to large changes in circular dichroism or activity, *i.e.* before formation of large structural units. Nucleation is followed by more extensive secondary structure formation with α-helix and β-sheet forming at the same rate. The final rate-limiting step is the reshuffling of disulphide bonds. A possible nucleating region close to a cysteine residue in carbonic anhydrase is identified by a rapid immobilization of spin label attached to this cysteine. This region is rich in hydrophobic residues.[134]

Discovery of a stable expanded intermediate H between the native and fully unfolded states of penicillinase has led to a proposed unfolding and refolding mechanism:[135]

$$N \leftrightharpoons H \rightleftharpoons U, \text{ when } N \rightleftharpoons H \text{ is rate limiting.}$$

A rapid stabilization of nucleated regions gives a compact but solvated intermediate state or group of states, whose secondary structure is stabilized by limited tertiary interaction. *In vivo* this structure might evolve progressively as the protein is synthesized before finally reshuffling to give the native structure on release from the ribosome (see also p. 187).

Residual Structure. More evidence has become available to support the belief that denatured proteins frequently contain significant amounts of residual structure. Such stable centres could act as possible candidates for nucleating sites in refolding experiments. Tyrosine and tryptophan charge-transfer experiments [136] on denatured proteins such as lysozyme, trypsin, and chymotrypsin show lower values of exposure of these residues than for the free amino-acid in solution. If disulphide bonds are reduced, exposure increases. This suggests that the aromatic residues may lie in the regions of residual structure and hence possibly be involved as instigators of protein folding. Two histidine residues of thermally unfolded ribonuclease A appear by proton magnetic resonance to be in regions of residual structure.[137] This is destroyed by GuCl and a cross-linked random coil is formed. The residual structure may consist of 'non-productive' clusters of neutral or

[131] C. Chothia, *Nature*, 1975, **254**, 304.
[132] I. D. Kuntz, *J. Amer. Chem. Soc.*, 1975, **97**, 4362.
[133] S. W. Schaffer, *Internat. J. Peptide Protein Res.*, 1975, **7**, 179.
[134] U. Carlsson, R. Aasa, L. Henderson, B. H. Jonsson, and S. Lindskog, *European J. Biochem.*, 1975, **52**, 25.
[135] B. Robson and R. H. Pain, *Biochem. J.*, 1976, **155**, 331.
[136] C. R. Coan, L. M. Hinman, and D. A. Deranleau, *Biochemistry*, 1975, **14**, 4421.
[137] C. R. Matthews and D. G. Westmoreland, *Biochemistry*, 1975, **14**, 4532.

hydrophobic groups minimizing interactions with solvent so that refolding would take place by first disrupting this structure. Disruption of the residual structure of ferricytochrome *c* by acidification and urea is a complex triphasic, non-co-operative process probably involving the haem-His-18 ligand.[138] Esterification of ribonuclease A in methanol–HCl is consistent with the retention of at least part of the native structure.[139] Extensive acetylation, up to 98% of the lysine residues of ovalbumin, still leaves residual structure identifiable by antigenic determinants.[140] The unfolding process is suggested to mimic that of thermal denaturation and probably involves changes in electrostatic free energy, loss of energy of solvation, and steric hindrance due to the substituent groups. Two different positions of spin labelling of leucine aminopeptidase are found which differ in their mobility in 6.2 M urea. One is strongly immobilized and hence in a region of residual structure, whereas the other is free and must be in an unfolded region.[141]

The Influence of Sequence on Conformation.—*Variation in Sequence.* The enhanced thermal stability of 6-phosphogluconate dehydrogenase from *Bacillus stearothermophilus* over the enzyme from *E. coli* must be due to specific side-chain interactions (*i.e.* sequence), since their conformations are similar.[142] The phenomenon of complementation whereby inactive enzymes with a mutation in one region can bind a mutant enzyme with the mutation in another region, thereby regaining activity, has been discussed in the context of protein folding.[143] Experiments suggest that the *N*-terminus must be free from mutations in order for the proteins to refold from GuCl.

Semi-synthetic ribonuclease S 1—20 peptides have been produced with Glu-9 replaced by leucine or glycine.[144] Both of these bind to ribonuclease S 21—124 and direct its refolding from a disulphide scrambled state. Although the leucine peptide binds more strongly than the one with glycine in line with its greater helix-forming potential, the fact that they both bind is evidence for the presence of the entire protein sequence being required for refolding. The fragment could bind to and stabilize ribonuclease S 20—124 when in its low probability 'native format', or it could bind in the random coil state and both S-peptide and S-protein refold together.

In occasional instances the amino-acid sequence does not appear to contain enough information for folding to take place. Thus, phosphofructokinase slowly forms a monomeric state P′ in GuCl which cannot refold to give monomers capable of forming the tetrameric active enzyme.[145] The alternative possibility exists that lack of reversibility is due to inter-domain entanglement within the large unfolded subunit. This would suggest sequential folding *in vivo*, domain by domain.

[138] T. Y. Tsong, *Biochemistry*, 1975, **14**, 1542.
[139] A. S. Acharya and P. J. Vithayathie, *Internat. J. Peptide Protein Res.*, 1975, **7**, 207.
[140] A. A. Ansari, S. A. Kidwai, and A. Salahuddin, *J. Biol. Chem.*, 1975, **250**, 1625.
[141] G. Lassmann, W. Damerau, G. Sklenar, D. Schwarz, M. Frohne, M. Ludewig, and H. Hanson, *European J. Biochem.*, 1975, **54**, 453.
[142] F. M. Veronese, E. Boccu, and A. Fontana, *Internat. J. Peptide Protein Res.*, 1975, **7**, 341.
[143] R. H. Pain, *Nature*, 1975, **256**, 366.
[144] B. M. Dunn and I. M. Chaiken, *J. Mol. Biol.*, 1975, **95**, 497.
[145] G. R. Parr and G. G. Hammes, *Biochemistry*, 1975, **14**, 1600.

The Role of Individual Amino-acids. Consideration of the role particular amino-acid residues play in forming secondary structures can throw light on their behaviour in globular proteins. A study of the torsional potentials of amino-acids in pancreatic trypsin inhibitor suggests that the amino-acids possess dihedral angles corresponding to those in free solution.[146]

Study of synthetic polypeptide transitions enables the propensity of certain amino-acids to form secondary structure to be studied. Valine, owing to its greater hydrophobicity and possibly its branched shape, has a greater propensity than leucine for forming β-sheet.[147] However, the value of K_{eq} of leucine for the helix to random coil transition of co-polymers of leucine and glutamine seems too low to attribute to leucine a special role as a helix former,[148] as proposed by Chou and Fasman. The helix stabilizing power for neutral amino-acids has been graded as Leu, Nle, Ile, Ala, Phe, Val, Gly in decreasing power.[149]

Three methods of helix initiation have been proposed. Initiation from a helix-forming residue in the centre of the helix seems to be the case for Leu-99 in synthetic peptides of cytochrome *c*.[150] Terminal initiation of helix can occur in two ways: by the clustering of polar amino-acids at either end, aspartic and glutamic acids at the amino-terminus, lysine, arginine, and histidine at the carboxy-terminus, and hence generation of an aggregate dipole along the helix,[151] or by β turns at the ends which similarly generate a dipole along the helix.

Prediction of Conformation from the Amino-acid sequence. Predictions made from sequence information can yield information on the importance of that sequence in the folding pathway. A lattice model using Monte Carlo techniques has been proposed for predicting native structure. This indicates that interunit interactions are the primary factors responsible for all or none transitions.[152] Empirical and ideal prediction algorithms have both been examined to find a proper starting conformation from which energy minimization should predict the native structure of proteins.[153] This was tried for pancreatic trypsin inhibitor.

Breaks in the Primary Sequence. RNA polymerase from *E. coli* may be cleaved specifically in the same region of the β subunit when bound to DNA by trypsin, chymotrypsin, or subtilisin. The cleaved enzyme with β fragments of molecular weight 124 000 and 52 000 can renature from 6M-GuCl.[154]

Similarly, staphylococcal enterotoxin β can be split into two fragments between residues Lys-97 and Thr-98 after reduction and carboxymethylation.[155] The separated peptides have no physiological activity but can bind antibodies. Following recombination of the GuCl-denatured peptides and removal of GuCl the structure and physiological activity are restored.

[146] B. R. Gelin and M. Karplus, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2002.
[147] R. Mandel and G. D. Fasman, *Biopolymers*, 1975, **14**, 1633.
[148] V. E. Bychkova, A. T. Gudkov, W. G. Miller, Yu. U. Mitin, O. B. Ptitsyn, and I. L. Shpungin, *Biopolymers*, 1975, **14**, 1739.
[149] H. A. Arfmann, R. Labitzke, and K. G. Wagner, *Biopolymers*, 1975, **14**, 1381.
[150] L. Moroder, B. Filippi, G. Borin, and F. Marchiori, *Biopolymers*, 1975, **14**, 2075.
[151] D. E. Blagdon and M. Goodman, *Biopolymers*, 1975, **14**, 241.
[152] H. Taketomi, Y. Ueda, and N. Gō, *Internat. J. Peptide Protein Res.*, 1975, **7**, 445.
[153] A. W. Burgess and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1221.
[154] P. A. Lowe and A. D. Malcolm, *Biochem. Soc. Trans.*, 1975, **3**, 652.
[155] L. Spero, J. F. Metzger, J. R. Warren, and B. Y. Griffin, *J. Biol. Chem.*, 1975, **250**, 5026.

By using contaminant proteinases, bovine plasma albumin can be cleaved once within the disulphide loop comprising residues 390—434. The helix content of the complex decreases by *ca.* 15% and the hydrodynamic volume increases by 20%, although tertiary structure is retained.[156] This change, which mimics the N ⇌ F transition of bovine plasma albumin, suggests that the molecule has two or more domains, one of which is damaged by cleavage, hence the loss of helix. Although the other domains remain intact the decrease in interdomain interactions due to the damage of one of them leads to the swelling of the molecule.

Peptide Fragments obtained from Proteins. Trypsin is able to refold even in the absence of the fragment cleaved in its conversion from trypsinogen.[157] This fragment may lie on the surface of the molecule and not be directly involved in the folding. Synthetic ribonuclease 21—124 with deletions of residues 21—25, 36—40, 58—73, and 113—114 retains some low activity when refolded. The hydrolysis step of the enzymic reaction is enhanced both in monomeric and dimeric forms of the synthetic peptide, by the addition of the S peptide 1—23.[158] Some local refolding at least must take place in the synthetic peptide. However, residues from the nucleating regions described by Burgess *et al.*[176] are missing and this may explain why greater amounts of activity are not recovered.

Limited proteolytic attack on bovine serum albumin produces 12 fragments. From c.d. studies, secondary structure has been shown to be substantially retained by individual fragments. Secondary structure must therefore be locally determined.[159]

The rate of unfolding of nuclease T′, the non-covalent complex formed by recombining fragments 6—48 and 50—149 of staphylococcal nuclease, has been studied by the rate of exchange of a ^{14}C-labelled fragment, nuclease T (50—149), incorporated in the structure of nuclease T′.[160] Refolding from the two fragments is independent of temperature and ligand binding, and hence the energy barrier of folding is entropic.

The peptide fragment of lysozyme, residues 62—68 and 74—94, containing two disulphide bonds, still possesses antigenic specificity although greatly unfolded compared to the intact molecule.[161] Retention of enough structure for this antigenicity must be due to the restrictive influence of the disulphide bonds.

Folding–Unfolding Transitions.—*Probes of Structural Change.* Circular dichroism is coming to the fore as a technique for probing the conformation of proteins. Resolution of protein structure with particular reference to the random coil state has been discussed.[162] A loosening of the aromatic residues of bovine luteinizing hormone prior to denaturation is revealed by c.d. and thermal difference spectroscopy.[163] Characterization of penicillinase as a suitable model for protein folding involved both c.d. and o.r.d. in the study of GuCl denaturation.[164] The N ⇌ F

[156] V. R. Zurawski, jun., W. J. Kohr, and J. F. Foster, *Biochemistry*, 1975, **14**, 5579.
[157] N. K. Sinha and A. Light, *J. Biol. Chem.*, 1975, **250**, 8624.
[158] B. Gutte, *J. Biol. Chem.*, 1975, **250**, 889.
[159] R. C. Reed, R. C. Feldhoff, O. L. Clute, and T. Peter, jun., *Biochemistry*, 1975, **14**, 4578.
[160] H. Taniuchi and J. L. Bohnert, *J. Biol. Chem.*, 1975, **250**, 2388.
[161] C. L. Lee and M. Z. Atassi, *Biochim. Biophys. Acta*, 1975, **405**, 464.
[162] J. Manhussen and A. Volund, *Internat. J. Peptide Protein Res.*, 1975, **7**, 47.
[163] S. J. Leach, E. Minasian, and L. E. Reichert, jun., *Biochim. Biophys. Acta*, 1975, **386**, 144.
[164] B. Robson and R. H. Pain, *Biochem. J.*, 1976, **155**, 331.

transition of bovine and human serum albumin has been characterized by study of the kinetics and equilibria of intrinsic fluorescence[165] and by fluorescence polarization.[166] An intermediate was found to be involved.

Thermal denaturation of serum albumin has been studied by scanning microcalorimetry. Whereas the 'low pH' form denatures *via* a two-state reaction the 'high pH' form unfolds non-co-operatively.[167] Similarly, thermal denaturation of *Mucor meihei* protease has been studied by differential calorimetry.[168]

Conformational changes may be followed by the change in ability of the molecule to react with antibodies. The amount of each conformational state of staphylococcal nuclease present during the transition has been quantitated in this way.[169] Burial of antigenic determinants during the folding of alkaline phosphatase has been observed.[170]

A significant change in the pH of unbuffered proteins occurs on the addition of GuCl and the subsequent structural transition. The transition may be followed by observing this change but not for some proteins such as lysozyme and trypsin inhibitor. Fourteen proteins have been investigated by this method.[171]

N.m.r. studies of ribonuclease revealed a loosening of one part of the molecule outside the major unfolding transition. The urea or GuCl denaturation involves at least two intermediates.[172] The alkaline denaturation of β-lactoglobulin has been studied by Raman spectroscopy. While α-helix remains constant, β-sheet disappears, though some β-structure remains resistant until pH 13.5 when the molecule becomes randomly coiled.[173]

Intrinsic viscosity is useful as a probe of conformational states.[174] The discrepancy in ovomucoid values for the random coil state is attributed to carbohydrate residues.

Susceptibility to Enzyme Digestion. Carboxypeptidase A bound to Sephadex can be used to probe the transition of ribonuclease at elevated temperatures. Comparing the rates of hydrolysis of oxidized (100% unfolded) ribonuclease and partially denatured ribonuclease allows the degree of unfolding of the latter to be calculated.[175] Since the accessibility of the *C*-terminus is being examined here it is shown that this region unfolds later than the region containing tyrosines 92 and 25.

Susceptibility of ribonuclease to different enzymes at various temperatures has enabled the same group to identify a six-stage pathway of folding.[176] This includes two initial nucleation sites, the first being formed by two chain reversals bringing together three extended chains which are stabilized by formation of two disulphide bonds. Short and medium range interactions involving one chain reversal form the second site. The *N*-terminus folds and interacts with the first site whilst the

[165] R. Rudolph, E. Holler, and R. Jaenicke, *Biophys. Chem.*, 1975, **3**, 226.
[166] M. Sogami, K. B. Itoh, and Y. Nemoto, *Biochim. Biophys. Acta*, 1975, **393**, 446.
[167] D. Ya. Leibman, E. Tiktopulo, and P. L. Privalov, *Biofizika*, 1975, **20**, 376.
[168] D. A. Ledward, A. Finch, and W. S. Rickert, *Biochim. Biophys. Acta*, 1975, **379**, 426.
[169] B. Furie, A. N. Schechter, D. H. Sachs, and C. B. Anfinsen, *J. Mol. Biol.*, 1975, **92**, 497.
[170] C. J. Lazdunski, J. M. Pages, and P. Louvard, *J. Mol. Biol.*, 1975, **97**, 309.
[171] H. B. Bull and K. Breese, *Biopolymers*, 1975, **14**, 2197.
[172] F. W. Benz and G. C. K. Roberts, *J. Mol. Biol.*, 1975, **91**, 345, 367.
[173] B. G. Frushour and J. L. Koenig, *Biopolymers*, 1975, **14**, 649.
[174] F. Ahmad and A. Salahuddin, *Internat. J. Peptide Protein Res.*, 1975, **7**, 417.
[175] A. W. Burgess, L. I. Weinstein, D. Gabel, and H. A. Scheraga, *Biochemistry*, 1975, **14**, 197.
[176] A. W. Burgess and H. A. Scheraga, *J. Theoret. Biol.*, 1975, **53**, 403.

C-terminus condenses between the two. Finally, α-helix 25—33 forms to give the native structure.

Disulphide Bond Formation. Creighton has studied the formation of disulphide bonds on refolding reduced pancreatic trypsin inhibitor. Of the four major two disulphide intermediates found, only that with bonds 30—51 and 5—55 can directly form a third disulphide bond to generate native inhibitor.[177] Thus, refolding must take place *via* this intermediate and hence follow an obligatory and unique pathway. The last step in folding of the inhibitor is the formation of disulphide bond 14—38.[178] All of the six cysteine residues are involved in formation of the first disulphide bond of the protein.[178]

When reduced proteins are rapidly reoxidized, incorrect structures may be formed with the wrong disulphide bonds. Such is the case for trypsin and its zymogen.[179] Regeneration is possible using a mixture of oxidized and reduced glutathione which promotes disulphide exchange. Disulphide formation is thus rate limiting, there not being enough time to test all possibilities when the protein is rapidly reoxidized. The probability of finding such 'knotted' structures has been calculated [180] and is 2.5% for ribonuclease and 10% for lysozyme. If these values can be found experimentally, disulphide bond formation is implicated as a driving force in folding. Pyrophosphatase denatures irreversibly at 1.5M-GuCl because of the oxidation of thiol groups.[181] At higher GuCl concentrations, the thiols are less reactive and hence oxidation is prevented and refolding can occur. Between 30 and 70% of rapidly reoxidised soya bean trypsin inhibitor cannot refold, even in the presence of disulphide exchange enzyme, because the conformational barriers are too high.[182]

When ribonuclease A is exposed to u.v. irradiation, the activity loss shows a linear dependence on destruction of disulphide bonds. C.d. shows a decrease of secondary and tertiary structure and a lower thermal stability on irradiation of the enzyme.[183] When denatured, the irradiated enzyme has a different state from the denatured unmodified enzyme since some regular structure is lost on destruction of the disulphide bond(s). However, *S*-methylated asparaginase has the same properties as the unmodified enzyme [184] and hence its disulphide bonds are not important in folding.

The Influence of Intermolecular Interactions on Folding. There is an active folding of lutropin dependent on the association of the subunits.[185] A simple folding model involves formation of a loose complex followed by slow formation of the native state. Both subunits are implicated in the unfolding of lutropin by acid since the isolated subunits have lower α-helix and β-sheet structure.[186] A similar

[177] T. E. Creighton, *J. Mol. Biol.*, 1975, **95**, 167.
[178] T. E. Creighton, *J. Mol. Biol.*, 1975, **96**, 767, 777.
[179] N. K. Sinha and A. Light, *J. Biol. Chem.*, 1975, **250**, 8624.
[180] G. C. Crippen, *J. Theoret. Biol.*, 1975, **51**, 495.
[181] Y. Yano and M. Irie, *J. Biochem.*, 1975, **78**, 1001.
[182] H. C. Hawkins and R. B. Freedman, *F.E.B.S. Letters*, 1975, **58**, 7.
[183] R. M. Schultz, A. J. Iammartino, and S. Aktipis, *Biochim. Biophys. Acta*, 1975, **386**, 120.
[184] K. Todokoro, T. Saito, M. Obata, S. Yamazaki, Y. Tamuara, and Y. Inade, *F.E.B.S. Letters*, 1975, **60**, 259.
[185] R. Salesse, M. Casteing, J. C. Pernollet, and J. Garnier, *J. Mol. Biol.*, 1975, **95**, 483.
[186] J. Garnier, J. C. Pernollet, C. Tertrin-Clary, R. Salesse, M. Casteing, M. Barnavon, P. De La Llosa and M. Jutisz, *European J. Biochem.*, 1975, **53**, 243.

folding is shown by the self-association of Apo II apoprotein. The monomers lack enough hydrophobic residues to allow folding, but can fold on dimer formation.[187]

Renaturation of hydroxypyruvate reductase from 6M-GuCl is a bimolecular reaction in line with the association of two subunits.[188] In common with other reductases folding is assisted by the presence of NADH, whereas NAD assists in the folding of dehydrogenases.

Association of trypsin with soya bean trypsin inhibitor or ovomucoid gives an increase in heat stability, probably due to kinetic factors. The complex may denature as a whole or either part may denature before dissociation.[189]

Metal Proteins and Haem Proteins. Cytochrome b_5 has two domains as evidenced by the biphasic denaturation. A hydrophilic domain, containing the haem and destabilized by its loss, denatures at 2.6M-GuCl.[190] The hydrophobic domain is more resistant and unfolds only at 5.0—5.5M-GuCl. The two domains thus fold independently.

The haem group is thought to influence protein folding by a competition between the protein trying to form helices and its tendency to wrap around the haem.[150] Acid unfolding of ferricytochrome *c* has been shown to be due to a two-stage loss of the axial co-ordinating ligands to the haem moiety. Chloride ions stabilize against loss of the Met-80 haem ligand by forming an electrostatic bridge.[191] Denaturation of the apo-enzyme of carbonic anhydrase β is biphasic, whereas that of the zinc-containing enzyme is monophasic. Refolding is also slower. Thus, zinc appears to stabilize the protein, increase co-operativity, and increase rate of fol ling. However, it is not a necessary part of the information for folding.[192]

Immunoglobulin Domains. β-Microglobulin has been used as a model for the constant (C) homology domain of immunoglobulin.[193] Disruption of the disulphide bond even in aqueous neutral solution causes unfolding; reform the disulphide and the molecule refolds. Nucleation, which may be directed by β-bends, must thus bring the cysteine residues close together. Once folding has reached an advanced stage disulphide formation is no longer obligatory. If immunoglobulins have several independently folding domains this would limit the formation of incorrect disulphide bonds. Conservation of disulphide-forming residues may thus have ensured kinetically accessible folding pathways in the face of variability in primary structure.

Analysis of the sequence of five constant regions of human and mouse immunoglobulin κ- and λ-chains shows that the invariant residues are often buried or in contact with the heavy chain and are usually hydrophobic in nature.[194] These could be important residues in the folding mechanism.

[187] J. C. Osborne, jun., G. Palumbo, H. B. Brewer, jun., and H. Edelhoch, *Biochemistry*, 1975 **14**, 3741.
[188] J. M. Utting and L. D. Kohn, *J. Biol. Chem.*, 1975, **250**, 5233.
[189] J. W. Donovan and R. A. Beardslee, *J. Biol. Chem.*, 1975, **250**, 1966.
[190] S. Tajima, K.-I. Enomoto, and R. Sato, *Arch. Biochem. Biophys.*, 1976, **172**, 90.
[191] E. Stellwagen and J. Babul, *Biochemistry*, 1975, **14**, 5135.
[192] K.-P. Wong and L. M. Hamlin, *Arch. Biochem. Biophys.*, 1975, **170**, 12.
[193] D. E. Isenman, R. H. Painter, and K. J. Dorrington, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 548.
[194] E. A. Kabat, E. A. Padlan, and D. R. Davies, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **72**, 2785.

On reassociation of alkylated heavy and light chains of human myeloma protein, slow recombination takes place during which a large conformational change may take place involving a slow folding process.[195]

Similarly, the kinetics of the approach to equilibrium of the GuCl-induced denaturation of an IgG myeloma Fab fragment are extremely slow.[196] This may possibly be due to a strong Fd–L interaction under conditions where monomeric domains are unstable. The data obtained are consistent with a two-region structure for Fab where the regions are independently folded.

Protein 315, a homogeneous antibody, has been studied by the circular polarization of luminescence.[197] The spectra change as the chains interact, *i.e.* there is a conformational change. Different parts of the spectra are altered at different urea concentrations, indicating that parts of the molecule unfold separately. Thus, Fv is more susceptible to urea than Fab which is more susceptible than immunoglobulin A.

Denaturing Conditions.—Protein transitions are very dependent on the conditions under which they are induced. Thus, phage T_4 lysozyme has a transition mid-point guanidine hydrochloride concentration C_m at 2.7M-GuCl and a value of n of 15.9 at pH 5.0. The apparent co-operativity index n can be obtained as the slope of a plot of log K (equilibrium constant of unfolding in guanidine chloride) against log [GuCl]. At pH 2.5, however, C_m decreases to 1.7M and n decreases to 10.7.[198] The normally irreversible thermal inactivation of α-chymotrypsin is rendered reversible by the presence of urea and other classical denaturants, presumably owing to the spatial estrangement of groups preventing formation of incorrect disulphide bonds, oxidation, *etc.*[199]

For proteolytic enzyme studies, autolysis must be prevented. For example, subtilisin may be inhibited by phenylmethylsulphonyl fluoride[200] or di-isopropyl-fluorophosphonate.[201] Its high stability (native at 6M-GuCl) is probably due to its tight structure preventing access to denaturant.[200, 201]

The apparent irreversibility of transitions is often a result of aggregation. This may be overcome by polyalanylation of the protein.[202] This technique has been used to find conditions for the renaturation of chymotrypsinogen. Moderate amounts of denaturant (*ca.* 2M-GuCl) and a mixture of oxidized and reduced glutathione are required for regeneration. The effect of denaturing conditions on free and matrix-bound oxyhaemoglobin has been studied.[203] The rate of unfolding is considerably diminished by matrix binding, although the mobility of a spin label was not affected.

Matrix binding of triose phosphate isomerase increases stability, as does the binding of the ligands and glycerophosphate and 2-phosphoglycolate.[204]

[195] T. Azuma, T. Isobe, and K. Hamaguchi, *J. Biochem.*, 1975, **77**, 473.
[196] E. S. Rowe, *Biochemistry*, 1976, **15**, 905.
[197] J. Schlessinger, I. Z. Steinberg, D. Givol, and J. Hochman, *F.E.B.S. Letters*, 1975, **52**, 231.
[198] M. Elwell and J. Schellman, *Biochim. Biophys. Acta*, 1975, **386**, 309.
[199] K. Martinek, V. S. Goldmacher, A. M. Klibanov, and I. V. Berezin, *F.E.B.S. Letters*, 1975, **51**, 152.
[200] C. C. Contaxis, P. A. Mcbride-Warren, and R. M. Epand, *Internat. J. Peptide Protein Res.*, 1975, **7**, 135.
[201] M. F. Brown and T. Schleich, *Biochemistry*, 1975, **14**, 3069.
[202] G. Orsini, C. Skrzynia, and M. E. Goldberg, *European J. Biochem.*, 1975, **59**, 433.
[203] G. Lassman, B. Ebert, D. Schwarz, W. Damerau, G. Sklenar, K. Pommerening, and K. Buttgereit, *Studia Biophysica*, 1974, **47**, 141.
[204] T. H. Sawyer and R. W. Gracy, *Arch. Biochem. Biophys.*, 1975, **169**, 51.

The effects of organic solvents have been investigated. The α-helix content of apomyoglobin is increased to 80—95% by added alcohols, so that the protein consists of fully extended α-helical rods.[205] In the formation of α-helical poly(γ-benzyl-L-glutamate) in chloroform a higher concentration of trifluoroacetic acid is needed to produce a transition in side-chain n.m.r. resonances than for backbone resonances. It is suggested that the side-chains take up their own orientation before the backbone forms the helix, *i.e.* there is an intermediate state between α-helical and random coil polypeptide.[206]

Unfolding of ribonuclease may be induced by methylation of methionine residues. Out of 15 possible methylated derivatives only three will refold, these being the molecules modified at Met-29 or Met-79, and at both of these. Any substitution of residues 13 or 30 which lie in the hydrophobic core makes refolding impossible.[207]

3 Nuclear Magnetic Resonance

Contributed by H. W. E. Rattle

Introduction.—After slackening for a year or so, the flow of publications on biological applications of n.m.r. is now greater than ever before. There has been a clear swing from studies of amino-acids and synthetic peptides (though these are still numerous) to studies of proteins, particularly enzymes where n.m.r. is capable, given the right level of effort, of supplying much useful information on the active site, especially in terms of substrate and inhibitor binding, the binding of ions, and the characteristics of active site histidines. It is now clear that n.m.r., once thought to be as revolutionary a technique for biological molecules as for smaller organic molecules, can only answer certain very specific questions about macromolecules, and that it is best used in conjunction with as many other physical techniques as possible, particularly *X*-ray crystallography, where n.m.r. can provide the dynamic measurements in solution which studies in the crystalline state cannot, by definition, produce. Reviews of the n.m.r. of biomolecules published in 1975 include two on the use of lanthanides [208] and metal ions [209] as probes, one on the conformations of peptides [210] and two on ^{13}C in conformational [211] and biosynthetic [212] studies.

Amino-acids.—The ^{1}H n.m.r. of amino-acids is a fairly well-worked seam by now, but some small nuggets remain. For example, the spectrum of a new amino-acid, L-*threo*-γ-hydroxylysine, from an antibiotic found in *Cillus* organisms is reported,[213] while the *exo–endo* equilibrium in L-proline has been studied at 250 MHz and found to shift towards the *endo* form in basic solution.[214] A

[205] T. T. Herskovits and N. J. Solli, *Biopolymers*, 1975, **14**, 319.
[206] Y. Suzuki, Y. Inoue, and R. Chujō, *Biopolymers*, 1975, **14**, 1223.
[207] G. R. Stark and T. P. Link, *Biochemistry*, 1975, **14**, 3476.
[208] J. Reuben, *Naturwiss.*, 1975, **62**, 172.
[209] S. J. Ferguson, in 'Tech. Top. Bioinorg. Chemistry', ed. C. A. McAuliffe, Wiley, New York, 1975.
[210] G. Nemethy, *Biochimie*, 1975, **57**, 483.
[211] I. C. P. Smith, H. J. Jennings, and R. Deslauriers, *Accounts Chem. Res.*, 1975, **8**, 306.
[212] A. G. McInnes and J. L. C. Wright, *Accounts Chem. Res.*, 1975, **8**, 313.
[213] J. Shoji and H. Hinoo, *J. Antibiotics*, 1975, **28**, 60.
[214] L. Pogliani, M. Ellenberger, and J. Valat, *Org. Magn. Resonance*, 1975, **7**, 61.

potentially useful chemical modification, the deuterium substitution of *one* of the β-methylene protons in the side-chain of an amino-acid, may have value in side-chain conformation analysis,[215] and some studies [216] of amino-acid–Mn^{2+} complexes suggest that the amino-acids complex in the outer co-ordination sphere of the metal. More work has been done on the ^{13}C resonance of amino-acids as part of the groundwork for future carbon magnetic resonance study of proteins. In particular, ^{13}C–^{1}H coupling constants [217] and ^{13}C–^{13}C coupling constants [218] have been reported, and a paper on the ^{13}C chemical shifts induced by protonation of amino-acids [219] gives a method for isolation of the linear electric field shift component of the effect; this may well be important in conformational analysis near protonation sites. It has been pointed out [220] that the ^{13}C resonances of tryptophan residues can be recognized in the partially relaxed spectra of proteins by virtue of their long longitudinal relaxation time T_1, and ^{13}C resonance has also been proposed for the identification of formaldehyde cross-linking sites between amino-acids [221] and used in studies of the *Z*,*E* isomerism of a number of blocked amino-acids.[222] ^{13}C Relaxation times can only be measured properly in solutions which have been carefully purged of paramagnetic ions. It has been pointed out [223] that even for the simple glycine molecule, earlier reported values of T_1 were considerably too short because of failings in this respect. Deliberate addition of paramagnetic ions in the form of Mn^{2+} has yielded the information that two histidine molecules form a complex with the metal in which it is octahedrally co-ordinated.[224]

Synthetic Peptides.—Synthetic polypeptides, whose n.m.r. spectra were once the source of much controversy, continue to attract some attention. The dynamics of the helix–coil transition in poly(γ-benzyl-L-glutamate) have been studied with reference to the effects of polydispersity,[225] and alanine oligopeptides have again been studied.[226, 227] The alignment of molecules of poly-L-lysine [228] and poly(γ-methyl-L-glutamate) [229] in the magnetic field of the n.m.r. spectrometer has been discussed, as have the longitudinal and transverse relaxation times of poly-L-lysine as functions of concentration, chain length, and temperature.[230] Of interest to palaeobiochemists is a report on the properties of polypeptides produced by pyrocondensation; poly-L-aspartic acid produced thermally showed

[215] M. Kainosho and K. Ajisaka, *J. Amer. Chem. Soc.*, 1975, **97**, 5630.
[216] E. Tiezzi, *J.C.S. Perkin II*, 1975, 769.
[217] P. E. Hansen, J. Feeney, and G. C. K. Roberts, *J. Magn. Resonance*, 1975, **17**, 249.
[218] Tran Dinh Son, S. Fermandjian, E. Sala, R. Mermet-Bouvier, and P. Fromageot, *J. Amer. Chem. Soc.*, 1975, **97**, 1267.
[219] J. G. Batchelor, J. Feeney, and G. C. K. Roberts, *J. Magn. Resonance*, 1975, **20**, 19.
[220] E. Oldfield and A. Allerhand, *J. Amer. Chem. Soc.*, 1975, **97**, 221.
[221] M. K. Dewer, R. B. Johns, D. P. Kelly, and J. F. Yates, *Austral. J. Chem.*, 1975, **28**, 917.
[222] M. Branik and H. Kessler, *Chem. Ber.*, 1975, **108**, 2176.
[223] H. Peason, D. Gust, I. M. Armitage, H. Huber, J. D. Roberts, R. E. Stark, R. R. Vold, and R. L. Vold, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1599.
[224] J. J. Led and D. M. Grant, *J. Amer. Chem. Soc.*, 1975, **97**, 6962.
[225] K. Nagayama and K. Wada, *Biopolymers*, 1975, **14**, 2489.
[226] M. Goodman, N. Ueyama, and F. Naider, *Biopolymers*, 1975, **14**, 901.
[227] G. Bemski, *Acta Cient. Venez.*, 1974, **25**, 38.
[228] E. G. Finer and A. Darke, *J.C.S. Faraday I*, 1975, **71**, 984.
[229] R. W. Filas and H. Stefanon, *J. Phys. Chem.*, 1975, **79**, 941.
[230] D. R. Woodhouse, W. Derbyshire, and P. Lillford, *J. Magn. Resonance*, 1975, **19**, 267.

only β-peptide linkages, a finding which must cast some doubt on the proposal that this mechanism was responsible for the synthesis of prebiotic peptides.[231]

In the field of small synthetic peptides, it has been shown that substitution of deuterium for hydrogen in -NH groups causes upfield shifts in the resonances of nearby ^{13}C nuclei in amino-acids and dipeptides.[232] This may be useful in assignments, as may ^{13}C chemical shift data on a whole series of 85% ^{13}C-enriched amino-acids and small peptides[233] and complete proton resonance parameters for all 20 naturally-occurring amino-acids in the sequence trifluoroacetyl-Gly-Gly-L-X-L-Ala-OMe.[234] Proton chemical shifts of aromatic residues in small peptides measured at 300 MHz have also been reported.[235] Slightly less usual applications of n.m.r. to peptides are found in the use of methyl ester chemical shifts to detect racemization in peptide preparations[236] and the sequence determination of up to nine residues in a peptide by the expedient of measuring the broadening, induced by a Gd^{3+} or Cu^{2+} ion at the *N*-terminus, in the ^{13}C resonance lines; the broadening diminishes as distance from the *N*-terminus increases.[237] A disadvantage of this method is that it requires 20 mg of sample. Other results presented for small peptides include second-order rate constants for base-catalysed exchange reactions in halogeno-substituted dipeptides,[238] *cis–trans* interconversion barriers for proline-containing dipeptides,[239] the detection of type 1 and β-bends in proline-containing tetrapeptides,[240] and a preference of some peptides for a *gauche* conformation of bulky side-chains relative to one another.[241] Attempts have been made to examine the conformational preferences of short peptide sequences which are synthetic versions of critical regions of proteins; in particular a repeating pentapeptide of elastin, HCO-(L-Val-L-Pro-Gly-L-Val-Gly)$_n$-L-Val-OMe where $n \sim 18$, gave evidence of β- and γ-turns and 11-atom rings closed by hydrogen bonds,[242] and tetrapeptides from a number of variant chymotrypsin sequences showed some propensity for the β-bend which is their conformation in the native protein.[243] Cyclic dipeptides studied include these of tryptophan with alanine, leucine, and valine[244] showing large changes in α-C<u>H</u> proton chemical shift with the *cis–trans* transition, studies of *cyclo*-(Thr-His)[245] and two reports of longitudinal relaxation time measurements.[246, 247]

[231] S. Andini, E. Benedetti, L. Ferrara, L. Paolillo, and P. A. Temussi, *Origins of Life*, 1975, **6**, 147.
[232] H. K. Ladner, J. J. Led, and D. M. Grant, *J. Magn. Resonance*, 1975, **20**, 530.
[233] S. Fermandjian, Tran Dinh Son, J. Sarrda, E. Sala, R. Mermet-Bouvier, E. Bricas, and P. Fromageot, *Biochim. Biophys. Acta*, 1975, **399**, 313.
[234] A. Bundi, C. Grathwohl, J. Hochmann, R. M. Keller, G. Wagner, and K. Wuthrich, *J. Magn. Resonance*, 1975, **18**, 191.
[235] R. Hintscher and B. Mehlis, *Z. Chem.*, 1975, **15**, 22.
[236] J. S. Davies, R. J. Thomas, and M. K. Williams, *J.C.S. Chem. Comm.*, 1975, 76.
[237] B. Warren and J. H. Bradbury, *J. Polymer Sci., Polymer Symposia*, 1975, **49**, 65.
[238] M. Sheinblatt and Y. Rahamin, *J.C.S. Perkin II*, 1975, 784.
[239] P. E. Young and C. M. Deber, *Biopolymers*, 1975, **14**, 1547.
[240] K. D. Kopple, A. Go, and D. R. Pilipauskas, *J. Amer. Chem. Soc.*, 1975, **97**, 6830.
[241] B. J. Dale and D. W. Jones, *Spectrochim. Acta* (*A*), 1975, **31**, 83.
[242] D. W. Urry, L. W. Mitchell, T. Ohnishi, and M. M. Long, *J. Mol. Biol.*, 1975, **96**, 101.
[243] J. C. Howard, A. Ali, H. A. Scheraga, and F. A. Momany, *Macromolecules*, 1975, **8**, 607.
[244] I. Z. Siemion, D. Konopinska, and W. A. Klis, *Roczniki Chem.*, 1975, **49**, 781.
[245] P. Ptak, *Inf. Chim.*, 1975, **147**, 237, 245, 251.
[246] R. Deslaurier, Z. Grzonka, K. Schaumburg, T. Shiba, and R. Walter, *J. Amer. Chem. Soc.*, 1975, **97**, 5093,
[247] E. T. Fossel, K. R. K. Easwaran, and E. R. Blout, *Biopolymers*, 1975, **14**, 927.

Other cyclic peptides studied include cyclic tetraglycine [248] and peptides of the form *cyclo*-(X-Gly-Gly)$_2$ and (X-Gly)$_3$.[249]

Growing interest is being shown in the possible uses of ^{15}N resonance. Papers published in 1975 restrict themselves mostly to reports of chemical shifts and relaxation times for simple systems: glycine,[250] glycylglycine,[251] phenylalanylglycine,[252] *cyclo*-(Gly-Pro) and Gly-Phe (including some vicinal ^{13}C–^{15}N coupling constants),[253] and a series of 16 ^{15}N-labelled dipeptides.[254] An attempt [255] to predict the ^{15}N spectrum of gramicidin, based on measurements of amino-acids and peptides, was only partially successful due to factors such as intramolecular hydrogen bonding, the lack of free carboxy-groups, and the cyclic nature of the antibiotic.

Natural Peptides.—Gramicidin-S is, of course, a well-studied molecule, and major progress has been made in recent years. Modified (*N*-methyl-leucine) gramicidin has recently been investigated and shown to retain the normal gramicidin-S structure in one half of the molecule only,[256] while the di-*N*-methyl-leucine derivative yields a proposed structure in MeOH which contains four β-turns, the hydrogen-bond patterns forming a partial turn of right-handed 3_{10} helix.[257] Interactions between gramicidin-S and solvent molecules have been studied by ^{13}C relaxation measurements [258] and by a transfer of saturation method which reveals contacts between the phenylalanine rings of the antibiotic and apolar (methyl or trifluoromethyl) groups of the solvents.[259]

Valinomycin, another antibiotic popular with n.m.r. spectroscopists, has been the subject of investigations into the nature of its interactions with metal ions [260] and ammonium ions.[261] Analogues of valinomycin have also been analysed conformationally by i.r. and c.d. as well as by n.m.r. techniques.[262] A ^{13}C-labelling method for oxytocin has been reported,[263] and from a laboratory noted for its advanced n.m.r. techniques comes a series of decoupling-difference cor-

[248] C. Grathwohl, A. Tun-Kyi, A. Bundi, R. Schwyzer, and K. Wuthrich, *Helv. Chim. Acta*, 1975, **58**, 415.
[249] V. T. Ivanov, I. A. Lavrinovich, G. A. Kogan, E. M. Mescheryakova, S. L. Portnova, M. D. Isabaev, P. V. Kostetskii, and Yu. A. Ovchinnikov, *Khim. prirod Soedinenii*, 1975, **11**, 202.
[250] T. K. Leipert and J. H. Noggle, *J. Amer. Chem. Soc.*, 1975, **97**, 269.
[251] C. S. Irving and A. Lapidot, *J. Amer. Chem. Soc.*, 1975, **97**, 5945.
[252] M. Kainosho, K. Ajisaka, M. Kamisaku, A. Murai, and Y. Kyogoku, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 425.
[253] R. Diblasi and K. D. Kopple, *J.C.S. Chem. Comm.*, 1975, 33.
[254] T. B. Posner, V. Markowski, P. Loftus, and J. D. Roberts, *J.C.S. Chem. Comm.*, 1975, 769.
[255] G. E. Hawkes, E. W. Randall, and C. H. Bradley, *Nature*, 1975, **257**, 767.
[256] N. G. Kumar, N. Izumiya, M. Miyoshi, H. Sugano, and D. W. Urry, *Biochemistry*, 1975, **14**, 2197.
[257] N. G. Kumar, N. Izumiya, M. Miyoshi, H. Sugano, and D. W. Urry, *J. Amer. Chem. Soc.*, 1975, **97**, 4105.
[258] R. A. Komoroski, R. I. Peat, and G. C. Levy, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 272.
[259] T. P. Pitner, J. D. Glickson, R. Rowan, J. Dadok, and A. A. Bothner-By, *J. Amer. Chem. Soc.*, 1975, **97**, 5917.
[260] D. G. Davis and D. C. Tosteson, *Biochemistry*, 1975, **14**, 3962.
[261] D. G. Davis, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 786.
[262] V. T. Ivanov, A. A. Sanasaryan, L. A. Fonina, L. B. Senyavina, E. I. Vinogradova, Yu. A. Ovchinnikov, I. I. Chervin, and G. J. Yakovlev, *Bioorg. Khim.*, 1975, **1**, 214.
[263] J. H. Griffin, R. Alazard, C. Dibello, E. Sala, R. Mermet-Bouvier, and P. Cohen, *F.E.B.S. Letters*, 1975, **50**, 168.

relation ^{1}H n.m.r. spectra of the decapeptide tyrocidine A, revealing for example the α-CH and β-CH protons of individual residues.[264]

Enzymes and other Non-haem Proteins.—Moving on to the magnetic resonance of larger proteins, a number of techniques for increasing the applicability of n.m.r. have been developed. An assay for free lysine ε-CH_2 groups by adding trifluorothiol acetate in DMSO solution and measuring the resulting ^{19}F spectral intensity has been described,[265] and ^{13}C-labelling of amino-acid side-chains with electrophilic groups [266] may have applications for enzyme active sites. Methods by which different pulse sequences may be used in Fourier transform n.m.r. to simplify the spectra of proteins have been described,[267] and strategies for the assignments of proton [268] and ^{13}C [269] spectra of proteins enumerated. Reviews published in 1975 include one on the study of protein histidine resonance,[270] on the active site of micrococcal nuclease among other topics,[271] and a review on current knowledge of phosphotransferases, which emphasizes common features found in these molecules such as the way in which each successive ligand bound progressively immobilizes the substrate and makes the active site less accessible to water.[272]

Ribonuclease, long a favourite molecule for n.m.r. investigation, continues to attract attention. A complete study of the pathway of its unfolding by heat and acid [273] and denaturing agents such as urea and guanidinium hydrochloride [274] has been reported, although other workers claim evidence, from relaxation and other measurements, for residual structure involving histidines 48 and 105 in thermally denatured RNase A.[275] The controversial assignment of the active-site histidines 12 and 119 has been re-studied using deuterium exchange, resulting in assignment of the four C-2 resonances to histidines 105, 119, 12, 48, respectively.[276] while another report assigns the lower pK of the two active-site residues to His-12.[277] Evidence is also presented that histidines 12 and 119 in fact interact electrostatically.[278] A resonance found in the aromatic region of the RNAse A spectrum and having a pK of 6.1 is now assigned to a proton involved in a hydrogen bond between His-12 and Thr-45.[279] The degree of exposure of various ribonuclease A histidines to solvent molecules has been established by means of titrations at various temperatures[280] showing that histidines 105 and 119 are well exposed to

[264] W. A. Gibbons, C. F. Beyer, J. Dadok, R. F. Sprecher, and H. R. Wyssbrod, *Biochemistry*, 1975, **14**, 420.
[265] J. E. Ramirez, J. R. Cavanaugh, K. S. Schweizer, and P. D. Hoagland, *Analyt. Biochem.*, 1975, **63**, 130.
[266] I. J. G. Climie, D. A. Evans, and M. Akhtar, *J.C.S. Chem. Comm.*, 1975, 160.
[267] I. D. Campbell, C. M. Dobson, R. J. P. Williams, and P. E. Wright, *F.E.B.S. Letters*, 1975, **57**, 96.
[268] I. D. Campbell, C. M. Dobson, and R. J. P. Williams, *Proc. Roy. Soc.*, 1975, **A345**, 23.
[269] E. Oldfield, R. S. Norton, and A. Allerhand, *J. Biol. Chem.*, 1975, **250**, 6381.
[270] J. L. Markley, *Accounts Chem. Res.*, 1975, **8**, 70.
[271] J. H. Griffin, B. Furie, and A. N. Schechter, *Biochimie*, 1975, **57**, 453.
[272] M. Cohn, *Ciba Found. Symposia*, 1975, **31**, 87.
[273] F. W. Benz and G. C. K. Roberts, *J. Mol. Biol.*, 1975, **91**, 345.
[274] F. W. Benz and G. C. K. Roberts, *J. Mol. Biol.*, 1975, **91**, 367.
[275] C. R. Matthews and D. G. Westmoreland, *Biochemistry*, 1975, **14**, 4532.
[276] J. L. Markley, *Biochemistry*, 1975, **14**, 3546.
[277] D. J. Patel, L. L. Canuel, and F. A. Bovey, *Biopolymers*, 1975, **14**, 987.
[278] J. L. Markley and W. R. Finkenstadt, *Biochemistry*, 1975, **14**, 3562.
[279] D. J. Patel, L. L. Canuel, C. Woodward, and F. A. Bovey, *Biopolymers*, 1975, **15**, 959.
[280] D. G. Westmoreland, C. R. Matthews, M. B. Hayes, and J. S. Cohen, *J. Biol. Chem.*, 1975, **250**, 7456.

solvent, while His-12 is more restricted of access. Titration curves of His-48 reveal an inflection, which two groups of workers [281, 282] attribute to an interaction with Asp-14; the latter report also shows that tyr-25 appears to be affected by the interaction. Some exchangeable peaks in the proton spectrum of RNAse A have been assigned to N-1 and N-3 protons of His-48,[283] and the modification in histidine titration patterns consequent upon methylation of lysine residues in the enzyme has been determined.[284]

Second only to ribonuclease for popularity in recent years has been lysozyme. Contributions to our knowledge of it in 1975 have included a summary of its now-known solution structure,[285] the kinetics of enzyme structural changes induced by binding *N*-acetylglucosamine trimer,[286] studies of its tyrosine [287] and exchangeable [288] protons, relaxation time changes through the denaturation process,[289] and ^{13}C studies of temperature-induced conformational changes,[290] and the denaturation of lysozymes and lactalbumins.[291] Histidine titrations of bovine α-lactalbumin give p*K* values and solvent accessibilities of the three histidines.[292] Equally varied have been approaches to carbonic anhydrase, the histidine titrations of which yield a single resonance which is apparently common between human carbonic anhydrase B and C and which may therefore be functionally significant.[293] A non-histidine catalytic group with a p*K ca.* 7 in human carbonic anhydrase B has been investigated [294] and found to be probably the ionization of a metal-co-ordinated water molecule. Other studies have concentrated on analogues of carbonic anhydrase in which the zinc ion has been replaced by various others: Mn^{2+},[295] Co^{2+},[296] and cadmium and mercury,[297] with consequent changes in relaxation times and the binding of anions to the protein.

Basic pancreatic trypsin inhibitor is a stable protein of 58 residues, including four tyrosine and four phenylalanine residues. These aromatic residues have been subjected to a thorough investigation, yielding their conformation-dependent chemical shifts,[298] their rotation rates,[299] the p*K* values for the tyrosine residues,[300] and some extremely well resolved spectra produced by resolution enhancement of 360 MHz spectra.[301] Another very stable protein is soya bean trypsin inhibitor,

[281] J. S. Cohen and H. Shindo, *J. Biol. Chem.*, 1975, **250**, 8874.
[282] J. L. Markley, *Biochemistry*, 1975, **14**, 3554.
[283] D. J. Patel, L. L. Canuel, and F. A. Bovey, *Biochim. Biophys. Acta*, 1975, **400**, 275.
[284] L. R. Brown and J. H. Bradbury, *European J. Biochem.*, 1975, **54**, 219.
[285] I. D. Campbell, C. M. Dobson, and R. J. P. Williams, *Proc. Roy. Soc.*, 1975, **A345**, 41.
[286] C. M. Dobson and R. J. P. Williams, *F.E.B.S. Letters*, 1975, **56**, 362.
[287] I. D. Campbell, C. M. Dobson, and R. J. P. Williams, *Proc. Roy. Soc.*, 1975, **B189**, 503.
[288] I. D. Campbell, C. M. Dobson, and R. J. P. Williams, *Proc. Roy. Soc.*, 1975, **B189**, 485.
[289] D. Pumpernik, B. Fajt, S. Lapanje, and A. Azman, *Z. Naturforsch.*, 1975, **30c**, 294.
[290] P. J. Cozzone, S. J. Opella, O. Jardetzky, J. Berthon, and P. Jolles, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2095.
[291] G. Van Binst, M. Biesemans, and A. O. Barel, *Bull. Soc. chim. belges*, 1975, **84**, 1.
[292] J. H. Bradbury and R. S. Norton, *European J. Biochem.*, 1975, **53**, 387.
[293] J. M. Pesando, *Biochemistry*, 1975, **14**, 675.
[294] S. Lindskog, *Chimia*, 1975, **29**, 65.
[295] A. Lanir, S. Gradstajn, and G. Navon, *Biochemistry*, 1975, **14**, 242.
[296] I. D. Campbell, S. Lindskog, and A. I. White, *J. Mol. Biol.*, 1975, **98**, 597.
[297] R. J. Smith and R. G. Bryant, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1281.
[298] G. Wagner and K. Wuthrich, *J. Magn. Resonance*, 1975, **20**, 435.
[299] K. Wuthrich and G. Wagner, *F.E.B.S. Letters*, 1975, **50**, 265.
[300] G. H. Snyder, R. Rowan, S. Karplus, and B. D. Sykes, *Biochemistry*, 1975, **14**, 3765.
[301] G. Wagner, A. DeMarco, and K. Wuthrich, *J. Magn. Resonance*, 1975, **20**, 565.

and deuterium exchange studies[302] using ^1H n.m.r. for assay have revealed that its two histidines have half-lives against deuterium exchange of 61 and 24 days! Similar methods reveal high stability in *Streptomyces* subtilisin inhibitor.[303]

Less common nuclei were used in two magnetic resonance studies of lactate dehydrogenases; ^{35}Cl resonance of chloride ions in a protein solution revealed two binding sites, one of them the active site,[304] and ^{31}P resonance of NAD^+ and NADH binding to a lactate dehydrogenase and a glyceraldehyde-3-phosphate dehydrogenase showed that the binding of the coenzyme is similar whether it is in the oxidized or reduced form, but differs from one enzyme to the other.[305] Further data on rabbit muscle glyceraldehyde-3-phosphate dehydrogenase have been provided by ^{19}F-labelling methods.[306] ^{31}P methods were used for the binding of $NADP^+$ and NADPH to dihydrofolate reductase, showing a conformational change in the coenzyme on complex formation,[307] while ^{13}C-labelling of the NADP enabled a rate constant for the binding to be established as $55 \pm 10\ s^{-1}$;[308] dihydrofolate reductase has also been studied by ^1H n.m.r.[309]

The use of manganese and calcium ions as probes is well illustrated by two studies of concanavalin A. A stopped-flow method was used[310] to observe three distinct conformational states of the protein with α-methyl-D-mannoside, and a tentative assignment[311] made of the C-2 proton resonance of the active site His-24 by using Mn^{2+} and Ca^{2+}.

Of course, the library of proteins which might some day be usefully examined by n.m.r. is vast, and the spectroscopists' search through the catalogues yields an ever greater variety of subjects. Not all is plain sailing, however: an odd and apparently significant resonance found during a study of histidine titrations in a serine protease from *Staphylococcus aureus*[312] was quickly attributed to formic acid impurity by another author.[313] Less contentious, perhaps, was the observation, in a ^{13}C-labelled histidine residue of another serine protease, of changes in environment on binding of inhibitor.[314] Paramagnetic probes continue to provide a major window on the active sites of metalloenzymes; a manganese ion at the active site of carboxypeptidase A, for example, revealed that when benzyloxycarbonylglycine was bound to the enzyme, it was with distances of 5.2 and 7.0 Å between the glycine and benzyl methylene groups, respectively, and the metal.[315] In aspartate transcarbamylase, there are four zinc ions which

[302] J. L. Markley and I. Kato, *Biochemistry*, 1975, **14**, 3234.
[303] K. Akasaka, H. Aoshima, H. Hatano, S. Sato, and S. Murao, *Biochim. Biophys. Acta*, 1975, **412**, 120.
[304] R. L. Ward, *Arch. Biochem. Biophys.*, 1975, **169**, 22.
[305] M. Blumenstein, *Biochemistry*, 1975, **14**, 5004.
[306] J. Bode, M. Blumenstein, and M. A. Raftery, *Biochemistry*, 1975, **14**, 1153.
[307] J. Feeney, B. Birdsall, G. C. K. Roberts, and A. S. V. Burgen, *Nature*, 1975, **257**, 564.
[308] J. L. Way, B. Birdsall, J. Feeney, G. C. K. Roberts, and A. S. V. Burgen, *Biochemistry*, 1975, **14**, 3470.
[309] G. C. K. Roberts, J. Feeney, A. S. V. Burgen, B. Yuferov, J. G. Dann, and R. Bjur, *Biochemistry*, 1974, **13**, 5351.
[310] J. J. Grimaldi and B. D. Sykes, *J. Biol. Chem.*, 1975, **250**, 1618.
[311] B. H. Barber and J. P. Carver, *Canad. J. Biochem.*, 1975, **53**, 371.
[312] J. L. Markley, W. R. Finkenstadt, H. Dugas, R. Leduc, and G. R. Drapeau, *Biochemistry*, 1975, **14**, 998.
[313] C. M. Dobson, *Biochemistry*, 1975, **14**, 4905.
[314] M. W. Hunkapiller, S. H. Smallcombe, and J. H. Richards, *Org. Magn. Resonance*, 1975, **7**, 262.
[315] T. Kushnir and G. Navon, in 'Proceedings of the 1974 Symposium on Protein–Ligand Interactions', de Gruyter, Berlin, 1975, p. 174.

can be replaced by manganese, with consequent broadening of the resonances from succinate bound at the active site; on the other hand, the protons of cytosine triphosphate bound at a regulatory site showed no broadening, indicating that the site is at least 20 Å from the main catalytic site.[316] The catalytic site of the same enzyme has also been probed by a deuteriated L-malate molecule.[317] An example of n.m.r. backing up crystal data is found in the location of the calcium-binding site of α- and β-trypsin, by substituting Gd^{3+}, as being between Asp-194 and Ser-190.[318] The use of this lanthanide is a less common event than one would have predicted a few years ago; manganese, rather than lanthanides, seems to be the probe ion of choice for many investigators. Examples are in the binding of deoxynucleoside triphosphate substrates to *E. coli* DNA polymerase I,[319] the binding of D-xylose to D-xylose isomerase,[320] some evidence for methionine sulphoximine as a transition-state analogue of glutamine synthetase,[321] and some observations of unusual histidine behaviour in porcine muscle adenylate kinase, coupled with clear evidence that ATP lies close to His-36 in its complex with this enzyme.[322]

Halogen nuclei are gaining favour for magnetic resonance studies of proteins; among the 1975 reports we find ^{35}Cl resonance used to study anion (perchlorate) binding sites in serum albumin,[323] $^{35}Cl^-$ binding to renal dipeptidase,[324] and $^{35}Cl^-$ and $^{81}Br^-$ in competition with tetracyanoplatinate [$Pt(CN)_4^{2-}$] and dicyanoaurate [$Au(CN)_2^-$] ions in various proteins,[325] including horse liver alcohol dehydrogenase.[326] ^{19}F Resonance was used to detect the binding rate constants of a trifluoroacetylated inhibitor to α-chymotrypsin.[327] Carbon-13 still makes very slow progress in macromolecule investigations, presumably because of signal-to-noise problems, and conclusions using natural abundance ^{13}C tend to be very general. We may mention relaxation studies on muscle calcium binding protein[328] and on elastin,[329] and some assignments of ^{13}C resonances from coenzyme A.[330] In fact, the naturally phosphate-containing substrates of many enzymes make ^{31}P as attractive as ^{13}C for many studies. Membrane-bound ATPase has been studied by this method,[331] and an interesting paper on phosphate bound to *E. coli* alkaline phosphatase reports a resonance assigned to a strained phosphoserine, presumably part of the activity of the enzyme.[332]

[316] S. Fan, L. W. Harrison, and G. G. Hammes, *Biochemistry*, 1975, **14**, 2219.
[317] H. I. Mosberg, P. G. Schmidt, and S. S. Seaver, *J. Magn. Resonance*, 1975, **20**, 82.
[318] F. Abbott, J. E. Gomez, E. R. Birnbaum, and D. W. Darnall, *Biochemistry*, 1975, **14**, 4935.
[319] D. L. Sloan, L. A. Loeb, A. S. Mildvan, and R. J. Feldmann, *J. Biol. Chem.*, 1975, **250**, 8913.
[320] J. M. Young, K. J. Schray, and A. S. Mildvan, *J. Biol. Chem.*, 1975, **250**, 9021.
[321] J. J. Villfranca, D. E. Ash, and F. C. Wedler, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1003.
[322] G. G. McDonald, M. Cohn, and L. Noda, *J. Biol. Chem.*, 1975, **250**, 6947.
[323] P. Reimarsson, T. Bull, and B. Lindman, *F.E.B.S. Letters*, 1975, **59**, 158.
[324] L. G. Ferren, R. L. Ward, and B. J. Campbell, *Biochemistry*, 1975, **14**, 5280.
[325] J. E. Norne, H. Lilja, B. Lindman, R. Einarsson, and M. Zeppezauer, *European J. Biochem.*, 1975, **59**, 463.
[326] T. E. Bull, B. Lindman, R. Einarsson, and M. Zeppezauer, *Biochim. Biophys. Acta*, 1975, **377**, 1.
[327] J. T. Gerig and A. D. Stock, *Org. Magn. Resonance*, 1975, **7**, 249.
[328] D. R. Bauer, S. J. Opella, D. J. Nelson, and R. Pecora, *J. Amer. Chem. Soc.*, 1975, **97**, 2580.
[329] J. R. Lyerla and D. A. Torchia, *Biochemistry*, 1975, **14**, 5175.
[330] S. B. W. Roeder, B. S. Master, and C. J. Stewart, *Physiol. Chem. Phys.*, 1975, **7**, 115.
[331] C. M. Grisham and A. S. Mildvan, *J. Supramol. Struct.*, 1975, **3**, 304.
[332] J. L. Bock and B. Sheard, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 24.

With their known dynamic role in chromosome structure, the histones continue to yield up information to n.m.r. spectroscopists. Aggregation of histone molecules has been studied by ^{13}C n.m.r.[333] although the high concentrations required for natural-abundance measurements can make interpretation in terms of specific interactions very difficult. Individual histones have been complexed with DNA,[334] and specific studies reported of histones H2A,[335] H1,[336] and the H1/DNA complex.[337]

Haem Proteins.—Work continues on the quaternary structure of haemoglobin, studied *via* exchangeable protons made visible by running spectra in water (rather than deuterium oxide) solution. In a study of sickle-cell haemoglobin [338] the haem environment and subunit interfaces appeared normal, although surface residues showed evidence of a more hydrophobic environment than normal. Comparison between normal and mutant haemoglobins permitted the assignment of a specific resonance at −9.4 p.p.m. (relative to the water resonance) to the proton of a hydrogen bond between tyrosine α42 and aspartic acid β99, and another, between aspartic acid α94 and asparagine β102, whose resonance moves from −6.4 p.p.m. in the deoxy form to −5.8 p.p.m. in the oxy form, giving a reliable indicator of the quaternary structure.[339] The same laboratory also studied the alkylation of haemoglobin S by nitrogen mustard.[340] ^{13}C Resonance is beginning to be applicable to haemoglobin: in a natural-abundance study, signals from individual carbon positions in the molecule could be resolved,[341] and in another, mouse haemoglobin was labelled *in vivo* with ^{13}C at the histidine C-2 position; subsequent measurement of relaxation time T, before and after haemolysis, gave indications of intracellular fluid viscosities which proved to be much the same as those outside the cell.[342] Solvent proton relaxation measurements of valency-hybrid haemoglobins [343] made possible comparisons between the solvent accessibility of haem groups in different valency states of human and canine haemoglobins.

Myoglobin has been studied using ^{13}C resonance, which gave a 22 ns rotational correlation time for sperm whale cyanoferrimyoglobin;[344] labelling of haem methyl groups with deuterium [345] and of methionyl residues with ^{13}C [346] have also been described. Proton magnetic resonance of myoglobins gave pK values for

[333] D. M. J. Lilley, O. W. Howarth, V. M. Clark, J. F. Pardon, and B. M. Richards, *Biochemistry*, 1975, **14**, 4590.
[334] O. A. Azizova, V. N. Bushuev, V. A. Korol, L. P. Kayushin, L. A. Sibel'dina, Yu. V. Nikolaev, and V. A. Kopylov, *Biofizika*, 1975, **20**, 327.
[335] E. M. Bradbury, P. D. Cary, C. Crane-Robinson, H. W. E. Rattle, M. Boublik, and P. Sautière, *Biochemistry*, 1975, **14**, 1876.
[336] E. M. Bradbury, P. D. Cary, G. E. Chapman, C. Crane-Robinson, S. E. Danby, H. W. E. Rattle, M. Boublik, J. Palau, and F. J. Aviles, *European J. Biochem.*, 1975, **52**, 605.
[337] E. M. Bradbury, S. E. Danby, H. W. E. Rattle, and V. Giancotti, *European J. Biochem.*, 1975, **57**, 97.
[338] L. W. M. Fung, K.-L. C. Lin, and C. Ho, *Biochemistry*, 1975, **14**, 3424.
[339] L. W. M. Fung and C. Ho, *Biochemistry*, 1975, **14**, 2526.
[340] L. W. M. Fung, C. Ho, E. F. Roth, and R. L. Nagel, *J. Biol. Chem.*, 1975, **250**, 4786.
[341] E. Oldfield and A. Allerhand, *J. Biol. Chem.*, 1975, **250**, 6403.
[342] R. E. London, C. T. Gregg, and N. A. Matwiyoff, *Science*, 1975, **188**, 266.
[343] B. Markovska, G. D. Efremov, S. Vuk-Parlovic, B. Benko, and S. Maricic, *Arch. Biochem. Biophys.*, 1975, **171**, 337.
[344] R. B. Visscher and F. R. N. Gurd, *J. Biol. Chem.*, 1975, **250**, 2238.
[345] O. Oster, G. W. Neireiter, A. O. Clause, and F. R. N. Gurd, *J. Biol. Chem.*, 1975, **250**, 7990.
[346] W. E. Jones, jun., T. M. Rothgeb, and F. R. N. Gurd, *J. Amer. Chem. Soc.*, 1975, **97**, 3875.

histidines (6.6, 7.0, 5.8, and 7.4),[347] and an energy barrier of 14.8 kcal mol^{-1} for hindered rotation of a methyl group on a side-chain of myoglobin haem,[348] and leads to a discussion of Curie spin relaxation and its effects on linewidth.[349] The aromatic resonances of ferri- and ferro-cytochrome *c* samples have been assigned by a variety of techniques,[350, 351] and water relaxation in solutions of ferricytochrome *c* at acid pH give an electronic correlation time of 6×10^{-11} s for the iron atom.[352] A new haem protein for n.m.r. investigation is horseradish peroxidase, whose haem environment has been shown to be consistent with a sedentary water molecule at the sixth ligand site of the iron [353] and whose isoenzyme shows evidence for a histidine proximal ligand.[354] A ^{13}C investigation of ferredoxins has determined the midpoint oxidation–reduction potential of each iron–sulphur cluster,[355] and an interesting study of living tunicate blood cells gives a broad signal from a labile vanadium–water complex, which disappears immediately on cell disruption and leads to a discussion of the vanadium chromogen.[356]

Hydration Studies.—The use of n.m.r. in the study of protein hydration has been reviewed.[357] Reports of such studies published during 1975 include measurements made on water molecules in frozen lysozyme solutions, [358] adsorbed on collagen and ovalbumin,[359] and in frog muscle [360] and mouse muscle with and without tumours.[361]

Conclusion.—Although there has been a large increase in papers published in the n.m.r. of amino-acids, peptides, and proteins during 1975, a disappointingly small proportion of them make contributions of real biological significance. Greater collaboration and interaction between first-class biochemists and spectroscopists, and a great deal of hard work, are needed to improve the situation.

4 Spin Labels

Contributed by P. J. Seeley

Covalent Spin Labels.—A classical use of spin labels (*e.g.* ref. 362), the application of covalently bound nitroxyl radicals to monitor protein conformation, has found elegant expression in studies of phosphofructokinase reported by Jones and coworkers.[363] The e.s.r. spectrum of rabbit muscle enzyme, which had been specific-

[347] M. B. Hayes, H. Hagenmaier, and J. S. Cohen, *J. Biol. Chem.*, 1975, **250**, 7461.
[348] I. Morishima and T. Iizuka, *Biochim. Biophys. Acta*, 1975, **386**, 542.
[349] K. Wuthrich, J. Hochmann, R. M. Keller, G. Wagner, M. Brunori, and C. Giacometti, *J. Magn. Resonance*, 1975, **19**, 111.
[350] C. M. Dobson, G. R. Moore, and R. J. P. S. Williams, *F.E.B.S. Letters*, 1975, **51**, 60.
[351] G. R. Moore and R. J. P. Williams, *F.E.B.S. Letters*, 1975, **53**, 334.
[352] A. Lanir and I. Aviram, *Arch. Biochem. Biophys.*, 1975, **166**, 439.
[353] S. Vuk-Pavlovic and B. Benko, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1154.
[354] R. J. P. Williams, P. E. Wright, G. Mazza, and J. R. Richard, *Biochim. Biophys. Acta*, 1975, **412**, 127.
[355] E. L. Packer, H. Sternlicht, E. T. Lode, and J. C. Rabinowitz, *J. Biol. Chem.*, 1975, **250**, 2062.
[356] R. M. K. Carlson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2217.
[357] L. J. Lynch and D. S. Webster, *J. Polymer Sci., Polymer Symposia*, 1975, **49**, 43.
[358] E. Hsi and R. G. Bryant, *J. Amer. Chem. Soc.*, 1975, **97**, 3220.
[359] N. V. Ratnikova, V. V. Ivanov, and Yu. N. Moskvich, *Biofizika*, 1975, **20**, 398.
[360] M. M. Civan and M. Shporer, *Biophys. J.*, 1975, **15**, 299.
[361] B. M. Fung, D. A. Wassil, D. L. Durham, R. W. Chesnut, N. N. Durham, and K. D. Berlin, *Biochim. Biophys. Acta*, 1975, **385**, 180.
[362] H. M. McConnell, S. Ogawa, and A. Horwitz, *Nature*, 1968, **220**, 787.
[363] R. Jones, R. A. Dwek, and I. O. Walker, *European J. Biochem.*, 1975, **60**, 187.

ally modified at its most reactive cysteine by 4-(2-iodoacetamido)-2,2,6,6-tetramethylpiperidino-oxyl, was altered by binding of both effectors and substrates to the protein. The results were interpreted in terms of concerted transitions between two conformational states of the kinase (Monod–Wyman–Changeux model). Addition of positive effectors (*e.g.* MgATP, ammonium ion) shifted the spectrum to that of the 'catalytic' form of the protein, characterized by a high proportion of an immobile spin-label signal. Inhibitors, ATP, and hydrogen ion pushed the equilibrium in the opposite direction (characterized by a signal from an immobile spin radical). There was also e.s.r.-detectable positive co-operativity in titrations with ATP, M^{2+}ATP, and fructose 6-phosphate, and effector and substrate binding sites for ATP were distinguished by a biphasic response to nucleotide triphosphate in the presence of fructose 6-phosphate. Whilst there was considerable agreement between the e.s.r. and kinetic data, the authors stressed anomalies in the two-state model and indicated difficulties involved in rationalizing homotropic interactions and polymerization behaviour on this basis.

A further spin-label study of the kinase family of enzymes has been reported: the labelling of pig muscle adenylate kinase with an iodoacetamide nitroxyl derivative.[364] Both protein thiol groups are reactive to the nitroxyl derivative. The e.s.r. spectrum of the modified protein consisted of signals from both strongly and weakly immobilized labels. It was the signal from the more mobile radical which responded to substrate (*e.g.* ATP, AMP) binding. Dipolar interaction between the *immobile* nitroxide and a manganous ion of MnATP substrate allowed the inter-spin distance to be quantified as 2 nm.

In vitro studies of the interaction of myofibrillar proteins have utilized the spin-label method. Chao and Holtzer[365] treated a maleimide derivative of piperidine oxide with rabbit muscle tropomyosin. Although there was a mixed reaction of label with both amino and thiol groups, the e.s.r. spectra were indicative of a complex route for protein denaturation: the nitroxide sites were disordered at lower concentrations of guanidine hydrochloride (1.5 mol l^{-1}) than were required for denaturation of the protein as a whole (3.5 mol l^{-1}). The same authors also reported e.s.r. spectra of fibres of tropomyosin drawn from solutions of spin-labelled protein. The spectra were dependent on the orientation of the fibres with respect to the applied magnetic field of the spectrometer and it was therefore concluded that spin-label sites were regularly arranged in the fibre, and that the normal to the nitroxide plane was inclined at an angle of 50° to the fibre axis. The development of this type of experiment appears attractive for studies of the orientation of subunits in proteinaceous fibres and the relationship between subunit topography and protein–protein interaction in the muscle system.

Attempts have been made to correlate e.s.r. and fluorescence data for myosin labelled with an iodoacetamide nitroxide on the S-1 thiols.[366] Modification of the enzyme reduced the magnitude of protein fluorescence enhancement on ATP binding. The fluorescence of labelled myosin was, however, critically dependent on conditions of pH and ionic strength, and these effects were closely followed by changes in e.s.r. parameters. Addition of ATP to nitroxide enzyme (in the

[364] N. C. Price, M. Cohn, and R. H. Schirmer, *J. Biol. Chem.*, 1975, **250**, 644.
[365] Y.-Y. H. Chao and A. Holtzer, *Biochemistry*, 1975, **14**, 2164.
[366] J. C. Seidel, *J. Biol. Chem.*, 1975, **250**, 5681.

presence of Mg^{2+}) increased the mobility of the label and this would seem to correspond to formation of the M**ADP-P_i complex (M = myosin). Although the initial burst of inorganic phosphate production on addition of ATP analogues to myosin was unrelated to spectral changes observed in both fluorescence and e.s.r., the extents of changes in the two sets of spectroscopic parameters did parallel each other. For example, ATP itself generated maximal enhancement in both protein fluorescence intensity and spin-label mobility.

The complementary partner in actin–myosin interaction has also been spin-labelled.[367] Actin monomers were doubly labelled with Mn^{2+} ion at a tight bivalent metal ion site, and with nitroxide by reaction between a maleimide derivative and a protein thiol group. G-Actin labelled with two spin centres showed identical polymerization kinetics to the native material. Changes in amplitude of the nitroxide e.s.r. signal due to variation in dipolar coupling between Mn^{2+} and N—O· spins served to monitor the complexing of actin fibres to myosin S1 heads in the absence of ATP. Identical e.s.r. responses were obtained from actin fibres formed from mixtures of native and paramagnetic G-actin monomers. It was concluded that changes in the e.s.r. spectrum in the course of the F-actin–myosin titration represent myosin-induced conformational changes in the actin monomers. There was not, however, a monotonic relation between e.s.r. amplitude and the fractional saturation of actin binding sites by myosin: the magnitude of the e.s.r. signal rose and then fell across the titration range. The authors concluded that there must be a *co-operative interaction* between actin monomers, even in the *absence* of tropomyosin.

Direct interpretation of the e.s.r. spectra of spin-labelled proteins in terms of the mobility of the label suggests a use for the technique in studies of protein denaturation. Mention has already been made of tropomyosin denaturation by guanidine hydrochloride.[365] Lassmann and colleagues[368] have observed the unfolding of bovine lens leucine aminopeptidase in 6.2M urea. The spectrum of spin-labelled native protein had components from both immobile (I) and (relatively) mobile (M_1) nitroxides. A second state of mobile nitroxide (M_2) formed on urea denaturation and there was a loss in intensity of I and M_1 signals. Urea did not completely mobilize the I nitroxide. The e.s.r. signals of 'native' protein were completely restored on removal of urea.

A novel method of modifying 'buried' cysteine residues has been described for human carbonic anhydrases B and C.[369] Unique, but distinct, thiol groups in the isozymic forms were labelled under denaturing conditions (in 5M-GuCl). Activity was restored by dilution of the denaturing medium. The modified C enzyme had only 50% esterase activity of native protein, but the nitroxide spectrum was altered by binding of benzene sulphonamide and iodide inhibitors. Conversely, the B isozyme had unimpaired catalytic efficiency and an invariant e.s.r. spectrum. The two results taken together imply that the change in conformation induced by the inhibitor molecules is limited in its spatial extent. Unfolding of spin-labelled B isozyme proceeded *via* a transient state for which the nitroxide was

[367] J. Loscalzo, G. H. Reed, and A. Weber, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3412.
[368] G. Lassmann, W. Damerau, G. Sklenar, D. Schwarz, M. Frohne, M. Ludewig, and H. Hanson, *European J. Biochem.*, 1975, **54**, 453.
[369] U. Carlsson, R. Aasa, L. E. Henderson, B.-H. Jonsson, and S. Lindskog, *European J. Biochem.*, 1975, **52**, 25.

more mobile than in either the initial or final states of folding. A two-state model for denaturation cannot therefore apply in this case. The refolding process was also rather rapid as judged by e.s.r. criteria since the 'native' nitroxide signals were generated within *ca.* 0.1 s of dilution.

Two further papers on covalent modification with spin labels should be mentioned. Daniel and Cohn [370] have specifically modified *E. coli* $tRNA_{f1}^{Met}$ at thiouridine (position 8) with *N*-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolidinyl)-bromoacetamide. They subsequently observed the perturbation of specific resonances in the ^1H n.m.r. spectrum of the tRNA caused by proximity of the nitroxyl radical. The C-5 methylene signal from a dihydrouridine residue and signals in the NH region of the spectrum were, for example, considerably broadened by the adjacent spin label. Comparison of spectra from modified tRNA's with paramagnetic and diamagnetic (reduced) forms of the spin label indicated perturbation of hydrogen-bonding by the modification of thiouridine and also served to implicate another proton signal in hydrogen-bonding to residue 8. The authors plan to examine structural perturbations induced in the region of the spin label on interaction of the tRNA with its aminoacyl-tRNA synthetase.

A spin-labelled derivative of the dipeptide Phe-Leu has been synthesized.[371] The molecule is a potent inhibitor of papain at pH 4 and competes with benzoyl-arginine ethyl ester in the hydrolysis reaction. A 'substrate-like' (acyl enzyme) binding of the nitroxyl-dipeptide was inferred and it was concluded that the specific nature of this paramagnetic modification of papain should allow accurate assignment and mapping of ^1H n.m.r. signals of the protein.

Non-covalent Spin Labels. A great diversity of spin-labelled substrate and effector analogues have been used to probe ligand–protein interactions. Dwek *et al.*[372] have examined by e.s.r. the interaction of a series of spin-labelled dinitrophenyl haptens with the Fv fragment of an homogeneous myeloma protein. The extent of motion of the spin label in the hapten–antibody complex increased with its distance from the central aromatic ring of the hapten. Detailed interpretation resulted in values of 1.1 nm × 0 9 nm × 0.6 nm for the dimensions of the combining site cleft and the conclusion that the pocket which contained the hapten was rigid. High-resolution ^1H n.m.r. spectroscopy on protein samples plus and minus ligand distinguished those proton signals of amino-acid residues lining the cleft from the broad envelope of protein signals. Approximately 30 aliphatic and 30 aromatic protons were assigned to the binding site.

The interactions of four spin-labelled analogues of biotin with hen egg-white avidin have been reported.[373] The avidin molecule is a tetramer of molecular weight 68 000 daltons and there is one biotin site per protomer. Fluorescence and optical absorption properties of the spin-label–avidin complex and competition with unmodified ligand suggested that the binding sites for the paramagnetic molecules were identical with those for biotin itself. Avidin which was fully

[370] W. E. Daniel and M. Cohn, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2582.
[371] A. Kalk, A. S. H. de Jong, and M. D. Barratt, *F.E.B.S. Letters*, 1975, **60**, 58.
[372] R. A. Dwek, J. C. A. Knott, D. Marsh, A. C. McLaughlin, E. M. Press, N. C. Price, and A. I. White, *European J. Biochem.*, 1975, **53**, 25.
[373] C. F. Chignell, D. K. Starkweather, and B. K. Sinha, *J. Biol. Chem.*, 1975, **250**, 5622.

saturated with biotinyl nitroxide gave an e.s.r. spectrum which was generated by *pairwise* dipolar interaction between electronic spins on adjacent cofactors. A distance of 1.6 nm between the radical centres was calculated from the 13.8 G splittings in the e.s.r. spectrum. The interaction of each biotin spin label with only one of its three neighbours was in agreement with a model for the avidin tetramer for which two biotin sites are much closer together than they are to their remaining neighbours. Mixing of two spin-label biotin equivalents with avidin (*i.e.* two biotins per tetramer) showed a competition between the kinetics and thermodynamics of binding. The e.s.r. spectrum immediately after mixing represented random association of spin labels with binding sites. The equilibrium state was one for which there was no dipolar interaction between nitroxyl spins, *i.e.* the adjacent pairs of biotin sites were only half-saturated.

The tight binding of steroids to corticosteroid binding protein (transcortin) has been studied by e.s.r.[374] Cortisol and testosterone spin-label analogues were assayed for their ability to compete with tritiated cortisol for the globulin. The cortisol analogue competed strongly with the unmodified steroid whereas the testosterone analogue was ineffective. Basset *et al.*[374] proposed the use of cortisol nitroxides and e.s.r. in 'spin assays' for steroid binding proteins.

Spin-label studies of fatty acid binding to bovine serum albumin have been carried out.[375, 376]

Membrane-associated Systems.—Devaux *et al.*[377] have examined the interaction of spin-labelled fatty-acyl-CoA molecules with the adenine nucleotide translocase of the mitochondrial inner membrane.[377] Stearoyl-CoA and palmitoyl-CoA nitroxide derivatives inhibited mitochondrial ADP transport at similar concentrations (100 nmol l^{-1}) to the simple long-chain acyl-CoA derivatives. The bound nitroxide generated an e.s.r. spectrum characteristic of an immobilized radical. The spectrum was only evident in preparations of the *inner* mitochondrial membrane and represented a nitroxide with substantially lower mobility than either free spin-labelled acyl-CoA or spin-labelled fatty acid. Specificity of interaction of acyl-CoA with the translocase was demonstrated by mobilization of the nitroxide by carrier ligands: adenine (but not guanosine or uridine) nucleotides, carboxyatractyloside and bongkrekic acid were effective agents. E.s.r. spectra recorded from samples for which the acyl-CoA spin label was in excess with respect to the translocase indicated that surplus label was incorporated into the mitochondrial membrane. The nitroxyl in the acyl-CoA–carrier complex was progressively less immobilized as the probe was moved towards the tail of the hydrocarbon chain. This suggests that binding of acyl-CoA to the translocase occurs principally *via* the polar end of the fatty acid chain.

A second study of molecular interactions in the mitochondrial inner membrane involved the use of a spin-labelled carbodi-imide, *N*-(2,2,6,6-tetramethylpiperidyl-1-oxyl)-*N'*(cyclohexyl)carbodi-imide.[378] The di-imide formed a strong complex

[374] M. Basset, G. Defaye, and E. M. Chambaz, *F.E.B.S. Letters*, 1975, **60**, 364.
[375] J. D. Morrisett, H. J. Pownall, and A. M. Gotto, *J. Biol. Chem.*, 1975, **250**, 2487.
[376] A. N. Kuznetsov, B. Ebert, G. Lassmann, and A. B. Shapiro, *Biochim. Biophys. Acta*, 1975, **379**, 139.
[377] P. F. Devaux, A. Bienvenue, G. Lauquin, A. D. Brisson, P. M. Vignais, and P. V. Vignais, *Biochemistry*, 1975, **14**, 1272.
[378] C. Montecucco and A. Azzi, *J. Biol. Chem.*, 1975, **250**, 5020.

with the inner membrane which could not be dissociated by solvent extraction (*e.g.* by ether or chloroform). Specific removal of F_1ATPase or F_1 inhibitor from the membrane caused no perturbation of the e.s.r. spectrum. Loss of a protein responsible for conferring oligomycin sensitivity on the ATPase did, however, alter the peak separation of nitroxide signals. An association between the carbodi-imide site and the oligomycin protein was inferred.

A specific interaction between 8-doxylpalmitoylcholine and the cholinergic receptor protein of electric tissue has been demonstrated.[379] The spin label was a substrate for acetylcholinesterase and reversibly blocked carbamylcholine induced depolarization of *Electrophorus* electroplaque (in the presence of an acetylcholinesterase inhibitor). Both [^{3}H]-acetylcholine and α[^{3}H]-toxin from *Naja nigricollis* were used in the assessment of the specificity of spin-label binding to the receptor protein of *Torpedo marmorata*. 8-Doxylpalmitoylcholine reduced the extent of acetylcholine binding to the membrane fragments, and a dissociation constant for the nitroxide–receptor complex of 150 nmol l^{-1} was derived (*cf.* K_d of 8 nmol l^{-1} for the acetylcholine–receptor complex). The spin label also reduced the initial *rate* of α-toxin binding to the receptor. The doxylcholine group appeared to be completely immobilized on interaction with the membranes and was only mobilized by addition of tight-binding cholinergic ligands such as carbamylcholine, decamethonium, or α-toxin. The spectra taken in the presence of ligands were not, however, those of a completely mobile label in an aqueous environment. They were characteristic of a lipid spin label in a fluid membrane and similar to e.s.r. spectra of 8-doxylpalmitic acid incorporated in *Torpedo* membrane fragments. The situation is analogous to that in spin-label studies of the mitochondrial adenine nucleotide carrier.[377]

Use of a spin-labelled cytoplasmic pentapeptide in the initial stage of peptidoglycan biosynthesis has been described by Johnston and Neuhaus.[380] The UDP-activated pentapeptide, UDP-*N*-acetylmuramyl-Ala-D-Glu-Lys-D-Ala-D-Ala, was spin-labelled at the ε-amino-group of its lysine residue. Complex formation between the spin-labelled pentapeptide and antibiotics vancomycin or ristocetin was apparent from isotropic broadening of the spin-label spectrum on addition of antibiotic. The broadening effect was reversed by addition of excess unlabelled pentapeptide to the solution. The failure of antibiotics such as bacitracin and moenomycin to interact with the spin label indicated a possible assay for exposure of the D-Ala-D-Ala section of the molecule during biosynthesis. The pentapeptide was covalently linked to undecaprenyl phosphate bound to the *Staphylococcus aureus* membrane by the pentapeptide translocase. Spin-label signals were broadened as the pentapeptide was transferred to the membrane, but this broadening could be reversed by addition of UMP which shifts the translocase-mediated equilibrium in favour of free pentapeptide. The membrane-associated undecaprenyl-diphosphate-*N*-acetylmuramylpentapeptide was still able to bind to vancomycin and ristocetin, though the strength of the interaction was reduced. The D-Ala-D-Ala terminus of the membrane peptide is not therefore located in the lipid bilayers or embedded in the peptidoglycan-synthesizing complex.

[379] A. D. Brisson, C. J. Scandella, A. Bienvenue, P. F. Devaux, J. B. Cohen, and J.-P. Changeux, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1087.

[380] L. S. Johnston and F. C. Neuhaus, *Biochemistry*, 1975, **14**, 2754.

Membrane Studies.—*Phase Separation.* Wu and McConnell [381] have extended observations of lateral phase separations in binary lipid mixtures.[382, 383] They employed a combination of the temperature dependence of e.s.r. observations using a phospholipid spin label and the Tempo partitioning technique [382, 383] for mixtures of lipids over a range of composition. In addition to previously reported forms of the phase diagram for which fluid and solid states of the lipid bilayer can coexist at equilibrium, the authors recorded observations on the immiscibility of *entirely fluid* lipid mixtures at elevated temperatures: dielaidoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine were immiscible at temperatures above 50 °C for mixtures with mole fractions of 0.1—0.5 in the ethanolamine. It is therefore possible for a completely fluid membrane to separate out laterally *or* across the plane of the bilayer. A likely consequence of such asymmetry across the plane of the bilayer would be curvature of the membrane.

Ito and co-workers [384] have extended previous examination of lateral phase separation [385, 386] by studies with a newly synthesized phosphatidylserine spin label containing a 12-doxylstearate chain at position 2. Solidification of phosphatidylserine membranes as a function of solution pH was observed from e.s.r. spectra of incorporated phosphatidylserine spin label. The proportions of fluid and solid phases of the membrane were estimated by linear combination of the spectra of totally solid and totally fluid states (at a given temperature). The membranes were found to be 50% solid phase at pH 3.5. Effects of bivalent metal ion co-ordination to seryl head-groups were studied similarly. Ca^{2+}, of the alkaline earth cations, was the most effective mediator of membrane solidification at concentrations of 10—50 mmol l^{-1}. Calcium-induced phase separation in phosphatidylserine–phosphatidylcholine mixtures was also examined. Even at relatively high mole fractions of seryl phospholipid, a significant proportion of the serine lipid remained dissolved in the fluid phosphatidylcholine region of the membrane. The relatively slow approach to phase equilibrium after addition of Ca^{2+} ($\sim$h) and the dependence of equilibration rate on lipid composition were stressed.

The spin-label technique and the concepts of lateral phase separation have been applied to study of anaesthetic interaction with membranes.[394] The inhalation anaesthetic 2,2-dichloro-1,1-difluoroethyl methyl ether reduced the phase transition temperature of dimyristoylphosphatidylcholine or dipalmitoylphosphatidylcholine vesicles by up to 5 °C. (The anaesthetic was dissolved in the membrane at concentrations appropriate to general anaesthesia and phase transitions were assessed by the Tempo partition method.) The phase transition temperatures of mixed bilayers of dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine were also altered by dissolution of anaesthetic in the membrane. Both phase transition and phase separation effects could be reversed by application of helium pressures of the order of 100 atm. Possible modifications of membrane protein function by anaesthetic-induced alterations in lipid–protein association are suggested.

[381] S. H. Wu and H. M. McConnell, *Biochemistry*, 1975, **14**, 847.
[382] E. J. Shimschick and H. M. McConnell, *Biochemistry*, 1973, **12**, 2351.
[383] W. Kleeman, C. W. M. Grant, and H. M. McConnell, *J. Supramol. Structure*, 1974, **2**, 609.
[384] T. Ito, S. Ohnishi, M. Ishinaga, and M. Kito, *Biochemistry*, 1975, **14**, 3064.
[385] S. Ohnishi and T. Ito, *Biochemistry*, 1974, **13**, 881.
[386] T. Ito and S. Ohnishi, *Biochim. Biophys. Acta*, 1974, **352**, 29.

Functional Correlations with Lipid Fluidity. Several techniques have been simultaneously applied to the observation of 'fluidity' transitions in natural membranes. Enzyme activities, pyrene excimer fluorescence, and e.s.r. signals from doxyl fatty acids were measured as a function of temperature for *E. coli* membranes enriched in elaidic acid.[387] Arrhenius plots of membrane NADH oxidase activity had slope discontinuities at 27 and 32 °C, whereas D-lactate oxidase activity showed discontinuities at 31 and 36 °C. The results of fluorescence and e.s.r. studies were similar: changes in the properties of the probes occurred over the range 26–35 °C. It was concluded that the two enzymes sampled different parts of the 'phase transition' and that they were perhaps associated with distinct regions of membrane lipid of differing composition and fluidity. Spin-labelled fatty acids also detected changes in membrane motion in the range 9—21 °C. Since spectroscopy of lipid extracts of the bacteria indicated bilayer transitions in the ranges 4—9, 14—20, and 25—36 °C, it seemed likely that the methods employed for the membranes recorded the properties of the lipid environment itself, rather than protein-induced perturbations in those properties.

Wunderlich *et al.*[388] have studied thermotropic effects in membranes of *Tetrahymena pyriformis.* They have added the techniques of freeze-etch electron microscopy, calorimetry, and ^1H n.m.r. to those mentioned above. Freeze-etch microscopy indicated emergence of large areas of smooth membrane at temperatures below *ca.* 17 °C. Microsomes prepared from *Tetrahymena* cells also experienced a form of structural transition around 17 °C. There were discontinuities at this temperature in parameter *versus* reciprocal temperature plots for glucose-6-phosphatase activity, 8-anilino-1-naphthalenesulphonate fluorescence, partitioning of 4-doxyldecane, and $2T_{\|}$ for incorporated 5-doxylstearate. ^1H N.m.r. and calorimetry provided no evidence for a gel $\rightleftharpoons$ liquid crystalline phase transition at 17 °C. Since 12-doxylstearate e.s.r. spectra indicated the presence of at least two lipid regions of differing fluidity, the transition associated with the temperature of 17 °C was interpreted in terms of *coalescence* of protein-excluding liquid crystalline regions of lipid.

Several communications, particularly from McConnell's laboratory, have examined the ramifications of lipid fluidity for membrane mediated processes. Humphries and McConnell[389] have investigated the relationship between antigen environment and complement fixing activity for liposomal systems. Cardiolipin was incorporated as univalent antigen (3 mole %) into liposomes containing cholesterol and dimyristoyl- or dipalmitoyl-phosphatidylcholine. Liposomes were treated with human syphilitic serum containing cardiolipin-specific antibodies and complement attack on these liposomes assessed as residual haemolytic activity against sheep erythrocytes. Complement fixation by liposomes increased markedly when the mole fraction of cholesterol exceeded 0.35. Phase diagrams for phospholipid–cholesterol–cardiolipin mixtures were determined by the Tempo partition method.[381–383] At the high cholesterol concentrations which promote efficient complement fixation, the univalent cardiolipin antigen is

[387] J. D. Morrisett, H. J. Pownall, R. T. Plumlee, L. C. Smith, Z. E. Zehner, M. Esfahani, and S. J. Wakil, *J. Biol. Chem.*, 1975, **250**, 6969.
[388] F. Wunderlich, A. Ronai, V. Speth, J. Seelig, and A. Blume, *Biochemistry*, 1975, **14**, 3730.
[389] G. M. K. Humphries and H. M. McConnell, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2483.

incorporated into a solid phase of the membrane, or else forms a phase which is rich in cholesterol–cardiolipin. Complement attack is thus enhanced by the limited lateral or segmental mobility of the antigen in a 'solid' membrane.

Observations of alterations in erythrocyte rheology as a result of interaction with physiological concentrations of prostaglandins and adrenaline [390, 391] have been extended by e.s.r. studies of lipid spin labels incorporated into red cells and red cell ghosts.[392] Very small changes of order 2% in the spin-label order parameter were induced by addition of prostaglandin E to membranes. Similar changes were produced by charging cells or membranes with cAMP or cGMP up to 10 μmol l^{-1} concentration. Cellular cyclic nucleotide effects were blocked by an isothiocyanate inhibitor of the anion channel. It was concluded that the small changes in mobility of the lipid spin label may be mediated by alterations in membrane-associated proteins and in particular by alteration of spectrin–membrane interaction at the cytoplasmic surface caused by phosphorylation of this protein.

Maeda *et al.*[393] have recorded red cell membrane–virus interactions using a phosphatidylcholine spin label. The viral envelope of haemagglutinating virus of Japan was loaded with spin label to an extent at which exchange-broadening of e.s.r. signals occurred. The mixing of viral lipid with that of the erythrocyte membrane was observed as a gradual reduction in nitroxide signal linewidth on incubation with cells: the final e.s.r. spectrum was that of phosphatidylcholine spin label added to red cell membranes. There was a strong temperature dependence of the processes of virus–cell interaction, erythrocyte fusion, and haemolysis. Lipid transfer was inhibited by cross-linking of erythrocytes, trypsin treatment of the virus, or the presence of concanavalin A. Viral catalysis of the transfer of spin-labelled phospholipids between erythrocytes was also observed.[394]

A further process of membrane fusion which has received attention using the spin-labelling technique is coalescence of dipalmitoylphosphatidylcholine vesicles induced by concanavalin A.[395] Substantial differences between the e.s.r. spectra of fatty acid spin-label incorporated into sonicated and unsonicated liposomes were recorded. Addition of concanavalin A to sonicated vesicles resulted in substantial time-dependent perturbations of spin-label spectra. The equilibrium situation was consistent with co-ordination of the lectin to *fused* lipid vesicles, and this view was confirmed by freeze-fracture electron microscopy. The fusogenic effect of concanavalin A was maximal in the range of the lipid phase transition temperature. A lower limit of 2000 was placed on the enhancement of vesicle fusion rate by protein.

Brûlet and McConnell [396] have reported a new form of relaxation theory appropriate to the two-dimensional diffusion occurring in lipid bilayers and to relaxation of nuclear spins by nitroxyl radicals. A diffusion rate constant (D) of

[390] J. E. Allen and H. Rasmussen, *Science*, 1971, **174**, 512.
[391] P. G. Kury, P. W. Ramwell, and H. M. McConnell, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 178.
[392] P. G. Kury and H. M. McConnell, *Biochemistry*, 1975, **14**, 2798.
[393] T. Maeda, A. Asano, K. Ohki, Y. Okada, and S. Ohnishi, *Biochemistry*, 1975, **14**, 3736.
[394] J. R. Trudell, D. G. Payan, J. H. Chin, and E. N. Cohen, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 210.
[395] J. van der Bosch and H. M. McConnell, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4409.
[396] P. Brûlet and H. M. McConnell, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1451.

4×10^{-8} cm^2 s^{-1} was calculated from the enhancement of ^{13}C relaxation of choline methyl groups by a spin-labelled choline lipid in *unsonicated* vesicles. The possibility of significantly higher rates of lateral diffusion in *sonicated* vesicles is suggested. Godici and Landsberger [397] performed experiments on ^{13}C relaxation in lecithin–cholesterol mixtures in the presence of nitroxyl radicals, but did not apply the Brûlet–McConnell theory to the interpretation of their data.

New Spin Labels.—Studies of photosynthesis by the nitroxyl-labelling technique are hindered by loss of e.s.r. signals on illumination of paramagnetically labelled chloroplasts. Sun and Calvin [398] found that a nitroxyl biradical, *NN*-bis-(1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxyl)-1,2-diaminoethane), could be stabilized in the presence of illuminated chloroplasts by addition of ferredoxin and NADP to the system at pH > 7. Time-dependent changes in the five-line e.s.r. spectrum on illumination implied an attachment of the biradical to a soluble macromolecule which was promoted by light energy.

The design of spin-label probes which localize on the outer surface of the plasma membrane has been discussed by Lepock *et al.*[399] They described the use of four probes and assessed their permeation into *Saccharomyces cerevisiae* by paramagnetic broadening of e.s.r. signals by *impermeant* Ni^{2+} and ferricyanide ions.

5 Infrared Spectroscopy

Contributed by R. M. Stephens

Amino-acids, Peptides, and Model Compounds.—Infrared spectroscopy of small peptides is used extensively to study the vibrational frequencies associated with the peptide group and relate these frequencies to peptide structure. The spectra from 10 pairs of L-α- and L-γ-glutamyl dipeptides and several related compounds have been measured using KBr disc techniques. Carbonyl stretching vibrations of α-glutamyl peptide bonds appeared at a higher wavenumber (1685—1665 cm^{-1}) than those of γ-glutamyl peptide bonds (1600—1675 cm^{-1}). The N—H stretching vibration of the α-glutamyl bond was observed at a lower wavenumber than that of the corresponding γ-glutamyl bond.[400] The conformation of dipeptides containing proline and 4-hydroxyproline has been examined and results obtained from i.r. and c.d. spectroscopy compared.[401] Spectral analysis has also been made on glycine hydrochloride, L-alanine, and derivatives of them containing ^{14}C.[402] ^{1}H N.m.r. and i.r. spectroscopy have been used to study the conformations of linear tetrapeptides containing proline in MeOH and $CHCl_3$ solutions. The results revealed that internal hydrogen-bonded structures were present indicative of β-structures.[403] Similar folded structures were found in cyclopeptide systems containing L-valine, L-leucine, L-norvaline, and glycine.[404] The structure

[397] P. E. Godici and F. R. Landsberger, *Biochemistry*, 1975, **14**, 3927.
[398] A. S. Sun and M. Calvin, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3107.
[399] J. R. Lepock, P. D. Morse, R. J. Mehlhorn, R. H. Hammerstedt, W. Snipes, and A. D. Keith, *F.E.B.S. Letters*, 1975, **60**, 185.
[400] T. Kasai and S. Sokamura, *Nippon Nogei Kagaku Kaishi*, 1974, **48**, 521.
[401] V. Justova and K. Blaha, *Coll. Czech. Chem. Comm.*, 1975, **40**, 662.
[402] L. Zilka, *Radioisotopy*, 1975, **16**, 215.
[403] K. D. Kopple, A. Go, and D. R. Pilipauskas, *J. Amer. Chem. Soc.*, 1975, **97**, 6831.
[404] V. T. Ivanov, I. A. Lavrinovich, C. A. Kogan, E. A. Meschcheryakova, S. L. Portnova, M. D. Isabaev, P. V. Kostetskii, and Yu. A. Ovchinnikov, *Khim. prirod Soedinenii*, 1975, **11**, 202.

and related properties of valinomycin cyclodepsipeptides have been examined using i.r. spectroscopy. The results have shown that although analogues of valinomycin had similar conformations they had different stabilities. Successive substitution of alanine for valine in certain complexes increased the interaction between solvent and the complex.[405] The far-i.r. spectra of five amino-acids and seven polyamino-acids have been shown to be extremely temperature dependent with the appearance of narrow absorption lines at 4.2 K. The lines broaden and eventually disappear as the temperature increases. The results were explained in terms of the crystal order of the samples.[406] Low temperatures have been used to isolate acetylglycine methylamide and acetyl-L-alanine methylamide in an argon matrix. Their i.r. spectra were fully assigned by comparison with those of isotopic homologues and model compounds. Two intramolecular hydrogen-bonded rings were shown to coexist. No semi-extended forms without intramolecular hydrogen-bonds were present in the matrix. Qualitative agreement between conformational states of dipeptides isolated in the argon matrix and results of certain conformational energy calculations were noted.[407] The i.r. spectra from 400—30 cm^{-1} of L- and DL-alanine and their *N*-deuteriated analogues have been recorded at temperatures ranging from 295 to 100 K. Highly dependent temperature bands were observed at 972 cm^{-1} for poly-L-alanine and at 975 cm for DL-alanine. These bands were assigned to the first overtone of the NH_3^+ torsional mode. The temperature dependence of the half-widths of these bands indicated that above 200 K the vibrational modes are considerably modulated by some motion, possibly by the breaking of hydrogen-bonds.[408] Spectra at room and liquid nitrogen temperature have been obtained from α-, β-, and γ-glycine and the corresponding *C*-dideuterio-, *N*-trideuterio-, and pentadeuterio-glycines. The bands in the spectra of the three crystal forms were correlated and an equation obtained which related the frequency shifts to the force constant matrix.[409] The interpretation of the i.r. spectrum of α-amino-isobutyric acid anhydride and its *NN'*-dideuteriated derivatives was aided by a normal co-ordinate analysis assuming C_{2h} symmetry and by treating the methyl groups as point masses. A simple Urey–Bradley force field was used and the normal frequencies calculated using force constants obtained from glycine anhydride. Assignments of fundamental frequencies were made by referring to isotopic frequency shifts, calculated frequencies, and potential energy distributions.[410] Many spectroscopic techniques, including i.r., have been used to study the internal rotation potentials about the nitrogen–α-carbon and α-carbon–carbonyl areas of the peptide backbone in the following models: MeCONHEt, EtCONHMe, $ClCH_2CONHMe$, $MeCONHCH_2CONHMe$, and MeCONHCHMeCONHMe. It was found that the $N—C_\alpha$ axis had a potential energy minimum near the *gauche* position. The $C_\alpha—CO$ axis had two potential energy minima, one *cis* and the other *trans*; the energy difference between the minima was small and dependent on intermolecular

[405] V. T. Ivanov, A. A. Sanasaryan, I. I. Chervin, G. I. Yakovlev, L. A. Fonina, L. B. Senyavina, S. V. Sychev, E. I. Vinogradova, and Yu A. Ovchinnikov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 2310.

[406] W. J. Shotts and A. J. Seviers, *Biopolymers*, 1974, **13**, 3593.

[407] I. Y. Grenie, M. Avignon, and C. Garrigou-Lagrange, *J. Mol. Structure*, 1975, **24**, 293.

[408] N. Kaneko, H. Takahazhi, and K. Higasi, *Bull. Chem. Soc. Japan*, 1975, **48**, 1961.

[409] J. Herranz and J. M. Delgado, *Spectrochim. Acta (A)*, 1975, **31**, 9, 1245.

[410] M. Asai, K. Noda, and A. Sado, *Sci. Rep. Kanazawa Univ. Japan*, 1974, **19**, 33.

interactions.[411] Force constants and i.r. absorption frequencies for *N*-methyl diacetamide isomers have been calculated and compared with experimental results.[412] The i.r. and Raman spectra of *NN*-dimethylselenoformamide and two deuteriated analogues were observed between 200 and 4000 cm^{-1}. Band assignments were made by comparison with frequencies calculated by a normal coordinate analysis using a 35-parameter generalized valence force field.[413]

Polypeptides.—Poly(γ-benzyl-glutamate) with strict alternation of L and D residues was shown to exist in two different helical conformations (αD-L and πD-L) specific to poly-D-L-peptides. *X*-Ray and electron diffraction results were given as well as those obtained from i.r. spectroscopy. The polymer was also capable of forming unusual pleated sheet structures, and the various conformations which this polymer could adopt were discussed from the point of view of the sequence–structure relationship.[414] Poly-L-valine samples with various degrees of polymerization were prepared from the *N*-carboxyanhydride of L-valine. The high molecular weight polymer was fibrous and capable of forming an oriented film when cast from trifluoroacetic acid solution. The i.r. and Raman spectra obtained from the high molecular weight polymer indicated that it might contain some α-helical conformation. The low molecular weight polymers had a mainly β-conformation.[415] Water-soluble polypeptides of L-valyl and L-isoleucyl residues flanked with DL-lysyl blocks have been investigated in water as well as in aqueous methanol solutions. The i.r. and c.d. spectral parameters observed were quite different from those of synthetic homopolypeptides in the β-conformation. The differences could be explained by the presence of a single isolated extended chain without either intra-, or inter-chain association.[416] The far-i.r. spectra of poly-L-proline I and II, between 190 and 35 cm^{-1}, were obtained at 300 and 110 K. They revealed a very intense band at 60 cm^{-1} in the spectrum obtained from poly-L-proline II which was absent in the spectrum from poly-L-proline I. This indicates the sensitivity of low-frequency vibrations to the differences in conformations assumed by both forms in the solid state. Additional bands observed in this region were correlated with the results obtained from normal mode calculations.[417]

Conformational studies of sequential polypeptides of L-leucine and glycine in solution were compared to the conformation of poly-L-leucine. The results indicated that $(Leu)_n$, $(Leu_3\text{-}Gly)_n$, $(Leu_2\text{-}Gly)_n$, and $(Leu\text{-}Gly)_n$ were α-helical in solution, but $(Leu\text{-}Gly_2)_n$ was not. The order of the stabilities of the α-helix of these polymers was $(Leu)_n > (Leu_2\text{-}Gly)_n \sim (Leu_2\text{-}Gly)_n > (Leu\text{-}Gly)_n > (Leu\text{-}Gly_2)_n$. This was attributed to interactions between side-chains of L-leucine residues, which were regularly arranged.[418] Peptides containing arginine, such as $(Ala\text{-}Ala\text{-}Arg)_n$ and $(Arg\text{-}Arg\text{-}Ala)_n$, were shown to possess a globular-like structure in water and a helical conformation in organic solvents.[419]

[411] Y. Koyama and T. Shimanouchi, in 'Proceedings of the 1974 Rehovot Symposium on Peptides, Polypeptides and Proteins', p. 396.
[412] S. A. Stepanyan, A. M. Naumova, and L. A. Gribov, *Izvest. Timiryazevsk Akad.*, 1975, 198.
[413] U. Anthou, L. Henriksen, P. H. Nielsen, G. Borch, and P. Klaboe, *Spectrochim. Acta* (*A*), 1974, **30A**, 1351.
[414] F. Heitz, B. Lotz, and G. Spach, *J. Mol. Biol.*, 1975, **92**, 1.
[415] S. Yamashita and T. Yamashita, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 941.
[416] S. Kubota and G. Fasman, *Biopolymers*, 1975, **14**, 605.
[417] J. F. Rabolt, W. Wedding, and K. W. Johnson, *Biopolymers*, 1975, **14**, 1615.
[418] I. Takayoshi and S. Takahashi, *Bull. Chem. Soc. Japan*, 1975, **48**, 1240.
[419] V. K. Burichenko, G. F. Kasymova, P. M. Marynov, and M. V. Vsabaev, *Khim. prirod Soedinenii*, 1975, **11**, 232.

The nature of co-ordinate bondings between Cu^{II} and Ni^{II} polyglycine chelates formed over a wide range of pH values has been established in aqueous (D_2O) solutions. Band assignments provide the first microscopic evidence for metal–polyglycine co-ordination sites in acid solutions. Shifts in peptide carbonyl and terminal carboxylate frequencies are indications of the conformational changes that occurred in both Cu^{II} and Ni^{II} chelates.[420]

Proteins.—The i.r. spectra of both light and heavy meromyosin have been shown to be similar to that obtained from myosin.[421] As with myosin the precipitation conditions determine the observed conformations. The conformation of the films prepared at pH 7.0 appears to be a mixture of α-helix and random coil. However, β-structures are observed in addition to the α-helical secondary structure if the films are precipitated at low pH or with 1 : 1 mixtures of chloroform and methanol. The occurrence of β-structures in both light and heavy meromyosin suggest that the β-structure occurs in the rod portion of the myosin molecule.[421] Using attenuated total reflection techniques the integrated intensities of the amide A and I bands have been calculated for the α-helical portions of tropomyosin.[422] These were found to be 1.94 and 1.83 × 10^{-4} l (mol residue)$^{-1}$ cm^{-2}. The i.r. spectra of films of partly denatured collagen cast from 0.05M acetic acid at temperatures of 4, 23, and 60 °C have been compared with the spectrum obtained from native collagen cast at 23 °C.[423] Native collagen gave rise to an absorption peak at 354 cm^{-1} which originated from the triple helical structure. The band was absent in the spectrum of the partly denatured film cast at 60 °C. An analogy between the various spectra allowed an estimate to be made of the percentage helical material present. Complexes of zinc triethylenediamine and keratin have been compared with isolated keratin. Shifts in the characteristic keratin absorption bands showed that co-ordination occurred between the nitrogen of the NH group and Zn^{2+} ions.[424]

Raman studies of the crystalline, solution and alkaline-denatured states of β-lactoglobulin have been carried out.[425] Spectra obtained from freeze-dried and crystalline β-lactoglobulin were almost indentical. The conformationally sensitive amide III band at 1242 cm^{-1} increased in intensity by 30% when the protein was in aqueous solution, and this was interpreted as a conformational change in the disordered chains of the protein. When the pH of the solution was raised from 6 to 11 this amide band shifted from 1242 to 1246 cm^{-1}, broadened, and decreased in intensity consistent with the conversion of extended sheet regions of β-lactoglobulin into the disordered form. At pH 13.5 the band shifted to 1257 cm^{-1}, characteristic of the completely disordered protein. Changes in the intensity of the tyrosine ring vibrations were also noted, and by comparing the position and intensity of these ring vibrations with those obtained from the spectrum of 95% glutamic acid : 5% tyrosine at pH 7.0, it was suggested that the average tyrosine in the denatured state of β-lactoglobulin may be in a more hydrophobic environ-

[420] A. E. Martell and M. K. Kim, *J. Coordination Chem.*, 1974, **4**, 9.
[421] A. L. Jacobson, V. Vejdovsky, and P. J. Krueger, *Internat. J. Biochem.*, 1974, **5**, 547.
[422] D. S. Bulatov, *Biofizika*, 1974, **19**, 797.
[423] C. Huang, R. C. Lord, and I. V. Yannas, *Macromolecules*, 1974, **7**, 954.
[424] F. K. Habib, J. H. Keighley, P. Rhodes, and C. S. Whewell, *Spectrochim. Acta* (*A*), 1975, **31A**, 1.
[425] B. G. Frushow and J. L. Koenig, *Biopolymers*, 1975, **14**, 649.

ment compared with its position in the native state.[425] I.r. spectra of myoglobulin, ribonuclease, lysozyme, α-chymotrypsin, α-lactalbumin, and β-lactoglobulin A have been obtained in D_2O solutions.[426] The spectra were resolved into Gaussian components by means of an iterative computer program, and resolved characteristic absorption peaks for the two i.r.-active amide I′-components of anti-parallel pleated sheets were obtained. Absorptivity values for the resolved β-structure peak at *ca.* 1630 cm^{-1} were established on the basis of the known structure of ribonuclease, lysozyme, and chymotrypsin. The β-structure content of β-lactoglobulin was established as *ca.* 48%, of α-lactalbumin as 18% and of α-casein as zero.[426] Water absorption in α- and β-micellar caseins takes place readily and results indicate that less than one water molecule per polar group is needed to cause changes in the i.r. spectra of α- and β-casein.[427]

The secondary structures of certain isolated proteins and membrane proteins have been determined using computer curve analysis of i.r. and c.d. spectra.[428] The percentages of α- and β-structures in insulin, serum albumin, ribonuclease, lysozyme, metmyoglobin, and synaptosomal membranes were obtained. Accurate spectra of several aqueous proteins have been obtained by subtraction of the solvent spectra from that of the solution with the aid of a minicomputer interfaced to the spectrometer. By using thin sampling regions in combination with signal-to-noise enhancing techniques, the amide I could be distinguished from the overlying water band. Examples were given for the solution spectra of 0.36% solution of 72% clottable bovine fibrinogen in 0.1M phosphate buffer.[429] The values of frequencies, intensities, and other spectral parameters of the main absorption bands of amino-acid side-chain groups have been obtained in the 1500—1800 cm^{-1} region for protein solutions in heavy water over a range of pD's varying from 1 to 12.[430] The results indicate that absorption from residues of asparagine, glutamine, aspartic acid, glutamic acid, and tyrosine must be taken into account when quantitative analysis has been made using the amide bands from the spectra of proteins in solution. The analysis was applied to ribonuclease A.[430]

6 Mössbauer Spectroscopy

Contributed by D. P. E. Dickson

The main source of literature information for this report is the half-yearly compilation 'The Index of Publications in Mössbauer Spectroscopy of Biological Materials', by Dr. Leopold May, Department of Chemistry, The Catholic University of America, Washington, D.C. 20017, U.S.A. Dr. May kindly made available an advance copy of the latest additions to the Index.

^{57}Fe Mössbauer spectroscopy continues to provide valuable data on the state of iron in various biological systems. In 1975 work has been reported on haem proteins, iron–sulphur proteins, and iron transport and storage proteins. In addition to this work on isolated biological molecules there have been a number of

[426] M. Ruegg, V. Metzzer, and H. Susi, *Biopolymers*, 1975, **14**, 1465.
[427] M. Ruegg and H. Haeni, *Biochim. Biophys. Acta*, 1975, **400**, 17.
[428] R. Grosse, J. Malur, and K. R. H. Rerke, Symposium paper, 4th International Biophysics Congress, 1974, Part 1, p. 91.
[429] J. S. Mattson, C. A. Smith, and K. E. Paulsen, *Analyt. Chem.*, 1975, **47**, 376.
[430] Yu. N. Chirgadze, O. V. Fedorov, and N. P. Trushina, *Biopolymers*, 1975, **14**, 679.

investigations of more complex systems. A study of certain thyroid hormones using ^{127}I Mössbauer spectroscopy has also been reported.

Haem Proteins.—A review of the considerable previous work in this area has been presented by Münck and Champion.[431] The 1975 work on haem proteins includes both experimental and theoretical investigations. There have also been a number of studies of model compounds containing the planar haem group which it is hoped will elucidate the structure of the more complex haem proteins.

Papaefthymiou *et al.*[432] and Chow and Mukerji[433] have reported Mössbauer spectra obtained from samples of both the α- and β-subunits of anhydrous deoxyhaemoglobin and of the complete protein at temperatures between 77 and 200 K. The Mössbauer spectra of both subunits and of the whole protein consist of a superposition of a ferrous high-spin quadrupole-split doublet and a ferrous low-spin quadrupole-split doublet, of approximately equal intensity. No significant differences could be detected between the chemical shifts or the quadrupole splittings of the three proteins. Consequently it was concluded that the model for anhydrous deoxyhaemoglobin in which the ferrous ions of the α- and β-chains are in different spin states is incorrect. Papaefthymiou *et al.*[432] analysed the temperature dependence of the quadrupole splitting using a ligand field approximation which yielded the electronic structure of the low-lying levels of the ferrous ion in the two spin sites. The principal component of the electric field gradient tensor was calculated to be normal to the haem plane at both sites. These authors have considered a possible interpretation of the origin of the spin-state mixture based on the disruption of the hydrophobic interaction upon dehydration.

Champion *et al.*[434, 435] have used Mössbauer spectra obtained in large applied magnetic fields to investigate some high-spin ferrous haem proteins. Their measurements on reduced samples of cytochrome P450 from *Pseudomonas putida*, chloroperoxidase from *Caldariomyces fumago*, horseradish peroxidase and deoxyhaemoglobin were obtained at temperatures down to 4.2 K and in applied magnetic fields up to 4.5 T. The applied magnetic field spectra show complex electric and magnetic hyperfine interactions of the high-spin ferrous haem iron which have been successfully parameterized in terms of a spin-Hamiltonian formalism. In the cytochrome the results imply a very low (triclinic) symmetry for the haem iron and the effects of the ligand environment and the spin–orbit coupling result in a large zero-field splitting of the electronic ground state. The temperature dependence of the quadrupole splitting was observed to be very small for the cytochrome and chloroperoxidase but much larger for the horseradish peroxidase and deoxyhaemoglobin. The spectral similarities that have been previously noted for chloroperoxidase and cytochrome P450 were further explored and substantiated in these high applied magnetic field measurements. In particular the parameters of the spin-Hamiltonian which were obtained by

[431] E. Münck and P. M. Champion, *Ann. New York Acad. Sci.*, 1975, **244**, 142.
[432] G. C. Papaefthymiou, B. H. Huynh, C. S. Yen, J. L. Groves, and C. S. Wu, *J. Chem. Phys.*, 1975, **62**, 2995.
[433] Y. W. Chow and A. Mukerji, *Biochem. Biophys. Res. Comm.*, 1975, **62**, 989.
[434] P. M. Champion, J. D. Lipscomb, E. Münck, P. Debrunner, and I. C. Gunsalus, *Biochemistry*, 1975, **14**, 4151.
[435] P. M. Champion, R. Chiang, E. Münck, P. Debrunner, and L. P. Hager, *Biochemistry*, 1975, **14**, 4159.

computer fitting of a calculated spectrum to the experimental data were found to be closely similar for these two proteins. It was concluded that in the reduced state the iron in chloroperoxidase has a very similar ligand environment to that in cytochrome P450. However, horseradish peroxidase and deoxyhaemoglobin show high-field spectra that differ considerably from each other and from those of the cytochrome P450 type, indicating a different ligand arrangement of the haem iron in each case. This work emphasizes how the application of a large magnetic field can appreciably increase the amount of information that can be obtained from Mössbauer spectroscopy and thus enhance the usefulness of the technique.

Ono *et al.*[436] have investigated cytochrome c_3 from *Desulphovibrio vulgaris* in its oxidized state. This electron transport protein contains four haem groups. The Mössbauer spectra obtained at 4.2 K in the absence of an applied magnetic field do not show any magnetic hyperfine structure even though the spectra obtained at higher temperatures indicate that the haem iron is in a low-spin ferric form. The lack of the expected magnetic hyperfine structure was interpreted as resulting from a spin–spin interaction between the iron atoms in the different haem groups. It was suggested that this could arise from a direct dipolar interaction, although this would require a distance between haem groups of only 1 nm, half of what would be expected for a cubic arrangement. As an alternative, the authors put forward the possibility of a superexchange mechanism between the ferric ions. The spectra obtained in applied magnetic fields show magnetic hyperfine structure and were computer fitted to a calculated spectrum, a procedure that yielded *g*-values in reasonable agreement with those obtained from e.p.r. measurements.

Chow and Mukerji[437] have studied the binding of oxygen to haemoglobin using Mössbauer spectroscopy as a means of monitoring the fractional saturation of oxygen on haemoglobin and its subunits.

The biological significance of the haem proteins results from their ability to change their ligand structure to take up either electrons or various atoms. These changes occur close to the haem iron and therefore ^{57}Fe Mössbauer spectroscopy constitutes a powerful means of investigating these important processes. However, the complexity of the proteins makes it useful to undertake, in parallel with work on the proteins themselves, studies of related organic molecules containing the haem group, which can be considered as model or analogue compounds of the proteins.

Spartalian *et al.*[438] have reported Mössbauer spectra obtained from a model complex for haemoglobin. The complex of *meso*-tetra-(αααα-*o*-pivalamidophenyl)porphyrin can bind oxygen reversibly as the sixth iron ligand. The Mössbauer spectra of the complex in its oxidized form, taken over a range of temperatures, yield chemical shifts and quadrupole splittings that are closely similar to those of oxyhaemoglobin, indicating a similar electronic state and ligand environment for the iron in the two molecules. *X*-Ray studies of this complex show a statistical disorder in the oxygen position which is believed to be

[436] K. Ono, K. Kimura, T. Yagi, and H. Inokuchi, *J. Chem. Phys.*, 1975, **63**, 1640.
[437] Y. W. Chow and A. Mukerji, *Bull. Amer. Phys. Soc. II*, 1975, **20**, 92.
[438] K. Spartalian, G. Lang, J. P. Collman, R. R. Gagne, and C. A. Reed, *J. Chem. Phys.*, 1975, **63**, 5375.

dynamic, and which accounts for the observed Mössbauer spectra. The authors suggested that this disorder may be related to the anomalously large temperature dependence of the quadrupole splitting in oxyhaemoglobin.

Papaefthymiou *et al.*[439] and Koch *et al.*[440] have investigated a number of ferric porphyrin thiolates by Mössbauer spectroscopy as well as by other techniques. The possible use of these compounds as analogues for cytochrome P450 was confirmed by the similarity of their Mössbauer parameters to those of the protein. In particular the authors hoped that evidence on the co-ordination in these compounds, obtained from Mössbauer spectroscopy, could be applicable to cytochrome P450. Papaefthymiou *et al.*[439] studied differences between oxygen and sulphur ligation in two of these compounds, as it has been suggested that the cytochrome may have axial sulphur ligation in one or more of its reaction states.

Loew and Kirchner[441] have performed molecular orbital calculations for oxyhaemoglobin and oxidized cytochrome P450 model compounds. Using the information from *X*-ray crystallographic analyses, these authors have obtained the electronic distributions and hence values for the Mössbauer quadrupole splittings. These calculations allow an investigation of any correlation between the nature of the iron–oxygen bond and the experimentally observed Mössbauer quadrupole splittings which are thought to be anomalous for low-spin ferrous haem complexes. The authors have concluded from their calculations that while the bond is formally $Fe^{II}—O_2$ it might be better described as $Fe^{III}—O_2^-$. The calculations confirm the sensitivity of the magnitude and sign of the principal component of the electric field gradient tensor to the oxygen geometry. Additional calculations for a model high-spin deoxygenated haem complex give good agreement with the experimental low-temperature values. It is found that in the oxyhaemoglobin model compound the main contribution to the electric field gradient comes from the iron–oxygen bonds, while in the cytochrome P450 model compound iron–oxygen and iron–sulphur bonds are important, affecting both the sign and the magnitude of the electric field gradient.

Sharma and Moutsos[442] have used the procedure previously developed for evaluating the electric field gradient tensor in various iron salts for calculating the Mössbauer quadrupole splitting for the ferric ion in haemin. Their method involves forming a multielectron molecular wavefunction in the Hartree–Fock approximation by means of a linear combination of polarized atomic orbitals. The calculated value agrees with that obtained experimentally, but is found to be particularly sensitive to the values chosen for the polarizability of the iron ligands.

Duff[443] has pointed out the problems inherent in using a linear combination of polarized atomic orbitals approach for calculating the Mössbauer chemical shifts in haem compounds.

Trautwein and Harris,[444] and Trautwein, Zimmermann, and Harris[445] have used molecular orbital theory to calculate electron densities and hence Mössbauer

[439] G. Papaefthymiou, R. B. Frankel, S. Koch, S. C. Tang, and R. H. Holm, *Bull. Amer. Phys. Soc. II*, 1975, **20**, 425.
[440] S. Koch, S. C. Tang, R. H. Holm, R. B. Frankel, and J. A. Ibers, *J. Amer. Chem. Soc.*, 1975, **97**, 916.
[441] G. H. Loew and R. F. Kirchner, *J. Amer. Chem. Soc.*, 1975, **97**, 7388.
[442] R. R. Sharma and P. Moutsos, *Phys. Rev.* (*B*), 1975, **11**, 1840.
[443] K. J. Duff, *J. Chem. Phys.*, 1975, **63**, 2259.
[444] A. Trautwein and F. E. Harris, *Theor. Chim. Acta*, 1975, **38**, 65.
[445] A. Trautwein, R. Zimmermann, and F. E. Harris, *Theor. Chim. Acta*, 1975, **37**, 89.

chemical shifts and quadrupole splittings in deoxy- and carboxy-myoglobin and haemoglobin.

Iron–Sulphur Proteins.—Anderson *et al.*[446] have investigated the two-iron ferredoxin from the alga *Synechococcus livida* by Mössbauer and magnetic resonance spectroscopies. Their results confirm the well established model for the active centre of these plant-type ferredoxins, consisting of two high-spin ferric ions in the oxidized state and a high-spin ferric ion and a high-spin ferrous ion in the reduced state. The spins are coupled to give a non-magnetic ground state for the oxidized centre and a total spin of 1/2 for the reduced centre. These authors have computer-fitted their experimental Mössbauer spectra obtained in applied magnetic fields to spectra calculated from a spin-Hamiltonian, using parameters obtained from the magnetic resonance measurements and in particular hyperfine coupling constants from electron nuclear double resonance.

Suzuki *et al.*[447] have reported Mössbauer spectra obtained from yeast aconitase, an enzyme which catalyses the interconversion of citric *cis*-aconitic acid and isocitric acid. The enzyme has a molecular weight of 68 500 daltons and is thought to contain two non-haem iron atoms per molecule. The Mössbauer spectra of the ^{57}Fe-enriched enzyme obtained at 77 and 4.2 K are similar to those of the plant-type two-iron ferredoxins and indicate the presence of two high-spin ferric atoms. There are in addition indications of a small amount of high-spin ferrous material which e.p.r. measurements suggest may be spin-coupled to the high-spin ferric ions. However, there is no evidence for any magnetic hyperfine splitting in the low-temperature Mössbauer spectra.

Mullinger *et al.*[448] have reported the first Mössbauer measurements on a four-iron ferredoxin, the bacterial ferredoxin from *Bacillus stearothermophilus*. This protein is particularly stable and a complete physicochemical characterization was carried out. All the measurements indicate that this type of ferredoxin has a similar active centre, consisting of four iron atoms and four labile sulphur atoms, to those found in the high-potential iron–sulphur proteins and the eight-iron bacterial ferredoxins, which have two such centres. At high temperatures both redox forms of *B. stearothermophilus* ferredoxin give Mössbauer spectra consisting of quadrupole-split doublets. In the reduced state two resolved doublets are observed indicating an appreciable localization of the additional reducing electron. The average Mössbauer chemical shifts indicate formal valences of two ferric and two ferrous in the oxidized state and one ferric and three ferrous in the reduced state, with varying degrees of electron delocalization over the iron atoms. At low temperatures and in applied magnetic fields the spectra of the oxidized protein show no indications of magnetic hyperfine interactions, evidence that this form is non-magnetic as a result of antiferromagnetic coupling between the iron atoms. At temperatures below 20 K the spectrum of the reduced form is a broad asymmetric pattern resulting from unresolved magnetic hyperfine interactions. This contrasts with the case of the reduced eight-iron ferredoxin from *Clostridium pasteurianum* which, even at 1.3 K, exhibits a spectrum which is

[446] R. E. Anderson, W. R. Dunham, R. H. Sands, A. J. Bearden, and H. L. Crespi, *Biochim. Biophys. Acta*, 1975, **408**, 306.
[447] T. Suzuki, Y. Maeda, H. Sakai, S. Fujimoto, and Y. Morita, *J. Biochem.*, 1975, **78**, 555.
[448] R. N. Mullinger, R. Cammack, K. K. Rao, D. O. Hall, D. P. E. Dickson, C. E. Johnson, J. D. Rush, and A. Simopoulos, *Biochem. J.*, 1975, **151**, 75.

essentially a quadrupole-split doublet, despite evidence from e.p.r. and high-field Mössbauer spectroscopy which indicates that each centre has an unpaired spin. This anomaly has been interpreted in terms of a spin-coupling between the two centres in the reduced eight-iron ferredoxin which is certainly confirmed by the lack of any such anomalous behaviour in the case of the reduced four-iron ferredoxin, which exhibits the expected magnetic hyperfine structure in its low temperature Mössbauer spectra. In large applied magnetic fields the spectra of the reduced four-iron and eight-iron ferredoxins are closely similar, as the field decouples the two centres. These spectra show evidence for both positive and negative hyperfine fields, further confirmation of the antiferromagnetic coupling between the iron atoms within each centre.

Iron Transport and Storage Proteins.—Spartalian *et al.*[449] have obtained Mössbauer spectra from the iron transport protein enterobactin (enterochelin) in the temperature range 4.2—77 K and in large applied magnetic fields. This protein is found in *E. coli* and other enteric bacteria. It has a high affinity for ferric iron and forms a stable ferric chelate, which places it among the siderochromes, which are known to contain iron in the high-spin ferric form. The Mössbauer spectra of enterobactin are characteristic of a high-spin ferric ion and were explained using a spin-Hamiltonian with rhombic symmetry including a fourth order cubic term in the electronic spin. At low temperatures the spectra are well resolved but at higher temperatures spin relaxation effects broaden the lines.

Interest in the iron transport protein transferrin has been concerned with the binding of iron to the protein. It has been suggested that bicarbonate or other low molecular weight chelators may be involved in this binding. Tsang *et al.*[450] have investigated a bicarbonate-free ferric transferrin complex by Mössbauer spectroscopy under anaerobic conditions. No evidence for specific binding was found. The spectra are different to those obtained in the presence of bicarbonate and indicate that the apparent binding is due to the formation of ferric hydroxide or ferric albumin polymers which are firmly attached to the transferrin molecule. The authors attributed the absence of a magnetic hyperfine interaction at 78 K to fast relaxation resulting from a spin–spin interaction between the ferric ions, although superparamagnetism could not be ruled out.

Spartalian *et al.*[451] have obtained Mössbauer spectra from different parts of the fungus *Phycomyces blakesleeanus* grown in an agar medium enriched in ^{57}Fe. The spectra indicate that the iron within the fungus exists primarily in two chemical states: one is the same as that of the iron in the growth medium while the other is that of the iron storage protein, ferritin. The conversion of iron from the chemical state of the nutrient to ferritin was monitored in different parts of the organism. The superparamagnetic behaviour observed in the Mössbauer spectra indicates that the iron clusters are smaller by a factor of two than those in mammalian ferritin. Mössbauer measurements taken in an applied magnetic field show antiferromagnetic coupling between the iron atoms at low temperatures.

Other Proteins.—Debrunner *et al.*[452] have investigated ^{57}Fe-enriched preparations of reaction centres from the photosynthetic bacterium *Rhodopseudomonas*

[449] K. Spartalian, W. T. Oosterhuis, and J. B. Neilands, *J. Chem. Phys.*, 1975, **62**, 3538.
[450] C. P. Tsang, A. J. F. Boyle, and E. H. Morgan, *Biochim. Biophys. Acta*, 1975, **386**, 32.
[451] K. Spartalian, W. T. Oosterhuis, and N. Smarra, *Biochim. Biophys. Acta*, 1975, **399**, 203.
[452] P. G. Debrunner, G. E. Schulz, G. Feher, and M. Y. Okamura, *Biophys. J.*, 1975, **15**, 226a.

spheroides in both the non-reduced and dithionite reduced forms. At 77 K and above the Mössbauer spectra of both forms are closely similar and consist of quadrupole-split doublets with large chemical shifts (~ 1.1 mm s^{-1}), and large quadrupole splittings (~ 2 mm s^{-1}) which have an appreciable temperature dependence. At low temperatures the reduced form shows line broadening indicative of a magnetic interaction. In applied fields the spectra of both forms show unresolved magnetic hyperfine splitting characteristic of a high-spin ferrous ion, with the reduced form having a slightly larger hyperfine field. The authors have suggested that the differences between the two species may result from a free radical coupling with the iron atom.

Nitrogenase is an enzyme capable of reducing nitrogen to ammonia. It contains a molybdenum–iron protein and an iron protein, both of which are required for the reduction. A Mössbauer spectroscopic investigation of the nitrogenase from *Klebsiella pneumoniae* has been previously reported.[453] Münck *et al.*[454] have investigated the molybdenum–iron protein from *Azotobacter vinelandii* using ^{57}Fe-enriched samples. Chemical analyses give values of 23 iron atoms, 25 labile sulphur atoms, and two molybdenum atoms per molecule of 300 000 daltons. E.p.r. signals are observed from two centres per molecule, each of which undergoes a one-electron reduction. Four iron species could be discerned in the Mössbauer spectra, one of which changes on reduction. This component has an intensity corresponding to eight iron atoms per molecule and is presumably associated with the two centres which give the e.p.r. signals. The Mössbauer measurements show that these two centres are closely similar, with the four iron atoms within each being equivalent at temperatures of 77 K and above. Spectra obtained at low temperatures and in applied magnetic fields show a magnetic inequivalence between the iron atoms within each centre. The Mössbauer and e.p.r. data suggest that each centre has a total spin of 3/2 in the non-reduced form. The authors have suggested that these centres may be a modification of the four-iron centres found in ferredoxins and high-potential iron–sulphur proteins. Another component of the Mössbauer spectra has an intensity corresponding to three or four iron atoms per molecule. These appear to be high-spin ferrous, giving spectra like those of reduced rubredoxin in zero applied field, but somewhat different in an applied field. There are also two diamagnetic components in the Mössbauer spectra. One has an intensity corresponding to approximately ten iron atoms per molecule. This may arise from either low-spin ferrous ions or antiferromagnetically coupled high-spin ferrous ions. The other diamagnetic component is of low intensity and may result from a contaminant, although a similar feature has been observed in the spectra of the *K. pneumoniae* protein.

Hormones.—Oberley and Erhardt [455] have reported a ^{127}I Mössbauer study of the thyroid compounds iodo-tyrosine (MIT) and di-iodo-tyrosine (DIT) and complexes of these compounds T3 and T4. These compounds are important metabolically and the amounts of them in the blood are intimately related to many of the thyroid diseases. The results obtained are consistent with previous ^{129}I

[453] B. E. Smith and G. Lang, *Biochem. J.*, 1974, **137**, 169.
[454] E. Münck, H. Rhodes, W. H. Orme-Johnson, L. C. Davis, W. J. Brill, and V. K. Shah, *Biochim. Biophys. Acta*, 1975, **400**, 32.
[455] L. W. Oberley and J. C. Erhardt, *J. Chem. Phys.*, 1975, **63**, 2329.

Mössbauer studies. The spectrum of MIT was statistically different from those of DIT and T4, while T3 had a spectrum intermediate between those of MIT and DIT. All three compounds exhibited a small *s* hybridization.

7 Dissociation and Association of Proteins

Contributed by E. J. Wood

Analytical Ultracentrifuge.—*Techniques.* In order to perform experiments at very high protein concentrations (*e.g.* >100 mg ml^{-1}) Williams and Kim[456] made extremely thin centre-pieces for ultracentrifuge cells from circles of 0.35 mm Nylon or PVC sheet in which a keyhole shape was cut. The solution to be examined was applied as a 1 μl drop in the 'round' part of the keyhole shape and the cell was assembled so that the sheet was sandwiched between two windows. Upon centrifugation the liquid moved into the 'straight' part of the keyhole shape which served as the cell.

Rowe[457] has introduced a new method for the determination of the absolute fringe number where Rayleigh interference optics have been used. There are many advantages of the low-speed method of equilibrium centrifugation over the high-speed or Yphantis method, but it has always been a problem to determine the absolute fringe number. The method involves a modified fringe relaxation procedure: after exposures have been taken at equilibrium, the rotor is accelerated to a much higher speed, so that a region of zero concentration is produced near the meniscus. The new procedure takes account of window distortion, which it was claimed occurred exclusively during acceleration, and involves extrapolating the apparent fringe count, relative to an arbitrary standard, to zero effective time of acceleration. The high-speed method may nevertheless be used to obtain accurate data. In a study of the molecular weights of the three non-identical subunits of *Klebsiella aerogenes* citrate lyase, Carpenter *et al.*[458] went to great lengths to extract as much data as possible from the fringe photographs. They used a projector and placed a light difference detector on the screen to locate the fringe centres. Eighty to one-hundred radial positions were measured per fringe and readings for five fringes were averaged. Collection of data was aided by the use of digital micrometers on the projector interfaced with a computer terminal. The ultracentrifuge computer system described in the last report[459] has been used to study a number of systems including the subunit dissociation in methaemoglobin,[460] and the acetylcholine receptor from *Torpedo* electric organs.[461] In the latter study the molecular weight was determined in the presence and in the absence of the detergent Triton X-100, and calculations were performed using information obtained from equilibrium dialysis experiments to correct for Triton binding.

[456] R. C. Williams and H. Kim, *Analyt. Biochem.*, 1975, **63**, 277.
[457] A. J. Rowe, *J. Mol. Biol.*, 1975, **97**, 202.
[458] D. E. Carpenter, M. Singh, E. G. Richards, and P. A. Srere, *J. Biol. Chem.*, 1975, **250**, 3254.
[459] E. J. Wood, this series, Vol. 7, p. 227.
[460] P. Hensley, K. Moffat, and S. J. Edelstein, *J. Biol. Chem.*, 1975, **250**, 9391.
[461] S. J. Edelstein, W. B. Beyer, A. T. Eldefrawi, and M. E. Eldefrawi, *J. Biol. Chem.*, 1975, **250**, 6101.

Data Evaluation. For associating–dissociating systems the evaluation of interacting components based on molecular weight distributions is a difficult problem, and much effort continues to be spent in simplifying procedures and overcoming disadvantages in existing methods of data analysis. Matrix rank analysis has been applied to the enumeration of species from diffusion measurements,[462] and now Chun and Kim [463] have discussed the use of principal component analysis as a more effective alternative for component enumeration. Milthorpe *et al.*[464] have presented theory for polymerizing systems which permits the analysis of results obtained with Rayleigh interference or absorption optics without recourse to differentiation or integration, thus avoiding the errors inherent in such procedures. They claim that the method can detect possible changes in volume occurring on polymer formation, and is capable of dealing with systems where activity coefficients of solute species are functions of total concentration, and of describing the system in terms of the relevant equilibrium constants.

Stevens [465] has presented a preliminary report on a method of data reduction and has applied it to the study of TMV protein. For a polymerizing system the most important data are those at low protein concentrations where the error is usually relatively high. Also the absolute concentration across the cell must be known, though the interference optical system only produces concentration differences. Stevens used a function of concentration difference for the association of identical molecules, which can be expanded in a power series. The expansion had a mathematical form characteristic of the polymerization and took a simple form for monomer–dimer, monomer–trimer, monomer–*n*-mer, and indefinite polymerization. Absolute concentration was obtained as a parameter of the fit and, for a reversible polymerization, stability constants were obtainable directly. It was necessary to know the molecular weight of the polymerizing unit, but this information is very often available from other procedures.

An area where sedimentation equilibrium analysis is finding considerable use is in the determination of the density and molecular weight distributions of lipoprotein. These systems are difficult to study because each class of lipoprotein exhibits a continuous distribution of density and molecular weight because of the large content of non-covalently bound lipid. Indeed the distributions for serum low-density lipoprotein varies with physiological state. Jeffrey *et al.*[466] used a procedure based on the Donnelly method (employing Laplace transforms) to analyse results obtained in the absence of a density gradient. However, a way was found to avoid the necessity for differentiating experimental results thus avoiding the error which this introduces. This analysis was of a non-interacting system. Stone and Reynolds [467] on the other hand studied the self-association of polypeptides of human serum high-density lipoprotein by sedimentation equilibrium. The polypeptide apo-Glu-I had a dimeric association constant of $1.3 \times 10^4\,\mathrm{l\,mol^{-1}}$, but another polypeptide, apo-Glu-II, was tetrameric at all the concentrations investigated.

[462] W. Godschalk, *Biochemistry*, 1971, **10**, 3284.
[463] P. W. Chun and S. J. Kim, *Biophys. Chem.*, 1975, **3**, 46.
[464] B. K. Milthorpe, P. D. Jeffrey, and L. W. Nichol, *Biophys. Chem.*, 1975, **3**, 169.
[465] C. L. Stevens, *F.E.B.S. Letters*, 1975, **56**, 12.
[466] P. D. Jeffrey, L. W. Nichol, and G. D. Smith, *J. Biol. Chem.*, 1975, **250**, 533.
[467] W. L. Stone and J. A. Reynolds, *J. Biol. Chem.*, 1975, **250**, 8045.

The self-association of human high-density lipoprotein was also studied by Osborne *et al.*[468] by sedimentation equilibrium. Important changes in secondary and tertiary structure accompanied dimerization.

Gel Chromatography.—The precision has been tested of the method of difference chromatography which is used to detect differences between the extent of reversible dissociation of two multisubunit proteins.[469] The net accumulation or deficiency of protein at the junction between the two layers is a direct measure of the difference between the elution volumes of the two proteins originally applied, layered one over the other, to the column. It was arranged that there was a steady flow of a protein (haemoglobin) through a column of gel particles. The flow was then interrupted momentarily by injecting a known weight of buffer solution thus simulating a small difference in elution volume. The area of the dip in the elution record was evaluated with a standard error of ± 0.02 g in an elution weight of *ca.* 50 g: this is comparable to the precision of difference ultracentrifugation. Difference gel filtration on Sephadex G-100 was used[470] to compare two haemoglobin variants, Hb Kansas (β102 $\rightarrow$ Thr) which has a low O_2 affinity, with Hb Richmond (β102 Asn $\rightarrow$ Lys) which has a normal O_2 affinity. Hb Richmond had an abnormal Bohr effect and the tetramer–dimer equilibrium showed such a strong pH dependence that protons promoted dissociation.

The theory of concentration-dependent migration in gel chromatography of reversibly polymerizing solutes has been extended.[471] It had previously been established[472] that the concentration dependence of elution volume of a non-interacting solute should not be expressed as a linear function of solute concentration. A more rigorous expression for the quantitative description of the concentration dependence has now been derived. The treatment is extended to include a solute undergoing rapid reversible polymerization, and analysis of simulated data for monomer–dimer systems showed the feasibility of this approach for evaluation of equilibrium constants from experimental results provided that the magnitude of the overall change in V_w (the weight-average elution volume) is large compared with the random error associated with each measurement of elution volume.

Many investigators have used gel chromatography to study protein associations and dissociations. Both Tivol *et al.*[473] and Benson *et al.*[474] studied Δ^5-3-keto-steroid dehydrogenase, which can exist as a dimer (monomer molecular weight, 13 400). Although the dimer appeared to be the active species and the enzyme was present in this form in the organism (*Pseudomonas testosteroni*), at concentrations greater than 1 mg ml^{-1} it underwent aggregation. In gel filtration experiments with a large zone of enzyme, the leading edge was sharpened and the trailing edge showed spreading, which is characteristic of a reversibly associating

[468] J. Gwynne, G. Palumbo, J. C. Osborne, H. B. Brewer, and H. Edelhoch, *Arch. Biochem. Biophys.*, 1975, **170**, 204; J. C. Osborne, G. Palumbo, H. B. Brewer, and H. Edelhoch, *Biochemistry*, 1975, **14**, 3741.
[469] G. A. Gilbert, L. M. Gilbert, and N. A. F. Shawky, *Biochim. Biophys. Acta*, 1975, **379**, 488.
[470] R. M. Winslow and S. Charache, *J. Biol. Chem.*, 1975, **250**, 6939.
[471] P. A. Baghurst, L. W. Nichol, A. G. Ogston, and D. J. Winzor, *Biochem. J.*, 1975, **147**, 575.
[472] L. W. Nichol, M. Janado, and D. J. Winzor, *Biochem. J.*, 1973, **133**, 15.
[473] W. F. Tivol, E. D. Beckman, and W. F. Benisek, *J. Biol. Chem.*, 1975, **250**, 271.
[474] A. M. Benson, A. J. Suruda, and P. Talalay, *J. Biol. Chem.*, 1975, **250**, 276.

system in rapid equilibrium. Below 0.05 mg ml^{-1} the dimer underwent dissociation. Ingham *et al.*[475] studied the ligand-induced self-association of human luteinizing hormone by gel filtration, as well as by sedimentation equilibrium and fluorescence methods. Gel filtration showed that the dimeric form had a single high affinity site ($K \gg 10^6$ l mol^{-1}) for 8-anilino-1-naphthalenesulphonate (ANS). These workers also studied human chorionic gonadotrophin[476] by similar methods. Whereas the luteinizing hormone was a monomer–dimer system showing negative co-operativity in the binding of ANS, the chorionic gonadotrophin was a monomer–dimer–tetramer system showing positive co-operativity of ANS binding.

Tan *et al.*[477] investigated the association–dissociation of oxyhaemerythrin by frontal gel chromatography on Sephadex G-75. Haemerythrin is an octamer (monomer molecular weight, 13 500) and the dissociation was strongly pH-dependent. Below pH 6.4 the rate of equilibration between species was slow relative to their time of passage through the column and they were thus resolved. Above pH 6.6 the rate was rapid and no resolution occurred. The effects of temperature and ionic strength were also investigated.

An interesting example of protein association is that of bovine β-casein. Barry and Creamer[478] showed by gel filtration that the monomer which is predominant at low temperatures associates at 36 °C. Molecular weights $> 1.34 \times 10^6$ were found at this temperature. The *C*-terminal region of the protein chain was important in the association: removal of two *C*-terminal amino-acids decreased the amount of polymer present, and removal of 20 *C*-terminal amino-acids completely destroyed the ability to form a polymer. The amino-acid sequence of bovine β-casein A^1 is known, and a 47 amino-acid sequence at the *N*-terminal end has a lower hydrophobicity than the rest of the molecule and a high negative charge. It was concluded from a fluorescence study of the temperature-dependent polymerization[479] that many of the properties observed could be explained by micelle formation.

Light Scattering.—The technique of determining translational diffusion coefficients by intensity fluctuation spectroscopy of scattered laser light was introduced less than 10 years ago.[480, 481] It is also called quasi-elastic light scattering, and an advantage of the technique is the small amount of experimental material required. Loewenstein and Birnboim[482] have described a technique in which 0.1 ml of solution of macromolecules is held in a capillary tube, and both sedimentation and diffusion coefficients are determined. The tubes are spun in a preparative ultracentrifuge, carefully removed, and placed in a light-scattering photometer equipped with a scanning mechanism. The intensity distribution of scattered light along the tube provides a measure of the rate of boundary migration if the

[475] K. C. Ingham, H. A. Saroff, and H. Edelhoch, *Biochemistry*, 1975, **14**, 4745.
[476] K. C. Ingham, H. A. Saroff, and H. Edelhoch, *Biochemistry*, 1975, **14**, 4751.
[477] K. H. Tan, S. Keresztes-Nagy, and A. Frankfater, *Biochemistry*, 1975, **14**, 4280, 4286.
[478] G. P. Barry and L. K. Creamer, *Biochemistry*, 1975, **14**, 3542.
[479] K. N. Pearce, *European J. Biochem.*, 1975, **58**, 23.
[480] S. B. Dubin, J. H. Lunacek, and G. B. Benedek, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **57**, 1164.
[481] R. Foord, E. Jakeman, C. J. Oliver, E. R. Pike, R. J. Blagrove, E. Wood, and A. R. Peacocke, *Nature*, 1970, **227**, 242.
[482] M. A. Loewenstein and M. H. Birnboim, *Biopolymers*, 1975, **14**, 419.

applied centrifugal field is known. At any point in the tube the scanner may be stopped and a diffusion coefficient determined from a time-autocorrelation analysis of the fluctuation in the intensity of the scattered light. The particle weights of two monodisperse systems, bacteriophage R17 and 28S ribosomal RNA, were determined from values of S and D obtained by this method. As such values of D have a high precision,[481] it is of great interest that this method is now being extended to cope with heterodisperse systems such as associating and dissociating proteins, and protein–small-molecule interactions. Dubin and Cannell [483] used a differential method with two cells in contact and a chopping wheel, and showed that when the enzyme aspartate transcarbamylase interacted with succinate the diffusion coefficient underwent a 4% decrease. Cohen *et al.*[484] studied the self-aggregation of bovine glutamate dehydrogenase by quasi-elastic light-scattering spectroscopy. They presented a mathematical model to explain their findings and concluded that in the experiments they performed, the effects of rotational diffusion, diffusion anisotropy, and electrostatically coupled diffusion would be negligible and that the data should be unaffected by thermodynamic non-ideality.

Chu *et al.*[485] studied the self-association of β-lactoglobulin A in acidic solution using the autocorrelation technique. The determination of diffusion coefficients from decay curves which are not single exponentials is not without difficulties, and the β-lactoglobulin data were analysed by the method of cumulants as devised by Kopple.[486] At a concentration of 21 $\mu g\,ml^{-1}$ and pH 4.58, the self-association could be represented by a simpler dimer–octamer equilibrium. If precise values of D can be obtained, then so can Stokes' radii, and it was possible to calculate that hydrodynamically the octamers behave like close-packed spheres with an effective radius of *ca.* 4.5 nm according to the Stokes–Einstein relation.

Mention may be made of the experiments of Fraser *et al.*[487] with muscle proteins. These workers obtained repeatable intensity fluctuation autocorrelations for actin, heavy meromyosin, and actin subfragment S-1 at scattering angles between 40 and 130°, and concluded that the non-exponential character of the actin autocorrelation was consistent with a large molecule having hindered diffusion, rotation, and perhaps flexion. Approximate diffusion coefficients were $10^{-7}\,cm^2\,s^{-1}$ for heavy meromyosin, and *ca.* $6 \times 10^{-7}\,cm^2\,s^{-1}$ for the S-1 preparation: actin could be said to diffuse about an order of magnitude more slowly. It was concluded from these and other measurements that under conditions of maximal actin activation of ATPase, most of the heavy meromyosin and S-1 occur in a refractory state unable to bind to actin.

Several studies using conventional (*i.e.* intensity) light scattering have appeared, including the investigation of the tetramer–dimer dissociation of haemoglobin and its derivatives,[488, 489] but other investigators have used kinetic techniques involving

[483] S. B. Dubin and D. S. Cannell, *Biochemistry*, 1975, **14**, 192.
[484] R. J. Cohen, J. A. Jedziniak, and G. B. Benedik, *Proc. Roy. Soc.*, 1975, **A345**, 73.
[485] B. Chu, A. Yeh, F. C. Chen, and B. Weiner, *Biopolymers*, 1975, **14**, 93.
[486] D. E. Kopple, *J. Chem. Phys.*, 1972, **57**, 4814.
[487] A. B. Fraser, E. Eisenberg, W. W. Kielley, and F. D. Carlson, *Biochemistry*, 1975, **14**, 2207.
[488] S. L. White, *J. Biol. Chem.*, 1975, **250**, 1263.
[489] R. K. Bhat and T. T. Herskovitz, *Biochemistry*, 1975, **14**, 1572.

light scattering. The self-association of bovine glutamate dehydrogenase has been mentioned above in relation to the measurement of *D*. This association has also been studied by light scattering, temperature-jump, and stopped-flow techniques.[490] The enzyme appears to undergo a rapid, reversible aggregation to form long rods of indefinite length, but the data were incompatible with the previously proposed single sequential model and suggested that the monomer did not play a critical role in the self-assembly. Rather, a 'random association' was favoured as an explanation in which association–dissociation reactions occur between all polymerizing forms of the oligomer. Large entropy effects and heat capacities indicated the participation of water, and it was suggested that the rate-determining step in the association is the 'melting' of a layer of ordered water structure between two hydrophobic contact sites.

Kegeles and Ke[491] have developed a light-scattering pressure-jump apparatus in which a stainless steel cell fitted with three quartz ultracentrifuge windows is pressurized by leading in a gas. The pressurization time was 2 min and the total volume in the cell was under 2 ml: pressures of over 2000 psi have been used. In preliminary experiments the cell was used to determine the relaxation curve for the α-haemocyanin of *Helix pomatia*, which has already been studied by light scattering at high pressures.[492]

Transport Studies.—Claverie *et al.*[493] have considered the chemical equilibria between interacting molecules during sedimentation taking into account boundary conditions, cell geometry, equilibrium constants, diffusion, *etc.* Their general treatment may be applied to boundary, band, or active enzyme sedimentation, and in a second paper[494] the latter is considered in more detail. The basic approach in these papers is to assume a certain type of equilibrium as a model, and to simulate, through a Fortran program, the data one should obtain, and to compare them with the real data. The same sort of approach was tried successfully by Frigon and Timasheff[495] in the study of the Mg^{2+}-induced self-association of calf-brain tubulin. A consideration of the sedimentation velocity patterns at different protein concentrations suggested a model for the association of the tubulin dimer (6S) to the polymer (41S). Generated sedimentation patterns expected for such a system were then compared with the actual patterns obtained.

Janado[496] has produced an analysis of macromolecular interactions studied by the moving boundary method. A constituent velocity equation was derived permitting analysis of macromolecule (P)–macromolecule (A) interactions in which a number of complexes, PA, PA_2, PA_3, ..., PA_n, are in rapid equilibrium. The velocity of the complex PA_i ($i = 1, 2, \ldots, n$) was expressed in the form of a polynomial function in i and a generalized equation was formulated. In another theoretical treatment, Schönert[497] has derived an approximate solution of the

[490] D. Thusius, P. Dessen, and J.-M. Jallon, *J. Mol. Biol.*, 1975, **92**, 413; D. Thusius, *J. Mol. Biol.*, 1975, **94**, 367.
[491] G. Kegeles and C.-H. Ke, *Analyt. Biochem.*, 1975, **68**, 138.
[492] W. N. Konings, R. J. Siezen, and M. Gruber, *Biochim. Biophys. Acta*, 1969, **194**, 376.
[493] J.-M. Claverie, H. Dreux, and R. Cohen, *Biopolymers*, 1975, **14**, 1685.
[494] R. Cohen and J.-M. Claverie, *Biopolymers*, 1975, **14**, 1701.
[495] R. P. Frigon and S. N. Timasheff, *Biochemistry*, 1975, **14**, 4559, 4567.
[496] M. Janado, *J. Biochem. (Tokyo)*, 1975, **78**, 199.
[497] H. Schönert, *Biophys. Chem.*, 1975, **3**, 161.

differential equation for the migration of a reversibly reacting substance. The concentration dependence of the constituent transport coefficients was approximated by polynomials, and features of the leading and trailing boundaries in monomer–trimer, and monomer–dimer–trimer systems were discussed.

Skerrett [498] has discussed the theory of the difference sedimentation technique, and the method has been used by a number of groups. Nagel and Schachman [499] used it to study hybrids of aspartate transcarbamylase containing succinylated regulatory chains, and Colman *et al.*[500] used it to study tryptophan oxygenase, a tetrameric cupro-haem enzyme from *Pseudomonas acidovorans*. They attributed a 3.9% increase in $s_{20,w}$ with pH change from 8.0 to 6.0 to a decrease in frictional coefficient.

Electron Microscopy.—The formation of microtubules from the protein tubulin has been studied by electron microscopy [501] amongst other methods, and a discussion of this process will be found below. However, two papers are worth mentioning here as examples of the use of electron microscopy to follow the rates of formation of macro-polymers. Binder *et al.*[502] used the electron microscope to determine the rate of microtubule formation using the negative staining technique. The process this group studied was the assembly of chick-brain tubulin onto flagellar microtubules from *Chlamydomonas* and sea urchin sperm. Similarly Kirschner *et al.*[503] examined the kinetics of microtubule assembly from purified porcine brain tubulin by quantitative electron microscopy. Quantitation was achieved by mixing the polymerizing protein solution with a suspension of tomato bushy stunt virus at known particle concentration, followed by spray-depositing with negative stain on the grids. In the electron micrographs the percentage of protein seen in distinguishable polymeric forms was calculated from the numbers, lengths, and known masses, and mass/unit lengths of these entities. Ring-like forms, microtubules, and aggregates of parallel protofilaments were found, and the amount of protein found as rings rapidly decreased from an initial 30% to a negligible amount at 2 min. The amount of microtubules increased asymptotically to an equilibrium value of 50% by 8 min (initial protein concentration *ca.* 1 mg ml^{-1}).

Other interesting extended polymerizations that have been studied by electron microscopy are the formation of three- and seven-stranded helical cables by the enzymic glutamine synthetase after treatment with cobaltous ion,[504] and the formation of infinite linear polymers by the β-haemocyanin of *Helix pomatia* after trypsin treatment.[505] In this latter case, the molecule is a 30 nm long cylinder, and trypsinization appears to remove some end-structure that normally stops the polymerization of subunits proceeding beyond a certain stage. The result is an extended cylinder, often up to 30 times the length of the native

[498] R. J. Skerrett, *Analyt. Biochem.*, 1975, **66**, 1.
[499] G. M. Nagel and H. K. Schachman, *Biochemistry*, 1975, **14**, 3195.
[500] P. D. Colman, S. P. Blanchet, E. Chow, and P. Feigelson, *J. Biol. Chem.*, 1975, **250**, 6208.
[501] L. H. Green, J. W. Brandis, F. R. Turner, and R. A. Raff, *Biochemistry*, 1975, **14**, 4487.
[502] L. I. Binder, W. L. Deutler, and J. L. Rosenbaum, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1122.
[503] M. W. Kirschner, L. S. Honig, and R. C. Williams, *J. Mol. Biol.*, 1975, **99**, 263.
[504] T. G. Frey, D. Eisenberg, and E. A. Eiserling, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3402.
[505] J. F. L. van Breemen, T. Wichertjes, M. F. J. Muller, R. van Driel, and E. F. J. van Bruggen, *European J. Biochem.*, 1975, **60**, 129.

molecule. Image reconstruction techniques were used to give information on the arrangement of the subunits in the cylinder wall.

Direct measurement of the size and shape of the fundamental subunit of rabbit muscle phosphofructokinase has been reported.[506] Native and dimethyl suberimidate cross-linked enzyme were examined using the negative staining technique. Accepting that some distortion may result from the staining process, it was concluded that the fundamental subunit, a prolate ellipsoid of molecular weight 80 000, had axes 6.7 and 2.5 nm. The dimer was formed by association along the 2.5 nm axis, and the tetramer appeared to be formed by an end-to-end aggregation of dimers.

Kinetic Studies.—*Protein–Protein Interactions.* Hybridization studies have been widely used to obtain information on the quaternary structures of protein. Osborne and Hollaway[507] developed a method for studying the kinetics of subunit exchange in glyceraldehyde 3-phosphate dehydrogenase. They measured rates of subunit exchange between rabbit enzyme (R_4), yeast enzyme (Y_4), and rabbit–yeast hybrid (R_2Y_2). The rate-limiting steps in the hybridization process were the dissociations of tetramers to dimers. When ligands such as substrates or coenzymes bind to the native enzymes and alter the free energy of interaction between subunits, it would be expected that the rate and possibly extent of hybrid formation would alter. The method has the advantage that changes in subunit interaction energy can be measured where oligomeric proteins are at concentrations at which they are in the associated state. However, a limitation to the technique is the use of electrophoresis to determine the composition of reaction mixtures. Rate constants could only be measured if they fell in the range 2×10^{-3} to 2×10^{-5} s^{-1}. Hybridization studies have also been performed with glutamate aspartate transaminase.[508]

Yagisawa[509] has devised a method for observing protein–protein interactions in which the pK of an ionizable reporter group attached to the molecule is altered by the electrostatic effect of another charged molecule which associates with the former. The method was used to study BSA and egg-white lysozyme self-association. Vallee and Williams[510] investigated the two-step reversible denaturation of lactate dehydrogenase. At low pH the tetrameric enzyme lost activity but this was completely restored on returning to pH 7. With long times of exposure to low pH, less and less activity was recovered. After a brief time at low pH the kinetics of recovery of activity were second order, but after several minutes at low pH the recovery or partial recovery was first order and slower. Gel filtration and sedimentation equilibrium studies showed the presence at low pH of monomer (which was capable of recovering activity) and of polymeric species (which were incapable). Low pH was also used to study the association of ovine luteinizing hormone.[511] The kinetics suggested that the α- and β-subunits combined rapidly to give a loose complex, $\alpha\beta$, still containing partially unfolded chains. This complex then slowly formed active hormone.

[506] J. N. Telford, P. M. Lad, and G. G. Hammes, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3054.
[507] H. H. Osborne and M. R. Hollaway, *Biochem. J.*, 1974, **143**, 651; 1975, **151**, 37.
[508] B. Boettcher and M. Martinez-Carrion, *Biochemistry*, 1975, **14**, 4528.
[509] S. Yagisawa, *J. Biochem.* (*Tokyo*), 1975, **77**, 605.
[510] R. B. Vallee and R. C. Williams, *Biochemistry*, 1975, **14**, 2574.
[511] R. Salesse, M. Casteing, J. C. Pernollet, and J. Garnier, *J. Mol. Biol.*, 1975, **95**, 483.

Various different methods were used to follow the rate of gel formation in sickle cell haemoglobin (HbS) (see also ref. 459). Ross *et al.*[512] used a light-transmission method to measure the optical birefringence resulting from the alignment of deoxy HbS fibres in the gel, and also measured the heat of gelation with a high sensitivity heat-flow calorimeter. Briehl and Salhany[513] used their previously reported ultracentrifuge method to study the gelation of NO-HbS, and Levinthal *et al.*[514] studied interactions in HbS fibres. Minton[515] has presented a theoretical thermodynamic analysis of the mechanism by which reagents (alkali halides, retinol, lysolecithin, H^+, ethylurea, tetrabutylammonium chloride) inhibit gelation of HbS, presumably by increasing the minimum gelling concentration by reacting preferentially with the isolated molecule relative to the aggregate and increasing the solubility of non-aggregated haemoglobin. The study of HbS should be aided by the knowledge of the crystal structure of deoxyHbS at 5 Å resolution.[516] In erythrocytes from heterozygotes, hybrids of HbS and HbA may well be present. Williams and Kim[517] used an equilibrium ultracentrifugation method to measure an apparent dimer–tetramer association constant for the hybrid tetramer $\alpha_2\beta^A\beta^S$, and made estimates of the fraction of AS hybrids likely to be present in erythrocytes of A-S heterozygotes.

Protein–Small-molecule Interactions. The binding of the β-1 : 4-linked trimer of *N*-acetyl-D-glucosamine to lysozyme has been measured by rapid reaction methods,[518] including differential stopped-flow fluorometry and *T*-jump fluorometry. Kinetic studies are often limited in that although a sequence of events in ligand binding may be revealed, and in favourable cases some sort of conformational change may be implicated in the catalytic mechanism, the kinetics by themselves give no information about the nature of the isomerization, and the spectrophotometric probes are not generally open to a molecular interpretation. In the present case it was found that discrete segments of the fluorescence-difference spectrum were perturbed at different times during the binding process. This was interpreted in terms of an initial fast phase of complex formation during which the environment of Trp-62 was disturbed, followed by a slower rearrangement of the initial complex perturbing the environment of Trp-108.

The kinetics of antibody–hapten interactions were investigated in a system involving [^{3}H]-labelled hapten, and dextran-coated charcoal was used for the rapid separation of free- from antibody-bound hapten.[519] The haptens used were digoxin and ouabain, and the association rate constants under both second- and pseudo-first-order conditions were in the range $0.87—1.7 \times 10^7$ l mol^{-1} s^{-1}, but the dissociation rate constants varied widely from 1.9×10^{-4} to 1.7×10^{-2} s^{-1}. These observations support the idea that the major kinetic variable governing antibody–hapten interaction is the rate of dissociation of the complex and that the strength of the antibody–hapten association is determined principally

[512] P. D. Ross, J. Hofrichter, and W. A. Easton, *J. Mol. Biol.*, 1975, **96**, 239.
[513] R. W. Briehl and J. M. Salhany, *J. Mol. Biol.*, 1975, **96**, 733.
[514] C. Levinthal, S. J. Wodak, P. Kahn, and A. K. Dadwanian, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1330.
[515] A. P. Minton, *J. Mol. Biol.*, 1975, **95**, 289.
[516] B. C. Wishner, K. B. Ward, E. E. Lattman, and W. E. Love, *J. Mol. Biol.*, 1975, **98**, 179.
[517] R. C. Williams and H. Kim, *Arch. Biochem. Biophys.*, 1975, **170**, 368.
[518] S. E. Halford, *Biochem. J.*, 1975, **149**, 411.
[519] T. W. Smith and K. M. Skubitz, *Biochemistry*, 1975, **14**, 1496.

by the activation energy for dissociation. Antibody–hapten kinetics studied at different times of the immune response by fluorescence polarization and intensity methods have confirmed this.[520]

Many studies of haemoglobin–ligand kinetics have appeared. A number of the kinetic parameters have been reported for the five principal components of human haemoglobin (A_{1a}, A_{1b}, A_{1c}, A_0, and A_2).[521] The effect of urea on the kinetics of ligand combination,[522] the pH dependence of the reaction of haemoglobin,[523] and the correlation between quaternary structure and ligand dissociation kinetics for fully liganded haemoglobin[524] have all been studied, as have the kinetics of binding of CO to the giant multisubunit haemoglobin of the earthworm, *Lumbricus*.[525] Gibson's group have reinvestigated the oxygen-pulse experiment[526] in which it was proposed to measure the O_2 off-rate from the deoxy quaternary structure by mixing O_2 with a solution of haemoglobin containing dithionite and adjusting the relative amounts of O_2 and dithionite so as to vary the peak percentage saturation of haemoglobin with oxygen. The aim of these studies was to explain the grossly biphasic O_2 release kinetics observed with this technique. This could be due to co-operativity, the fast phase being O_2 release from the low affinity (*T*) state and the slow phase being O_2 release from molecules that have switched to the high affinity (*R*) state. Alternatively it could be that there is a large difference in rate of O_2 release from the two types of subunit in haemoglobin. The new experiments with haemoglobin Kansas (which in the presence of inositol hexaphosphate is stabilized in the *T* state even when fully liganded) indicated that the first of these two hypotheses was the correct one.

Protein–Small-molecule Equilibria.—A number of theoretical papers have dealt with the binding of small molecules to proteins.[527–530] Levitski *et al.*[531] have presented a model in which a co-operative membrane response can be generated by a cluster of oligomeric receptors for ligand, and Paulus and DeRiel[532] considered negative co-operativity in oligomeric enzymes. Cornish–Bowden and Koshland[533] have investigated the possible shapes encountered in the Hill plot when it is used for the analysis of ligand binding, and similarly Endrenyi *et al.*[534] have looked at the case of the evaluation of Hill slopes and coefficients when the saturation binding or velocity is not known. Hunston[535] has proposed two

[520] S. A. Levison, A. N. Hicks, A. J. Portmann, and W. B. Dandlikier, *Biochemistry*, 1975, **14**, 3778.
[521] R. C. Steinmeier and L. J. Parkhurst, *Biochemistry*, 1975, **14**, 1564.
[522] V. S. Sharma, J. Everse, and H. M. Ranney, *J. Mol. Biol.*, 1975, **95**, 385.
[523] R. P. May and A. Mayer, *European J. Biochem.*, 1975, **52**, 589.
[524] J. M. Salhany, S. Ogawa, and R. G. Shulman, *Biochemistry*, 1975, **14**, 2180.
[525] G. M. Giacometti, A. Foresi, B. Giardina, M. Brunori, and J. Wyman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4313.
[526] J. M. Salhany, C. L. Castillo, M. J. McDonald, and Q. H. Gibson, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3998; Q. H. Gibson, *ibid.*, 1973, **70**, 1.
[527] J. Wyman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1464, 3983.
[528] M. Dourlent, *Biopolymers*, 1975, **14**, 1717.
[529] J. A. Schellman, *Biopolymers*, 1975, **14**, 999.
[530] I. M. Klotz and D. L. Hunston, *J. Biol. Chem.*, 1975, **250**, 3001.
[531] A. Levitzki, L. A. Segel, and M. L. Steer, *J. Mol. Biol.*, 1975, **91**, 125.
[532] H. Paulus and J. K. DeRiel, *J. Mol. Biol.*, 1975, **97**, 667.
[533] A. Cornish-Bowden and D. E. Koshland, *J. Mol. Biol.*, 1975, **95**, 201.
[534] L. Endrenyi, C. Fajszi, and F. H. F. Kwong, *European J. Biochem.*, 1975, **51**, 317.
[535] D. L. Hunston, *Analyt. Biochem.*, 1975, **63**, 99.

techniques for the evaluation of multiple binding in complex systems which can be applied where more than one class of binding site is present or where there are interactions between equivalent sites. One technique uses a Klotz or Scatchard plot to evaluate three stoicheiometric binding constants and the total number of sites, and the second enploys a continuous distribution of binding constants to analyse the reaction. Several articles by Weber have appeared[536] on ligand interactions in globular proteins, and two papers by Kolb and Weber[537] deal with the reciprocity of effects of two ligands simultaneously bound to a protein. The two cases Kolb and Weber consider are the ternary complex of heart lactate dehydrogenase with NAD^+ and oxalate, and the binding of 8-anilino-1-naphthalene-sulphonate and 3,5-dihydroxybenzoate to serum albumin, which involves multiple interactions. The finding of co-operative binding effects in a single-chain protein, induced by the binding of a second ligand, seems to negate the idea that these effects are to be found only in oligomeric proteins or that structural symmetry is necessarily involved in their production. These findings are of interest in relation to the report that the co-operative binding of O_2 by *Levantina hierosolima* haemocyanin arose from O_2–Ca^{2+} interactions and was not directly related to changes in protein aggregation.[538] Bardsley and Childs[539] applied the theory of plane curves to the graphical methods used in enzyme kinetics and considered the mathematical significance of sigmoid curves and non-linear double reciprocal plots with special reference to steady-state data.

Experimentally many different systems have been investigated by a variety of techniques. Calorimetry (and equilibrium dialysis) allowed the study of the binding of NADH to lactate dehydrogenase,[540] and the same technique was used to study antigen and hapten binding to antibody,[541] trypsin–trypsin inhibitor equilibria,[542] and the binding of CTP and ATP to aspartate transcarbamylase.[543] This latter enzyme has also been investigated by a fluorescence energy transfer technique to measure the distances between sites,[544] by tetraiodofluorescein binding,[545] and by a variety of other methods including hybrid formation.[546–548] Fluorescence and c.d. were used to investigate the binding of ligands to the α subunit of tryptophan synthetase.[549] Though the binding of substrates induced extrinsic Cotton effects, there was no evidence in the far-u.v. c.d. for any conformation change upon binding indole or D-glyceraldehyde 3-phosphate. Other techniques that have been used are resonance Raman spectroscopy to study

[536] G. Weber, in 'Protein–Ligand Interactions', ed. H. Sund and G. Blauer, de Gruyter, Berlin, 1975, p. 15; G. Weber, *Adv. Protein Chem.*, 1975, **29**, 1.
[537] D. A. Kolb and G. Weber, *Biochemistry*, 1975, **14**, 4471, 4476.
[538] A. Klarman, N. Shaklai, and E. Daniel, *Biochemistry*, 1975, **14**, 102, 105.
[539] W. G. Bardsley and R. E. Childs, *Biochem. J.*, 1975, **149**, 313.
[540] H.-J. Hing and R. Jaenicke, *Biochemistry*, 1975, **14**, 24.
[541] J. F. Halsey and R. L. Biltonen, *Biochemistry*, 1975, **14**, 800.
[542] R. Turner, J. E. Liener, and R. E. Lovrien, *Biochemistry*, 1975, **14**, 275.
[543] N. M. Allewell, J. Friedland, and K. Niekamp, *Biochemistry*, 1975, **14**, 224.
[544] S. Matsumoto and G. G. Hammes, *Biochemistry*, 1975, **14**, 214.
[545] L. B. Jacobsberg, E. R. Kantrowitz, and W. N. Lipscomb, *J. Biol. Chem.*, 1975, **250**, 9238.
[546] I. Gibbons, J. E. Flatgaard, and H. K. Schachman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4298.
[547] J. S. Mort and W. W. C. Chan, *J. Biol. Chem.*, 1975, **250**, 653.
[548] D. R. Evans, S. C. Pastra-Landis, and W. N. Lipscomb, *J. Biol. Chem.*, 1975, **250**, 3571.
[549] M. P. Heyer and W. O. Weischet, *Biochemistry*, 1975, **14**, 2962.

haemerythrin–ligand complexes,[550] and affinity labelling of the regulatory site of glutamate dehydrogenase with the adenosine analogue, 3′-*p*-fluorosulphonylbenzoyladenosine.[551] Negative co-operativity was found in the binding of thyroxine to human serum prealbumin [552] and under certain conditions with fructose 6-phosphate and fructose 1,6-bisphosphate to phosphofructokinase.[553] The malate dehydrogenase from pig heart supernatant showed antico-operative binding of NADH and L-malate.[554]

Benesch and Rubin [555] studied the interaction of haemoglobin with three ligands and showed that in the presence of allosteric effectors such as 2,3-diphosphoglycerate, the assumption that the Bohr coefficient ($\Delta \log P_{50}/\Delta pH$) is equal to the Haldane coefficient (ΔH^+ upon oxygenation) is not correct. Szabo and Karplus [556] have proposed an allosteric model for the structure–function relations in normal haemoglobin which can be used to interpret the behaviour of high-spin and low-spin valency hybrids, the methaemoglobin ligand replacement reactions, and the haemoglobin redox reaction. Bull and Hoffman [557] have examined the redox equilibria of azidomethaemoglobin (low-spin) and fluoromethaemoglobin (high-spin). Deal [558] has analysed the effects of chloride and 2,3-diphosphoglycerate on the co-operative binding of O_2 by haemoglobin. Ackers *et al.*[559] studied the dependence of oxygenation curves upon protein concentration and concluded that the presence of very small amounts of haemoglobin dimer that are necessarily present at the low protein concentrations normally used may have a devastating effect on the reliability of Adair constant determinations. Such studies on these are aided by the determination of precise O_2-equilibrium curves.[560]

Quantitative Affinity Chromatography. A new technique for studying ligand–protein (and indeed protein–protein) interactions is quantitative affinity chromatography. Dunn and Chaiken [561] used this method in a study of the binding of staphylococcal nuclease to thymidine 3′-(*p*-Sepharose-aminophenyl phosphate) 5′-phosphate. The elution volume of nuclease was proportional to matrix-bound ligand concentration (at constant soluble ligand concentration), proportional to soluble ligand concentration (at constant matrix-bound ligand concentration), and inversely proportional to dissociation constant of soluble ligand (at constant concentrations of soluble and matrix-bound ligand). These relationships were expressed in an equation describing the competition of soluble and matrix-bound ligand for nuclease binding. The experimental results obtained agreed well with values obtained by both equilibrium dialysis and kinetics of

[550] J. B. R. Dunn, D. F. Shriver, and I. M. Klotz, *Biochemistry*, 1975, **14**, 2689.
[551] P. K. Pal, W. J. Wechter, and R. F. Colman, *Biochemistry*, 1975, **14**, 707.
[552] R. N. Fergusson, H. Edelhoch, H. A. Saroff, and J. Robbins, *Biochemistry*, 1975, **14**, 282.
[553] D. E. Hill and G. G. Hammes, *Biochemistry*, 1975, **14**, 203.
[554] F. A. Mueggler, F. W. Dahlquist, and R. G. Wolfe, *Biochemistry*, 1975, **14**, 3490.
[555] R. E. Benesch and H. Rubin, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2465.
[556] A. Szabo and M. Karplus, *Biochemistry*, 1975, **14**, 991.
[557] C. Bull and B. M. Hoffman, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3382.
[558] W. J. Deal, *Biopolymers*, 1975, **14**, 1273.
[559] G. K. Ackers, M. L. Johnson, F. C. Mills, H. R. Halvarson, and S. Shapiro, *Biochemistry*, 1975, **14**, 5128.
[560] K. Imai and T. Yonetani, *J. Biol. Chem.*, 1975, **250**, 7093.
[561] B. M. Dunn and I. M. Chaiken, *Biochemistry*, 1975, **14**, 2343.

inhibition of nuclease activity. Kasai and Ishii[562] used quantitative affinity chromatography to estimate the dissociation constants for trypsin and the peptide Gly-Gly-Arg coupled to agarose. Denizot and Delage[563] have developed the theory of affinity chromatography in terms of the statistical approach. A simple procedure was described for expressing the fundamental constants of the model in terms of experimentally determinable quantities. They claim that affinity chromatography can be used to determine rate constants of association and dissociation in the range usually considered as the domain of stopped-flow methods.

Determination of Protein Subunit Structures by Detergent–Gel Electrophoresis.—Great use continues to be made of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS) as a quick and easy method for estimating protomer molecular weights, and several investigators have looked again at the principles behind the method. It is well known for example that glycoproteins can behave anomalously, and Frank and Rodbard[564] have investigated the precision of the method in the case of a membrane glycoprotein, rhodopsin. Here the apparent molecular weight decreased from 40 400 to 34 500 as the acrylamide concentration increased from 4 to 10%. The most reliable plot appeared to be a weighted linear regression for the retardation coefficient against molecular weight, but even so values must be viewed cautiously because of uncertainties regarding the detergent-binding properties of the protein. Wright *et al.*[565] used the method of transient electric birefringence to study the conformation of protein-SDS complexes. In contrast with other models (rod-like, 'necklace') they propose a model of a deformable prolate ellipsoid for the protein-SDS complex. A number of binding studies have appeared involving detergents other than SDS,[566] and for SDS itself Takagi *et al.*[567] found that the binding isotherm was biphasic, there being different isotherms for different proteins. The actual amount of SDS present in polyacrylamide gels appears to affect the electrophoretic mobility of protein-SDS complexes.[568] The structure of polyacrylamide gels has been visualized in the scanning electron microscope.[569]

Of the many proteins that have been studied by this method, space limitation prevents more than a few being mentioned. Several phycocyanins and phycoerythrins have received attention. These protein complexes often constitute up to 20% of the soluble proteins in algal cells. Most have two types of polypeptide chain (α and β, 1 : 1) but many homologies, both between chains, and between proteins from different species, have been found.[570] The molecular weights of both polypeptide chains are in the region of 15 000—20 000, but the native pro-

[562] K. Kasai and S. Ishii, *J. Biochem.* (*Tokyo*), 1975, **77**, 261.
[563] F. C. Denizot and M. A. Delage, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4840.
[564] R. N. Frank and D. Rodbard, *Arch. Biochem. Biophys.*, 1975, **171**, 1.
[565] A. K. Wright, M. R. Thompson, and R. L. Miller, *Biochemistry*, 1975, **14**, 3224.
[566] M. N. Jones, H. A. Skinner, and E. Tipping, *Biochem. J.*, 1975, **147**, 229.
[567] T. Takagi, K. Tsujii, and K. Shirahama, *J. Biochem.* (*Tokyo*), 1975, **77**, 939.
[568] J. T. Stoklosa and H. W. Latz, *Analyt. Biochem.*, 1975, **68**, 358; K. Kubo, T. Isemura, and T. Tagaki, *J. Biochem.* (*Tokyo*), 1975, **78**, 349.
[569] R. Rüchel and M. D. Brager, *Analyt. Biochem.*, 1975, **68**, 415.
[570] J. Takemoto and L. Bogorad, *Biochemistry*, 1975, **14**, 1211; R. F. Troxler, J. A. Foster, A. S. Brown, and C. Franzblau, *ibid.*, p. 268; A. S. Brown, J. A. Foster, P. V. Voynow, C. Franzblau, and R. F. Troxler, *ibid.*, p. 3581.

teins are very much bigger, hexamers $(\alpha\beta)_6$, and possibly duodecamers being present in some cases.[571]

Of the many enzymes studied one may mention a fungal L-phenylalanine ammonia lyase (molecular weight of 330 000 by gel filtration), which has the structure $\alpha_2\beta_2$ where the molecular weights of α and β are 70 000 and 90 000, respectively,[572] and the citrate synthase of *E. coli* K_{12} which has a subunit molecular weight of 43 000—46 000 but in 0.1M-KCl exists entirely as hexamer.[573] The 4-deoxy-5-oxoglucarate hydrolyase from *Pseudomonas acidovorans* has a molecular weight of 30 500 by detergent–gel electrophoresis.[574] Sedimentation equilibrium experiments on the enzyme (native molecular weight 113 000) in 6M-GuCl in the absence of thiol reagents gave an apparent molecular weight of 56 000, but this fell to 30 400 in the presence of 2-mercaptoethanol. Detergent–gel electrophoresis has been especially valuable in the determination of the extremely complicated subunit structures of the DNA-dependent RNA polymerase enzymes.[575, 576] These exist in three major classes which differ in function. For example, a class II polymerase of molecular weight 554 000 from a mouse tumour had polypeptide chains of molecular weights 240 000, 140 000, 41 000, 30 000, 25 000, 22 000, 20 000, and 16 000. One chain of each size was present with the exception of the 20 000 chain, of which there were two.

The annelid haemoglobins and mollusc erythrocruorins also have complex structures, and are very different from vertebrate haemoglobins. Annelid haemoglobins have molecular weights of over 3×10^6 and characteristically appear as a stack of two hexagonal plates in electronmicrographs. Furthermore the haem content indicates a minimum molecular weight of 22 000—27 000 rather than 17 000 as is found with vertebrate haemoglobins. A number of studies have appeared[577–581] in which the molecular weights of polypeptide chains derived from annelid haemoglobins were determined by detergent–gel electrophoresis. Most of the annelid haemoglobins that have been investigated contain four to six types of polypeptide chain with molecular weights in the region 13 000—25 000, but there is as yet no clue to how the pieces fit together, and with the haem, to form the native molecule. Waxman[582] has speculated that if the apparent lack of stoicheiometry between the number and molecular weights of the polypeptide chains and the haem content is not an artifact of isolation (as it could be), it may reflect an inability of these proteins to bind all haem groups with equal affinity, or may even be attributable to an inefficient mechanism for inserting haem into newly synthesized globin chains. The mollusc haemoglobins,

[571] O. H. W. Kao, M. R. Edwards, and D. S. Berns, *Biochem. J.*, 1975, **147**, 63.
[572] K. K. Kalghatgi and P. V. Subba Rao, *Biochem. J.*, 1975, **149**, 65.
[573] E. K. Tong and H. W. Duckworth, *Biochemistry*, 1975, **14**, 235.
[574] R. Jeffcoat, *Biochem. J.*, 1975, **145**, 305.
[575] V. E. F. Sklar, L. B. Schwartz, and R. G. Roeder, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 348.
[576] L. B. Schwartz and R. G. Roeder, *J. Biol. Chem.*, 1975, **250**, 3221.
[577] R. C. Terwilliger, N. B. Terwilliger, and R. Roxby, *Comp. Biochem. Physiol.*, 1975, **50B**, 225.
[578] R. L. Garlick and R. C. Terwilliger, *Comp. Biochem. Physiol.*, 1975, **51A**, 849.
[579] S. N. Vinogradov, S. L. Hevsey, and R. Shukuya, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 505.
[580] J. M. Shlom, L. Amesse, and S. N. Vinogradov, *Comp. Biochem. Physiol.*, 1975, **51B**. 389.
[581] M. R. Andonian, A. S. Barrett, and S. N. Vinogradov, *Biochim. Biophys. Acta*, 1975, **412**, 202.
[582] L. Waxman, *J. Biol. Chem.*, 1975, **250**, 3790.

or erythrocruorins, are different from the annelid and the vertebrate haemoglobins and seem to have polypeptide chains of molecular weight >220 000, and are thus rather like mollusc haemocyanins in this respect. *Planorbis* erythrocruorin [583] had a molecular weight of about 1.65×10^6 and was extremely stable to pH change, dissociating to mixtures of products only above about pH 9.9 and below about pH 2.0. It is curious that some freshwater snails, *e.g. Planorbis*, *Biomphalaria*, should have an erythrocruorin, whilst others, *e.g. Lymnaea*, have a haemocyanin.[584] Even more remarkable is the report [585] that haemolymph of the mollusc, *Aplysia californica*, contains both a haemocyanin *and* an erythrocruorin. Interestingly, a haemoglobin from a holothurian had a subunit molecular weight of 18 000 by detergent–gel electrophoresis.[586] The native oxy-form was a dimer, but upon deoxygenation reversible tetramerization took place. This O_2-linked association–dissociation was studied by sedimentation velocity and by gel filtration in the presence and in the absence of dithionite.

Examples of Associating–Dissociating Systems.—One of the most important features of many proteins is their ability to associate to produce an extended macrostructure, often in conjunction with other proteins or with nucleic acids. Virus coat protein is an example of self-association, and microtubule formation, multi-enzyme complex, and ribosome assembly are examples of more complicated systems. The indefinite head-to-tail polymerization of a haemocyanin [505] and of the enzyme glutamine synthetase [504] have already been mentioned.

Microtubule Formation. The magnesium-induced self-association of calf brain tubulin has been mentioned above.[495] A number of other factors besides magnesium influence the formation of microtubules, however. One of these factors seems to be an accessory protein of high molecular weight in the absence of which tubulin will not polymerize. The usual way of preparing tubulin is to form microtubules, and to separate them by centrifugation, and then to dissociate them to obtain tubulin. The high molecular weight protein co-purifies in this procedure. Keates and Hall [587] found that the protein had a molecular weight of *ca.* 360 000 by SDS–gel electrophoresis. Other workers have reported lower molecular weights, and have also suggested that two or more proteins are involved. Murphy and Borisy [588] separated a high molecular weight fraction from tubulin by DEAE–Sephadex chromatography which promoted ring formation at 5 °C and microtubule formation at 37 °C. This fraction appeared to facilitate the formation of ring structures which may be intermediates in polymerization, and was itself incorporated into the microtubules as a structural component projecting from the surface of the microtubule wall. Weingarten *et al.*[589] and also Bryan *et al.*[590] found a heat-stable protein, which they called τ, in tubulin from pig

[583] E. J. Wood and L. J. Mosby, *Biochem. J.*, 1975, **149**, 437.
[584] R. L. Hall, J. S. Pearson, and E. J. Wood, *Comp. Biochem. Physiol.*, 1975, **52B**, 211.
[585] F. A. Bevelagna, K. S. Kim, M. H. Kumarasiri, and J. H. Schwartz, *J. Biol. Chem.*, 1975, **250**, 731.
[586] R. C. Terwilliger, *Biochim. Biophys. Acta*, 1975, **386**, 62.
[587] R. A. B. Keates and R. H. Hall, *Nature*, 1975, **257**, 418.
[588] D. B. Murphy and G. G. Borisy, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2696.
[589] M. D. Weingarten, A. H. Lockwood, S.-Y. Hwo, and M. W. Kirschner, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1858.
[590] J. Bryan, B. W. Nagle, and K. H. Doenges, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3570.

brain, which was essential for microtubule formation. Sloboda *et al.*[591] found there to be two proteins associated with microtubules from chick brain. One of these, molecular weight 300 000, was the preferred substrate for an endogenous cAMP-dependent protein kinase, which appeared to be an integral component of the microtubule. cAMP stimulates phosphorylation of tubulin, and the idea that cAMP plays a role in microtubule formation is supported by data from a number of laboratories. Shikagawa and Olsen[592] also detected a cAMP-dependent kinase, and Sheterline and Schofield[593] obtained a preparation of pituitary microtubules that contained both a protein kinase and a protein phosphatase. The degree of phosphorylation of microtubules from HeLa cells was different at different times during the cell cycle,[594] and Ohtsubo *et al.*[595] separated the α- and β-subunits of tubulin by electrophoresis after S-sulphonation, and found that both could be phosphorylated *in vitro* at least.

The mitosis-arresting alkaloid, vinblastine, not only causes the dissociation of microtubular structures *in vivo*, but can also induce the formation of different molecular organizations of tubulin. It has been used a great deal in the study of microtubule formation.[596] Lee *et al.*[597] found that vinblastine induced the stable tubulin dimers to dimerize further to form tetramers and they studied this reaction by sedimentation velocity. They also measured the binding of vinblastine by gel filtration and spectrofluorometry. There were two vinblastine-binding sites per tubulin dimer of molecular weight 110 000 and the association constant was 2.3×10^4 l mol^{-1} at 25 °C. Binding of vinblastine, however, did not induce any significant conformational changes in tubulin as monitored by circular dichroism.

Virus Assembly. The theory of length regulation in linear protein aggregates has been explored in terms of statistical thermodynamics,[598] and this should be useful in understanding the assembly not only of viruses but also of multisubunit proteins. Two models were considered, the length-determining factor model, and the cumulated strain model, and calculations were made with particular reference to bacteriophage T4 tail-core polymerization. The self-association of bacteriophage T4 gene 32 protein was studied by analytical ultracentrifugation by Carroll *et al.*[599] and is interesting because the process is not one of indefinite association with one association constant (*i.e.* monomer $\overset{k_2}{\rightleftharpoons}$ dimer $\overset{k_2}{\rightleftharpoons}$ trimer $\overset{k_2}{\rightleftharpoons}$ *etc.*). In fact there was evidence for more than one type of protein–protein interaction, and this may explain the previous observation that T4 gene 32 protein bound tightly to DNA at high concentrations but more loosely at lower concentrations (called 'co-operative binding' by Alberts *et al.*).[600] Such different interactions may be involved in a physiological function such as recombination.

[591] R. D. Sloboda, S. A. Rudolph, J. L. Rosenbaum, and P. Greengard, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 177.
[592] B. L. Shigekawa and R. W. Olsen, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 455.
[593] P. Sheterline and J. G. Schofield, *F.E.B.S. Letters*, 1975, **56**, 297.
[594] R. Piras and M. M. Piras, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1161.
[595] K. Ohtsubo, H. Sakai, H. Murofushi, and R. Kuriyama, *J. Biochem.* (*Tokyo*), 1975, **77**, 17.
[596] M. Ventilla, C. R. Cantor, and M. L. Shelanski, *Arch. Biochem. Biophys.*, 1975, **171**, 154.
[597] J. C. Lee, D. Harrison, and S. N. Timasheff, *J. Biol. Chem.*, 1975, **250**, 9276.
[598] T. Wagenknecht and V. A. Bloomfield, *Biopolymers*, 1975, **14**, 2297.
[599] R. B. Carroll, K. Neet, and D. A. Goldthwaite, *J. Mol. Biol.*, 1975, **91**, 275.
[600] B. M. Alberts, F. J. Amodio, M. Jenkins, E. D. Gutman, and F. L. Ferris, 'Cold Spring Harbor Symposium Quantitative Biology', 1968, **33**, 289.

Another interesting virus protein is the coat protein of bacteriophage f1.[601] This protein resembles many membrane proteins and exists as a dimer even in the presence of SDS showing the existence of unusually strong forces of self-association.

Much progress has been made in the study of tobacco mosaic virus (TMV) assembly utilizing a number of techniques. There is now *X*-ray crystallographic data at 5 Å resolution enabling prediction of the structure of the TMV disc.[602, 603] The disc seemed to have a more open structure, apparently designed for rapid interaction with the RNA. It has been proposed[604] from an electron micrographic examination of the assembly process that the protein subunits interact with the RNA tail protruding from the growing rod and become incorporated into the rod-shaped structure. A study of the pH-dependent formation of the double disc from the A protein by the stopped-flow method[605] indicated a triphasic reaction sequence: nucleation, followed by propagation, followed by dissociation of overshoot and redistribution (slow). No first-order process was observed under the conditions used. Proton binding to TMV protein exhibited hysteresis resulting from the metastable nature of the high molecular weight helical, virus-like rods.[606] The assembly of cowpea chlorotic mottle virus also showed evidence of metastable states.[607]

Multienzyme Complexes. A number of the enzyme systems found in cells are highly organized assemblies of proteins often containing several enzymic activities, and sometimes they are associated with membranes. The study of these macromolecular particles has recently been most fruitful: the number and size of the components may be determined by detergent–gel electrophoresis, and the interrelation between the different components may, as with ribosomal proteins (see below), sometimes be determined by cross-linking. Many groups have studied the copper- and haem-containing complex, cytochrome oxidase.[608, 609] This appears to contain six or seven proteins, and intriguingly some of these are synthesized on mitochondrial ribosomes and some on cytoplasmic ribosomes. In yeast, cytochrome oxidase subunits I—III (mitochondrial synthesis) had molecular weights of 40 000, 33 000, and 22 000, and were relatively hydrophobic, while subunits IV—VII (cytoplasmic synthesis) had weights 14 500, 12 700, 12 800, and 4600 and were all relatively hydrophobic.[609] The *Candida utilis* enzyme contained *ca.* 9 nmol haem *a* per mg, and had a molecular weight of *ca.* 200 000 as measured by gel filtration in the presence of 2% cholate.[610] The molecular weights of the six component proteins were 49 000, 32 000, 28 000, 20 000, 13 500, and 8000, but not much could be said about the proportions in the complex due to the variable amount of lipid present. (The association with

[601] S. Makino, J. L. Woolford, C. Tanford, and R. E. Webster, *J. Biol. Chem.*, 1975, **250**, 4327.
[602] A. C. H. Durham and P. J. G. Butler, *European J. Biochem.*, 1975, **53**, 397.
[603] J. N. Champness, A. C. Bloomer, G. Bricogne, P. J. G. Butler, and A. Klug, *Nature*, 1976, **259**, 20.
[604] Y. Okada, T. Ohno, and Y. Nonomura, *J. Biochem.* (*Tokyo*), 1975, **77**, 1157.
[605] A. L. Adiarte, D. Vogel, and R. Jaenicke, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 432.
[606] R. B. Scheele and T. M. Schuster, *J. Mol. Biol.*, 1975, **94**, 519.
[607] B. Jacrot, *J. Mol. Biol.*, 1975, **95**, 433.
[608] T. Kuronen, M. Saraste, and N. Ellfolk, *Biochim. Biophys. Acta*, 1975, **393**, 48.
[609] R. O. Poynton and G. Schatzm, *J. Biol. Chem.*, 1975, **250**, 752, 767.
[610] J. Keyhani and E. Keyhani, *Arch. Biochem. Biophys.*, 1975, **167**, 588, 596.

membranous structures means that detergents have to be used in the preparation of the complex from mitochondria.[611]) Eytam *et al.*[612] have made a start on studying the topology of the enzyme complex in relation to the membrane. They coupled exposed subunits to a radio-labelled, membrane impermeable reagent, then lysed the membrane and obtained the cytochrome oxidase by immunoprecipitation. The six subunits were separated by detergent–gel electrophoresis and their radioactivity was determined. On the basis that only exposed subunits would become labelled they were able to conclude that subunits II, V, and VI were on the outer side of the membrane, subunit III was on the matrix side (because it became labelled only in submitochondrial particles), and that subunits I and IV are buried inside the membrane.

The next steps in such investigations are the identification of activities associated with the different components and the reassembly of dissociated complexes from the components so that the factors necessary for assembly can be studied. The fact that many of the component proteins are fairly small gives some hope that chemical studies may eventually reveal the structural features necessary for complex formation. In several instances preparative separation and enzymic activity determinations have been performed. The citrate lyase from *Klebsiella aerogenes* was separated into the three component chains by DEAE–cellulose chromatography in the presence of 5M urea.[613] The native complex has the structure $(\beta\alpha\gamma)_6$. The α-chain catalyses the formation of the citryl thioester and the liberation of acetate, the β-chain catalyses the cleavage of citryl-*S*-acetyl carrier protein to yield oxaloacetate, and the γ-chain *is* acyl carrier protein. In an extensive series of studies[614] the pyruvate dehydrogenase complex of *Azotobacter vinelandii*, molecular weight $1—1.2 \times 10^6$, was investigated and a model presented for the arrangement of the different enzyme functions. Reed *et al.*[615] studied *E. coli* pyruvate dehydrogenase using sedimentation equilibrium analysis and other techniques. Their results indicated that the transacetylase which consists of 24 identical polypeptide chains organized into a cubic structure could bind 24 pyruvate dehydrogenase dimers in the absence of flavoprotein and 24 flavoprotein dimers in the absence of pyruvate dehydrogenase. In the native complex, however, the stoicheiometry appeared to be transacetylase : pyruvate dehydrogenase dimer : flavoprotein dimer, 24 : 12 : 6.

Other enzyme complexes that have been examined are rat liver pyruvate carboxylase,[616] a tetramer containing 4 mol biotin, *E. coli* succinyl CoA synthetase,[617] structure $\alpha_2\beta_2$, where the α-chain has a molecular weight of 25 500 and the β-chain 38 500, chicken liver fatty acid synthetase,[618] a dimer of two (inactive) polypeptide chains of molecular weight 250 000, bacteriophage 4B RNA

[611] M. Briggs, P.-F. Kamp, N. C. Robinson, and R. A. Capaldi, *Biochemistry*, **14**, 5123.
[612] G. D. Eytan, R. C. Carroll, G. Schatz, and E. Racker, *J. Biol. Chem.*. 1975, **250**, 8598.
[613] P. Dimroth and H. Eggerer, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3458.
[614] T. W. Bresters, R. A. DeAbreau, A. De Kok, J. Visser, and C. Veeger, *European J. Biochem.*, 1975, **59**, 335, 347, 355.
[615] L. J. Reed, F. H. Pettit, M. E. Eley, L. Hamilton, J. H. Collins, and R. M. Oliver, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3068.
[616] K. Nakashima, F. B. Rudolph, T. Wakabayashi, and H. A. Lardy, *J. Biol. Chem.*, 1975, **250**, 331.
[617] P. H. Pearson and W. A. Bridger, *J. Biol. Chem.*, 1975, **250**, 4451.
[618] J. K. Stoops, M. J. Asslanian, Y. H. Oh, K. C. Aune, T. C. Vanamar, and S. J. Wakil, *Proc. Nat. Acad. Sci. U.S.A* , 1975, **72**, 1940.

replicase,[619] which has subunits of molecular weight 70 000, 65 000, 45 000, and 35 000, and *Euglena* acetyl CoA carboxylase,[620] of molecular weight 360 000.

Ribosomal Assembly. If viruses and multienzyme complexes are complicated, ribosomes are much more so, being systemic clusters of up to 20 proteins with RNA.[621] Several different approaches are being used in the study of these entities. These range from experiments in which protein components are removed and then replaced,[622] to neutron-scattering measurements. Treatment of *E. coli* 50S ribosomes with 2M-LiCl washes out a group of 18 proteins.[623] One of these could be shown to be essential for the reconstitution of peptidyl transferase activity. Another three proteins were shown to be essential for assembly to give a 70S ribosome.[624] The primary structure of ribosomal protein L27 from the peptidyl-tRNA binding site has been determined.[625] Interactions between various ribosomal proteins have been investigated by analytical ultracentrifugation. Rohde *et al.*[626, 627] determined the molecular weights of proteins S3, S4, S5, S6, S7, S8, and S20 from the 30S subunit of the *E. coli* ribosome under the conditions of subunit assembly. All exhibited molecular weights consistent with monomeric behaviour except S8, which tended to self-associate. When S3 and S5 were mixed under the same conditions they associated with a stoicheiometry of 1 : 1 and an association equilibrium constant of 5.7×10^5 l mol^{-1}. Proteins S5 and S10 also interacted with a similar association equilibrium constant. The kinetics of assembly of proteins into a 30S ribosome have been analysed by preparing fluorescent derivatives of the proteins.[628] Re-assembly was a first-order process and similar rate constants were obtained in all four cases, suggesting that the rate-limiting step is a co-operative folding or organization of many proteins.

Attempts have been made to determine the arrangement of proteins in ribosomes by electron microscopy using antibodies to ribosomal protein[629] and by the use of cross-linking reagents followed by electrophoretic identification of products.[630] An extension of this latter technique involves the use of reversible bifunctional cross-linking reagents such as dimethyl 3,3′-dithiobispropionimidate.[631] Kenny *et al.*[632] treated *E. coli* 50S ribosomal subunits with 4-mercaptobutyrimidate and then promoted the formation of disulphide bonds by gentle oxidation. Electrophoretic analysis of the proteins afterwards revealed three pairs of cross-linked proteins. The electrophoresis was performed in one direction on a polyacrylamide slab gel which was then soaked in 2-mercaptoethanol

[619] R. A. Young and T. Blumenthal, *J. Biol. Chem.*, 1975, **250**, 1829.
[620] J. S. Wolpert and M. L. Ernst-Fonberg, *Biochemistry*, 1975, **14**, 1095, 1103.
[621] S. Mizushima and M. Nomura, *Nature*, 1970, **226**, 1214.
[622] J. H. Highland and G. A. Howard, *J. Biol. Chem.*, 1975, **250**, 831.
[623] V. G. Moore, R. E. Atchison, G. Thomas, M. Morau, and H. F. Noller, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 844.
[624] M. Kazemie, *European J. Biochem.*, 1975, **58**, 501.
[625] R. Chen, L. Munde, and V. Arfsten, *F.E.B.S. Letters*, 1975, **59**, 96.
[626] M. F. Rohde, S. O'Brien, S. Cooper, and K. C. Aune, *Biochemistry*, 1975, **14**, 1079.
[627] M. F. Rohde and K. C. Aune, *Biochemistry*, 1975, **14**, 4344.
[628] K.-H. Huang and C. R. Cantor, *J. Mol. Biol.*, 1975, **97**, 423.
[629] G. W. Tischendorf, H. Zeichhardt, and G. Stöffler, *Proc. Nat., Acad. Sci. U.S.A.*, 1975, **72**, 4820.
[630] A. Expert-Bezançon, D. Barritault, J. C. S. Clegg, M. Milet, Y. Khouvine, and D. H. Hayes, *F.E.B.S. Letters*, 1975, **59**, 64.
[631] A. Ruoho, P. A. Bartlett, A. Dutton, and S. J. Singer, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 417.
[632] J. W. Kenny, A. Sommer, and R. R. Traut, *J. Biol. Chem.*, 1975, **250** 9434.

solution before electrophoresis at right angles. All unaffected proteins lay on the diagonal, any proteins unlinked by the 2-mercaptoethanol lay off the diagonal and so stood out.

Serdyuk and Grenader [633] used light, *X*-ray, and neutron scattering to investigate RNA and protein distributions in *E. coli* 50S particles, and concluded that the RNA was in the centre. Engelman *et al.*[634] used neutron scattering to measure the separation and shape of proteins in 30S particles. They specifically deuteriated pairs of proteins which enabled distances between the centres of gravities of these pairs to be determined. For example the S2—S5 distance was 10.5 nm and the S5—S8 distance 3.5 nm. They also demonstrated a method for extracting shape information and concluded that S5 had a compact configuration but S2 was extended.

[633] I. N. Serdyuk and A. K. Grender, *F.E.B.S. Letters*, 1975, **59**, 133.
[634] D. M. Engelman, P. B. Moore, and B. P. Schoenbom, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3888; D. R. Engelman and P. B. Moore, *ibid.*, 1972, **69**, 1997.

3
Peptide Synthesis

BY E. ATHERTON AND R. C. SHEPPARD
Appendices compiled by
G. A. FLETCHER AND J. H. JONES

1 Introduction

The past year has been one of modest achievement in peptide and protein synthesis. No pinnacles comparable to the Ciba-Geigy group's human insulin synthesis (1974) or the solid-phase ribonuclease synthesis (1969) have been surmounted, though efforts of equal magnitude continue in several laboratories. Indeed, reports from the conferences suggest that the final steps in some synthetic strategies – removal of protecting groups from the completed polypeptide chain – may yet provide formidable barriers to the generation of large biologically active products. There have been some encouraging developments in solid-phase synthesis, both in methodology and achievement. Again it may be the final step, cleavage of the completed polypeptide from the resin, which will need to be made less brutal before the full potential of the method for protein synthesis will be realized. This year, more papers on partially synthetic operations on proteins are reviewed than previously, perhaps indicating a slowly developing trend. On the methodology side, base-labile protecting groups have achieved practical importance, and there have been a number of particularly ingenious proposals for novel protecting groups and coupling methods. Doubtless some of these will fall by the wayside for lack of simplicity and practicality, but the continued flow of new ideas augurs well in a field which has been active since the turn of the century.

The general format of this chapter is the same as that for previous volumes. The Proceedings of the 13th European Peptide Symposium[1] were published during the period under review and selected papers are discussed as appropriate. However, despite commendably rapid publication, the Proceedings of the 4th American Peptide Symposium[2] were received in England just too late to be included, and only one or two papers available to the reporters in preprint form are reviewed. The present chapter includes the now customary lists of synthetic peptides and useful intermediates. Other lists have been published by Fletcher and Jones,[3] and by Pettit.[4]

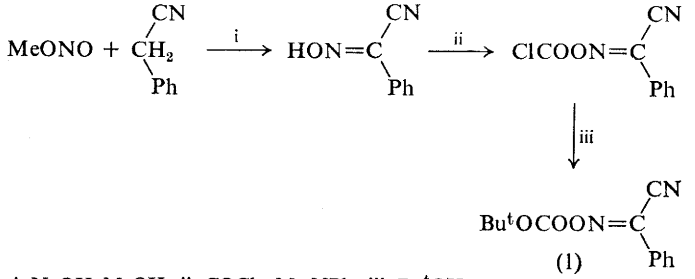
This Report contains several references to the published papers of the late Joseph Rudinger. All who have been associated with peptide synthesis during the

[1] 'Peptides 1974.' Proceedings of the Thirteenth European Peptide Symposium, Israel, 1974, ed. Y. Wolman, Wiley, New York, 1975.
[2] 'Peptides: Chemistry, Structure and Biology.' Proceedings of the Fourth American Symposium, New York, 1975, ed. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, Ann Arbor, 1975.
[3] G. A. Fletcher and J. H. Jones, *Internat. J. Peptide Protein Res.*, 1975, 7, 91.
[4] G. R. Pettit, 'Synthetic Peptides', Academic Press, New York, Vol. 3, 1975.

past 25 years will know of the influence Dr. Rudinger has had on the development of the subject during this period, through his research contributions, as founder of the European Peptide Symposium, and as an international ambassador. All will have been saddened by his untimely death.

2 Methods

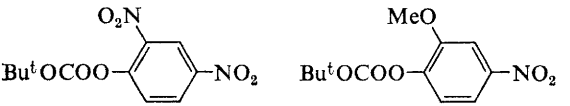
Protective Groups.—*Established Methods of Amino-group Protection.* New methods for the preparation of t-butoxycarbonylamino-acids continue to be introduced. An interesting reagent is the oximino derivative (1), prepared from benzyl cyanide by the route shown (Scheme 1).[5] It is a stable crystalline solid



Reagents: i, NaOH–MeOH; ii, $COCl_2$, Me_2NPh; iii, Bu^tOH, py

Scheme 1

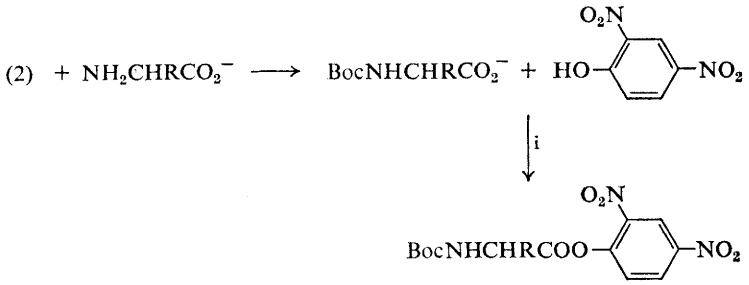
which reacts with the triethylammonium salts of amino-acids within 1 h at 45 °C – more rapidly than does t-butoxycarbonyl azide. The method has also been applied to the preparation of *p*-methoxybenzyloxycarbonyl derivatives. The dinitrophenyl (2)[6] and 4-nitroguiacyl (3)[7] derivatives join the list of mixed



carbonates useful for the preparation of t-butoxycarbonyl and other urethane-type protected amino-acids. Both reagents lend themselves to the one-step preparation of t-butoxycarbonylamino-acid active esters by recombining the phenol liberated in the initial acylation reaction[8] (*e.g.* Scheme 2).

A note of caution has been sounded in the activation of t-butoxycarbonyl-amino-acids for peptide synthesis.[9] After reaction between t-butoxycarbonyl-alanine and dicyclohexylcarbodi-imide for 10 min, ninhydrin-reacting side-products could be detected by chromatography. These have been tentatively identified as free alanine, alanylalanine, alanine t-butyl ester, and possibly

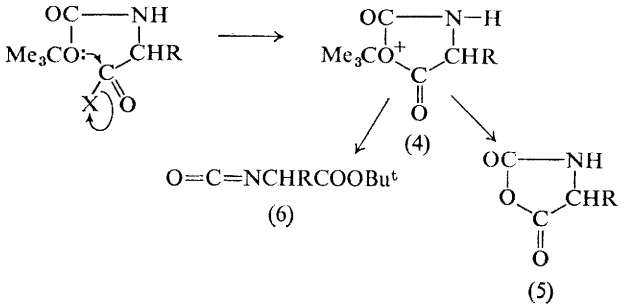
[5] M. Itoh, D. Hagiwara, and T. Kamiya, *Tetrahedron Letters*, 1975, 4393.
[6] V. F. Pozdnev, *Khim. prirod Soedinenii*, 1975, 437.
[7] S. Klimkiewicz and S. Drabarek, *Roczniki Chem.*, 1975, **49**, 959.
[8] W. Broadbent, J. S. Morley, and B. E. Stone, *J. Chem. Soc.* (*C*), 1967, 2632.
[9] M. Bodanszky, Y. S. Klausner, and A. Bodanszky, *J. Org. Chem.*, 1975, **40**, 1507.



Reagent: i, DCC

Scheme 2

polymer. The same impurities were observed when solvents ranging from methylene chloride to dimethylformamide were used, and activation was carried out with ethyl chloroformate, Woodward's reagent K, or EEDQ. None of these methods of activation produced comparable contaminants from benzyloxycarbonylamino-acids. A plausible explanation[9] is that some attack by the urethane oxygen takes place (Scheme 3, arrows), and that the intermediate (4)



Scheme 3

decomposes in the two ways giving the *N*-carboxyanhydride (5) and isocyanate t-butyl ester (6). Under the conditions of chromatography, these could give rise to the observed products. There are no indications of the seriousness of these side-reactions under the conditions investigated, but since, in solid-phase synthesis, t-butoxycarbonylamino-acids are now commonly activated (to form symmetrical anhydrides) in the absence of the amino nucleophile, the results should be noted.

It has been reported that toluene-*p*-sulphonic acid in ethanol–ether mixtures is capable of cleaving t-butoxycarbonyl groups selectively in the presence of *C*-terminal and side-chain t-butyl and *p*-methoxybenzyl esters.[10] Likewise, trimethylsilyl perchlorate in benzene or benzene–acetonitrile mixtures is claimed to cleave t-butoxycarbonyl groups selectively in the presence of side-chain t-butyl esters within 5 min at room temperature.[11]

[10] J. Goodacre, R. J. Ponsford, and I. Stirling, *Tetrahedron Letters*, 1975, 3609.
[11] H. Vorbruggen and K. Krolikiewicz, *Angew. Chem. Internat. Edn.*, 1975, **14**, 818.

A number of additional 2-phenylisopropoxycarbonylamino-acids (7) have been prepared. These derivatives are marginally less acid-labile than the established biphenylyl derivatives (8), but are more easily accessible. They have been used



(7) R = Ph
(8) R = C_6H_4Ph

successfully in a briefly reported solid-phase synthesis of the 18-residue toxin apamin.[12]

A full account of the kinetic studies of acidolysis in trifluoroethanol solution of trityl and other acid-labile protecting groups has now been published.[13] Previously, efficient differentiation between trityl (Trt) and biphenylyl (Bpoc) or phenylisopropoxycarbonyl (Ppoc) derivatives has not been possible since their relative stabilities (in 80% acetic acid) are of the order of 1 : 7 : 30. In 90% trifluoroethanol, however, differentiation between trityl and the urethane-type amino-protecting groups is much enhanced (Table 1). The reaction is carried out by titration (pH-stat) of solutions in 90% trifluoroethanol with 0.1N-HCl in the same solvent to an apparent 'pH' indicated by a glass electrode. At low apparent 'pH's', this procedure also provides an efficient differentiation between Bpoc or Ppoc and Boc derivatives (Table 1).

Table 1 *Acidolysis of protected dipeptide esters by hydrogen chloride in* 90% *trifluoroethanol at various apparent* 'pH's' [13]

	'pH' 4		'pH' 1		'pH' 0	
Compound	$t_{\frac{1}{2}}$/min	K_{rel}	$t_{\frac{1}{2}}$/min	K_{rel}	$t_{\frac{1}{2}}$/min	K_{rel}
Trt-Leu-Val-OMe	0.8	2000				
Bpoc-Leu-Val-OMe		5–10	1.1	2500		
Ppoc-Leu-Val-OMe		1	10.5	300	1.2	1500
Boc-Leu-Val-OMe				1		1

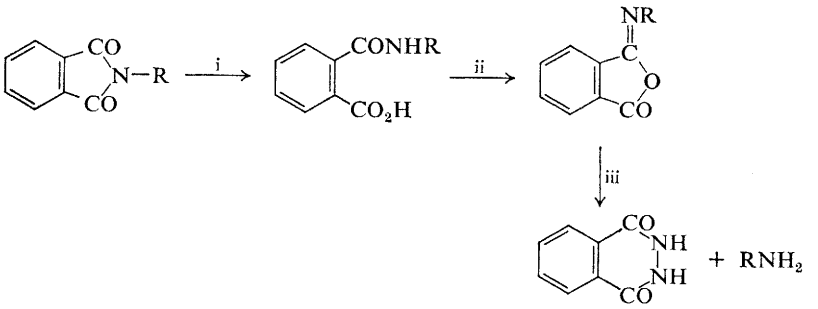
Trityl derivatives of amino-acids with bulky side-chains are apparently destabilized by steric compression, and their cleavage rates vary widely (Table 2). Similar variations were not found for biphenylyl and phenylisopropoxycarbonyl derivatives.

Table 2 *Acidolysis of* N-*trityl derivatives by hydrogen chloride in* 90% *trifluoroethanol* [13]

	'pH' 4	
Compound	$t_{\frac{1}{2}}$/min	K_{rel}
Trt-Val-Val-OMe	0.3	20
Trt-Leu-Val-OMe	0.8	7.5
Trt-Gly-Val-OMe	5.6	1

[12] B. E. B. Sandberg and U. Ragnarsson, *Internat. J. Peptide Protein Res.*, 1975, **7**, 503.
[13] B. Riniker, B. Kamber, and P. Sieber, *Helv. Chim. Acta*, 1975, **58**, 1086.

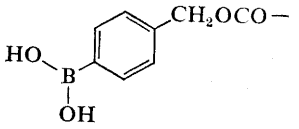
A new but multistage procedure for the cleavage of phthaloyl groups from acid- and base-sensitive compounds has been described.[14] This involves mild hydrolysis to the phthalamic acid, recyclization to the isoimide, and hydrazinolysis (Scheme 4). In contrast to the direct hydrazinolysis of phthalimides, the last step takes place with 1 equivalent of hydrazine at −20 °C.



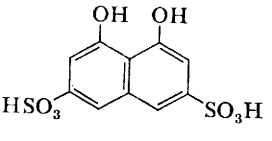
Reagents: i, sodium sulphide in aq. acetone or THF; ii, ethyl chloroformate–NEt_3, or DCC, or trifluoroacetic anhydride–NEt_3; iii, 1 equiv N_2H_4, −20 °C, 20—30 min in THF

Scheme 4

New Methods of Amino-group Protection. This year, emphasis can be seen on the design of new amino protective groups cleavable under specific (non-acidolytic) conditions, and on groups which confer favourable solubility or other purification properties on synthetic peptides. A particularly interesting example is provided by the boronic acid derivative (9).[15] This, the *p*-dihydroxyborylbenzyloxycarbonyl or Dobz protecting group, retains the general character of benzyloxycarbonyl derivatives (cleaved by hydrogenolysis, hydrogen bromide in acetic acid) but has special features associated with the ability of the boronic acid to complex reversibly with diols. Thus Dobz derivatives complex at pH 7 with the hydrophilic diol, chromotropic acid (10), and protected peptides, *e.g.* (11), can thus be



(9)



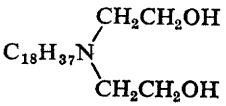
(10)

Dobz-Lys(Z)-Ala-Val-OEt

(11)

Bpoc-Asn-Phe-Phe-Trp-Lys(Dobz)-OBu^t

(12)

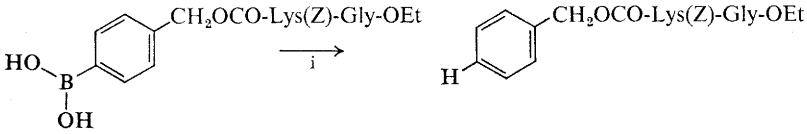


(13)

[14] S. Kukolja and S. R. Lammert, *J. Amer. Chem. Soc.*, 1975, **97**, 5582.
[15] D. S. Kemp and D. C. Roberts, *Tetrahedron Letters*, 1975, 4629.

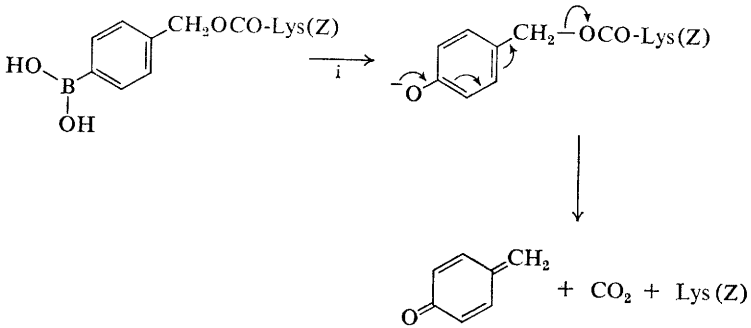
purified by extraction into neutral aqueous solution. An exception is the extremely lipophilic peptide (12). The complexes are decomposed at pH 1—2 or by excess boric acid. Complex formation can also occur with hydrophobic diols leading to enhanced solubility in non-polar solvents, *e.g.* Dobz-alanine is soluble in carbon tetrachloride containing *N*-octadecyldiethanolamine (13). In principle, the boronic acid substituent provides a potential ligand for affinity chromatography on diol-containing (polysaccharide ?) supports, but practical conditions have yet to be established.

Protection of the boronic acid moiety by complexation is necessary during introduction of the group *via* the chloroformate, and also in mixed anhydride condensations. This is unnecessary with other coupling methods (azide, dicyclohexylcarbodi-imide–hydroxybenzotriazole). The boronic acid substituent is removed by silver diammine cation at pH 7.5—8 giving the unsubstituted benzyloxycarbonyl derivative (*e.g.* Scheme 5), and oxidatively with hydrogen



Reagents: i, $AgNO_3$–NH_4OH, pH 7.7, 24 h, 25 °C, yield 90%

Scheme 5



Reagents: i, 1.1 equiv H_2O_2, 5 equiv SO_3^{2-}, borate buffer pH 9.5, 25 °C

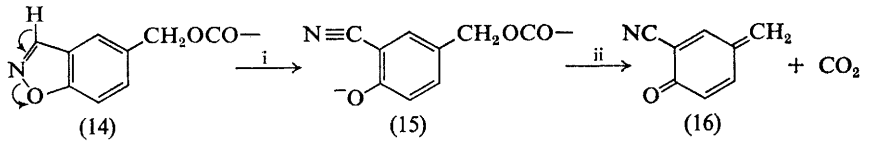
Scheme 6

peroxide at pH 9.5 to give the phenolate anion (Scheme 6). Spontaneous fragmentation of the latter to the quinone methide, carbon dioxide, and free amine provides an exceptionally mild and specific deprotection of Dobz-peptides. The conditions indicated enable removal of the protecting group without appreciable destruction of methionyl or tryptophyl residues and without further reaction between the liberated amine and quinone methide molecules.

A second modified benzyloxycarbonyl derivative from the same laboratory [16] is the 5-benzisoxazolylmethyleneoxycarbonyl (Bic) protecting group (14). Bic-protected amino-acids and peptides show exceptional base lability, and maybe

[16] D. S. Kemp and C. F. Hoyng, *Tetrahedron Letters*, 1975, 4625.

cleaved in a two-step process *via* the cyanophenolate (15) and quinone methide (16) (*e.g.* Scheme 7). The sensitivity to tertiary base is not sufficient to interfere with the usual neutralization processes of peptide synthesis (*e.g.* in the formation of mixed anhydrides) providing excess base is avoided. As expected, the group



(14) (15) (16)

Bic-Arg(NO_2)-Val-Tyr-Ile-His-Pro-Ala-ONB $\xrightarrow{i,ii}$ H-Arg(NO_2)-Val-Tyr-Ile-His-Pro-Ala-ONB

Reagents: i, NEt_3 (3 equiv), DMF, 30 min, 25 °C; ii, Na_2SO_3 in aq. EtOH, pH 7, 40 °C, 3 h, yield 91%

Scheme 7

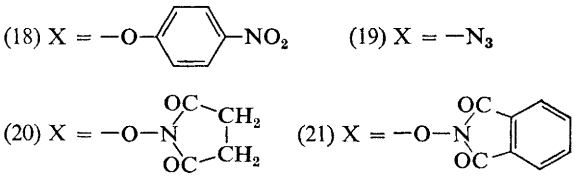
is cleaved by hydrogenolysis and hydrogen bromide, but is stable to trifluoroacetic acid.

Methylsulphonylethyloxycarbonyl (Msc) (17) is now well established as a useful amino-protecting group removable under resonably mild alkaline conditions.[17, 18] It can be introduced using a variety of mixed carbonate reagents, *e.g.* the nitrophenyl ester (18), azide (19), and succinimide (20) and phthalimide

$MeSO_2(CH_2)_2OCO-$

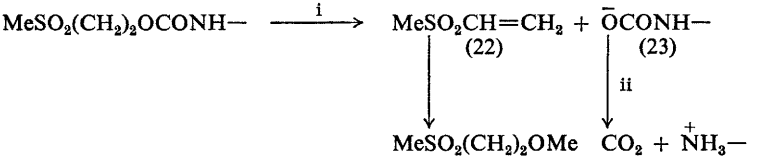
(17)

$MeSO_2(CH_2)_2OCOX$



(21) derivatives. The nitrophenyl ester appears to be the reagent of first choice. Msc-derivatives are very stable to acidic conditions, being unaffected by 90% trifluoroacetic acid during two weeks. The preferred reagent for cleavage is 0.1N-NaOH in a mixture of dioxan, methanol, and water. The β-elimination step (Scheme 8) appears to be complete within a few seconds. Two factors which may contribute to the cleanness and apparent speed of the reaction under these particular cleavage conditions are (i) the rapid quenching of the vinyl sulphone (22) by addition of methanol, and (ii) the stability of the carbamate (23) until the solution is acidified. There is thus little chance of irreversible back-addition of the liberated amine to the activated vinyl derivative – a potential hazard with all urethane-type amino-protecting groups cleaved by base-catalysed

[17] G. I. Tesser, ref. 1, p. 53.
[18] G. I. Tesser and I. C. Balvert-Geers, *Internat. J. Peptide Protein Res.*, 1975, **7**, 295.

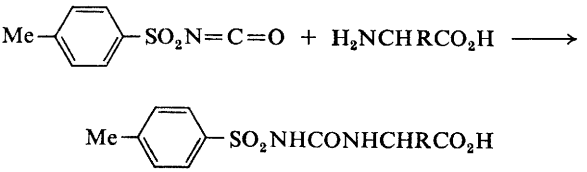


Reagents: i, dioxan (7.5), methanol (2.25), 4N-NaOH (0.25); ii, H^+

Scheme 8

elimination reactions. Side-chain t-butoxycarbonyl and t-butyl ester functions appear to be stable to the cleavage conditions, but *C*-terminal esters, even t-butyl esters, show some hydrolysis. The Msc group has found application for side-chain and α-amino protection [19, 20] (in one case the paper [19] also includes use of the related tosylethyloxy-group for carboxyl protection). It has also been used for the temporary protection of α- and ε-amino-groups in partially synthetic transformations of insulin [21] (p. 283).

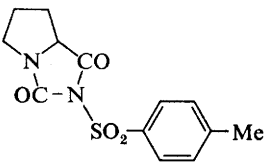
The utility of tosylaminocarbonyl (Tac)-amino-acids (24) in peptide synthesis has recently been examined.[22] These derivatives are obtained by reaction between tosyl isocyanate and free amino-acids (Scheme 9), but only in modest yield,



(24)

Scheme 9

presumably because the resulting carboxylic acids (24) are themselves capable of reaction with tosyl isocyanate.[23] The Tac-derivative of proline could not be obtained, and only the cyclized product (25) was isolated. This same cyclization



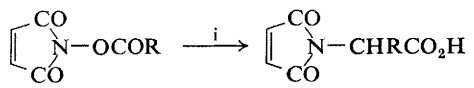
(25)

occurred during attempts to prepare activated ester derivatives of other Tac-amino-acids. Good yields of simple dipeptides were obtained by the dicyclohexylcarbodi-imide method, but the formation of cyclized by-products could be

[19] A. Eberle, J.-L. Fauchere, G. I. Tesser, and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 2106.
[20] J. W. van Nispen and G. I. Tesser, *Internat. J. Peptide Protein Res.*, 1975, **7**, 57.
[21] R. Geiger, K. Geisen, H.-D. Summ, and D. Langer, *Z. physiol. Chem.*, 1975, **356**, 1635.
[22] H. Künzi and R. O. Studer, *Helv. Chim. Acta*, 1975, **58**, 139.
[23] J. Rudinger, *Pure Appl. Chem.*, 1963, **7**, 335.

detected by thin-layer chromatography. The distinctive feature of the protecting group is its cleavage by solvolysis under neutral conditions, *e.g.* by heating in aqueous butanol or acetonitrile. Mercaptoethanol is also an effective nucleophile. It seems unlikely that the Tac group will become widely used for α-amino protection, but its neutral cleavage and stability to acidic (CF_3CO_2H and $HBr–CF_3CO_2H$) and basic (NaOH, KOH, N_2H_4) reagents may suggest application to side-chain protection. The neutral cleavage conditions may also be applicable to other acyl sulphonamide derivatives, *e.g.*—NHCHRCONHSO$_2$Ar, which have been suggested for *C*-terminal protection [23] and for solid-phase peptide–polymer linkage.[24] Conversely, Tac derivatives may be labilized to cleavage by mildly basic reagents by prior methylation of the acidic acylsulphonamide nitrogen atom.[23, 24]

Maleimido derivatives (26) of amino-acids which have previously been accessible only with difficulty have now been prepared *via* *N*-alkoxycarbonyl derivatives (27) (Scheme 10).[25] The carboxy-group of maleimido-acids can be activated,



(27) R = alkyl, aralkyl (26)

Reagents: i, $\overset{+}{N}H_3CHRCO_2^-$, $NaHCO_3$

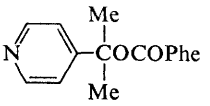
Scheme 10

e.g. as the succinimido-ester, and t-butyl-based protecting groups cleaved without damage to the maleimide ring. Maleimido derivatives are more sensitive to alkali than the analogous phthalimido compounds, and may be cleaved *via* the maleamic acid by successive mildly alkaline (pH 10) and mildly acidic (pH 2—3) treatments. Like phthalimido derivatives they are also cleaved by hydrazine. The generally high reactivity of maleimido derivatives may preclude their use as general protecting groups, but the reactivity of the olefinic bond towards thiols suggests application to the preparation of a variety of conjugates of biochemical interest, *e.g.* in immunology, affinity chromatography, *etc.*

A number of new basic analogues of protected amino-acid derivatives have been prepared.[26] These contain aromatic (pyridyl) or aliphatic tertiary amine groupings with potential value as 'handles' in purification procedures. Examples are the pyridyl derivative (28) (analogous to Ppoc-Phe), and the protected hydrazides (29) and (30). As expected, the aromatic tertiary amine substituent with the basic nitrogen conjugated with the incipient carbonium ion has a greater stabilizing effect towards acidic cleavage.

In an attempt to improve the solubility properties of protected peptides, new protected amino-acid derivatives have been prepared containing tertiary amide (dimethylcarbamoyl) substituents.[26] Examples are the phenylalanine derivatives (32) and (34), analogues of the Boc and Z-derivatives. Introduction of the new

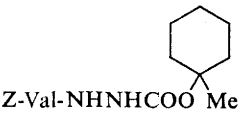
[24] G. W. Kenner, J. R. McDermott, and R. C. Sheppard, *Chem. Comm.*, 1971, 636.
[25] O. Keller and J. Rudinger, *Helv. Chim. Acta*, 1975, **58**, 531.
[26] S. Coyle, O. Keller, and G. T. Young, *J.C.S. Chem. Comm.*, 1975, 939.



(28)

Z-Val-NHNHCOOCPh$_2$(CH$_2$)$_2$NEt$_2$

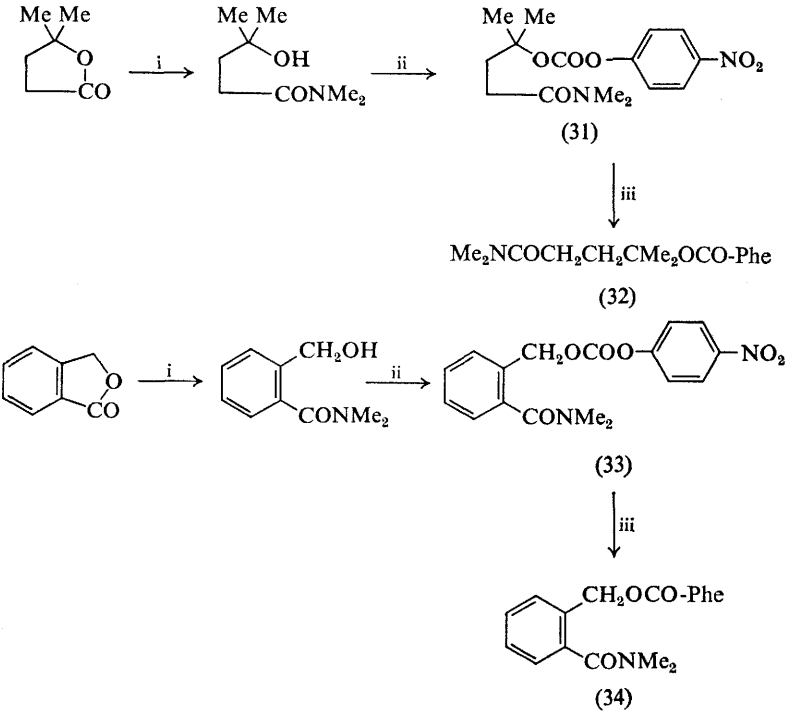
(29)



(30)

protecting groups is by way of the crystalline carbonates (31) and (33), prepared as indicated (Scheme 11).

An ingenious scheme of peptide synthesis using an enzymic deprotecting agent has been reported.[27] Activated amino-acids are N-protected as their benzyloxycarbonylarginyl derivatives as in the nitrophenyl esters (35). In the example described, the glutamine derivative (35; X = Gln) was coupled with the cystine



Reagents: i, Me_2NH; ii, $O_2NC_6H_4OCOCl$, 1-methyl morpholine; iii, Phe, tetramethylguanidine

Scheme 11

[27] C. Meyers and J. D. Glass, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2193.

peptide (36), excess nitrophenyl ester removed by addition of the glycine derivative of chloromethylated polystyrene, and the water-soluble peptide deprotected using trypsin at pH 8. No evidence was adduced concerning the possibility of racemization during the coupling reaction, but the tryptic hydrolysis was shown to possess a possibly useful degree of stereospecificity against any D-isomer formed. The basic guanidine group of the protecting arginine residue also provides a potential 'handle' for purification purposes, which, because of the large pK_a differences, may be useful for the separation from unchanged amino component.

Z-Arg-X-ONp
(35) X = Gln, Ile, Tyr, D-Gln

Asn-Cys-Leu-Pro-Gly-NH_2
|
Asn-Cys-Leu-Pro-Gly-NH_2
(36)

$Cl(CH_2)_2OCO-$
(37)

$PhCH_2OCH_2OCR$
(38)

The 2-chloroethyloxycarbonyl group (37) (introduced *via* the stable chloroformate) can be cleaved by cobaloxime [bis(dimethylglyoximato)cobalt complex] in the presence of sodium borohydride.[28] The reaction is thought to proceed by attack of cobaloxime supernucleophile on the chloroethyl group with formation of a labile alkylated intermediate containing a carbon–cobalt bond.

Protection of Terminal Carboxy-groups. There seems to have been little activity in this area during the past year. Benzyloxymethyl esters (38) have been suggested for general carboxyl protection.[29] They are prepared from benzyloxymethyl chloride and the sodium salt of the carboxylic acid, and are cleaved by mild acid treatment (1.5N-HCl in aqueous tetrahydrofuran) or by hydrogenolysis. They are thus comparable in reactivity to the isomeric *p*-methoxybenzyl esters. The liberation of formaldehyde during cleavage of benzyloxymethyl derivatives may cause complications in the peptide series.

Benzyl esters are conveniently prepared under non-acidic conditions by brief pyrolysis of the benzyldimethylphenylammonium salts (Scheme 12).[30] The

$$RCO_2^- \quad \begin{matrix} Me \\ +/ \\ N \\ \backslash \\ Bzl \end{matrix} \begin{matrix} Me \\ \diagup \\ \diagdown \\ Ph \end{matrix} \longrightarrow RCOOCH_2Ph + PhNMe_2$$

Conditions: 110 °C (boiling toluene), 5 min

Scheme 12

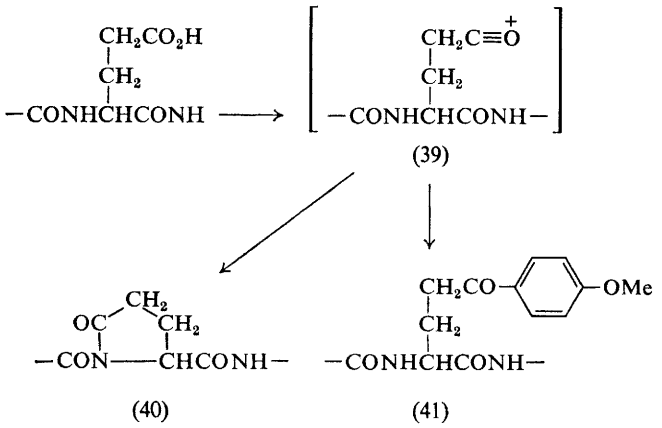
method has been applied particularly to tryptophan derivatives. A new method for the preparation of diphenyldiazomethane may be of value for the preparation of benzhydryl esters.[31]

[28] H. Eckert, G. N. Schrauzer, and I. Ugi, *Tetrahedron*, 1975, **31**, 1399.
[29] P. A. Zoretic, P. Soja, and W. E. Conrad, *J. Org. Chem.*, 1975, **40**, 2962.
[30] K. Williams and B. Halpern, *Austral. J. Chem.*, 1975, **28**, 2065.
[31] J. R. Adamson, R. Bywood, D. T. Eastlick, G. Gallagher, D. Walker, and M. Wilson, *J.C.S. Perkin I*, 1975, 2030.

Full details of the use of the 4-picolyloxycarbonyl group for the introduction and protection of *C*-terminal hydrazides have been published.[32] It provides an additional basic 'handle' facilitating isolation of products. A new method for the cleavage of acylhydrazides re-opens the possibility of using hydrazides as carboxy-protecting groups.[33] The conditions employed for cleavage (60% perchloric acid, 48 °C, 24 h) seem rather vigorous, although high yields of optically pure products were obtained with the simple model compounds studied.

Protection and Reactions of Side-chain Carboxy and Carboxamide Groups. A novel, high-yield synthesis of γ-esters of glutamic acid and β-esters of aspartic acid has been described [34] which uses the copper-catalysed hydrolysis of readily prepared diesters. Chelation of the α-ester to copper is thought to favour its selective hydrolysis. Isolation as the insoluble copper complex of the α-amino-acid monoester may further enhance the apparent selectivity. A synthesis of pteroyl-γ-glutamyl-γ-glutamyl-γ-glutamic acid using methyl esters for carboxy protection has been reported.[35] The product is claimed to be free from α-isomers even though protecting groups were removed by saponification.

Side-reactions occurring when γ-esters of glutamyl peptides are treated with liquid hydrogen fluoride–anisole mixtures have been thoroughly investigated.[36, 37] A number of decomposition products have been isolated and characterized, of which the most indicative were peptides containing γ-*p*-methoxybenzoyl-α-aminobutyric acid residues (41) in place of glutamic acid. Isolation of compounds of type (41) provides strong presumptive evidence for the formation of side-chain acylium ions (39) through the action of anhydrous hydrogen fluoride on γ-ester or carboxy groups (Scheme 13). Other compounds identified included



Scheme 13

[32] R. Macrae and G. T. Young, *J.C.S. Perkin I*, 1975, 1185.
[33] J. Schnyder and M. Rottenberg, *Helv. Chim. Acta*, 1975, **58**, 521.
[34] R. L. Prestidge, D. R. K. Harding, J. E. Battersby, and W. S. Hancock, *J. Org. Chem.*, 1975, 3287.
[35] P. K. Sengupta, J. H. Bieri, and M. Viscontini, *Helv. Chim. Acta*, 1975, **58**, 1374.
[36] S. Sano and S. Kawanishi, *J. Amer. Chem. Soc.*, 1975, **97**, 3480.
[37] R. S. Feinberg and R. B. Merrifield, *J. Amer. Chem. Soc.*, 1975, **97**, 3485.

further decomposition products of (41) and pyroglutamyl derivatives (40).[37] Fortunately, the formation of acylium ions appears to be strongly temperature dependent and can be minimized by operating at a suitably low temperature. However, the potential seriousness of these side reactions can be gauged from the observations that cleavage of the hexapeptide–polystyrene resin derivative (42) with hydrogen fluoride–anisole at 24 °C for 2 h gave only 10% of the desired hexapeptide, 79% of the γ-*p*-methoxybenzoyl derivative and 11% of cyclized pyroglutamyl compound. In the absence of anisole, the last product was formed to the extent of 66%. When cleaved at 0 °C for 30 min, the resin-bound peptide gave only 1.5% of by-products.[37] Remarkably, the aspartic acid analogue of (42) gave no evidence for acylation of anisole when cleaved at 24 °C, although 7% of succinimide derivative was formed. These results are clearly most immediately relevant to solid-phase synthesis (*q.v.*), but since hydrogen fluoride and other anhydrous strong acids (*e.g.* fluorosulphonic acid, see p. 262) may be used

Bpoc-Leu-Lys(Z)-Glu(OBzl)-Ala-Val-Gly-resin

(42)

$$\begin{array}{c} O^- \\ | \\ \text{Trp-Met-Asp-Phe-NH}_2 \end{array}$$

(43)

for general deprotection reactions in solution, they should be noted in a wider context.

A study of the active ester acylation of peptides bearing a free aspartyl β-carboxy-group, *e.g.* (43), has shown that ring closure to succinimide derivatives (and thence potentially to β-peptides) may occur.[38] The reaction was found to be particularly serious when the active ester was derived from Boc-glycine, but was independent of the nature of the phenolic component (*o*-nitrophenol, *p*-nitrophenol, 2,4,5-trichlorophenol). Neutralization of the side-chain carboxy-group with tertiary base favoured the cyclization reaction and in fact was unnecessary for efficient acylation of the terminal amino-group. It is suggested [38] that a mixed anhydride between Boc-glycine and the side-chain carboxy-group is an intermediate in succinimide formation, but transesterification to the side-chain active ester is also plausible.

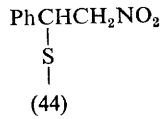
Active esters of benzyloxycarbonyl-asparagine have been shown to decompose in dimethylformamide at 25 °C (pentafluorophenyl ester, $t_{\frac{1}{2}}$ = 117 min; pentachlorophenyl ester, $t_{\frac{1}{2}}$ = 222 min; *p*-nitrophenyl ester, $t_{\frac{1}{2}}$ = 1296 min) with the formation of both nitrile and succinimide derivatives.[39]

Protection of the Arginine Guanidine Group. A study [40] of the reductive cleavage of ω-nitroarginine and a nitroarginyl peptide by dissolving zinc has shown that the nature of the acidic medium is critically important. Zinc in 85% acetic acid gave 60% ω-acetamidoarginine, and in 2N phosphoric acid 34% ω-aminoarginine

[38] M. Bodanszky and S. Natarajan, *J. Org. Chem.*, 1975, **40**, 2495.
[39] L. Kisfaludy, I. Schön, M. Rénejei, and S. Gorog, *J. Amer. Chem. Soc.*, 1975, **97**, 5588.
[40] A. Turan, A. Patthy, and S. Bajusz, *Acta Chim. Acad. Sci. Hung.*, 1975, **85**, 327.

and 6% ornithine. Side-product formation was minimal in 2N trifluoroacetic acid, which gave results rather comparable to those from hydrogenolysis.

Protection of Thiol Groups and the Synthesis of Cystine Peptides. Further studies have been reported relating to the preparation, stability, or use of various *S*-tetrahydropyranyl,[41, 46] diphenylmethyl,[41, 44] trityl,[41, 44] ethylsulphenyl,[42, 43] t-butylsulphenyl,[41] and *S*-t-butyl cysteine[45] derivatives. The *S*-methoxycarbonylsulphenyl group, originally introduced as a labilizing function for selective disulphide bond formation, has been shown to be sufficiently stable to function as a sulphur-protecting group.[47] A new (asymmetric) protecting and chiroptical reporter group for cysteine peptides is 2-nitro-1-phenylethyl (44), introduced with

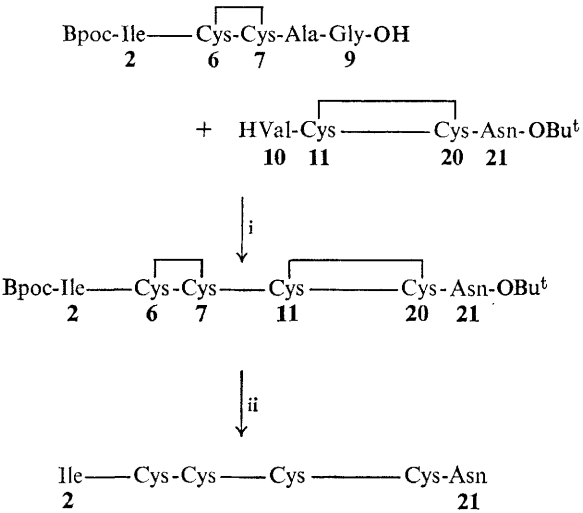


(44)

2-nitrostyrene and removed under mildly alkaline conditions by β-elimination, apparently without hydrolysis of methyl or benzyl esters.[48] Fontana has shown[49] that in addition to acetamidomethyl, several other S-protecting groups capable of giving stabilized cations are rapidly cleaved by *o*-nitrophenylsulphenyl chloride in acetic acid, *e.g.* trityl, diphenylmethyl, and 4,4′-dimethoxydiphenylmethyl. The free thiol is subsequently liberated by reduction (sodium borohydride, mercaptoethanol) of the intermediate *S*-nitrophenylsulphenyl derivative. *S*-Sulphonates of cysteine peptides are quantitatively cleaved by tributylphosphine;[50] the total reaction products can be air-oxidized to cystine peptides without isolation. With a mixture of the insulin A and B chain *S*-sulphonates, the overall yield of purified insulin was 8.5%.[50] A further report has appeared[44] on the thiocyanogen–sulphenyl thiocyanate method for the preparation of asymmetrical disulphides. Enzymic methods were used to establish the pairing of cysteine residues which showed that no disulphide interchange occurred during the reaction. Use of cyclic cystine peptide derivatives in synthesis effectively dispenses with the need for side-chain protection of cysteine residues. It may also confer favourable solubilities and purification properties on the intermediates. New syntheses of the insulin A chain residues 1—21 and 2—21 (Scheme 14) have been described using this approach.[51]

Protection of the Histidine Imidazole Group. A comparative study of the incorporation of a range of side-chain protected histidine derivatives during solid-phase peptide synthesis has been reported (see p. 271).[52]

[41] E. Schwertner, H. Berndt, H.-G. Gielen, and H. Zahn, *Annalen*, 1975, 581.
[42] H. Kunzek, C. Seidel, and G. Frache, *Z. Chem.*, 1975, **15**, 58.
[43] H. Kunzek, C. Seidel, E. Klauschenz, and K. D. Kaufmann, *Z. Chem.*, 1975, **15**, 20.
[44] R. G. Hiskey, C.-D. Li, and R. R. Vunnan, *J. Org. Chem.*, 1975, **40**, 3697.
[45] H. Kofod, ref. 1, p. 57.
[46] E. Schwertner, H. Klostermeyer, and H. Zahn, *Annalen*, 1975, 1092.
[47] R. G. Hiskey, N. Muthukumaraswany, and R. R. Vunnan, *J. Org. Chem.*, 1975, **40**, 950.
[48] G. Jung, H. Fouad, and G. Heusel, *Angew. Chem. Internat. Edn.*, 1975, **14**, 817.
[49] A. Fontana, *J.C.S. Chem. Comm.*, 1975, 976.
[50] U. T. Ruegg and H.-G. Gattner, *Z. physiol. Chem.*, 1975, **356**, 1527; U. T. Ruegg and J. Rudinger, *Israel J. Chem.*, 1974, **12**, 391.
[51] H. Berndt and H. Zahn, *Annalen*, 1975, 1601.
[52] O. Schou, K. Brunfeldt, I. Rubin, and L. Hansen, *Z. physiol. Chem.*, 1975, **356**, 1451.



Conditions: i, *N*-methylmorpholine, isobutyl chloroformate; ii, trifluoroacetic acid, mercaptoethanol

Scheme 14

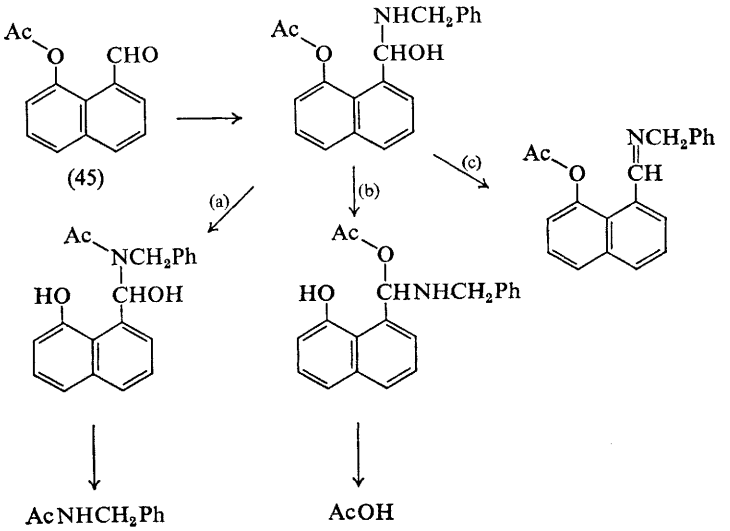
General Deprotection of Peptides. The use of trifluoromethane sulphonic acid for deprotection of synthetic peptides discussed last year (these Reports, Vol. 7, p. 258) has been applied to the synthesis of somatostatin.[53] The same laboratory has now reported similar surveys of the stability of protecting groups to fluorosulphonic acid and methane sulphonic acid.[54] Fluorosulphonic acid cleaves a wide range of acid-labile protecting groups [*o*-nitrophenylsulphenyl, t-butoxycarbonyl, benzyloxycarbonyl, t-butyl and benzyl esters, nitro (arginine), tosyl (arginine), and *p*-methoxybenzyl (cysteine)] within 30 min at 20 °C. A range of benzyl and t-butyl based protecting groups, as well as *o*-nitrophenylsulphenyl derivatives, are also cleaved by methane sulphonic acid under the same conditions.

Formation of the Peptide Bond.—A number of mechanistic and kinetic investigations of peptide bond formation have been reported. Rebek [55] has summarized his studies of carbodi-imide-mediated couplings and concludes that the major acylating species are *O*-acylisoureas in solution but symmetrical anhydrides under the conditions of solid-phase synthesis. Resin probe analysis [56] (see also 'solid-phase synthesis' section, p. 275) is proving a useful tool in rate studies. The rates of aminolysis (by *N*-methylbenzylamine) of a wide range of activated ester derivatives of benzyloxycarbonylglycine have been measured by n.m.r. spectroscopy in chloroform solution.[57] A ratio of 5000 : 1 was found for the rate constants of the most reactive (hydroxysuccinimide) ester to the least reactive (*p*-

[53] N. Fujii and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1596.
[54] H. Yajiman, Y. Kiso, H. Ogawa, N. Fujii, and H. Irie, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1164.
[55] J. Rebek, ref. 1, p. 27.
[56] A. M. Tometsko, M. Schreiner, and T. Cormstock, *Analyt. Biochem.*, 1975, **67**, 182.
[57] H. R. Kricheldorf, E. Stengele, and W. Regel, *Annalen*, 1975, 1379.

chlorophenate). Benzyloxycarbonylglycine imidazolide was included in this survey, and was the only compound to give exactly second-order kinetics for the acylation reaction. Divergence from second-order dependence for the activated ester derivatives may be due to protonation of the exceptionally basic amino component by the acidic phenol or alcohol, and ion-pair formation. A series of papers in the Russian literature [58–60] reports reaction rates for active ester acylation of t-butyl glycinate. Little effect of amino-acid side-chain reaction rate was found for the limited range of acylamino-acids studies (glycine, alanine, and phenylalanine).[59] Bifunctional catalysis of acylation by 2-pyridone was 5000 times greater in a non-polar solvent (cyclohexane) than in acetonitrile.[60] In chlorobenzene, acetic acid was a better catalyst.[60]

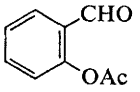
It has long been recognized that amide bond formation through first-order intramolecular rearrangment might provide a smoother pathway for peptide synthesis than the conventional biomolecular reaction. Kemp and his colleagues have now published [61–63] some thought-provoking studies in this area which bring the realization of a practical method a stage nearer. 8-Acetoxy-1-naphthaldehyde (45) reacts rapidly ($t_{\frac{1}{2}} < 1$ min) with benzylamine in dimethylformamide or dimethyl sulphoxide to yield some 70% of *N*-benzylacetamide and 30% of acetate ion.[61] Formation of these two products is rationalized by initial carbinolamine formation followed by O → N (path a) or O → O (path b) acyl transfer (Scheme 15). In less polar solvents dehydration of the carbinolamine to



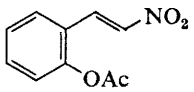
Scheme 15

[58] L. M. Litvinenko, V. A. Savelova, and L. P. Drizhel, *J. Org. Khimi* (*Russ.*), 1975, **11**, 1841.
[59] L. M. Litvinenko, V. A. Savelova, and L. P. Drizhel, *J. Org. Khimi* (*Russ.*), 1975, **11**, 1848.
[60] L. M. Litvinenko, L. P. Drizhel, V. A. Savelova, and V. I. Engei, *J. Org. Khimi* (*Russ.*), 1975, **11**, 1853.
[61] D. S. Kemp and F. Vellaccio, jun., *J. Org. Chem.*, 1975, **40**, 3003.
[62] D. S. Kemp and F. Vellaccio, jun., *J. Org. Chem.*, 1975, **40**, 3464.
[63] D. S. Kemp, J. A. Grattan, and J. Reczek, *J. Org. Chem.*, 1975, **40**, 3465.

Schiff base (path c) is also important, and this route is exclusive when benzylamine was replaced by alanine methyl ester or other substituted peptide amines. With the related *o*-acetoxybenzaldehyde (46), glycine ethyl ester gave 75—90% amide but alanine, phenylalanine, and valine methyl esters gave only dehydration products. A very marked steric dependence for acyl transfer was also found when the nitrostyrene derivative (47) was used for initial amine capture.[62]

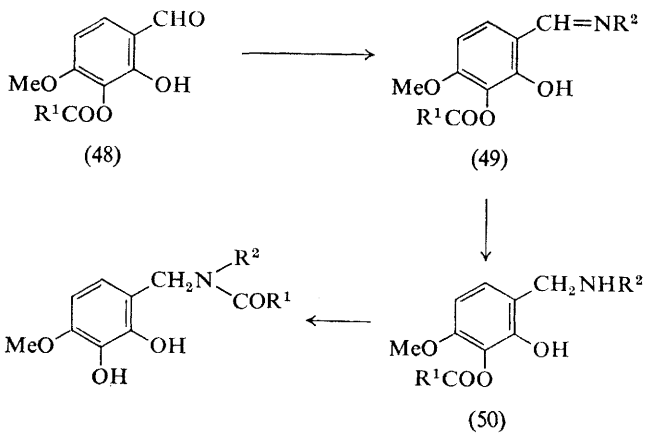


(46)



(47)

A longer but sterically less sensitive and more reliable route was developed based on Schiff base formation from substituted salicylaldehydes (Scheme 16).[63] Thus (48; R^1 = Z-$NHCH_2$-) reacted rapidly ($t_{\frac{1}{2}}$ 4—5 min) with the methyl esters of alanine, leucine, or phenylalanine forming the Schiff bases (49; R^1 = Z-$NHCH_2$-),



(48) (49) (50)

Scheme 16

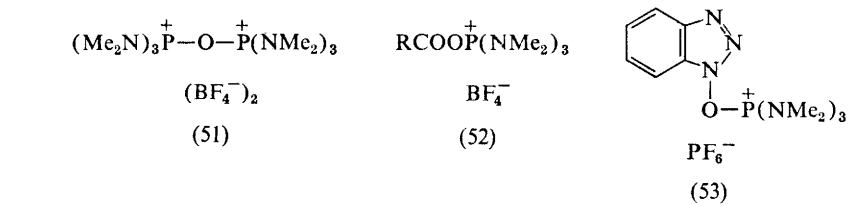
which were quantitatively reduced by pyridine borane to the substituted benzylamines (50). The O → N acyl transfer across the intervening free hydroxyl was relatively slow, with $t_{\frac{1}{2}}$ in the range 0.2—4 h in non-polar and longer in polar solvents. Cleavage of the 4-methoxy-2,3-dihydroxybenzyl substituent (which may confer favourable solubility properties on synthetic peptides) was readily achieved with trifluoroacetic acid.

Peptide bond formation also occurs intramolecularly in the four-component condensation (Ugi reaction). Four further papers[64–67] have been published

[64] R. Urban and I. Ugi, *Angew. Chem. Internat. Edn.*, 1975, **14**, 61.
[65] G. Zinner and O. Hantelmann, *Arch. Pharm.*, 1975, **305**, 321.
[66] I. Ugi, A. Arora, H. Burghard, G. Eberle, H. Eckert, G. George, G. Gokel, H. Herlinger, E. von Hinrichs, P. Hoffmann, H. Kleimann, H. Klusacek, H.-L. Lam, D. Marquarding, H.-S. Nah, K. Offermann, D. Rehn, S. Stuber, M. Tamasi, R. Urban, L. Wackerle, S. Zahr, and H. von Zychlinski, ref. 1, p. 71.
[67] R. Charles, B. Feibush, and E. Gil-Av, ref. 1, p. 93.

reviewing and describing further development of this reaction, which was discussed in detail in last year's Report (p. 262).

Full details have been published of the use of the bisphosphonium salt (51) in peptide synthesis.[68] This is a stable crystalline solid readily prepared by the action of toluene-*p*-sulphonic anhydride on hexamethylphosphoramide at 55 °C, and subsequent anion exchange. A careful n.m.r. study using both ^{31}P and ^{13}C resonances revealed no evidence for accumulation of the expected acyloxyphosphonium salts (52) in reactions of (51) with carboxylic acids. Evidently the initial attack on (51) is slow and rate-limiting, and is followed immediately by further reaction either with carboxylate to form symmetrical anhydrides, or with other nucleophiles present in the solution (hydroxybenzotriazole, peptide amine, *etc.*). In the presence of hydroxysuccinimide or hydroxybenzotriazole, the bisphosphonium salt (51) provides a practical reagent for fragment coupling in cases



$(Me_2N)_3\overset{+}{P}-O-\overset{+}{P}(NMe_2)_3$ $(BF_4^-)_2$ (51)

$RCOO\overset{+}{P}(NMe_2)_3$ BF_4^- (52)

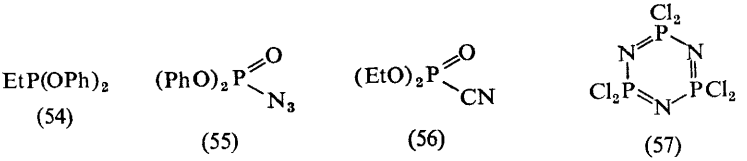
PF_6^- (53)

where racemization is not a problem. Results for a number of such couplings in the lysozyme series were favourable, particularly with respect to the ease of isolation of products.[68]

The oxybenzotriazolyltrisdimethylaminophosphonium cation may conceivably be an intermediate in reactions of the above type, and the hexafluorophosphate salt (53) of this cation has now been prepared and used directly in peptide synthesis.[69] It converts benzoic acid quantitatively into the hydroxybenzotriazolyl ester and has given good results in simple coupling reactions. Racemization in the Young test (Bz-Leu-Gly-OEt) was nearly complete, in contrast to the 3% racemization observed in the Izumiya test (Z-Gly-Ala-Leu-OBzl) with (51) in the presence of HOBt. This difference may reflect the different sensitivities of the two racemization tests or the different reaction conditions (base strength) employed, rather than indicate different reaction pathways.

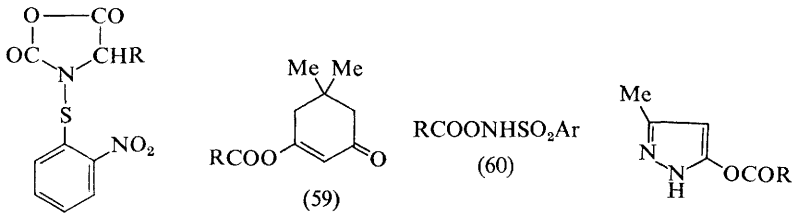
Earlier work on the use of phosphonium derivatives has been reviewed.[70] Other phosphorus derivatives investigated during the past year include diphenyl ethylphosphonite (54),[71] diphenyl phosphorazidate (55) and diethyl phosphorocyanidate (56)[72] (see also Vol. 6, p. 324), triphenyl phosphite–imidazole[73] (see Vol. 6, p. 313), and triphenylphosphine–carbon tetrachloride[74] (see Vol. 7, p. 265). Hexachlorotriphosphatriazene (57) is useful for the preparation of activated ester derivatives.[75]

[68] A. J. Bates, I. J. Galpin, A. Hallett, D. Hudson, G. W. Kenner, R. Ramage, and R. C. Sheppard, *Helv. Chim. Acta*, 1975, **58**, 688.
[69] B. Castro, J. R. Dormoy, G. Even, and C. Selve, *Tetrahedron Letters*, 1975, 1219.
[70] B. Castro and J. R. Dormoy, ref. 1, p. 43.
[71] N. Yamazaki, M. Niwano, J. Kawabata, and F. Higashi, *Tetrahedron* , 1975, **31**, 665.
[72] S. Yamada, N. Ikota, T. Shiori, and S. Tachibana, *J. Amer. Chem. Soc.*, 1975, **97**, 7174.
[73] A. T. Gudkov, *Zhur. obshchei Khim.*, 1975, **45**, 1893.
[74] R. Appel, G. Baumer, and W. Strüver, *Chem. Ber.*, 1975, **108**, 2680.
[75] J. Martinez and F. Winternitz, *Tetrahedron Letters*, 1975, 2631.

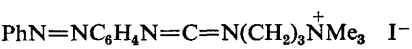


(54) (55) (56) (57)

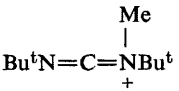
Further papers have appeared [76, 77] on the use of *o*-nitrophenylsulphenyl-*N*-carboxy-anhydrides (58). Despite the known optical lability of these derivatives, several simple peptides have been prepared in aqueous media without extensive racemization. Unsubstituted *N*-carboxy-anhydrides have also been used in aqueous phase couplings with a soluble polyethyleneimine support.[78] Cleavage of the synthetic *C*-terminal arginine peptide from the resin was achieved quantitatively with trypsin.[78]



(58) (59) (60) (61)



(62)



(63)

Enol esters (59) derived from dimedone have been prepared in good yield and used in simple couplings.[79] These derivatives may also be regarded as vinylogues of mixed anhydrides. Other activated derivatives investigated include esters of *N*-arylsulphonylhydroxylamines (60)[80] and of 5-hydroxy-3-methyl-1-phenylpyrazole (61).[81] Two further papers have appeared on the use of *N*-hydroxymorpholine esters.[82, 83] The new water-soluble and coloured carbodi-imide (62) has been prepared,[84] and the carbodi-imidium ion (63) shown to be modestly effective in peptide bond formation.[85] A quantitative study of the formation of

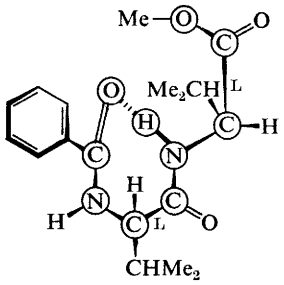
[76] R. Kataki and M. Oya, *Biopolymers*, 1975, **14**, 2507.
[77] R. Kataki, *J. Org. Chem.*, 1975, **19**, 2702.
[78] P. Pfaender, H. Pratzel, H. Blecher, G. Gorka, and G. Hansen, ref. 1, p. 137.
[79] T. Saito, *Chem. Letters*, 1975, 729.
[80] J. Przybylski and G. Kupryszewski, *Roczniki Chem.*, 1975, **49**, 529.
[81] H. G. Gorg and K. K. Shaunak, *Indian J. Chem.*, 1975, **13**, 301.
[82] K. Okamoto and S. Shimamura, *J. Pharm. Soc. Japan*, 1975, **95**, 269.
[83] K. Okamoto, H. Tanaka, and S. Shimamura, *J. Pharm. Soc. Japan*, 1975, **95**, 1138.
[84] G. N. Balandina, E. N. Lysogorskaya, E. A. Morozova, and V. M. Stepanov, *Khim. prirod Soedinenii*, 1975, **11**, 198.
[85] R. C. Schnur and E. E. van Tamelen, *J. Amer. Chem. Soc.*, 1975, **97**, 464.

N-acylureas in the reaction of various N-protected glycines with glycine ethyl ester and dicyclohexylcarbodi-imide gave the worst results with NPS-glycine (24.5%) and phthaloylglycine (18%). No acylurea was detected with Boc-glycine and only 5.5% with Z-glycine.[86]

Racemization.—^{1}H N.m.r. spectroscopy has been used to estimate the degree of racemization for a number of well-used coupling methods. The difference in chemical shifts of the methyl ester protons between the diastereoisomers (64) and

PhCO-Val-Val-OMe (64) PhCO-D-Val-Val-OMe (65)

(65) in deuteriochloroform is sufficient to allow accurate determination of the amount of racemate formed in a particular coupling reaction.[87] The difference in chemical shifts is solvent-dependent and also dependent on the presence of the benzoyl group, since no separation of the methyl ester signals was observed when the benzyloxycarbonyl group was used for N-protection. These separate ester signals can be explained by the hydrogen-bonded structure (66), since changes in



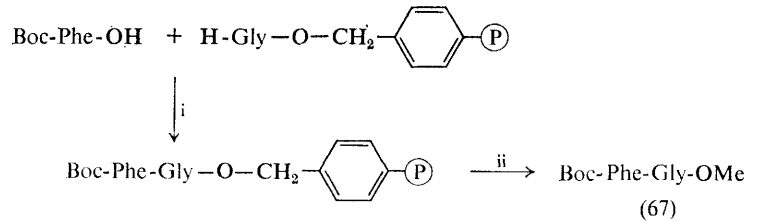
(66)

the stereochemistry of the *N*-terminal asymmetric centre would influence the position of the methyl ester relative to the benzene ring.

The preparation of the dipeptide (67) has been advocated as a model to assess racemization by the isotope dilution technique.[88] The amount of racemization showed a linear correlation with the concentration of the reactants in dichloromethane. By making use of this model the racemization was also determined with polymer-bound glycine mimicking typical solid-phase peptide synthesis conditions. The dipeptide was removed from the resin by diazomethane-catalysed transesterification (Scheme 17), and the amount of racemate determined to be 0.03%.

Isolation of the product formed from the Young racemization test, benzoyl-leucylglycine ethyl ester, by t.l.c. on silica plates developed in chloroform ethyl acetate (80 : 20), has been used to assess dicyclohexylcarbodi-imide (DCC) mediated couplings with and without additives.[89] The dipeptide was visualized

[86] J. Izdebski, S. Drabarek, and M. Lebek, *Roczniki Chem.*, 1975, **49**, 1535.
[87] J. S. Davies, R. J. Thomas, and (in part) M. K. Williams, *J.C.S. Chem. Comm.*, 1975, 76.
[88] J. Rebek and D. Feitler, *Internat. J. Peptide Protein Res.*, 1975, **7**, 167.
[89] J. Izdebski, *Roczniki Chem.*, 1975, **49**, 1097.



(67)

Reagents: i, DCC; ii, MeOH–CH_2N_2

Scheme 17

by spraying the plate with water, and after scraping from the plate was extracted with ethyl acetate. Examination of the product from several reactions in which *N*-hydroxy-compounds had been added to carbodi-imide couplings revealed that the additive 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine gave the highest yield of the L-isomer.

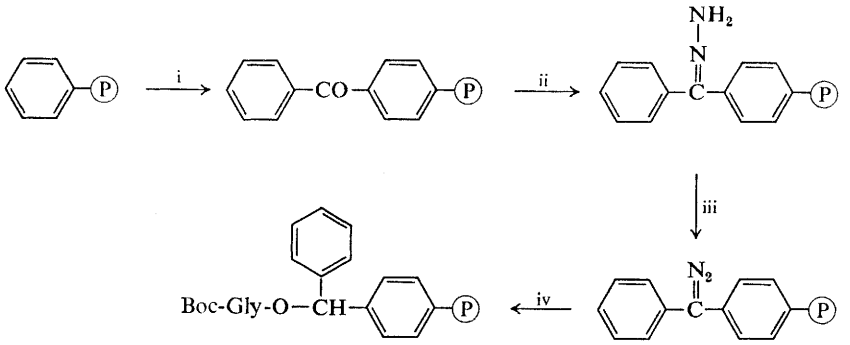
Repetitive Methods of Peptide Synthesis.—*Solid-phase Synthesis.* A book which collates papers important to the development of the use of solid supports in organic chemistry has appeared,[90] as has a critical review of the potential pitfalls associated with solid-phase peptide synthesis.[91]

Since an increasing number of peptides are now being prepared by solid-phase techniques relative to the classical approach, strenuous efforts must be made to establish the integrity of the final product. Small amounts of difficult to detect contaminants can lead to anomalous biological results. A good example where every effort has been made to establish the purity of a solid-phase product is the synthesis of camel β-MSH.[92] The product was found to be indistinguishable from the natural hormone by gel filtration, partition chromatography, paper electrophoresis (at pH 3.7 and 9.0), polyacrylamide gel electrophoresis, amino-acid analysis (acidic and enzymatic hydrolysis), tryptic and chymotryptic digestion, optical rotation, and biological activity. Obviously the more analytical tests a solid-phase product is subjected to the more convincing the final conclusions relating to its use will be.

An improved procedure for the chloromethylation of resins by use of boron trifluoride etherate and chloromethyl methyl ether has been reported.[93] The method offers easy control of the amount of chloromethylation in the range 0.1—0.6 meq g^{-1}. Resins prepared in this manner are claimed to swell to a greater extent than when conventional chloromethylation techniques are used, indicating a lower degree of concomitant cross-linking.

A new resin has been prepared which allows linkage of the first amino-acid as its diphenylmethyl ester.[94] Benzoylation of polystyrene, reaction with hydrazine and oxidation to the diazo derivative (Scheme 18), and reaction with Boc-glycine gave the diphenylmethyl ester. The Boc group was removed by conventional methods and a further glycine residue added in 84.5% yield.

[90] 'Solid Phase Synthesis', ed. E. C. Blossey and D. C. Neckers, Dowden, Hutchinson, and Ross, Stroudsburg, Pennsylvania, 1975.
[91] P. Fankhauser, P. Fries, R. Herlan, and M. Brenner, ref. 1, p. 191.
[92] C. H. Li, D. Yamashiro, and S. Lemaire, *Biochemistry*, 1975, **14**, 953.
[93] J. T. Sparrow, *Tetrahedron Letters*, 1975, **52**, 4637.
[94] P. H. Chapman and D. Walker, *J.C.S. Chem. Comm.*, 1975, 690.



Reagents: i, $PhCOCl$–$AlCl_3$; ii, N_2H_4,H_2O in Bu^nOH; iii, $MeCO_3H$, I_2 (catalyst), tetramethylguanidine; iv, Boc-Gly-OH

Scheme 18

Functionalization of polystyrene with *p*-(aminomethyl)phenoxymethyl groups allowing preparation of peptide amides has been described.[95] The method involves reaction of the chloromethyl resin with *p*-cyanophenol followed by reduction with lithium aluminium hydride in the presence of ammonia. The three peptide amides (68—70) were synthesized using Ppoc for N-protection and

Z-Pro-Leu-Gly-NH_2 (68)

Z-Ala-Phe-Gly-Leu-Met-NH_2 (69)

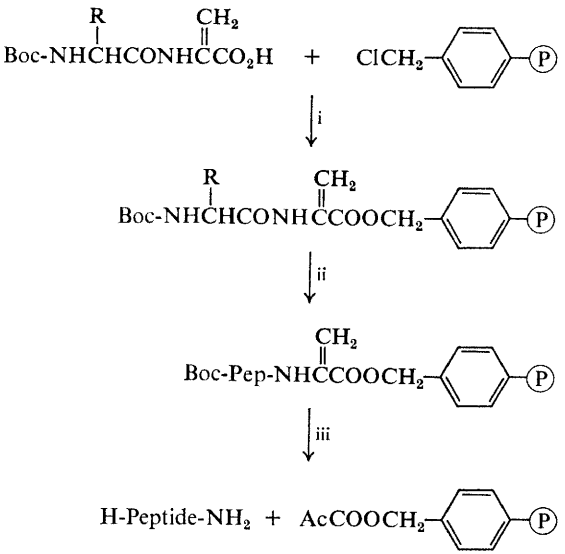
Z-Gln(Dmb)-Gly-Leu-Val-NH_2 (70)

50% trifluoroacetic acid in methylene chloride for final removal from the resin. This resin should prove to be a useful addition to the well-established benzhydrylamine polymer for the preparation of peptide amides.

Another resin linkage which can be cleaved to give peptide amides is provided by inserting a dehydroalanine residue between the peptide and the polymer.[96] t-Butoxycarbonyldipeptides with *C*-terminal dehydroalanine residues can be attached to the resin in the usual manner and cleaved at the end of the synthesis to release the peptide amide (Scheme 19). Protection of ω-amido functions of asparagine and glutamine residues can be accomplished in a like manner with dehydroalanine methylamide. Using this method several peptide amides have been prepared, including TRH.

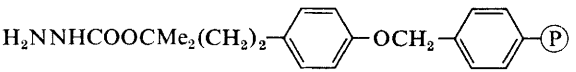
A detailed account of the synthesis of the hydrazide resin (71) has been published.[97] In conjunction with Bpoc for amino-group protection several protected peptide hydrazides were made. It was also noted that protected peptide hydrazides produced by hydrazinolysis of peptide bound through benzyl esters were more readily crystallized when hydroxymethyl polymer was used in place of the conventional chloromethyl polymer (see also p. 273).

[95] P. Pietta and O. Brenna, *J. Org. Chem.*, 1975, **40**, 2995.
[96] E. Gross, K. Noda, and S. Matsuura, ref. 1, p. 403.
[97] S.-S. Wang, *J. Org. Chem.*, 1975, **40**, 1235.



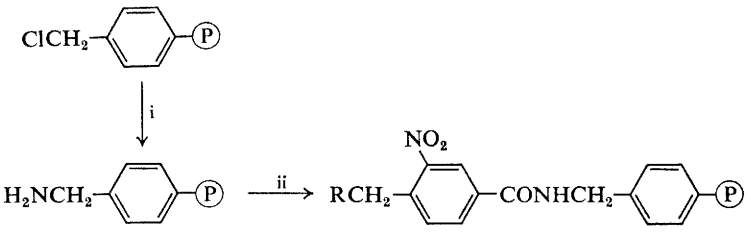
Conditions: i, DMF–Et_3N; ii, normal solid-phase cycles; iii, 1M-HCl–AcOH, 1 equiv H_2O, 50 °C for 30 min

Scheme 19



(71)

A resin incorporating a photolabile functionalizing group has been described, thus allowing the synthesis of protected peptide fragments which could be potentially useful in the fragment condensation procedure.[98] The resin (72; R = Br) was synthesized by ammonolysis of chloromethyl polystyrene, followed by a DCC-mediated coupling with 4-bromomethyl-3-nitrobenzoic acid (Scheme 20). Boc-amino-acids can be attached and peptides assembled in the normal



(72)

Conditions: i, NH_3; ii, 4-bromomethyl-3-nitro-benzoic acid (R = Br) or 4-t-butoxycarbonyl-aminomethyl-3-nitrobenzoic acid (R = Boc-NH)

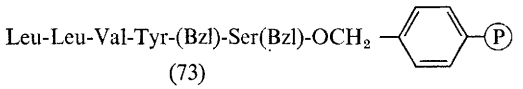
Scheme 20

[98] D. H. Rich and S. K. Gurwara, *J. Amer. Chem. Soc.*, 1975, **97**, 1575.

manner. Removal is affected by irradiation of a suspension of peptide resin in anhydrous methanol or ethanol. Several protected peptides, all with *C*-terminal glycine residues, have been made in overall yields of up to 64%.

Peptide amides can also be similarly prepared using the resin (72; R = Boc-NH-).[99] After removing the Boc group, peptide synthesis can proceed in the usual manner and photolysis at the completion of the assembly of the sequence yields the peptide amide directly. LH-RH in a pure protected form was isolated in an overall yield of 60% by this method.

The coupling yields in solid phase of several histidine derivatives have been estimated by potentiometric titration with perchloric acid.[100] N^{α}-Boc-protected histidine derivatives were coupled to (73) with DCC, in some cases with an *N*-hydroxy additive. Highest yields were obtained when the benzyl group was used for imidazole protection in the presence of *N*-hydroxybenzotriazole or *N*-hydroxysuccinimide, but considering the difficulties in final removal of this protecting group, Boc-His(Ztf) seems to be preferred. In the synthesis of camel β-MSH, Boc-His(Boc) was incorporated successfully.[92]



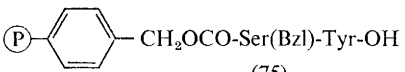
(73)

Boc-Ile-OPcp

(74)

Although the main method of coupling in solid-phase synthesis is *via* DCC, more groups are turning to the use of pre-formed symmetrical anhydrides. These offer the advantage of very rapid acylation without the necessity of adding DCC to the actual resin and are reputed to reduce the amount of chain termination in a synthesis.[101] An illustration of the use of trichlorophenyl active esters in conjuction with *N*-hydroxybenzotriazole in solid-phase synthesis has been described in a rapid synthesis of oxytocin.[102] Couplings were completed within 90 min. Incorporation of (74) took 210 min (completeness of acylation was monitored by the Kaiser ninhydrin test).

A synthesis of ACTH (1—24) employing the oxidation–reduction condensation of fragments onto a solid support in a reverse manner, *i.e.* extension of the chain from the *N*-terminus, has been described.[103] Starting with a benzylchloroformate type polymer, Ser(Bzl)-Tyr(Bu^t)-OBu^t was attached to give (75) after treatment with trifluoroacetic acid. Five fragments (76)—(80) were then sequentially coupled in the presence of 30-fold excesses of the reagents, triphenylphosphine,



(75)

Ser(Bzl)-Met-Glu(OBzl)-His-Phe-OBu^t

(76)

Arg(Tos)-Trp-Gly-OBu^t

(77)

[99] D. H. Rich and S. K. Gurwara, *Tetrahedron Letters*, 1975, 301.
[100] O. Schou, K. Brunfeldt, I. Rubin, and L. Hansen, *Z. physiol. Chem.*, 1975, **356**, 1451.
[101] J. Blake and C. H. Li, *Internat. J. Peptide Protein Res.*, 1975, **7**, 495.
[102] S. A. Khan and K. M. Sivanandaiah, *Tetrahedron Letters*, 1976, 199.
[103] R. Matsueda, H. Maruyama, E. Kitazawa, H. Takahagi, and T. Mukaiyama, *J. Amer. Chem. Soc.*, 1975, **97**, 2573.

Lys(Z)-Pro-Val-Gly-OBu^t
(78)

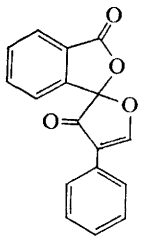
Lys(Z)-Lys(Z)-Arg(Tos)-Arg(Tos)-Pro-OBu^t
(79)

Val-Lys(Z)-Val-Tyr(Bu^t)-Pro-OBu^t
(80)

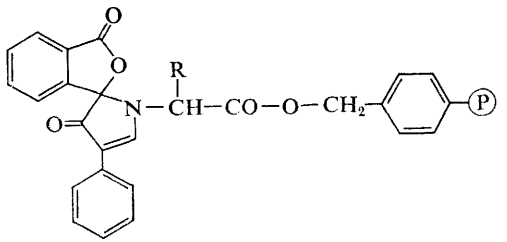
2,2′-bipyridyl disulphide, and 2-mercaptopyridine (except with a resin peptide terminating in glycine or proline) in dichloromethane for 6 h at −15 °C. Coupling rates were monitored by using high-pressure liquid chromatography on an aminated Hitachi Gel 3101, and taken as complete when no further uptake of a fragment was recorded. After treatment with hydrogen fluoride and purification, the ACTH (1—24) was obtained in a 25% yield based on the starting resin (75) and had a biological activity identical with the natural molecule.

Diethyl phosphorocyanidate, DEPC (56), and diphenyl phosphorazidate, DPPA, (55) have been used in a fragment condensation synthesis of motilin.[104] Trial experiments established that the rates of coupling of Boc-isoleucine to glycyl-polymer were much faster in dimethylformamide using (55) and (56) than with DCC. In the Izumiya test,[105] the two reagents gave 2.0 and 2.5% racemate respectively under appropriate conditions. In the motilin synthesis, DEPC was used to condense three fragments, one of which had an asymmetric methionine *C*-terminus. After extensive purification, the product was judged to be optically pure and possessed biological activity similar to that of the natural compound. An impressive conventional fragment condensation of insulin A-chain is described later (p. 290).

For couplings that do not go to completion, fluorescamine (81) has been added to the growing number of terminating reagents in use for blocking unchanged amino-groups.[106] Fluorescamine reacts smoothly and efficiently with resin-bound primary amino-groups under mild conditions and is converted by acid treatment in the next cycle into the non-fluorescent derivative (82). The superiority



(81)



(82)

Leu-Ala-Gly-Val

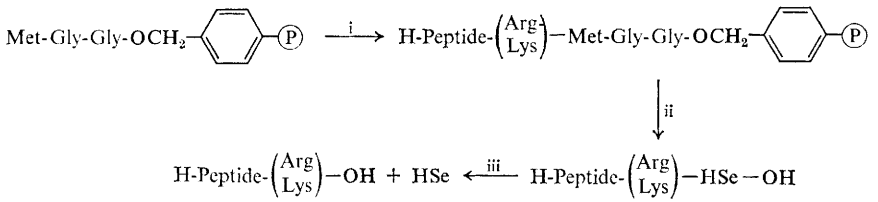
(83)

[104] S. Yamada, N, Ikota, and T. Shioiri, *J. Amer. Chem. Soc.*, 1975, **97**, 7174.
[105] N. Izumiya, M. Muraoka, and H. Aoyagi, *Bull. Chem. Soc. (Japan)*, 1971, **44**, 3391.
[106] A. M. Felix, M. H. Jimenez, R. Vergona, and M. R. Cohen, *Internat. J. Peptide Protein Res.*, 1975, **7**, 11.

of the fluorescamine procedure over acetylation was adequately demonstrated by comparative preparations of (83). However, in similar syntheses of angiotensin II the products could not be distinguished analytically, but that from the synthesis using fluorescamine termination had higher biological activity.

On the other hand, there is no general reason to suppose that residual amino-groups unreactive to acylation by amino-acid derivatives will react quantitatively with any other reagent, whether it be for deliberate chain termination or analytical purposes. The aim in solid-phase synthesis must still be to seek reaction conditions in which amino-termini are reliably accessible to all reagents.

Removal of peptides from phenacyl ester type resins by hydrazinolysis in dimethylformamide is claimed to be smoother than from benzyl ester resins [107] (see also ref. 97). The cyanogen bromide reaction widely used for the cleavage of proteins and peptides at methionine residues has been exploited in solid-phase peptide synthesis for final cleavage from the resin.[108] This reaction is specific and mild and can be incorporated into a programmed cycle. Presumably because of steric hindrance the methionine residue needed to be separated from the polymer by two glycine residues. After assembly, the peptide is removed from the resin with cyanogen bromide in propionic acid (Scheme 21). Removal of the



Conditions: i, normal solid-phase assembly; ii, excess CNBr in propionic acid; iii, digestion with carboxypeptidase A

Scheme 21

C-terminal homoserine residue is accomplished by treatment with carboxypeptidase A to which protected arginine or lysine residues are resistant. This is an interesting approach which provides an alternative to the almost universally used cleavage by hydrogen fluoride. Its general utility needs to be demonstrated in the synthesis of larger peptides since there was a notable fall of in cleavage yield as the chain was extended from di- to hexa-peptide. It is becoming evident that the use of hydrogen fluoride is fraught with difficulties (see p. 259) and other milder, less abrasive means of removing solid supports from assembled peptides will be very useful.

Supports used for Solid-phase Synthesis. A possibly important new development is the preparation of functionalized polydimethylacrylamide resins specifically designed for peptide synthesis.[109, 110] Hitherto, the overwhelming majority of

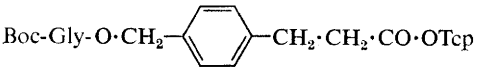
[107] Y. Maki, H. Sugiyama, K. Kikuchi, and S. Seto, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 929.
[108] W. S. Hancock and G. R. Marshall, *J. Amer. Chem. Soc.*, 1975, **97**, 7488.
[109] E. Atherton and R. C. Sheppard, ref. 1, p. 123.
[110] E. Atherton, D. L. J. Clive, and R. C. Sheppard, *J. Amer. Chem. Soc.*, 1975, **97**, 6584.

solid-phase syntheses have utilized polystyrene supports selected for their ready availability rather than for their complete chemical suitability. With such non-polar supports, differences in solvation between the peptide and polymer may be a cause of local collapse either of the resin matrix or of the peptide chain, leading in both cases to reduced steric accessibility. The solution envisaged for this problem was the use of resins readily solvated by polar organic solvents (*e.g.* dimethylformamide) in which protected peptides are most soluble. Preliminary results confirming the validity of this approach were obtained using chemically modified commercial polyacrylamide.[109] The final resin was obtained[110] by emulsion copolymerization of the monomer, dimethylacrylamide (84) in the presence of cross-linking agent, *NN'*-bisacryloylethylene diamine (85) (in commercial polyacrylamide the cross-linking agent is methylene bisacrylamide), and

$H_2C{=}CHCONMe_2$ (84)

$H_2C{=}CHCONH(CH_2)_2NHCOCH{=}CH_2$ (85)

$BocNHCH_2CH_2CONH(CH_2)_6NHCOCH{=}CH_2$ (86)



(87)

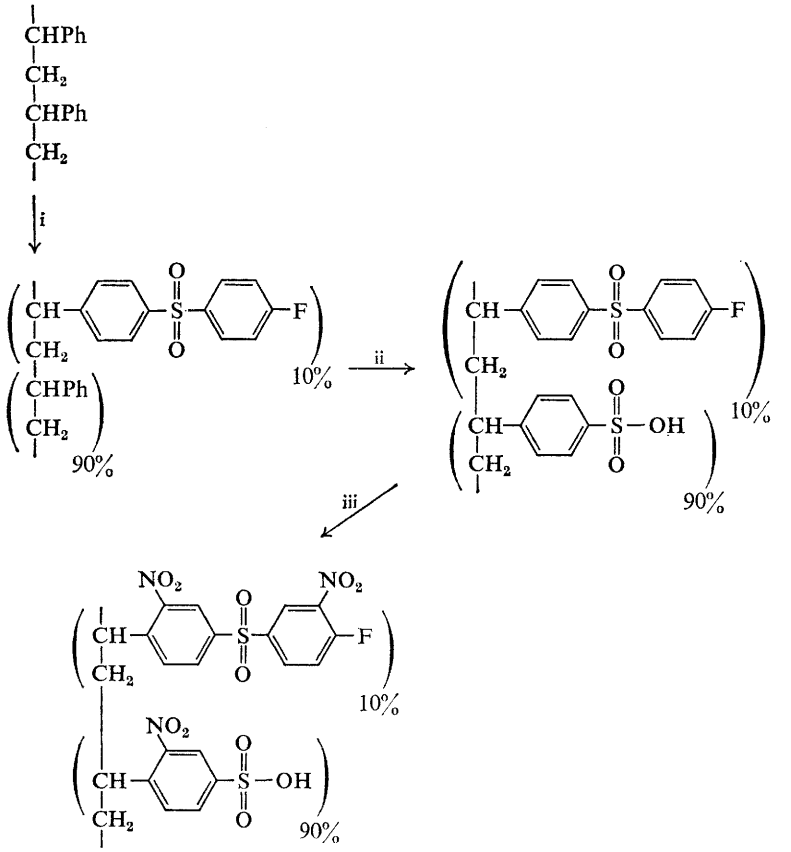
functionalizing agent (86). The latter provided a (protected) functional site, a spacer unit, and an internal reference amino-acid for evaluating the course of the synthesis. The labile resin–peptide linkage was contained in a novel benzyl ester activated ester derivative, *e.g.* (87), introduced under reaction conditions similar to those of subsequent amino-acid incorporation cycles. This resin has been shown to be superior to polystyrene in the synthesis of the difficult sequence of acyl carrier protein, residues 65–74.

In connection with this polydimethylacrylamide resin it is interesting to note that recent solution studies[111] on the aminolysis of *p*-nitrophenyl esters bound to soluble polystyrene, polymethylacrylate, or poly-*NN*-dimethylacrylamide gave much faster rates in the poly-*NN*-dimethylacrylamide system.

In an effort to produce a support suitable for use in aqueous conditions a polymer possessing 4-fluoro-3-nitrophenylsulphonyl groups on a sulphonated polystyrene matrix has been synthesized (Scheme 22).[112] This resin is compatable with the bidirectional approach to solid-phase synthesis in which the linkage to the resin is through a histidine side-chain (see Vol. 7 of these reports, p. 271).

The Kel-F grafted styrene polymer developed by Tregear is much denser than conventional polystyrene resin and is easily separated from the latter by floatation in chloroform. This has been exploited by the simultaneous synthesis of substance P and an analogue of substance P in the same reaction vessel.[113]

[111] H. Morawetz, ref. 2, p. 385.
[112] J. D. Glass, C. Meyers, I. L. Schwartz, and R. Walter, ref. 1, p. 141.
[113] J. Van Rietschoten, G. W. Tregear, S. Leeman, D. Powell, H. Niall, and J. T. Potts, jun., ref. 1, p. 113.



Conditions: i, $ClSO_2C_6H_4F$–$AlCl_3$; ii, Cl_2SO_2 followed by H_2O; iii, fuming HNO_3

Scheme 22

Techniques for monitoring Solid-phase Synthesis. The use of fluorescamine as a reagent for quantitative measurement of resin-bound amines has been suggested.[114] The method involves removal of the peptide from an aliquot of the resin with sodium methoxide, reaction of the free amine in solution with fluorescamine and measurement of fluorescence at 480 nm. The results are comparable with those obtained from amino-acid analysis but less time and sample are required. In a second paper this method for the removal of peptide from the resin was used to investigate the effects of reaction times, triethylamine concentration, and amino-acid concentration on DCC-mediated couplings.[115] However, the extent of coupling in these experiments was estimated by amino-acid analysis and not by the use of the fluorescamine technique.[114]

A detailed description of the automatic monitoring of solid-phase peptide synthesis by means of the spectrophotometric picrate method has appeared.[116]

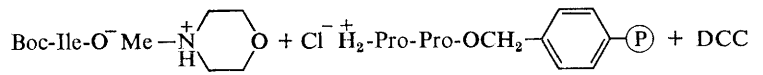
[114] A. M. Tometsko and E. Vogelstein, *Analyt. Biochem.*, 1975, **64**, 438.
[115] A. M. Tometsko, M. Schreiner, and J. Comstock, *Analyt. Biochem.*, 1975, **67**, 182.
[116] R. S. Hodges and R. B. Merrifield, *Analyt. Biochem.*, 1975, **65**, 241.

The absorbing properties of the Ddz group have also been used to monitor the course of solid-phase synthesis.[117] Simultaneous application of perchloric acid titration and radioisotope labelling allowed a detailed analysis of the course of the solid-phase synthesis of antanimide.[118]

A study of the rates of reactions on solid-phase supports by continuous monitoring of the absorbance of the liquid phase, and taking into account such factors as the distribution of reagents between the polymeric phase and liquid phase, allowed 'true' rate coefficients to be calculated under differing conditions.[119] In an analogous study the effects of phase distribution of reagents were not taken into account.[120]

Analysis of resin-bound peptides by the Edman method has been applied to synthetic insulin B chain residues 22—30.[121] Anilinothiazolinones were converted directly into phenylthiohydantoins[122] and quantitated by high-pressure liquid chromatography.[123] The results were interpreted as indicating 96.5% uniformity of the synthetic peptide. This should not be taken as indicating the efficiency of the synthesis since the analytical method ignores truncated sequences not containing free amino-groups, and nitrophthalic acid was used as a blocking agent after each amino-acid addition.

Side-reactions in Solid-phase Synthesis. Diketopiperazine formation by resin-bound dipeptides can be suppressed by the addition of the *N*-methylmorpholinium salt of the Boc-amino-acid to the protonated resin-bound dipeptide (Scheme 23).[124]



Scheme 23

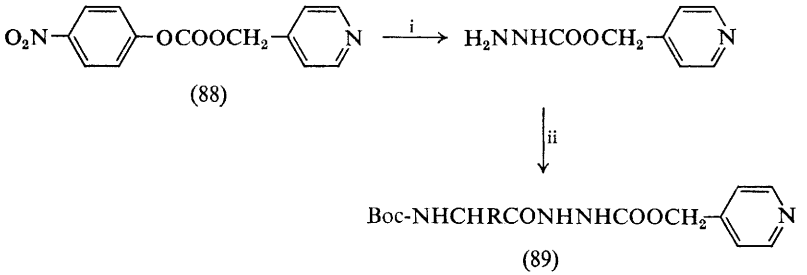
In a synthesis of substance P, a fall in incorporation of residues after a glutamine coupling was accounted for by pyroglutamyl formation during incubation of the resin with the following Boc-amino-acid and *N*-hydroxybenzotriazole prior to the addition of DCC.[125] This was prevented by pre-mixing of the Boc-amino-acid, *N*-hydroxybenzotriazole, and DCC. In the same synthesis a fall in incorporation after a proline attachment was noted when using a chloromethyl polymer but not with a benzydrylamine polymer. Boron trifluoride etherate has been used for the removal of the t-butoxycarbonyl groups from asparagine and glutamine in a solid-phase synthesis of oxytocin, presumably to prevent cyclization reactions.[102] In a synthesis of neurotensin, it was found that cleavage from the resin with hydrogen bromide in trifluoracetic acid led to extensive cyclization of a terminal glutamine residue.[126] However, problems associated with glutamine deprotection

[117] C. Birr, ref. 1, p. 117.
[118] K. Brunfeldt, D. Bucher, T. Christensen, O. Schou, and I. Rubin, ref. 1, p. 227.
[119] J. Rudinger and R. Buetzer, ref. 1, p. 211.
[120] V. Gut, *Coll. Czech. Chem. Comm.*, 1975, **40**, 129.
[121] C. Birr and R. Frank, *F.E.B.S. Letters*, 1975, **55**, 68.
[122] C. Birr and R. Frank, *F.E.B.S. Letters*, 1975, **55**, 61.
[123] J. X. de Vries, R. Frank, and C. Birr, *F.E.B.S. Letters*, 1975, **55**, 65.
[124] K. Suzuki, K. Nitta, and W. Endo, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 222.
[125] J. L. M. Syrier and H. C. Beyerman, ref. 1, p. 105.
[126] R. Carraway and S. E. Leeman, *J. Biol. Chem.*, 1975, **250**, 1912.

may not always be due to cyclization but to incomplete t-butoxycarbonyl group removal.[127] The modification of glutamic acid-containing peptides by hydrogen fluoride–anisole has been fully investigated (see p. 259). Acylated derivatives of anisole may be formed, and it is interesting to note that in a synthesis of human parathyroid hormone (1—34), a less acidic and u.v.-absorbing by-product was isolated which may have this origin.[127] Also in this synthesis a peptide which was more acidic than the target peptide was identified and could have arisen through deamidation of glutamine or asparagine in the hydrogen fluoride reaction.

Other Repetitive Methods. The repetitive excess mixed anhydride (REMA) method has been applied to a successful synthesis of porcine secretin.[128] Difficulties were encountered only in the coupling of BOC-Arg(NO_2) residues and the yield of protected peptide was 13%, an average yield of 93% for each step. After deprotection and purification on SP-Sephadex, no significant racemization was detected and a tryptic digest was indistinguishable from that of a sample prepared by a classical fragment condensation solution synthesis.

The picolyl ester method has been extended to produce fragments suitable for further coupling by the azide technique. Reaction of the mixed carbonate (88) with hydrazine hydrate followed by acylation with a t-butoxycarbonyl amino-acid gave the compound (89) (Scheme 24)[129] (see p. 259 and Vol. 7 of these Reports, p. 278).



Conditions: i, N_2H_4,H_2O; ii, Boc-amino-acid DCC HOBt

Scheme 24

Synthesis could then be carried out utilizing the picolyl moiety as an aid to purification and the model peptide (83) was synthesized. The picolyl group can be removed by catalytic hydrogenation or electrolytic reduction, thus giving the hydrazide which can be converted into the azide for further coupling. The 1—13 fragment of porcine gastric inhibitory polypeptide (90) was synthesized by this method using additional picolyl groups for side-chain protection of glutamyl and tyrosyl residues. These groups helped in the purification but did not alleviate solubility problems.

The soluble polymer method is claimed to have 'decisive advantages compared to the methods used to date in peptide synthesis'.[130] Further details have appeared

[127] G. W. Tregear, in ref. 1, p. 177.
[128] A. van Zon and H. C. Beyerman, ref. 1, p. 165.
[129] R. Macrae and G. T. Young, *J.C.S. Perkin I*, 1975, 1185.
[130] M. Mutter, R. Uhmann, and E. Bayer, *Annalen*, 1975, 901.

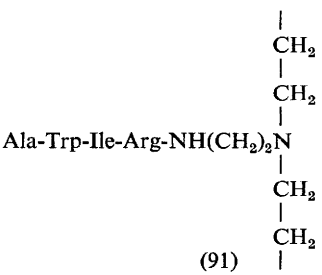
Boc-Tyr(Pic)-Ala-Glu(OPic)-Gly-Thr(Bzl)-Phe-Ile-Ser(Bzl)-Asp(OBzl)-
Tyr(Pic)-Ser(Bzl)-Ile-Ala-NHNH-Picoc

(90)

on the rates of coupling of t-butoxycarbonylglycine *p*-nitrophenyl esters to poly-(ethylene glycol)-bound glycine.[131] Details of attachment of the first residue to the polymer, chain elongation, crystallization of intermediates, analytical control of the synthesis, and detachment from the polymer have appeared as well as a comparison of polymers of varying molecular weights in the synthesis of the model peptide (83).[130] In its present form the method suffers from the limitation that attachment to the resin is *via* an alkyl ester [use of compounds such as (87) could provide wider scope for the method]. Therefore, apart from ammonolysis or hydrazinolysis, alkaline hydrolysis is the method for removal. The solubilizing effect of the poly(ethylene glycol) on a peptide is quite significant but may prove a limiting factor as chain length is increased.

The method was employed in the synthesis of deamido-calcitonin M residues 19—32.[132] Assembly of the peptide was monitored by both fluorescamine and ninhydrin and yields were estimated to be greater than 99.6% at each stage. After purification the synthetic peptide was attached to a benzhydrylamine-polystyrene support with DCC and *N*-hydroxybenzotriazole in an attempt to complete the total synthesis of calcitonin M. Other examples of the use of poly(ethyleneglycol) as a *C*-terminal solubilizing support are in syntheses of [Orn$^{12, 14}$]-secretin (9—14) hydrazide,[133] [Orn$^{18, 21}$]-secretin (15—27)[134] and secretin (3—8).[135]

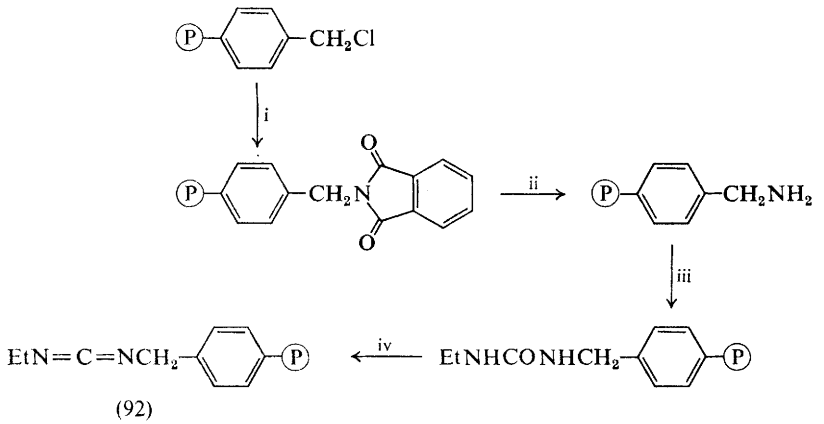
A water-soluble polyethyleneimine support has been developed[136] for use with *N*-carboxyanhydrides. Arginine was attached to the polymer and subsequent residues added by the NCA method to produce (91). Complete cleavage



(91)

from the resin is affected by treatment with trypsin at 27 °C for 2 h. Incubation with carboxypeptidase B at pH 7.5 for 1 h can then be used to remove the undesired *C*-terminal arginine residue. Further examples of the use of the insoluble active ester approach with (4-hydroxy-3-nitro)benzylated polystyrene have appeared.[137] An insoluble carbodi-imide reagent has been described and used in

[131] E. Bayer, M. Mutter, J. Polster, and R. Uhmann, ref. 1, p. 129.
[132] H. Hagenmaier, *Z. physiol. Chem.*, 1975, **356**, 777.
[133] W. Göhring and G. Jung, *Annalen*, 1975, 1776.
[134] W. Göhring and G. Jung, *Annalen*, 1975, 1765.
[135] W. Göhring and G. Jung, *Annalen*, 1975, 1781.
[136] P. Pfaender, H. Pratzel, H. Blecher, G. Gorka, and G. Hansen, ref. 1, p. 137.
[137] D. Atlas, R. Kalir, and A. Patchornik, *F.E.B.S. Letters*, 1975, **58**, 179.



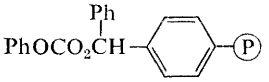
(92)

Conditions: i, potassium phthalimide; ii, N_2H_4,H_2O; iii, EtNCO; iv, toluene-*p*-sulphonyl chloride

Scheme 25

the preparation of several dipeptides.[138] Ethylcarbodi-imidomethylated polystyrene (92) was prepared according to Scheme 25; (92) gave 0.43 mmol of dipeptide formation per gram of resin.

A preliminary account of an alternating liquid–solid phase system for peptide synthesis has appeared.[139] In this approach the carboxyl component in a condensation is immobilized by reaction with the polystyrene-based support (93) and

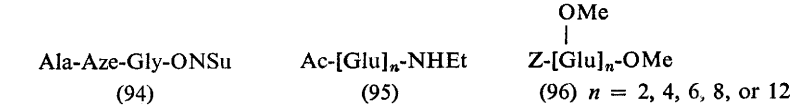


(93)

after coupling using DCC–HOBt the resultant peptide is released into solution, by treatment with trifluoroacetic acid. After purification and characterization, the process is repeated by coupling with another insoluble carboxyl component.

Synthesis of Polymeric Models for Studies in Protein Chemistry.—The proceedings of the Polyamino-acid Symposium held in Israel have appeared.[140]

A series of polymers containing azetidine carboxylic acid have now been made from monomer tripeptide units.[141] *p*-Nitrophenyl, pentachlorophenyl, and *N*-hydroxysuccinimide esters were used and good yields were obtained in all cases except for the tripeptide active ester (94).



(94) (95) (96) n = 2, 4, 6, 8, or 12

[138] H. Ito, N. Takamatsu, and I. Ichikizaki, *Chem. Letters*, 1975, 577.
[139] H. Frank and H. Hagenmaier, *Experientia*, 1975, **31**, 131.
[140] 'Peptides, Polypeptides and Proteins', ed. E. R. Blout, F. A. Bovey, M. Goodman, and N. Lotan, John Wiley, New York, 1974.
[141] R. Boni, E. De Gregoriis, R. Di Blasi, and A. S. Verdini, *Biopolymers*, 1975, **14**, 2211.

A series of homo-oligomers of glutamic acid of the form (95) have been prepared.[142] Polymerization of γ-benzyl glutamic acid *N*-carboxyanhydride was initiated with ethylamine and terminated by addition of *p*-nitrophenylacetate.

By a combination of solid-phase and classical solution synthesis, a series of oligopeptides having the general formula (96) have been prepared.[143, 144] C.d. studies indicated the expected occurrence of helical structures at the octamer level, and this was confirmed by air–water interface studies. It was also noted that on t.l.c. a fall in intensity of the colour developed with the chlorine–tolidine reagent occurred with increasing chain length. The most dramatic change was between hexamer and octamer implying that a change from disordered to ordered structure had reduced the accessibility of the reagent to the peptide bonds.

In a study on the polymerization of the monomers (97) and (98) it was shown over a range of concentrations that higher yields of polymer were obtained from

Val-Ala-Gly-ONSu	Ala-Val-Gly-ONSu
(97)	(98)
Val-Val-Gly-ONSu	Val-Gly-Val-Gly-ONSu
(99)	(100)

(98) which is as expected considering the order of the amino-acids in the sequence.[145] However, the influence of the *N*-terminal amino-acid on the rate of polymerization may not be the overriding factor since nearly quantitative yields of polymer were obtained from the monomers (99) and (100). It was suggested that the overall conformation of the growing polymer may exert an effect on the polymerization. I.r. studies of the polycondensation of valine *N*-carboxyanhydride suggest that the polymerization proceeds initially in the β-form and after a critical point changes to an α-helix-like conformation.[146]

Water-soluble polymers of valine and isoleucine flanked by poly-DL-lysine have been prepared in order to study the requirements for the adoption of β-conformations.[147] This paper gives a useful list of the physical data of polymers with β-structures. A series of homo-oligomers of alanine has been prepared and a c.d. study carried out.[148, 149] Conventional synthesis of co-oligomers of tryptophan and glycine with varying distances between the aromatic residues has been reported; the physical properties of the compounds were studied.[150]

Polylysine with the N^{ε}-group derivatized with stearyl or pelargonyl groups has been synthesized[151] and also statistical copolymers of lysine and alanine as histone models.[152, 153] The pentachlorophenyl ester method was used to prepare

[142] M. Rinaudo and A. Domard, *Biopolymers*, 1975, **14**, 2035.
[143] J. Caspers, W. Hecq, and A. Loffet, ref. 1, p. 97.
[144] J. Caspers, W. Hecq, and A. Loffet, *Biopolymers*, 1975, **14**, 2263.
[145] R. Katakai, M. Oya, and Y. Iwakura, *Biopolymers*, 1975, **14**, 1315.
[146] K. Itoh and G. D. Fasman, *Biopolymers*, 1975, **14**, 1755.
[147] S. Kubota and G. D. Fasman, *Biopolymers*, 1975, **14**, 605.
[148] G. M. Bonora, D. Nisato, and C. Toniolo, *Makromol. Chem.*, 1975, **176**, 2535.
[149] G. Toniolo and G. M. Bonora, *Makromol. Chem.*, 1975, **176**, 2547.
[150] R. Guarnaccia, G. P. Lorenzi, V. Rizzo, and P. L. Luisi, *Biopolymers*, 1975, **14**, 2329.
[151] V. Shibaev, M. Palumbo, and E. Peggion, *Biopolymers*, 1975, **14**, 73.
[152] Š. Štokrová, J. Šponar, M. Havránek, B. Sadláček, and K. Bláha, *Biopolymers*, 1975, **14**, 1231.
[153] J. Šponar, I. Frič, and K. Bláha, *Biophys. Chem.*, 1975, **3**, 255.

poly-[Asp(OBzlNO_2)-Asp-OBzl] in order to study whether the polymer gave a left-handed or right-handed helix.[154] Polymeric β-1-napthylmethyl aspartate and a copolymer with γ-benzyl glutamate have been prepared.[155] Poly-(Tyr-Glu-Ala-Gly) and poly-(Lys-Glu-Ala) have been prepared and characterized.[156] The former was made by polycondensation *via* the pentachlorophenyl active ester and the latter by the azide technique. Synthesis of poly-(Met-D-Met) and poly-(Met-D-Met-Met) has been described.[157] As a model for hydrolytic enzymes the monomer (101) was assembled on a solid support and polymerized by the *N*-hydroxysuccinimide ester method.[158] Attempts to make the *p*-nitrophenyl ester of (101) with or without *N*-hydroxybenzotriazole failed, as did an attempt at polymerization by a mixed anhydride technique.

The sequential polypeptide elastin models poly-(Gly-Val-Gly-Val-Pro) and poly-(Gly-Val-Pro-Gly)[159] and a series of polymeric collagen models have been

Ddz-Cys(Acm)-Ser(Bu^t)-Phe-Glu(OBu^t)-Glu(OBu^t)
(101)

Z-Lys(Boc)-Gly-Pro-OPcp
(102)

$Br^- \overset{+}{H}_2$(Pro-Leu-Gly)$_{10}$OH
(103)

Boc-Pro-Leu-Gly
(104)

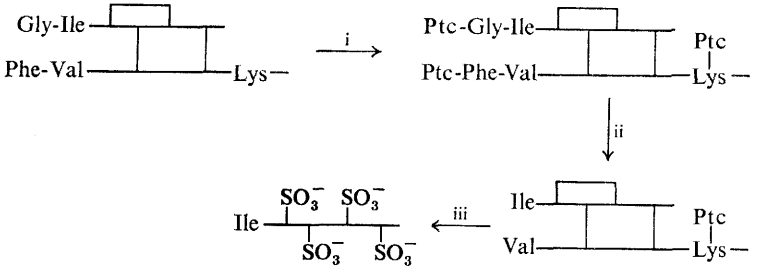
Pro-Leu-Gly-OCH_2-(P)
(105)

described.[160] In the synthesis of poly-(Lys-Gly-Pro) difficulty was encountered in the removal of the N-protecting group by hydrogenolysis from the tripeptide active ester (102) without removal of the t-butoxycarbonyl group. This problem was circumvented by hydrogenolysis in glacial acetic acid.[160] Other polymers relating to collagen models have been synthesized,[161–166] the synthesis of (103) was carried out by sequential couplings of the fragment (104) onto the solid-phase peptide (105).[167]

Synthetic Operations with Peptides of Biological Origins.—A brief article defining the term 'semisynthesis' and discussing achievements in the field has appeared.[168]

Several analogues of insulin have been prepared by chemically manipulating the parent molecule. In one approach des-Gly-A chain *S*-sulphonate was prepared from bovine insulin by controlled Edman degradation according to Scheme 26. Alternatively the A-chain S-sulphonate was isolated and degraded

[154] J.-P. Aubert and M.-H. Loucheux-Lefebvre, *Biopolymers*, 1975, **14**, 2429.
[155] A. Ueno, T. Ishiguro, F. Toda, K. Uno, and Y. Iwakura, *Biopolymers*, 1975, **14**, 353.
[156] A. R. Zeiger, C.-H. Lai, and P. H. Maurer, *Biopolymers*, 1975, **14**, 2281.
[157] E. Dellacherie and J. Neel, *Biopolymers*, 1975, **14**, 1437.
[158] Y. Trudelle and C. Birr, *Internat. J. Peptide Protein Res.*, 1975, **7**, 403.
[159] J. R. Bell, R. C. Boohan, J. H. Jones, and R. M. Moore, *Internat. J. Peptide Protein Res.*, 1975, **7**, 229.
[160] J. R. Bell, J. H. Jones, and T. C. Webb, *Internat. J. Peptide Protein Res.*, 1975, **7**, 237.
[161] M. Palumbo, R. L. Rodin, and M. Goodman, *Biochemistry*, 1975, **14**, 485.
[162] P. Burckner, B. Rutschmann, J. Engel, and M. Rothe, *Helv. Chim. Acta*, 1975, **58**, 1276.
[163] B. R. Shaw and J. M. Schurr, *Biopolymers*, 1975, **14**, 1951.
[164] R. S. Bhatnagar and R. S. Rapaka, *Biopolymers*, 1975, **14**, 597.
[165] R. S. Rapaka and R. S. Bhatnagar, *Internat. J. Peptide Protein Res.*, 1975, **7**, 475.
[166] R. S. Rapaka and R. S. Bhatnagar, *Internat. J. Peptide Protein Res.*, 1975, **7**, 119.
[167] A. Scatturin, A. M. Tamburro, G. Vidali, and E. Bordignon, *Internat. J. Peptide Protein Res.*, 1975, **7**, 221.
[168] D. G. Smyth, *Nature*, 1975, **256**, 699.



(106)

Conditions: i, PhNCS; ii, TFA; iii, Na_2SO_3, followed by preparative electrophoresis or ion exchange chromatography

Scheme 26

with a large excess of phenyl isothiocyanate followed by reaction with trifluoroacetic acid.[169]

Des-Gly-insulin was obtained by combination of the sulphonated derivative (106) with B-chain bis-*S*-sulphonate giving a molecule with very low activity. This low activity was associated with a loss of ordered secondary structure indicated by c.d.[169] Reaction of (106) with t-butoxycarbonyl-amino-acid active esters followed by combination with the B-chain sulphonate allowed the preparation of several semisynthetic analogues in which glycine in position 1 of the A-chain were replaced by leucine, t-butoxycarbonylvaline, valine, proline, lysine, and glutamic acid.[170] An alternative approach involved the combination of the $N^{\alpha B1}$,$N^{\epsilon B29}$-bis-t-butoxycarbonyl B-chain *S*-sulphonate with des-Gly-A-chain

[desPhe^{B1}] insulin (107)

[des(Phe-Val)^{B1,2}] insulin (108)

[des(Phe-Val-Asn)$^{B1-3}$, Glp^{B4}] insulin (109)

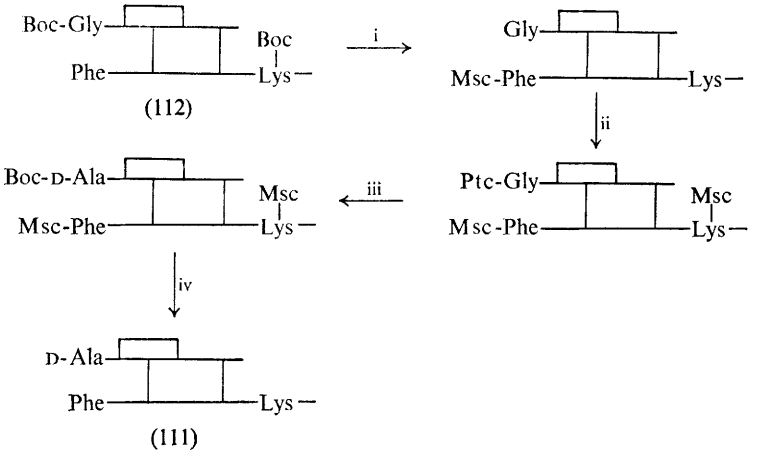
[des(Phe-Val-Asn-Gln)$^{B1-4}$] insulin (110)

S-sulphonate followed by acylation of the exposed isoleucine amino-group with active esters.[170] All analogues exhibited considerably reduced *in vitro* activity and c.d. indicated disruption of the secondary structure. The same group have used the previously described $N^{\alpha A1}$,$N^{\epsilon B29}$-bis-t-butoxycarbonyl insulin (112) [171] as an amino component for elongation of the chain at the B1 position with glycyl, lysyl, and glutamyl residues.[172] $N^{\alpha A1}$,$N^{\epsilon B29}$-bis-t-butoxycarbonyl des-B1-Phe pig insulin has also been used to prepare tritium-labelled insulin with a high specific activity.[173]

[169] D. Brandenburg, M. Biela, L. Herbertz, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 961.
[170] G. Krail, D. Brandenburg, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 981.
[171] R. Geiger, H.-H. Schone, and W. Pfaff, *Z. physiol. Chem.*, 1971, **352**, 1487.
[172] G. Krail, D. Brandenburg, and H. Zahn, *Makromol. Chem.*, 1975, **176**, 7.
[173] P. A. Halban and R. E. Offord, *Biochem. J.*, 1975, **151**, 219.

The surprising observation[174] that insulin was stable when exposed to hydrazine in 80% phenol for 20 h at 40 °C (which is contrary to a previous observation regarding the stability of insulin to hydrazine[175]), led to the preparation of analogues in which the B-chain amino-terminus was degraded by the Edman technique. Phthaloylation of insulin with *N*-ethoxycarbonylphthalimide gave $N^{\alpha A1}$,$N^{\varepsilon B29}$-bis-phthaloyl insulin which was then subjected to successive Edman degrative cycles. Removal of the phthaloyl groups by treatment with hydrazine in phenol allowed the isolation of the analogues (107)—(110). The compound (109) being obtained by cyclization of the glutamine residue during Edman degradation. All the compounds retained a substantial amount of insulin activity.[174]

The methylsulphonylethoxycarbonyl group (Msc) (see p. 254) can also be introduced specifically onto the $N^{\alpha A1}$,$N^{\varepsilon B29}$ groups of insulin, and can be removed to give insulin which is crystallizable and possesses full biological activity.[21] The base lability of this group was exploited in a preparation of D-alanine^{A1} insulin (111) (Scheme 27) starting from the $N^{\alpha A1}$,$N^{\varepsilon B29}$-bis-t-butoxycarbonyl derivative (112).[176]

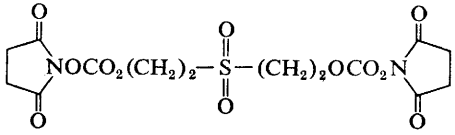


Conditions: i, Msc-ONSu, followed by TFA; ii, PhNCS, followed by Msc-ONSu; iii, TFA, followed by coupling with Boc-D-Ala-OTcp-HOBt; iv, NaOH, followed by TFA

Scheme 27

Shortening of the B-chain of bovine insulin from the *C*-terminus has been described. Binding of bovine insulin to sulphoethylcellulose, followed by digestion with pepsin, yielded des[pentapeptide B26—30] derivative.[177] Likewise, des[pentapeptide B26—30] and des[Phe B1, pentapeptide B26—30] camel insulin was prepared.[178] The boron trifluoride–methanol procedure has been adopted to esterify insulin at room temperature.[179] Bis-[2-(succinimido-oxycarbonyloxy)ethyl] sulphone (113) is another reagent which will cross-link

[174] R. Geiger and D. Langner, ref. 1, p. 159.
[175] F. Borras and R. E. Offord, *Nature*, 1970, **227**, 716.
[176] R. Geiger, K. Geisen, H.-D. Summ, and D. Lagner, *Z. physiol. Chem.*, 1975, **356**, 1635.
[177] H.-G. Gattner, *Z. physiol. Chem.*, 1975, **356**, 1398.
[178] W. O. Danho, H.-G. Gattner, D. Nissen, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 1405.
[179] H.-G. Gattner, E. W. Schmitt, and V. K. Naithani, *Z. physiol. Chem.*, 1975, **356**, 1465.



(113)

insulin between $N^{\alpha A1}$ and $N^{\varepsilon B29}$ amino-groups. Reduction and reoxidation of the cross-linked insulin restores 60% activity.[180]

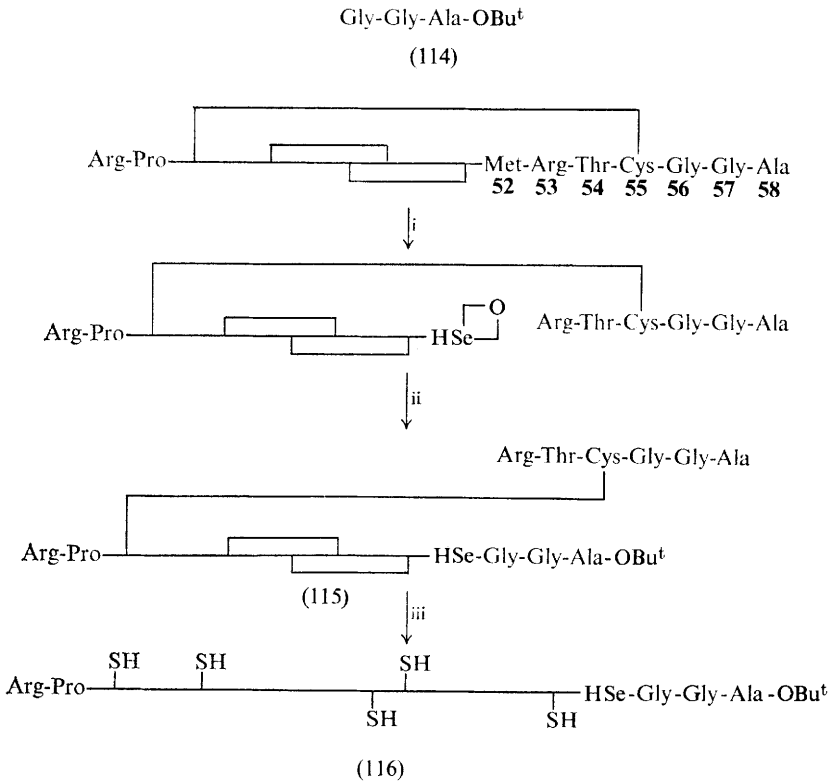
Further studies on S-peptide S-protein interaction using S-peptide analogues prepared by solid-phase synthesis have appeared. The compounds synthesized were the fluorinated derivative [His(4F)]S-peptide-(1—15) and the ^{13}C-enriched derivative (^{13}C-Gly^{6})S-peptide-(1—15).[181, 182] It was established that the fluorinated analogue bound to the S-protein fraction but the complex was inactive although it had the ability to bind cytidine-2′-monophosphate. ^{1}H N.m.r. studies on the complex showed that the pK_a of His^{119} had substantially increased, thus indicating that the His(4F)^{12} has significant effect on the electronic environment of His^{119}. This is consistent with *X*-ray data showing the closeness of these two histidine residues. The ^{13}C-enriched complex allowed some general conclusions regarding chemical shifts and signal broadening on going from coil to helix.[181]

A preliminary report has appeared on a semisynthetic approach to analogues of staphylococcal nuclease. This involves blocking the P_2 fragment 6—48 by trifluoroacetylation followed by phenzyldiazomethane esterification to give the fully protected derivative.[183] Since there is only one arginine residue in this fragment at position 35, tryptic hydrolysis yielded the protected sequence 6—35. Assembly of the 36—47 fragment on a solid support then allowed the coupling of the protected natural fragment using DCC–HOBt. However, the resultant crude peptide, obtained after removal from the resin with hydrogen fluoride and treatment with 1M piperidine in 8M urea gave only 5—10% activity when assayed with P_3 natural fragment.

The cyanogen bromide-mediated cleavage of basic pancreatic trypsin inhibitor to produce the homoserine lactone derivative (see Vol. 6, p. 351) has been used to produce a semisynthetic analogue in which the *C*-terminal hexapeptide has been replaced by the tripeptide (114) (Scheme 28).[184]

Denaturing conditions were established in which the intramolecular attack by the free amino-group of arginine on the homoserine lactone was slowed down sufficiently to allow reaction with the tripeptide (114). The product (115) was isolated by gel filtration and ion exchange chromatography. Reduction, followed by carboxymethylation and then tryptic digestion, characterized the semisynthetic analogue (116).[184] Since proteins can be subjected to very few chemical reactions that are mild and selective, this type of approach towards semisynthesis is considerably more attractive than other approaches where multiple chemical manipulations need to be carried out.

[180] R. Obermeier and R. Geiger, *Z. physiol. Chem.*, 1975, **356**, 1631.
[181] I. M. Chaiken and B. M. Dunn, ref. 1, p. 299.
[182] B. M. Dunn, C. Di Bello, K. L. Kirk, L. A. Cohen, and I. M. Chaiken, *J. Biol. Chem.*, 1974, **249**, 6295.
[183] C. Di Bello, ref. 1, p. 173.
[184] D. F. Dyckes and R. C. Sheppard, ref. 2, p. 515.



Conditions: i, CNBr; ii, (114) in DMSO; iii, dithiothreitol

Scheme 28

The action of cyanogen bromide on glucagon led to the isolation of des-[Met^{27},Asn^{28},Thr^{29}][homoserine lactone^{27}] glucagon in a highly purified form.[185] Previously the preparation had been contaminated with glucagon to the extent of 2—3%. Des-His-glucagon has also been prepared by attaching glucagon to an isothiocyanate resin, and treatment with trifluoroacetic acid. The difference between the pK_a's of the α- and ε-amino-groups is too small to allow selective reaction of phenyl isothiocyanate with the *N*-terminal histidine amino-group only, so this method provides a neat alternative route to des-His-glucagon since molecules in which the ε-amino-group of lysine has reacted will remain permanently attached to the solid support. This seems to be a generally applicable approach to the removal of single amino-terminal residues.

Protection of the ε-amino-groups of lysine by amidination with methyl acetimidate allowed a ^{13}C-enriched glycine residue to be added to the amino-terminal of sperm whale myoglobin.[186]

[185] M. C. Lin, D. E. Wright, V. J. Hruby, and M. Rodbell, *Biochemistry*, 1975, **14**, 1559.
[186] W. H. Garner and F. R. N. Gurd, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 262.

3 Syntheses Achieved

ACTH and MSH.—A new synthesis of the full ACTH molecule has been reported.[187] The synthesis involved the fragment condensation of residues 1—24 (117) with 25—39 (118). Two synthetic routes to the 1—24 sequence were described,[188] one involved condensation of fragments 1—10 to 11—24. Several

Z-Ser-Tyr-Ser-Met-Glu(OBu^t)-His-Phe-Arg-Trp-Gly-Lys(Z)-Pro-Val-Gly-Lys(Z)-Lys(Z)-Arg(NO_2)-Arg(NO_2)-Pro-Val-Lys(Z)-Val-Tyr-Pro

(117)

Asn-Gly-Ala-Glu(OBu^t)-Asp(OBu^t)-Glu(OBu^t)-Ser-Ala-Glu(OBu^t)-Ala-Phe-Pro-Leu-Glu(OBu^t)-Phe-OBu^t

(118)

reagents were tried to couple these two fragments, the trichlorophenyl ester giving the best results with an impressive coupling yield of 82.7%. The ester derived from *N*-hydroxy-5-norbornene-2,3-dicarboximide gave a coupling yield of 45.4% and this reagent was used extensively to form active esters in the synthesis of the 25—39 fragment.[187] The two fragments 1—24 and 25—39 were condensed with a DCC–*N*-hydroxy-5-norbornene-2,3-dicarboximide coupling over two days at room temperature. Removal of all protecting groups was accomplished in hydrogen fluoride with the belt and braces addition of anisole, skatole, methyl ethyl sulphide, and thioglycollic acid as scavengers. Purification was effected by carboxymethyl cellulose chromatography followed by desalting on Amberlite XAD2 and Biogel P6. The Amberlite XAD2 column was useful for removing tryptophan degradation products. The final compound had an activity of 145 units per mg. Porcine ACTH (18—39) (porcine CLIP) has been prepared by a classical fragment-condensation approach.[189] For the coupling of the fragments (119) and (120) *N*-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ) was used and gave results comparable with DCC–HOBt.

Z(OMe)-Leu-Ala-Glu(OBzl)-Ala-Phe-Pro

(119)

Leu-Glu(OBzl)-Phe-OBzl

(120)

H_2^+

MSc-Phe-Arg-Pmp-Gly-OMe

(121)

In an investigation into the possibility that the tryptophan residue in position 9 of ACTH acts as a charge-transfer moiety, two analogues of ACTH have been prepared with pentamethylphenylalanine (Pmp) replacing tryptophan.[190] In the synthesis of the key intermediate (121) the Msc group (p. 254) was used for protection of the phenylalanine, and the synthesis proceeded more smoothly when the guanidino-group of arginine was protected by protonation. Measurement of the donor characteristics of [Pmp^9] ACTH (1—24) using paraquat as an

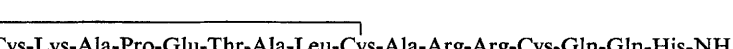
[187] O. Nishimura, C. Hatanaka, and M. Fujino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1212.
[188] C. Hatanaka, O. Nishimura, and M. Fujino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1017.
[189] K. Koyama, Y. Mori, Y. Kiso, S. Hirabayashi, and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2301.
[190] J. W. van Nispen and G. I. Tesser, *Internat. J. Peptide Protein Res.*, 1975, **7**, 57.

acceptor indicated that it has similar characteristics to ACTH (1—24); however, the analogue had only 0.2—0.4% of the steroidogenic activity of ACTH (see Vol. 7, p. 289). In another study of the structural significance of the tryptophan residue, [β-(1-naphthyl)alanine^9] ACTH (1—19), [N^{in}-formyltryptophan^9] ACTH (1—19), and [Lys^8,Phe^9] ACTH (1—19) have been synthesized by standard solid-phase procedures.[191] All showed diminished biological activity. [Ile^9] ACTH (1—24), synthesized by conventional solution techniques, was a competitive antagonist of cyclic AMP production by ACTH.[192] Twenty-three analogues of ACTH (4—10) have been made by solid-phase synthesis, leading to the observation that the glutamic acid residue in position 5 plays an important role as a 'spacer'.[193]

The synthesis of ACTH (1—24) by fragment condensation onto a solid support using the oxidation–reduction method is discussed earlier on p. 271.

Solution syntheses of porcine[194] and equine[195] β-MSH and a solid-phase preparation of camel β-MSH[92] have been described. The demonstration of a second active-site sequence in α-MSH[196] led to the preparation of a large number of fragments of α-MSH.[197]

Apamin.—Apamin is a neurotoxic octadecapeptide (122) which has been isolated from bee venom. A preliminary account of a solid-phase synthesis in which the



(122)

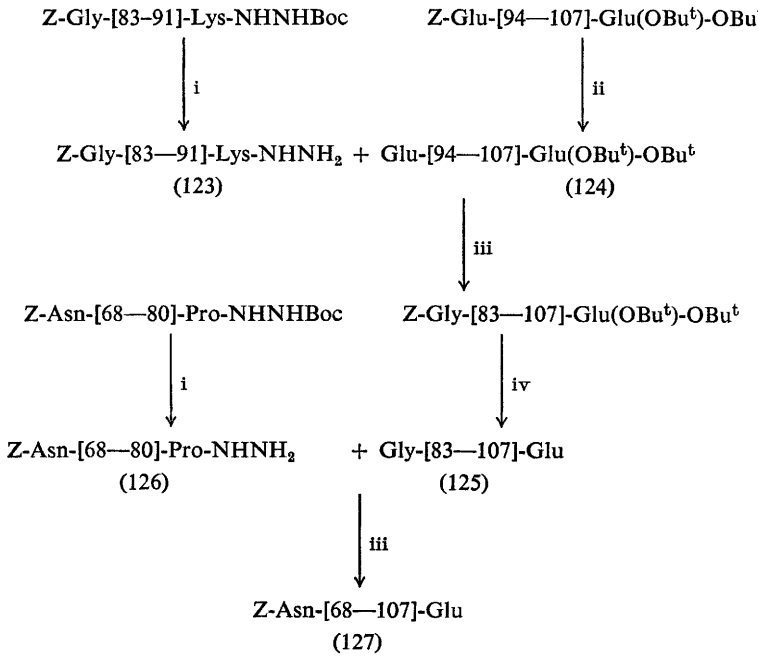
Ppoc group was used for N^{α}-protection has appeared,[12] as has a detailed account of its synthesis on a Kel-F support substituted to the extent of 0.034 mmol benzhydrylamine per g.[198] The Boc group was used for N^{α}-protection in all cases apart from arginine, when the Aoc group was used to increase its solubility. Histidine and arginine side-chains were protected by the tosyl group and the cysteine residues by the t-butylsulphenyl group. A double coupling programme was used, and when fluorescamine indicated the presence of unchanged amine on the resin, a third coupling was carried out. If the fluorescamine test was still positive, residual free amino-groups were blocked by acetylation with acetyl imidazole. This procedure was employed when incomplete acylation of His^{18} and Asn^2 was detected. Cleavage from the resin and removal of side-chain protecting groups was accomplished with hydrogen fluoride in the presence of anisole. This gave a polymeric product* which was reduced with tributylphosphine and

[191] J. Blake and C. H. Li, *J. Medicin. Chem.*, 1975, **18**, 423.
[192] S. Kumar, *Biochem. and Biophys. Res. Comm.*, 1975, **66**, 1063.
[193] M. W. Draper, M. A. Rizack, and R. B. Merrifield, *Biochemistry*, 1975, **14**, 2933.
[194] H. Watanabe, H. Ogawa, and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 375.
[195] F. Tamura, Y. Kiso, H. Yajima, A. Tanaka, and M. Nakamura, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2405.
[196] A. Eberle and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 1528.
[197] A. Eberle, J.-L. Fauchère, G. I. Tesser, and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 2106.
[198] J. van Rietschoten, C. Granier, H. Rochat, S. Lissitzky, and F. Miranda, *European J. Biochem.*, 1975, **56**, 35.

* Several other unpublished reports suggest that this sort of observation may be rather general for large cysteine-containing target sequences, and that reduction by conventional means is sometimes ineffective.

then oxidized at high dilution in 0.1M-tris buffer at pH 8.0. Carboxymethyl cellulose chromatography followed by filtration on Biogel P4 gave a homogeneous product. Edman degradation was effective up to step 13 and gave the correct sequence with no evidence for failure sequences. The synthetic compound was equipotent with the natural molecule.

Cytochrome *c*.—Studies continue on the total synthesis of bakers' yeast iso-1-cytochrome *c*, with reports on the synthesis of the *N*-benzyloxycarbonyl-protected fragment (127) (residues 67—108), with threonine replacing cysteine at position 107.[199, 200] The three fragments (123), (124), and (126) were joined as in Scheme 29 using a strategy of limited side-chain protection; only the side-chains



Conditions: i, TFA; ii, H_2-Pd; iii, Bu^{t}NO$_2$; iv, HF-anisole-methionine

Scheme 29

of lysine (Tfa) and glutamic acid (OBu^t) were protected. The condensation of (123) and (124) was carried out at 5 °C for 4 days. After isolation the product was deprotected with hydrogen fluoride and passed through a Sephadex G25 column to give a yield of 50%. T.l.c. and diagnostic amino-acid ratios from acid hydrolysates indicated the purity of the product (125). Coupling of fragments (125) and (126) was again accomplished by the azide method over 5 days at 5 °C in the powerful ternary solvent mixture, dimethylformamide, dimethyl sulphoxide, and hexamethylphosphoramide. The resultant product mixture was incubated

[199] L. Moroder, G. Borin, B. Filippi, and F. Marchiori, ref. 1, p. 1.
[200] L. Moroder, B. Filippi, G. Borin, and F. Marchiori, *Biopolymers*, 1975, **14**, 2061.

with 1M piperidine to remove the trifluoroacetyl groups and then purified by gel filtration on Sephadex G25 and G50, and finally chromatographed on carboxymethyl cellulose. Although incomplete removal of the Tfa groups was diagnosed, the final product was obtained in 25% yield.

A nonapeptide corresponding to residues 14—22 of cytochrome *c* has been synthesized by classical methods and also on a solid phase.[201]

Gastric Inhibitory Polypeptide (GIP).—A total synthesis of GIP from nine fragments (128)—(136) has been reported.[202] 5-Chloro-8-quinolyl esters were

Z(OMe)-Ile-Thr-Gln (128)

Z(OMe)-Lys(Z)-His-$NHNH_2$ (129)

Z(OMe)-Lys(Z)-Lys(Z)-Ser-$NHNH_2$ (130)

Z(OMe)-Gln-Gln-Lys(Z)-Gly-$NHNH_2$ (131)

Z(OMe)-Leu-Leu-Ala-$NHNH_2$ (132)

Z(OMe)-Phe-Val-$NHNH_2$ (133)

Z(OMe)-Lys(Z)-Ile-Arg(Tos)-$NHNH_2$ (134)

Z(OMe)-Tyr-Ser-Ile-Ala-Met-$NHNH_2$ (135)

Z(OMe)-Tyr-Ala-Glu(OBu^t)-Gly-Thr-Phe-Ile-Ser-$NHNH_2$ (136)

used extensively in the preparation of the fragments which were joined by the azide method. Asparagine (two residues), β-benzyl aspartate (three residues), and tryptophan (two residues) were added singly as *p*-nitrophenyl esters. The final deblocking was achieved with hydrogen fluoride in the presence of anisole, 2% ethanedithiol, and skatole. Purification after conversion into the acetate form was accomplished by gel filtration Sephadex G25 and carboxymethyl cellulose ion-exchange chromatography. This is a briefly reported synthesis and further details in a full paper will be of interest.

A synthesis of a protected (1—13) fragment of GIP has also been reported (see p. 277).

Human Growth Hormone(HGH).—Several synthetic attempts to locate an active fragment of HGH have appeared. The triacontapeptide fragment HGH (95—124) has been synthesized by the condensation of three fragments (137), (138), and (139) by the azide procedure employing the strategy of minimal side-chain protection.[203] Fragment (137) was condensed with (138) in 57% yield and, after

[201] G. A. Vasiléva, A. F. Mironov, L. F. Markova, T. A. Mednikova, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1975, **45**, 921 (*Chem. Abs.*, 1975, **83**, 79 573).

[202] H. Yajima, H. Ogawa, M. Kubota, T. Tobe, M. Fujimura, K. Henmi, K. Torizuka, H. Adachi, H. Imura, and T. Tominato, *J. Amer. Chem. Soc.*, 1975, **97**, 5593.

[203] R. Camble and R. Cotton, ref. 1, p. 17.

Z-Ser-Val-Phe-Ala-Asn-Ser-Leu-Val-Tyr(Bu^t)-Gly-NHNHBoc
(137)

Z-Ala-Ser-Asn-Ser-Asp(OBu^t)-Val-Tyr(Bu^t)-Asp(OBu^t)-Leu-Leu-NHNH-Boc
(138)

Z-Lys(Boc)-Asp-Leu-Glu-Glu-Gly-Ile-Gln-Thr-Leu-NH_2
(139)

purification and hydrogenolysis, the product was coupled with (139) in 62% yield. After gel filtration on Sephadex G25 diagnostic amino-acid ratios were excellent. After deprotection and final purification the product was devoid of biological activity. However, in earlier studies, the fragments HGH (88—124) and HGH (125—156) derived from solid-phase syntheses exhibited some growth-promoting activity,[204] and in order to obtain products with a 'higher degree of purity' solution classical syntheses were instigated.[205] The protected fragments HGH (139—146) and HGH (147—156) have now been described.[205]

A report on a solid-phase synthesis of HGH (140—191) with the cysteine residues in positions 165, 182, and 189 blocked by carboxyamidomethyl groups has appeared.[101] Symmetrical anhydrides were used for acylation with dimethylformamide and di-isopropylethylamine added during the 45 min coupling reaction. The claimed advantages of this procedure are that t-butoxycarbonyl glutamine can be incorporated as its symmetrical anhydride and that chain termination due to irreversible blocking of amino groups was reduced. Cleavage from the resin was effected by hydrogen fluoride and the product purified by partition chromatography and isoelectric precipitation. This latter procedure involved incubation of the peptide mixture at neutral pH; the target molecule was insoluble and precipitated while smaller contaminating fragments remained in solution. Synthetic product was less active than the natural fragment.

Seven fragments spanning the sequence 65–135 of HGH have been synthesized by a mixture of solid-phase and solution techniques and their purities impressively demonstrated.[206]

Insulin and Proinsulin.—An impressive solid-phase fragment condensation synthesis of [Glu^5,Ala^{12},Ala^{18},Ala^{21}] A-chain sheep insulin has been reported from the four fragments (140), (142), (143), (144), and a single residue (141).[207, 208]

Boc-Gly-Ile-Val-Glu(OBu^t)-Glu(OBu^t)
(140)

Boc-Cys(SPr^i)
(141)

Boc-Cys(SPr^i)-Ala-Gly-Val
(142)

Boc-Cys(SPr^i)-Ala-Leu-Tyr-Gln-Leu
(143)

Boc-Glu(OBu^t)-Ala-Tyr(Bzl)-Cys(SPr^i)-$NHNH_2$
(144)

[204] F. Chillemi, A. Aiello, and A. Pecile, *Nature New Biol.*, 1972, **238**, 243; F. Chillemi, *Gazzetta*, 1973, **103**, 657.
[205] F. Chillemi, *Internat. J. Peptide Protein Res.*, 1975, **7**, 143.
[206] S.-S. Wang and I. D. Kulesha, *J. Org. Chem.*, 1975, **40**, 1227.
[207] U. Weber and M. André, *Z. physiol. Chem.*, 1975, **356**, 701.
[208] U. Weber and M. André, ref. 1, p. 153.

Using a two-fold excess, (144) was coupled by the azide method to alanyl polystyrene resin, in 65% yield. Residual amino-groups were blocked by acetylation. The remaining three fragments and the single residue were coupled by the DCC–HOBt method using two-fold excesses in dimethylformamide in yields (143) 80—85%, (142) 96%, (141) 96%, and (140) 70—75%. Cleavage from the resin and removal of side-chain protecting groups (apart from the cysteine *S*-isopropylsulphenyl groups) was achieved by hydrogen bromide in trifluoroacetic acid. The cysteine protecting groups were removed by sulphitolysis and the *S*-sulphonated product purified on diethylaminoethyl-Sephadex and Biogel P4 giving the A-chain analogue in an overall yield of 9%. Combination with natural B-chain gave insulin with an activity of 86%.

One of the problems encountered in this synthesis was the attempted coupling of the fragment (140) containing *C*-terminal *S*-isopropylsulphenyl cysteine. This fragment was very insoluble and consequently the single residue coupling strategy had to be adopted. This general problem of insolubility of cysteine peptides has been neatly circumvented in a synthesis of insulin A-chain where internal disulphides were used for *S*-protection, greatly enhancing the solubility and ease of purification of protected peptide fragments (see p. 261). In another synthesis of the A-chain of sheep insulin the tetrahydropyranyl group was used for *S*-protection in combination with the Bpoc groups for N^{α}-protections and t-butyl esters and ethers for side-chain protection of carboxylic and hydroxy functions.[209] Total deblocking was achieved by treatment with trifluoroacetic acid thiocyanogen–acetic acid mixture, and after purification and combination with natural B-chain a crystallizable, insulin was obtained.

Several analogues of insulin which have been made by classical solution synthesis are [Lys^{B22}] human insulin,[210] [Ser^{A1}] ovine insulin,[211] [D-Asn^{A21}] ovine insulin,[212] and [$Hcy^{A7,20}$] ovine insulin.[213] The homocysteine analogue in which the intrachain disulphides have been elongated was completely devoid of insulin activity in the mouse convulsion assay.

An improved synthesis of human proinsulin connecting peptide and its benzyloxycarbonyl derivative from five fragments has been recorded.[214] Condensation was achieved by a combination of the DCC–HOBt, succinimide ester, and azide coupling methods. In immunological studies the synthetic compound showed responses similar to that of the natural fragment but different from those of an earlier synthesis. This is associated with the possibility of racemization during the coupling of fragments, or side reactions occurring with the azide method of coupling in the first synthesis. Tyrosine-C-peptide and its *N*-benzyloxycarbonyl derivative have also been prepared by condensing fragments Tyr-(33—39) with (40—63).[215] Synthesis of the sequence (28—34) of hog proinsulin has also been studied by a solid-phase stepwise and fragment condensation techniques.[216]

[209] E. Schwertner, H. Klostermeyer, and H. Zahn, *Annalen*, 1975, 1092.
[210] P. G. Katsoyannis, J. Ginos, A. Cosmatos, and P. G. Schwartz, *J.C.S. Perkin I*, 1975, 464.
[211] Y. Okada and P. G. Katsoyannis, *J. Amer. Chem. Soc.*, 1975, **97**, 4366.
[212] A. Cosmatos, S. Johnson, B. Breier, and P. G. Katsoyannis, *J.C.S. Perkin I*, 1975, 2157.
[213] A. Cosmatos and P. G. Katsoyannis, *J. Biol. Chem.*, 1975, **250**, 5315.
[214] V. K. Naithani, M. Dechesne, J. Markussen, and L. G. Heding, *Z. physiol. Chem.*, 1975, **356**, 997.
[215] V. K. Naithani, M. Dechesne, J. Markussen, L. G. Heding, and U. D. Larsen, *Z. physiol. Chem.*, 1975, **356**, 1305.
[216] K. Neubert and H. D. Jakubke, *J. prakt. Chem.*, 1975, **317**, 448.

Motilin.—A detailed analysis [217] of products obtained from a projected synthesis of [Nle^{13}] motilin has appeared.[218] In the final purification step on sulphoethyl-Sephadex C25, two fractions were obtained, one of which corresponded to the desired product and was fully active. The other was completely inactive. Comparison of D-amino-acid oxidase digests of acid hydrolysates with aminopeptidase M digests of the intact peptides, for both the active and inactive products, established that the latter was [D-Phe^{5},Nle^{13}] motilin. Racemization had evidently occurred either during the preparation of (145), when *N*-methylmorpholine was

Boc-Phe-Val-Pro-Ile-Phe-ONSu (145)

Thr(Bu^{t})-Tyr(Bu^{t})-Gly (146)

added to make the succinimide ester, or on coupling with (146). The o.r.d. and c.d. spectra of the correct synthetic target compound showed differences when compared to natural motilin. These are probably due to differences in their primary structures, particularly since the supposed glutamic acid residue in position 14 of motilin was later shown to be glutamine. The compound synthesized is therefore actually [Nle^{13},Glu^{14}] motilin. A total synthesis of the natural sequence has appeared.[219–221] Condensation of four fragments (147), (148), (149), and (150) was achieved by the azide method and (151) was introduced individually between fragments (149) and (150). Construction of the fragments was generally accomplished in a stepwise manner from the *C*-terminal residue without many problems. However, in the synthesis of fragment (150), the valine

Z(OMe)-Gln-Glu(OBzl)-Lys(Z)-Glu(OBzl)-Arg(Tos)-Asn-Lys(Z)-Gly-Gln (147)

Z(OMe)-Leu-Gln-Arg(Tos)-Met-$NHNH_2$ (148)

Z(OMe)-Thr-Tyr-Gly-$NHNH_2$ (149)

Z-Phe-Val-Pro-Ile-Phe-$NHNH_2$ (150)

Z(OMe)-Glu(OBzl)-ONp (151)

Z-Phe-Val-Pro (152)

to proline bond was difficult to form. Succinimide esters, DCC, and DCC–HOBt all failed. The tripeptide (152) was eventually prepared by a 2 + 1 condensation *via* the azide of *C*-terminal valine. Treatment with pentachlorophenyl trichloroacetate then produced the active ester which allowed the preparation of (150). Extensive use was made throughout the synthesis of column chromatography on silica using $CHCl_3$: MeOH : H_2O (8 : 3 : 1 v/v) as eluent. The hydrogen fluoride deprotected peptide exhibited characteristic contractile activity. A fragment condensation synthesis on a solid support has also been successful (see p. 272).

[217] E. Wünsch, E. Jaeger, R. Scharf, M. Deffner, and S. Knof, ref. 1, p. 239.
[218] E. Wünsch, J. C. Brown, K.-H. Deimer, F. Drees, E. Jaeger, J. Musiol, R. Scharf, H. Stocker, P. Thamm, and G. Wendlberger, *Z. Naturforsch.*, 1973, **28c**, 235.
[219] Y. Kai, H. Kawatani, and H. Yajima, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 2339.
[220] H. Yajima, Y. Kai, and H. Kawatani, *J.C.S. Chem. Comm.*, 1975, 159.
[221] K. Kai, H. Kawatani, H. Yajima, and Z. Itoh, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 2346.

Ribonuclease.—Perhaps the most ambitious synthetic project reported in 1975 is the solid-phase preparation of a 70 amino-acid residue analogue of ribonuclease A.[222] The *X*-ray data on ribonuclease A show several loops on the surface of the molecule, and this synthesis was initiated to study the influence these regions have on the correct folding of the enzyme. The target sequence (153) was arrived

H-*Leu*-Asn-Gln-Met-Met-
Lys-Ser-Arg-Asn-Leu————Ala————Lys-Pro-Val-Asn-Thr-
Phe-Val-His-Glu-Ser-Leu-Ala-Asp-Val-Gln-Ala-Val————
————Ala-Ala————Gln-Ser-
Tyr-Ser-Thr-Met-Ser-Ile-Thr-Asp-*Ala*-Arg-Glu————
————Gly————Tyr-Lys-Thr-Thr-Gln-Ala-Asn-Lys-His-
Ile-Ile-Val-Ala-*Ala*-Glu-Gly————Tyr-Val-Pro-Val-His-Phe-
Asp-Ala-Ser-Val-OH

(153)

at by omitting these loops and bridging the gaps with alanine or glycine residues; all cysteine residues were likewise replaced.

Protecting groups used were benzyl for the side-chains of serine, threonine, tyrosine, aspartic and glutamic acids, benzyloxycarbonyl for lysine, nitroarginine, and tosylhistidine. A normal solid-phase assembly of the sequence was carried out, with a double coupling procedure and after addition of the methionine residue at position 36, 10% anisole was added to the 20% trifluoroacetic acid–methylene chloride reagent to prevent alkylation during removal of Boc groups. Forty percent of the peptide was retained on the resin after assembly of the complete sequence. Hydrogen fluoride-containing anisole was used to liberate the free peptide over a period of 80 min during which time the temperature was allowed to rise to 14 °C. Gel filtration on Sephadex G50 gave polymeric material, a dimer, and a monomer, plus some shorter sequences. The monomer and dimer were further purified by ion exchange chromatography on carboxymethyl cellulose and the yield of the 70-residue S-protein analogue based on the amount of valine esterified to the resin was 12%.

Both the dimer and monomer alone were active to the extent of 4 and 0.1%, respectively in the transphosphorylation step using RNA as substrate. Addition of natural S-peptide or S-protein did not increase this activity. However, using cyclic cytidine 2′,3′-monophosphate as substrate, addition of natural S-peptide to both the monomeric and dimeric analogue gave 8% activity in both instances. Even higher activity was achieved with this substrate when natural S-protein was added to the synthetic compounds. In the case of the dimer 74% activity was generated and with the monomer, at a molar ratio of natural S-protein to synthetic 70-residue S-protein of 2 : 1, 151% activity was obtained based on comparisons with natural S-protein S-peptide mixtures. These are surprising results considering that histidine at position 12, which is thought to play a key role in the catalytic action of ribonuclease A, is missing in both these mixtures. Possible explanations of these observations, in terms of an overall mechanism of ribonuclease A, are discussed fully in the paper.

Solid-phase synthesis of an S-peptide (1—14) analogue in which histidine in position 2 has been replaced by homohistidine has appeared.[223] The analogue

[222] B. Gutte, *J. Biol. Chem.*, 1975, **250**, 889.
[223] O. D. van Bottenburg, J. Raap, K. E. T. Kerling, and E. Havinga, *Tetrahedron Letters*, 1975, 4591.

binds to S-protein to give practically full biological activity. S-peptide analogues have also been made by conventional solution synthesis to evaluate the role of the lysine residue in position 7.[224]

In continuing investigations into the reactivation of ribonuclease (1—118) by ribonuclease *C*-terminal tetradecapeptide, the role of serine-123 has been investigated.[225] The natural tetradecapeptide was made by solid-phase synthesis as were analogues in which serine-123 was replaced by Ser(Me) and alanine. After cleavage from the resin, purification was accomplished on Aminex 50W-X4 using a seven chambered gradient maker. A product deficient in tyrosine and containing the 3-benzyltyrosine, HF-rearrangment product of Tyr(Bzl) was characterized. Formation of other very minor products was also attributed to the action of hydrogen fluoride.

4 Appendix I: A List of Syntheses Reported during 1975

Natural Peptides, Proteins, Analogues, and Partial Sequences.—Syntheses are listed under the name of the peptide or protein to which they relate.

In previous lists we have normally followed tradition and included Greek prefixes when referring to partial sequences, as in, *e.g.* -(1—20)-eicosapeptide. However, if the residue numbers are given, the Greek prefix adds nothing but a bogus classical aura and we have dropped the practice this year.*

Peptide	*Ref.*
Acyl carrier protein	
Acyl carrier protein-(65—74)-peptide	3
Adrenocorticotropic hormone (ACTH)	
ACTH-(1—24)-peptide	4, 5
[Cys^4]-ACTH-(4—24)-peptide	6
A series of 23 analogues of ACTH-(4—10)-peptide	7
The human hormone	8
[9-(β-naphth-1-yl)alanine]-, [Trp(CHO)^9]-, and [Lys^8, Phe^9]-ACTH-(1—19)-peptides	9
[Ile^9]-ACTH-(1—24)-peptide	10
Miscellaneous related short peptides	11—13

[224] G. Borin, E. Marchiori, L. Moroder, and E. Scoffone, *Gazzetta*, 1975, **105**, 137.
[225] R. S. Hodges and R. B. Merrifield, *J. Biol. Chem.*, 1975, **250**, 1231.
[1] J.-P. Aubert and M.-H. Loucheux-Lefebvre, *Biopolymers*, 1975, **14**, 2429.
[2] E. Dellacherie and J. Neél, *Biopolymers*, 1975, **14**, 1437.
[3] E. Atherton, D. L. J. Clive, and R. C. Sheppard, *J. Amer. Chem. Soc.*, 1975, **97**, 6584.
[4] C. Hatanaka, O. Nishimura, and M. Fujino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1017.
[5] R. Matsueda, H. Maruyama, E. Kitazawa, and H. Takahagi, *J. Amer. Chem. Soc.*, 1975, **97**, 2573.
[6] J.-L. Fauchère and G.-M. Pelican, *Helv. Chim. Acta*, 1975, **58**, 1984.
[7] M. W. Draper, M. A. Rizack, and R. B. Merrifield, *Biochemistry*, 1975, **14**, 2933.
[8] O. Nishimura, C. Hatanaka, and M. Fujino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1212.
[9] J. Blake and C. H. Li, *J. Medicin. Chem.*, 1975, **18**, 423.
[10] S. Kumar, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1063.
[11] G. I. Tesser and I. C. Balvert-Geers, *Internat. J. Peptide Protein Res.*, 1975, **7**, 295.
[12] B. Penke and C. Birr, *Annalen*, 1974, 1999.
[13] G. I. Tesser in ref. 222, p. 53.

* This modest step in the direction of simplification will perhaps be thought rather boring as some authors have recently shown a taste for innovation and variety in the nomenclature of the field. During our work on these appendices we came across several rather bizarre inventions, the oddest being the use [1, 2] of $(cHxN{=})_2C$ for a reagent which already has a well-established and shorter abbreviation.

Peptide	*Ref.*
Angiotensin	
[Val^5]-Angiotensin II analogues modified in the *N*-terminal region	14
[Gly^4, Val^5]- and [Val^5, Gly^7]-Angiotensins II	15
Apamin	16
Bradykinin	
[7-(2-Carboxymethyl)-pyrrolidine*]-bradykinin	17, 18, 19
Alkylating analogues	20
Analogues containing α-methylamino-acids	21
[7-(3-amino-4-phenyl-butyric acid)]-bradykinin	19
Some protected fragments of bradykinin analogues containing pipecolic acid	22
Bradykinin-potentiating peptides	23, 24
Calcitonin	
Calcitonin M-(19—32)-peptide derivatives	28
Cholecystokinin	
A protected *C*-terminal pentapeptide sequence	29
Cytochrome *c*	
[Thr^{107}]-Baker's yeast iso-1-cytochrome *c*-(67—108)-protected peptide	25, 26
A protected nonapeptide fragment	27
Eledoisin	
Miscellaneous related peptides	30—32
Gastric inhibitory polypeptide (GIP)	
A protected porcine GIP-(1—13)-peptide and related peptides	33
Gastrin	
Analogues of the *C*-terminal tetrapeptide	34
[Leu^5]-Gastrin I-(5—13)-peptide	12

[14] P. J. Romanovskii, Z. P. Auna, and G. I. Cipens, *Bioorg. Khim.*, 1975, **1**, 182 (*Chem. Abs.*, 1975, **83**, 114 873).

[15] A. Pavars, I. Bite, G. Avotins, and G. Cipens, *Zhur. obshchei Khim.*, 1975, **45**, 1632 (*Chem. Abs.*, 1975, **83**, 193 700).

[16] J. van Rietschoten, C. Granier, H. Rochat, S. Lissitzky, and F. Miranda, *European J. Biochem.*, 1975, **56**, 35.

[17] U. Ragnarsson, S. M. Karlsson, and U. Hamberg, *Internat. J. Peptide Protein Res.*, 1975, **7**, 307.

[18] L. Balaspiri, B. Penke, Gy. Papp, Gy. Dambi, and K. Kovács, *Helv. Chim. Acta*, 1975, **58**, 969.

[19] M. A. Ondetti and S. L. Engel, *J. Medicin. Chem.*, 1975, **18**, 761.

[20] J. Turk, P. Needleman, and G. R. Marshall, *J. Medicin. Chem.*, 1975, **18**, 1135.

[21] J. Turk, P. Needleman, and G. R. Marshall, *J. Medicin. Chem.*, 1975, **18**, 1139.

[22] L. Balaspiri, B. Penke, G. Papp, and K. Kovacs, *Acta Phys. Chem.*, 1974, **20**, 465 (*Chem. Abs.*, 1975, **83**, 10 811).

[23] M. Tominaga, J. M. Stewart, T. B. Paiva, and A. C. M. Paiva, *J. Medicin. Chem.*, 1975, **18**, 130.

[24] G. A. Vardel, N. N. Monapova, N. A. Krit, M. P. Filatova, Yu I. Lisunkin, and V. T. Ivanova, *Khim. prirod. Soedinenii*, 1975, **11**, 47 (*Chem. Abs.*, 1975, **83**, 79 583).

[25] L. Moroder, B. Filipi, G. Borin, and F. Marchiori, *Biopolymers*, 1975, **14**, 2061.

[26] L. Moroder, G. Borin, B. Filipi, and F. Marchiori, in ref. 222, p. 1.

[27] G. A. Vasil'eva, A. F. Mironov, L. F. Markova, T. A. Mednikova, and R. P. Evstigneeva, *Zhur. obshchei Khim.*, 1975, **45**, 921 (*Chem. Abs.*, 1975, **83**, 79 573).

[28] H. Hagenmaier, *Z. physiol. Chem.*, 1975, **356**, 777.

[29] M. Bodanszky and S. Natarajan, *J. Org. Chem.*, 1975, **40**, 2495.

[30] M. Miyoshi and H. Sugano, in ref. 222, p. 355.

[31] M. M. Badawi, M. M. Kader, and M. K. Madkour, *J. Drug. Res.*, 1974, **6**, 157 (*Chem. Abs.*, 1975, **83**, 179 562).

[32] F. Cuiban, S. Cilianu, and V. Badea, *Rev. Roumaine Chim.*, 1974, **19**, 1521 (*Chem. Abs.*, 1975, **82**, 73 448).

[33] R. Macrae and G. T. Young, *J.C.S. Perkin I*, 1975, 1185.

[34] C. F. Hayward and J. S. Morley, in ref. 222, p. 287.

* This imino-acid is rather confusingly called α-homoproline by one group [18] and β-homoproline by another.[19] Both names seem unsatisfactory as the prefix homo has previously been used widely to indicate the extension of α-amino-acid side-chains.

Peptide	*Ref.*
Glutathione	35
Gonadotropin	
A partially blocked peptide with 20 residues related to the *C*-terminal sequence of the β-subunit of human chorionic gonadotropin	36
Growth hormone	
A set of seven protected peptides which span the 'active core' region of ovine pituitary growth hormone	37
[165,182,189-*S*-carbamidomethylcysteine]-Human growth hormone-(140—191)-peptide	38
Human growth hormone-(139—146)- and -(147—156)-protected peptides	39
Human growth hormone-(95—124)-peptide	40
Insulin*	
[Sar^{A1}] Ovine insulin	41
[Lys^{B22}] Human insulin	42
[D-Asn^{A21}] Ovine insulin	43
[Hcy^{A7,20}] Ovine insulin	44
A-chains and partial A-chains	45—48
B-chain sequences	49—51
Litorin (a nonapeptide related to bombesin *etc.* from the skin of an Australian frog)	56
Luteinizing hormone releasing hormone	
A series of analogues in which each residue is replaced in turn by proline	59
A series of analogues designed with regard to so-called 'pseudosymmetry'	60
A set of analogues with two or three modifications each	61

[35] M. M. Yuji, *Yamanouchi Seiyaku Kenkyu Hokoku*, 1974, **2**, 89 (*Chem. Abs.*, 1975, **83**, 193 702).
[36] C. H. Schneider, K. Blaser, C. Pfeuti, and E. Gruden, *F.E.B.S. Letters*, 1975, **50**, 272.
[37] S. S. Wang and I. D. Kulesha, *J. Org. Chem.*, 1975, **40**, 1227.
[38] J. Blake and C. H. Li, *Internat. J. Peptide Protein Res.*, 1975, **7**, 495.
[39] F. Chillemi, *Internat. J. Peptide Protein Res.*, 1975, **7**, 143.
[40] R. Camble and R. Cotton, in ref. 222, p. 17.
[41] Y. Okada and P. G. Katsoyannis, *J. Amer. Chem. Soc.*, 1975, **97**, 4366.
[42] P. G. Katsoyannis, J. Ginos, A. Cosmatos, and G. P. Schwartz, *J.C.S. Perkin I*, 1975, 464.
[43] A. Cosmatos, S. Johnson, B. Breier, and P. G. Katsoyannis, *J.C.S. Perkin I*, 1975, 2157.
[44] A. Cosmatos and P. G. Katsoyannis, *J. Biol. Chem.*, 1975, **250**, 5315.
[45] E. Schwertner, H. Klostermeyer, and H. Zahn, *Annalen*, 1975, 1092.
[46] H. Berndt and H. Zahn, *Annalen*, 1975, 1601.
[47] U. Weber and M. André, in ref. 222, p. 153.
[48] U. Weber and M. André, *Z. physiol. Chem.*, 1975, **356**, 701.
[49] G. Weitzel, K. Eisele, and W. Stock, *Z. physiol. Chem.*, 1975, **356**, 583.
[50] K. Eisele, *Z. physiol. Chem.*, 1975, **356**, 845.
[51] C. Birr and R. Frank, *F.E.B.S. Letters*, 1975, **55**, 68.
[52] H.-G. Gattner, *Z. physiol. Chem.*, 1975, **356**, 1397.
[53] W. O. Danho, H.-G. Gattner, D. Nissen, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 1405; H.-G. Gattner, E. W. Schmitt, and V. K. Naithani, *Z. physiol. Chem.*, 1975, **356**, 1465.
[54] H. Berndt, H.-G. Gattner, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 1469.
[55] D. Brandenburg, M. Biela, L. Herbertz, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 961.
[56] F. Angelucci and R. de Castiglione, *Experientia*, 1975, **31**, 507.
[57] D. H. Coy, E. J. Coy, and A. V. Schally, *Methods in Enzymology*, 1975, **37**, Part B, 416.
[58] D. H. Rich and S. K. Gurwana, *Tetrahedron Letters*, 1975, 301.
[59] H. Yajima, M. Kuroke, I. Yo, N. Fujii, and Y. Baba, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1622.
[60] C. R. Beddell, P. J. Fraser, D. Gilbert, P. J. Goodford, L. A. Lowe, and S. Wilkinson, *J. Medicin. Chem.*, 1975, **18**, 417.
[61] S. Kobayashi, S. Shinagawa, M. Obayashi, T. Fukuda, and M. Fujino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2033.

* Only analogues prepared by methods involving *in vitro* synthesis of peptide bonds are listed: several analogues have been prepared by chemical modification or degradation of native insulins, *e.g.* refs. 52—55.

Peptide	*Ref.*
Three antagonistic analogues	62
[Ser(Bu^t)^4]- and [Leu^4]-LH-RH	63
[2-(β-pyrazol-l-yl)alanine]-LH-RH	64
[Des-Gly-NH_2^{10}, Pro-NHEt^9]-LH-RH	65
[MeLeu^7]- and [D-Ala^6, MeLeu^7]-LH-RH	66
Miscellaneous analogues and related peptides	5, 57, 58, 63, 67
Lysozyme	
Lysozyme-(122—125)-tetrapeptide	68
Motilin	69
[Nle^{13}]-Motilin	70
Melanocyte-stimulating hormone (MSH)	
α-MSH and partial sequences thereof	71
Specifically tritiated α-MSH prepared by catalytic tritiation of the 3,5-dibromotyrosine analogue	72
Camel β-MSH	73
Porcine β-MSH	74
Myoglobin	
Partial sequences	75, 76
N-Terminal glycyl sperm whale myoglobin	77
Neurotensin	78
Oxytocin	79, 80
[1-β-mercapto-β,β-pentamethylenepropionic acid]-Oxytocin	81
[1-Penicillamine,Leu^4]-Oxytocin	82
[Phe^4]-Oxytocin	83
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[62] K. V. Prasad, R. W. Roeske, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 336.
[63] D. J. Schafer, A. D. Black, J. D. Bowen, and D. B. Crighton, *J. Medicin. Chem.*, 1975, **18**, 613.
[64] D. H. Coy, Y. Hirotsu, T. W. Redding, E. J. Coy, and A. V. Schally, *J. Medicin. Chem.*, 1975, **18**, 948.
[65] S. Shinagawa and M. Fujino, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 229.
[66] N. Ling and W. Vale, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 801.
[67] D. H. Rich and S. K. Gurwara, *J. Amer. Chem. Soc.*, 1975, **97**, 1575.
[68] P. Pfaender, H. Pratzel, H. Blecher, G. Gorka, and G. Hansen, in ref. 222, p. 137.
[69] H. Yajima, Y. Kai, and H. Kawatani, *J.C.S. Chem. Comm.*, 1975, 159.
[70] E. Wünsch, E. Jager, R. Scharf, M. Deffner, and S. Knof, in ref. 222, p. 239.
[71] A. Eberle, J.-L. Fauchère, G. I. Tesser, and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 2106.
[72] K. Nikolics, I. Telphan, and K. Medzihradszky, *Strahlentherapie, Sonderb.*, 1975, **74**, 282 (*Chem. Abs.*, 1975, **83**, 10 813).
[73] C. H. Li, D. Yamashiro, and S. Lemaire, *Biochemistry*, 1975, **14**, 953.
[74] H. Watanabe, H. Ogawa, and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 375.
[75] R. Uhmann and E. Bayer, *Annalen*, 1974, 1955.
[76] M. Mutter, R. Uhmann, and E. Bayer, *Annalen*, 1975, 901.
[77] W. H. Garner and F. R. N. Gurd, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 262.
[78] R. Carraway and S. E. Leeman, *J. Biol. Chem.*, 1975, **250**, 1912.
[79] J. D. Glass, C. Meyers, I. L. Schwartz, and R. Walter, in ref. 222, p. 141.
[80] L. Kisfaludy and I. Schon, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 227 (*Chem. Abs.*, 1975, **82**, 156 718).
[81] J. J. Nestor, M. F. Ferger, and V. du Vigneaud, *J. Medicin. Chem.*, 1975, **18**, 284.
[82] M. F. Ferger and W. Y. Chan, *J. Medicin. Chem.*, 1975, **18**, 1020.
[83] J. J. Nestor, M. F. Ferger, and W. Y. Chan, *J. Medicin. Chem.*, 1975, **18**, 1022.
[84] E. O. Lundell and M. F. Ferger, *J. Medicin. Chem.*, 1975, **18**, 1045.
[85] B. M. Ferrier and L. A. Branda, *Canad. J. Biochem.*, 1975, **53**, 21.
[86] H. W. Bossert and P.-A. Jaquenoud, in ref. 222, p. 233.

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[87] J. Roy, R. T. Havran, I. L. Schwartz, and R. Walter, *Internat. J. Peptide Protein Res.*, 1975, **7**, 171.
[88] I. Frič, M. Kodiček, Z. Procházka, K. Jöst, and K. Bláha, *Coll. Czech. Chem. Comm.*, 1975, **39**, 1290.
[89] D. H. Rich, P. D. Gesellchen, A. Tong, A. Cheung, and C. K. Buckner, *J. Medicin. Chem.*, 1975, **18**, 1004.
[90] L. Kisfaludy and I. Schon, *Acta Chim. Acad. Sci. Hung.*, 1975, **84**, 227 (*Chem. Abs.*, 1975, **82**, 156 717).
[91] M. A. El-Maghraby, *Egypt. J. Chem.*, 1974, **17**, 1, 5 (*Chem. Abs.*, 1975, **82**, 86 626, 98 384).
[92] V. J. Hruby and L. E. Barstow, *Macromol. Syn.*, 1972, **3**, 91 (*Chem. Abs.*, 1975, **83**, 10 819).
[93] G. W. Tregear, in ref. 222, p. 177.
[94] P. Rivaille, J. F. Staub, A. Julliene, D. Raulais, and G. Milhaud, in ref. 222, p. 221.
[95] K. Channabasavaiah and K. M. Sivanandaiah, *Indian J. Chem.*, 1974, **12**, 1203 (*Chem. Abs.*, 1975, **82**, 86 620).
[96] V. K. Naithani, M. Dechesne, J. Markussen, L. G. Heding, and U. D. Larsen, *Z. physiol. Chem.*, 1975, **356**, 1305.
[97] V. K. Naithani, M. Dechesne, J. Markussen, and L. G. Heding, *Z. physiol. Chem.*, 1975, **356**, 997.
[98] D. Yamashiro, M. Rigbi, T. A. Bewley, and C. H. Li, *Internat. J. Peptide Protein Res.*, 1975, **7**, 389.
[99] N. Nishino, H. Aoyagi, T. Kato, and N. Izumiya, *Experientia*, 1975, **31**, 410.
[100] R. Rocchi, C. A. Benassi, M. Guarneri, A. Guggi, and R. Tomatis, in ref. 222, p. 9.
[101] S. Wiejak and B. Rzeszotarska, *Roczniki Chem.*, 1974, **48**, 2207 (*Chem. Abs.*, 1975, **83**, 10 785).
[102] R. S. Hodges and R. B. Merrifield, *J. Biol. Chem.*, 1975, **250**, 1231.
[103] O. D. van Batenburg, J. Raap, K. E. T. Kerling, and E. Havinga, *Tetrahedron Letters*, 1975, 4591.
[104] G. Borin, F. Marchiori, L. Moroder, and E. Scoffone, *Gazzetta*, 1975, **105**, 137.
[105] B. Gutte, *J. Biol. Chem.*, 1975, **250**, 889.
[106] I. M. Chaiken and B. M. Dunn, in ref. 222, p. 299.
[107] H. N. Guttman, B. Weinstein, R. M. Bartschot, and P. S. Tam, *Experientia*, 1975, **31**, 285.
[108] A. van Zon and H. C. Beyerman, in ref. 222, p. 165.
[109] W. Göhring and G. Jung, *Annalen*, 1975, 1765.
[110] W. Göhring and G. Jung, *Annalen*, 1975, 1776.
[111] W. Göhring and G. Jung, *Annalen*, 1975, 1781.

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[112] N. Fujii and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1596.
[113] J. Rivier, M. Brown, and W. Vale, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 746.
[114] J. Rivier, P. Brazeau, W. Vale, and R. Guillemin, *J. Medicin. Chem.*, 1975, **18**, 123.
[115] J. L. M. Syrier and H. C. Beyerman, in ref. 222, p. 105.
[116] J. van Rietschoten, G. W. Tregear, S. Leeman, D. Powell, H. Niall, and J. T. Potts, jun. in ref. 222, p. 113.
[117] J. Rivier, *Methods in Enzymology*, 1975, **37**, Part B, 408.
[118] G. Mattalia and U. Bucciarelli, *Experientia*, 1975, **31**, 874.
[119] E. Gross, K. Noda, and S. Matsuura, in ref. 222, p. 403.
[120] K. Bankowski and S. Drabarek, *Roczniki Chem.*, 1975, **49**, 1043 (*Chem. Abs.*, 1975, **83**, 79 607).
[121] K. Channabasavaiah and K. M. Sivanandaiah, *Internat. J. Peptide Protein Res.*, 1975, **7**, 281.
[122] H. Yajima, H. Ogawa, H. Watanabe, N. Fujii, M. Kurobe, and S. Miyamoto, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 371.
[123] M. Walti and D. B. Hope, *J.C.S. Perkin I*, 1975, 1691.
[124] C. W. Smith, M. F. Ferger, and W. Y. Chan, *J. Medicin. Chem.*, 1975, **18**, 822.
[125] E. G. G. Lindberg, *Internat. J. Peptide Protein Res.*, 1975, **7**, 395.
[126] M. Zaoral, I. Laine, and F. Brtnik, *Coll. Czech. Chem. Comm.*, 1975, **39**, 2975.
[127] M. Palumbo, R. L. Rodin, and M. Goodman, *Biochemistry*, 1975, **14**, 485.
[128] R. Boni, E. De Gregorüs, R. Di Blasi, and A. S. Verdini, *Biopolymers*, 1975, **14**, 2211.
[129] B. R. Shaw and J. M. Schurr, *Biopolymers*, 1975, **14**, 1951.
[130] H. Daoust and S. St.-Pierre, *Canad. J. Chem.*, 1975, **53**, 1861.
[131] A. R. Zeiger, C.-H. Lai, and P. H. Maurer, *Biopolymers*, 1975, **14**, 2281.
[132] J. Šponar, Š. Štokrova, I. Koruna, and K. Bláha, *Coll. Czech. Chem. Comm.*, 1975, **39**, 1625.
[133] P. Bruckner, B. Rutschmann, J. Engel, and M. Rothe, *Helv. Chim. Acta*, 1975, **58**, 1276.

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[134] R. S. Rapaka and R. S. Bhatnagar, *Internat. J. Peptide Protein Res.*, 1975, **7**, 475.
[135] V. A. Shibner and M. I. Ismoilov, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1974, 2328 (*Chem. Abs.*, 1975, **82**, 86 605).
[136] Y. Trudelle and C. Birr, *Internat. J. Peptide Protein Res.*, 1975, **7**, 403.
[137] A. B. Zegel'man, T. Yu. Yusupov, A. Azizov, and S. Nizamiddinov, *Doklady Akad. Nauk Tadzh. S.S.R.*, 1974, **17**, 36 (*Chem. Abs.*, 1975, **82**, 140 489).
[138] J. R. Bell, R. C. Boohan, J. H. Jones, and R. M. Moore, *Internat. J. Peptide Protein Res.*, 1975, **7**, 227.
[139] J. R. Bell, J. H. Jones, and T. C. Webb, *Internat. J. Peptide Protein Res.*, 1975, **7**, 235.
[140] R. S. Rapaka and R. S. Bhatnagar, *Internat. J. Peptide Protein Res.*, 1975, **7**, 119.
[141] A. B. Zegel'man, T. Yu. Yusupov, and T. Dostov, *Doklady Akad. Nauk Tadzh S.S.R.*, 1975, **18**, 30 (*Chem. Abs.*, 1975, **83**, 79 567).
[142] A. Yaron, A. Yanovsky, M. Constantini-Sourojon, and S. Ben-Efraim, in ref. 222, p. 311.
[143] R. Katukai, M. Oya, and Y. Iwakura, *Biopolymers*, 1975, **14**, 1315.
[144] S. Sano and S. Kawanishi, *J. Amer. Chem. Soc.*, 1975, **97**, 3480.
[145] R. S. Feinberg and R. B. Merrifield, *J. Amer. Chem. Soc.*, 1975, **97**, 3485.
[146] S. S. Wang, *J. Org. Chem.*, 1975, **40**, 1235.
[147] R. G. Hiskey, N. Muthukumanaswamy, and R. R. Vunnam, *J. Org. Chem.*, 1975, **40**, 950.
[148] W. Y. Chen and R. K. Olsen, *J. Org. Chem.*, 1975, **40**, 350.
[149] R. Katakai, *J. Org. Chem.*, 1975, **40**, 2697.
[150] O. Keller and J. Rudinger, *Helv. Chim. Acta*, 1975, **58**, 531.
[151] M. Hariharan, R. J. Motekaitis, and A. E. Martell, *J. Org. Chem.*, 1975, **40**, 470.
[152] H. Künzi and R. O. Studer, *Helv. Chim. Acta*, 1975, **58**, 139.
[153] B. Riniker, B. Kamber, and P. Sieber, *Helv. Chim. Acta*, 1975, **58**, 1086.
[154] A. J. Bates, I. J. Galpin, A. Hallett, D. Hudson, G. W. Kenner, R. Ramage, and R. C. Sheppard, *Helv. Chim. Acta*, 1975, **58**, 688.

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[155] P. K. Sengupta, J. H. Bieri, and M. Viscontini, *Helv. Chim. Acta*, 1975, **58**, 1374.
[156] E. Escher and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 1465.
[157] Z. J. Kaminski, M. T. Leplawy, and V. Slomczynska, *Synthesis*, 1975, 593.
[158] D. H. Rich, J. Tam, P. Mathiaparanam, and J. Grant, *Synthesis*, 1975, 402.
[159] E. Schwertner, H. Berndt, H.-G. Gielen, and H. Zahn, *Annalen*, 1975, 581.
[160] R. Appel, G. Baumer, and W. Struver, *Chem. Ber.*, 1975, **108**, 2680.
[161] G. W. Phillips and S. J. Norton, *J. Medicin. Chem.*, 1975, **18**, 482.
[162] H. T. Nagasawa, J. A. Elberling, and F. N. Shirota, *J. Medicin. Chem.*, 1975, **18**, 826.
[163] G. Buse, H. Klostermeyer, and G. Steffens, *Z. physiol. Chem.*, 1975, **356**, 895.
[164] A. Kolodziejczak and A. Arendt, *Roczniki Chem.*, 1975, **49**, 777 (*Chem. Abs.*, 1975, **83**, 164 550).
[165] M. Tejima, M. Takeuchi, E. Ichishima, and S. Kobayashi, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 1423 (*Chem. Abs.*, 1975, **83**, 179 558).
[166] I. Tomida, M. Konno, H. Tokumori, T. Yasui, H. Kayahara, and R. Iriye, *Agric. and Biol. Chem. (Japan)*, 1975, **39**, 469.
[167] R. Livingston, D. G. Doherty, and H. Zeldes, *J. Amer. Chem. Soc.*, 1975, **97**, 3198.
[168] B. Castro, J. R. Dormoy, G. Evin, and C. Selve, *Tetrahedron Letters*, 1975, 1219.
[169] S. I. Hong, M. K. Rho, and Y. J. Kim, *Taehan Hwahak Hoechi*, 1975, **19**, 134 (*Chem. Abs.*, 1975, **83**, 59 256).
[170] A. S. Molokoedov, E. I. Filippovich, and R. P. Evstigneeva, *Trudy Moskov. in-ta torkoi khim. tekhnol.*, 1974, **4**, 64.
[171] J. Photaki, A. Ferderigos, and L. Zervas, in ref. 222, p. 47.
[172] J. Photaki, I. Samoulidis, and L. Zervas, in ref. 222, p. 415.
[173] C. N. C. Drey, J. Lowbridge, and R. J. Ridge, in ref. 222, p. 419.
[174] A. Eisele and K. Eisele, *F.E.B.S. Letters*, 1975, **55**, 153.
[175] T. Sugihara, Y. Imanishi, and T. Higashimura, *Biopolymers*, 1975, **14**, 723.
[176] J. Caspers, W. Hecq, and A. Loffet, *Biopolymers*, 1975, **14**, 2263.
[177] J. Caspers, W. Hecq, and A. Loffet, in ref. 222, p. 97.
[178] R. Guarnaccia, G. P. Lorenzi, V. Rizzo, and P. L. Luisi, *Biopolymers*, 1975, **14**, 2329.

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[179] R. Katakai and M. Oya, *Biopolymers*, 1975, **14**, 2507.
[180] G. P. Sachder and J. S. Fruton, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3424.
[181] L. A. Maksimova, G. L. Grigoryan, and E. G. Rozantsev, *Izvest. Akad. Nauk S.S.S.R., Ser. khim.*, 1975, 945 (*Chem. Abs.*, 1975, **83**, 97 892).
[182] V. I. Tsetlin, E. N. Shepel, V. T. Ivanov, and Yu. A. Ovchinnikov, *Bioorg. Khim.*, 1975, **1**, 407 (*Chem. Abs.*, 1975, **83**, 92 907).
[183] Ts. E. Agadzhanyan and K. L. Amboyan, *Armyan Khim. Zhur.*, 1974, **27**, 890, 896 (*Chem. Abs.*, 1975, **83**, 114 860, 114 861).
[184] B. Weinstein, R. M. Bartschot, R. M. Cook, P. S. Tam, and H. N. Guttman, *Experientia*, 1975, **31**, 754.
[185] K. Zech and W. Voelter, *Z. Naturforsch.*, 1974, **29b**, 818.
[186] E. M. S. Salem, H. I. El Sissi, and A. M. El Naggar, *Indian J. Chem.*, 1974, **12**, 1071 (*Chem. Abs.*, 1975, **82**, 98 378).
[187] L. I. Sholudchenko, L. G. Kovalenko, and N. Ya. Krasnobrizhii, *Zhur. obshchei Khim.*, 1974, **44**, 2337 (*Chem. Abs.*, 1975, **82**, 86 606).
[188] N. Ya. Krasnobrizhii, L. G. Kovalenko, and L. I. Sholudchenko, *Zhur. org. Khim.*, 1975, **11**, 310 (*Chem. Abs.*, 1975, **82**, 140 490).
[189] L. V. Fedorova, E. M. Skrynik, and N. Ya. Krasnobrizhii, *Zhur. obshchei Khim.*, 1975, **45**, 216 (*Chem. Abs.*, 1975, **82**, 156 699).
[190] L. V. Fedorova and N. Ya. Krasnobrizhii, *Ukrain. Khim. Zhur.*, 1974, **40**, 1295; 1975, **41**, 188 (*Chem. Abs.*, 1975, **82**, 156 700; 156 701).
[191] N. Ya. Krasnobrizhii, L. V. Fedorova, and L. G. Kovalenko, *Zhur. org. Khim.*, 1975, **11**, 307 (*Chem. Abs.*, 1975, **82**, 156 702).
[192] L. V. Fedorova, L. G. Kovalenko, and N. Ya. Krasnobrizhii, *Khim. prirod. Soedinenii*, 1975, **11**, 41, 44 (*Chem. Abs.*, 1975, **83**, 79 581, 79 582).
[193] H. Kunzek, C. Seidel, E. Klauschenz, and K. D. Kaufmann, *Z. Chem.*, 1975, **15**, 20.
[194] H. Kunzek, C. Seidel, and G. Frache, *Z. Chem.*, 1975, **15**, 58.
[195] R. Paegle, V. Krisane, I. Lulle, and M. Lidaks, *Khim. geterotsikl. Soedinenii*, 1975, 114 (*Chem. Abs.*, 1975, **82**, 156 719).
[196] D. H. Rich and J. P. Tam, *Tetrahedron Letters*, 1975, 211.
[197] B. Penke, L. Balaspiri, P. Pallai, and K. Kovacs, *Acta Phys. Chem.*, 1974, **20**, 471.
[198] I. Photaki, C. Sakarellos, A. Yotakis, and L. Zervas, in ref. 222, p. 65.
[199] M. Goodman and C. Gilon, in ref. 222, p. 271.
[200] D. Chang, T. Greibrokk, R. Knudsen, G. Howard, J. Humphries, and K. Folkers, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 1208.
[201] A. M. El-Naggar, E. M. S. Salem, and M. R. Zaher, *Indian J. Chem.*, 1974, **12**, 1161 (*Chem. Abs.*, 1975, **82**, 86 625).

5 Appendix II: Amino-acid Derivatives Useful in Synthesis

The preamble to last year's list applies. The compounds listed are not all new: in some cases the citation is made because useful new physical constants are reported. We have used the same abbreviations as Schafer did last year, together with a few additional ones. For complete clarity all the substituent abbreviations used are incorporated into the list at the front of the book. Residue abbreviations are standard throughout.

Coded Amino-acids

Compound	*m.p./°C*	$[\alpha]_D$*	*Conc.*	*Solvent*	*Ref.*
Alanine					
Ala-NHNHPicoc,2CF_3CO_2H	hygroscopic	+7	1.1	H_2O	33
Ala-OBu^t,TosOH	222	+1.7	1.62	MeOH	202
Ala-OPh(SMe),HCl	180—182	+4	1.5	EtOH	203
Boc-Ala-OBu^t	b.p. 166/2 mm†	−32.1	1.49	MeOH	202
Boc-Ala-ONp(*o*)	84—86	−75.5	2	DMF	204
Boc-Ala-OPcp	166	−22	5	$CHCl_3$	204
Boc-Ala-OPh(SMe)	93—95	−56	1.5	EtOH	203
Boc-Ala-$NHNH_2$	94.5—96	−25	0.4	MeOH	33
Boc-Ala-$NHNH_2$	96—97	−27	1	MeOH	33
Boc-Ala-NHNHPicoc	173—175	−23	1	DMF	33
Msc-Ala, Dcha	180	+3.9	1	MeOH	11
Nps-Ala NCA	176—178	+22.7	1	THF	149
Ppoc-Ala,Dcha	112—114	−3.9	1	MeOH	205
Tac-Ala	165—166	−8.3	2	EtOH	152
Z-Ala-ONp(*o*)	92—94	−51	2	DMF	204
Arginine					
Arg(Trt_2)-OBzl, HCl	176—178	+10.3	2	MeOH	198
Boc-Arg(NO_2)	123—125	—	—	—	206
Msc-Arg	oil	—	—	—	11
Ppoc-Arg(Tos)	122.5—123.5	+3.9	1	MeOH	205
Tac-Arg(Tos)	182—183	—	—	—	152
Trt-Arg(Trt_2)	136—139(d)	−29.8	2	$CHCl_3$	198
Asparagine					
Boc-Asn	167—168(d)	—	—	—	206
Boc-Asn[Bzh(OMe_2)]	144.5—145.0(d)	—	—	—	102
Msc-Asn,Dcha	171	+2.5	1	MeOH	11
Ppoc-Asn[Bzh(OMe_2)]	amorphous	+9.8	1	MeOH	205
Z-Asn-OBzl(NO_2)	—	−15	1	DMF	43
Z-Asn-OQ(5-Cl)	176—179	—	—	—	122
Z-(OMe)-Asn-OQ(5-Cl)	173—176	—	—	—	122
Aspartic acid					
Asp(OMe),HCl	188—190	—	—	—	207
Asp[OBzl(NO_2)]	193—195	—	—	—	207

[202] J. Goodacre, R. J. Ponsford, and I. Stirling, *Tetrahedron Letters*, 1975, 3609.
[203] W.-Y. Chen, M.-L. Hsu, and R. K. Olsen, *J. Org. Chem.*, 1975, **40**, 3110.
[204] J. Martinez and F. Winternitz, *Tetrahedron Letters*, 1975, 2631.
[205] B. E. B. Sandberg and U. Ragnansson, *Internat. J. Peptide Protein Res.*, 1975, **7**, 503.
[206] M. Itoh, D. Hagiwara, and T. Kamiya, *Tetrahedron Letters*, 1975, 4393.
[207] R. L. Prestidge, D. R. K. Harding, J. E. Battersby, and W. S. Hancock, *J. Org. Chem.*, 1975, **40**, 3287.

* The specific rotations listed were all determined at 20—25 °C and all except those distinguished by superscripts as follows are for the D-line: [a] 365 nm, [b] 546 nm, [c] 578 nm.

† $n_D^{22} = 1.4310$.

Coded Amino-acids (*cont.*)

Compound	*m.p./°C*	$[\alpha]_D$	*Conc.*	*Solvent*	*Ref.*
Asp(OBzl)	220—222	—	—	—	207
Asp(OBu^t)-OEt,HCl	152—153	+20.3	1	EtOH	75
Boc-Asp(OBzl)-NHNHPicoc	168—170	−17	1	DMF	33
Msc-Asp, $Dcha_2$	176	+7.8	1	MeOH	11
Nps-Asp(OBzl) NCA	137—138	+70.8	2	THF	149
Z(OMe)-Asp(OBzl)-OQ(5-Cl)	100—101	—	—	—	122
Cysteine					
Cys(Acm),H_2O	194(d)	−41.9	1	H_2O	136
Cys(Acm),H_2O		−49.6[b]	1	H_2O	136
Cys(SPr^i)-OMe,HCl	117—120	−53	1	MeOH	48
Boc-Cys(Ec)	137.5—138.5	−24	0.9	EtOH	124
Boc-Cys(Spr^i)-ONSu	126	−108	1	EtOAc	48
Boc-Cys(Scm)-OMe	78	+27.7	1	$CHCl_3$	147
Bpoc-Cys(Bzh),Cha	113—118	+8.3	1	MeOH	159
Bpoc-Cys(SBu^t),Cha	120—122	−46.2	1	MeOH	159
Bpoc-Cys(Trt),Dcha	173—176	+26.9	1	MeOH	159
Bpoc-Cys(Thp),Dcha	147—150	+76.2	1	MeOH	159
Ddz-Cys(Acm),Dcha	58—75	−20.6	1	MeOH	136
Ddz-Cys(Acm),Dcha		−25.0[b]	1	MeOH	136
Ppoc-Cys(Bzl),Dcha	134—135	+4.4	1	MeOH	205
Tac-Cys(Bzl)	177—179	−13.5	1	EtOH	152
Z-Cys(Bzl)-ONp	89—90	−38.5	4	EtOH	204
Z-Cys(Scm)-OMe	61	+17.6	1	$CHCl_3$	147
Z(OMe)-Cys(Bzl)-OQ(5-Cl)	125—126	—	—	—	122
Glutamic acid					
Glu(OBzl)	169—170	+19.3	5.49	AcOH	207
Glu[OBzl(NO_2)]	158—159	—	—	—	207
Glu(OMe)	180	—	—	—	207
Glu(OEt)	192—194	—	—	—	207
Boc-Glu(OBzl)-ONSu	103—104	+23.67	1	THF	37
Msc-Glu(OBu^t),Dcha	172	+8.0	1	MeOH	11
Msc-Glu,$Dcha_2$	150	−1.1	1	MeOH	11
Nps-Glu(OMe)NCA	113—115	+31.9	2	THF	149
Nps-Glu(OBzl)NCA	oil	+18.7	2.5	THF	149
Ppoc-Glu(OBzl),Cha	134—136	+19.4	1	MeOH	205
Z-Glu(OBu^t)-OMe	oil: t.l.c. and n.m.r. data only				208
Z-Glu(OBu^t)-ONSu	103—104	−32.8	1	MeOH	71
Z(OMe)-Glu(OBzl)-OQ(5-Cl)	92—93	—	—	—	122
Glutamine					
Gln-NHNHZ	160	—	—	—	107
Msc-Gln,Dcha	151	+3.0	1	MeOH	11
Ppoc-Gln[Bzh(OMe_2)]	amorphous	+5.0	1	MeOH	205
Z-Gln-OQ(5-Cl)	216—217	—	—	—	122
Z(OMe)-Gln-OQ(5-Cl)	152—154	—	—	—	122
Glycine					
Gly-OTse,HCl	no data given	—	—	—	71
Boc-Gly-OTse	no data given	—	—	—	71
Boc-Gly-NHNHPicoc	163.5—165	—	—	—	33
Bpoc-Gly-ONSu	136—138	—	—	—	159
Mal=Gly	105—106	—	—	—	150
Mal=Gly-ONSu	183—184	—	—	—	150

[208] W. Märki and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 1471; N. T. Boggs, tert., R. E. Gawley, K. A. Kochler, and R. G. Hiskey, *J. Org. Chem.*, 1975, **40**, 2850.

Coded Amino-acids (*cont.*)

Compound	*m.p./°C*	$[\alpha]_D$	*Conc.*	*Solvent*	*Ref.*
Msc-Gly,Dcha	167	—	—	—	11
Nps-Gly NCA	168—170	—	—	—	149
Ppoc-Gly-ONp	oil	—	—	—	153
Tac-Gly	197—198	—	—	—	152
Z-Gly-NHEt	99—100	—	—	—	61
Z-Gly-ONp(*o*)	74	—	—	—	204
Z-Gly-imidazolide	105—107	—	—	—	209
Histidine					
Boc-His(Boc),Dcha*	155—157	+18.8	2	$CHCl_3$	73
Boc-His[Bzl(2-NO_2)]	90(d)	+12.9	2	MeOH	210
Boc-His(Dnp)	92—94	—	—	—	75
Boc-His(Dnp)-ONp	164—165	−6.1	0.5	THF	39
Boc-His(Ztf)	146.5—147.5	—	—	—	211
Z(OMe)-His(Bzl)-OQ(5-Cl)	147—148	—	—	—	122
Isoleucine					
Boc-Ile-NHNHPicoc	amorphous	−21	1	DMF	33
Bpoc-Ile-ONSu	119—120	−30.9	1	dioxan	159
Msc-Ile	94	+5.8	1	MeOH	11
Nps-Ile NCA	101—103	+32.9	1	THF	149
Z-Ile	51—54	+13	2	EtOH	212
Leucine					
Boc-Leu-ONp(*o*)	54—57	−62	2	DMF	204
Boc-Leu-OPcp	110	−35	5	EtOAc	204
Boc-Leu-NHNHPicoc	136.5—137.5	−21	1	DMF	33
Mal-Leu,Dcha	193—194	−12.2	1	MeOH	150
Msc-Leu,Dcha	175	−6	1	MeOH	11
Msc-Leu,Dcha		−9.6	1	H_2O	11
Ppoc-Leu-ONp	97—100	—	—	—	153
Ppoc-Leu	108—110	−20.4	1	MeOH	205
Ppoc-Leu	119—120	—	—	—	153
Nps-LeuNCA	97—99	+44.8	1	THF	149
Tac-Leu	175—176	−5.1	2	EtOH	152
Z-Leu-ONp(*o*)	87—88	−46	2	DMF	204
Lysine					
Lys(Pz)-OMe,HCl	201	—	—	—	75
Lys(Pz)	244—246	—	—	—	75
Lys(Msc)	217—224	+13.5	1	1N-HCl	11
Lys(Z)-OPcp,HCl	146—148	+12.5	0.5	DMF	132
Lys(Z)-NH_2,HCl	211	+10.8	1	MeOH	71
Ac-Lys(Z)-NH_2	148	−1.8	1	MeOH	71
Ac-Lys-NH_2,AcOH	oil	−8.8	0.85	MeOH	71
Boc-Lys(Pz)	108—110	+7.3	2	MeOH	75
Boc-Lys(Pz)		+8.1	2	MeOH	75
Boc-Lys(Pz)-OMe	82	—	—	—	75
Boc-Lys(Msc)	85	+3.6	2	EtOAc	11
Boc-Lys(Msc)	83—86	—	—	—	71
Boc-Lys(Msc),Dcha	135	−2.4	1.22	MeOH	71
Boc-Lys(Msc)-ONp	94—96	−23.6	1	MeOH	71
Boc-Lys(Z)-NH_2	142	+1.6	1	MeOH	71

[209] H. R. Kricheldorf, E. Stengele, and W. Regel, *Annalen*, 1975, 1379.
[210] S. M. Kalbag and R. W. Roeske, *J. Amer. Chem. Soc.*, 1975, **97**, 440.
[211] O. Schon, K. Brunfeldt, I. Rubin, and L. Hansen, *Z. physiol. Chem.*, 1975, **356**, 1451.
[212] G. Flouret and S. H. Nakagawa, *J. Org. Chem.*, 1975, **40**, 2635.

* Improved procedure.

Coded Amino-acids (*cont.*)

Compound	*m.p./°C*	$[\alpha]_D$	*Conc.*	*Solvent*	*Ref.*
Nps-Lys(Z)-OPcp	118—119	−32.8	0.5	DMF	132
Nps-Lys(Z)NCA	121—123	+23.5	2	THF	149
Ppoc-Lys(Z),Dcha	87—89	+12.7	1	MeOH	205
Tac-Lys(Z)	amorphous: no data given				152
Z-Lys(Msc)	126	+4.3	1	MeOH	11
Z-Lys(Bpoc)-OMe	amorphous: no data given				153
Z-Lys(Trt)-OMe	amorphous: no data given				153
Z-Lys(=Mal),Dcha	140—142	+11.7	4	$CHCl_3$	150
Z-Lys(Z)-ONp	oil	−12.4	0.48	dioxan	204
Z(OMe)-Lys(Z)-OQ(5-Cl)	136—137	—	—	—	122
Methionine					
Met-ONp,HCl	154	+3.1	1.5	DMF	2
Boc-Met,Dcha	137—139	—	—	—	206
Bpoc-Met-ONp	90—92	−71.1	1	MeOH	37
Msc-Met,Dcha	155	+4.2	1	MeOH	11
Msc-Met,Dcha		−7.2	1	H_2O	11
Ppoc-Met	75.5—77	−18.2	1	MeOH	205
Z(OMe)-Met-OQ(5-Cl)	128—129	—	—	—	122
Proline					
Pro-OMe,Tos_2NH	157—158	−12.6	0.5	MeOH	132
Pro-OPcp,HCl	191—192(d)	16.7*	0.6	MeOH	128
Boc-Pro-OPcp	113—115	−36.5	1	$CHCl_3$	128
Bpoc-Pro-ONSu	185	+27.4	2	dioxan	159
Msc-Pro,Dcha	171	−29.6	1	MeOH	11
Z-Pro-NHEt	104—105	−38.6	0.94	EtOH	65
Z-Pro-NHEt	104—106	—	—	—	60
Z-Pro-OPcp	94—96	−46	1	DMF	204
Phenylalanine					
Boc-Phe-ONp(*o*)	144—145	−65.2	2	DMF	204
Boc-Phe-OPcp	145—148	−46.5	5	EtOAc	204
Boc-Phe-NHNHPicoc	177—178	−5	1	DMF	33
Ddz-Phe,Dcha	113—115	−45.5	1	MeOH	136
Mal=Phe	168—169	−124	5	MeOH	150
Msc-Phe-ONp	141—142	−18.7	1.5	MeCN	213
Msc-Phe-ONp	140	−20.1	1.03	MeCN	11
Msc-Phe	113	−2.6	1	MeOH	11
Msc-Phe		−4.0	1	pyridine	213
Msc-Phe		+6.0	1.5	AcOH	213
Nps-Phe NCA	151—153	+31.2	2	THF	149
Nps-Phe-OTcp	133—135	−70.1	0.5	DMF	88
Tac-Phe	186—187	+46.5	1	MeOH	152
Tac-Phe-OMe	158—160	+18.4	1	MeOH	152
Z-Phe-ONp(*o*)	107—109	−62	2	DMF	204
Z-Phe-NH_2	163—164	−6.3	2	DMF	29
Serine					
Ser(Bzl)-OEt,HCl	147—149	−7.8 [c]	0.6	H_2O	111
Msc-Ser,Dcha	156	−8.7	1	MeOH	11
Tac-Ser(Bzl)	160—161	+31.5	1	EtOH	152
Z-Ser(Tos)-OMe	117—118	−10.3	1.1	DMF	208
Threonine					
Bpoc-Thr(Bzl)-ONSu	no data given				37

[213] J. W. van Nispen and G. I. Tesser, *Internat. J. Peptide Protein Res.*, 1975, **7**, 57.

* Sign not stated.

Coded Amino-acids (*cont.*)

Compound	*m.p.*/°C	$[\alpha]_D$	*Conc.*	*Solvent*	*Ref.*
Msc-Thr	105—110	+1.6	1	MeOH	11
Ppoc-Thr(Bzl),Cha	131—133	+29.0	1	MeOH	205
Tryptophan					
Boc-Trp-ONp(*o*)	150—156	−58.6	2	DMF	204
Msc-Trp,Dcha	150	−5.4	1	MeOH	11
Z-Trp-OQ(5-Cl)	122—123	—	—	—	122
Z-Trp-OPcp	167—168	−52	1	$CHCl_3$	204
Z-Trp-ONp(*o*)	116	−63	2	DMF	204
Z(OMe)-Trp-OQ(5-Cl)	96—97	—	—	—	122
Tyrosine					
Boc-Tyr(Bzl)-NHNHPicoc	188—190	+18 [a]	1	DMF	33
Msc-Tyr(Bu^t)	130	+4.0	1	MeOH	11
Msc-Tyr(Bu^t)		+10.3	1	AcOH	11
Tac-Tyr(Bzl)	180—181	+69.6	1	EtOH	152
Valine					
Val-NH_2,HCl	262—263	+33.7	1	MeOH	71
Boc-Val-ONp(*o*)	53—55	−44	2	DMF	204
Boc-Val-OPcp	124—126	−38	5	EtOAc	204
Boc-Val-NHNHPicoc	amorphous	−18	1	DMF	33
Bpoc-Val-ONp	102—105	—	—	—	153
Msc-Val	128	−2.2	1	AcOH	11
Nps-ValNCA	150—152	+16.9	1	THF	149
Ppoc-Val	101—103	—	—	—	153
Ppoc-Val-ONp	oil	—	—	—	153
Tac-Val	169—171	−1.9	2	EtOH	152
Trt-Val-ONSu	70—72	—	—	—	153
Z-Val-OQ(5-Cl)	93—94	—	—	—	122

Other Amino-acids

β-Alanine					
Mal=β-Ala	106—107	—	—	—	150
Mal=β-Ala-ONSu	160—163	—	—	—	150
ε-Aminocaproic acid (εAhx*)					
Msc-εAhx	83				11
4-Aminocyclohexanecarboxylic acid					
The Boc-*cis*- (m.p. 167—169) and Boc-*trans*- (m.p. 147—150) derivatives					148
2-Amino-4-guanidinobutyric acid					
The methyl ester hydrochloride	162				214
2-Amino-3-guanidinopropionic acid					
The methyl ester hydrochloride	172				214
3-Amino-4-phenylbutyric acid					
The Boc-derivative	98—100	−13.3	2	DMF	19
D-Asparagine					
Z-D-Asn-OBzl(NO_2)	167—169	+14.1	1	DMF	43
Z-D-Asn	162—164	+6.5	1	DMF	43
α-Aza-amino-acids (carbazic acids)					
Several useful derivatives					215
γ-Carboxyglutamic acid					
Several useful derivatives					208

[214] F. Schwegler and W. Stock, *Z. physiol. Chem.*, 1975, **356**, 839.
[215] A. S. Dutta and J. S. Morley, *J.C.S. Perkin I*, 1975, 1712.

* This is now the recommended abbreviation: see Vol. 4, p. 444.

Coded Amino-acids (*cont.*)

Compound	*m.p.*/°C	$[\alpha]_D$	*Conc.*	*Solvent*	*Ref.*
2-Carboxymethylpyrrolidine* (Cmp)					
Z-Cmp-NH_2	129—130	−27	1	MeOH	18
Z-Cmp-ONSu	92—94	−20	1	MeOH	18
Z-Cmp,Dcha	145—146	−6	1	MeOH	18
Boc-Cmp	99—101	−41.6	1.9	DMF	19
Citrulline (Cit)					
Boc-Cit,Dcha	118—120	+14 [b]	1	EtOH	50
Boc-Cit,Dcha		+12 [c]	1	EtOH	50
Boc-Cit-ONp	85—86	−18.5 [b]	1	CH_2Cl_2	50
Boc-Cit-ONp		−16.0 [c]	1	CH_2Cl_2	50
Dehydroalanine					
The Z-, methyl ester	33—35				208
Diaminobutyric acid (Dab)					
Tac-Dab(Z)	164—165				152
Diaminopimelic acid					
Several useful derivatives					216
Homoarginine (Har)					
Har-OMe,2HCl	120—125	+14.2	2	H_2O	214
Homocysteine					
Several useful derivatives					217
Homocystine					
The bis-Z(OMe)-derivative	137—139				217
Homoserine					
Several useful derivatives					218
Hydrazino-acids (*N*-amino amino-acids)					
The acids and some derivatives					219, 220
Iodoalanine					
The Z-, methyl ester	67—68				208
allo-Isoleucine					
Z-*a*lle	oil	+15.2	2	Me_2CO	212
Several other useful derivatives					212
D-*allo*-Isoleucine					
Boc-D-*a*lle,Dcha	138—139	−11.3	1.5	DMF	212
Lanthionine					
Several useful derivatives					172
β-Lysine					
Z-β-Lys(Z)	155	+1.0	1	DMF	221
Boc-β-Lys(Boc),Dcha	106—107	−3.5	1	95% EtOH	221
D-Methionine					
Nps-D-Met,Dcha	195—196	+33.8	1.1	MeOH	2
N-Methyl-leucine					
Boc-MeLeu	57—58	−25.3	1	DMF	66

[216] A. Arendt, A. Kolodziejczyk, T. Sokolowska, and E. Szulfer, *Roczniki Chem.*, 1974, **48**, 1501.
[217] K. Jošt, Z. Procházka, J. H. Cort, T. Barth, J. Škopková, Z. Prusik, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1975, **39**, 2835.
[218] J. Schnyder and M. Rottenberg, *Helv. Chim. Acta*, 1975, **58**, 518.
[219] W. Streicher and H. Reinshagen, *Chem. Ber.*, 1975, **108**, 813.
[220] K. Achiwa and S. Yamada, *Tetrahedron Letters*, 1975, 2701.
[221] T. Wakamiya, H. Uratani, and T. Teshima, *Bull. Chem. Soc. Japan*, 1975, **48**, 2401.

* See footnote under bradykinin in Appendix I.

Other Amino-acids (*cont.*)

Compound	*m.p.*/°C	$[\alpha]_D$	*Conc.*	*Solvent*	*Ref.*
O-Methyltyrosine					
Boc-Tyr(Me)-OTcp	109—110	−31.8	0.5	DMF	88
Boc-Tyr(Me),Dcha	151—152	+34.2	0.5	DMF	88
β-(Napth-l-yl)alanine					
The Boc-,Dcha salt	154—156				9
Norleucine (Nle)					
Mal=Nle	89—90				150
Mal=Nle-ONSu	62—65(d)				150
Ornithine					
Orn(Z)	258—260	—	—	—	109
Boc-Orn(=Mal),Dcha	141—142	+13.7	1	MeOH	150
Boc-Orn(Z),Dcha	109—111	—	—	—	109
Boc-Orn(Z)	94—96	−3.1 [c]	1	AcOH	109
Boc-Orn(Boc)	120—121	−6.0	1	DMF	221
Z-Orn(Z)-ONp	118—119	−15.5 [b]	1	EtOAc	50
α-Phenylglycine (Phg)					
Z-Phg	128—129.5	112.7*	0.56	EtOAc	127
Sarcosine					
Boc-Sar	93				175
β-(Thien-2-yl)alanine					
The Boc-, ONp ester	145.5—146				124

[222] 'Peptides 1974.' Proceedings of the Thirteenth European Peptide Symposium, Israel, 1974, ed. Y. Wolman, 1975, Wiley, New York and Toronto, and Israel Universities Press, Jerusalem, 1975.

* Sign not specified.

4

Peptides with Structural Features not Typical of Proteins

BY B. W. BYCROFT AND C. M. WELS

1 Introduction

The choice of topics reviewed in this chapter follows a similar pattern to that adopted in previous years, although this year biosynthetic aspects, particularly with respect to the penicillins and cephalosporins, have been covered more fully.

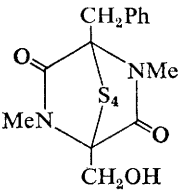
New cyclic peptides and depsipeptides with unusual structural features continue to be isolated and characterized. But once again the major effort in this area has centred on conformational studies and structure–activity relationships for the biologically active molecules. A significant advance has been the publication of the full molecular geometry for both valinomycin and its potassium complex. The increasing number of linear peptides which have been reported is noteworthy and probably reflects the broader biological screens which are now employed in the search for new antibiotics and antimetabolites.

2 Cyclic Peptides (Homodetic Peptides)

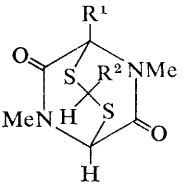
2,5-Dioxopiperazines (Cyclic Dipeptides).—An excellent review which comprehensively deals with all aspects relating to natural and synthetic 2,5-dioxopiperazines has been published.[1] Unfortunately the literature is covered only up to the end of 1973 and therefore it is already out of date, but nevertheless offers an important source of information on this rapidly growing group of compounds. Dioxopiperazines are often isolated as hydrolysis or thermolysis products from peptides or proteins. However, their widespread isolation from extracts and culture filtrates illustrates their genuine metabolic character. *cyclo*-L-Prolyl-L-valine, previously isolated from a number of micro-organisms, has also been observed in the culture broth of a *Streptomyces* strain.[2] Ten simple cyclic dipeptides have been identified in roasted cocoa beans.[3] These are clearly thermolysis products, since they are not present in the unroasted beans. The typical bitterness of cocoa is due to an interaction of the purine theobromine and the cyclic dipeptides.

The sulphur-bridged dioxopiperazines still continue to attract considerable attention. The epitetrathio derivative (1), originally isolated from *Penicillium turbatum*, has been detected in a *Hyalodendron* species, but with the enantiomeric

[1] P. G. Sammes, *Fortschr. Chem. org. Naturstoffe*, 1975, **32**, 51.
[2] H. Ogura, K. Furuhata, and K. Furuhata, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2474.
[3] W. Pickenhagen, P. Dietrich, B. Keil, J. Polonsky, F. Nouaille, and E. Lederer, *Helv. Chim. Acta*, 1975, **58**, 1078.

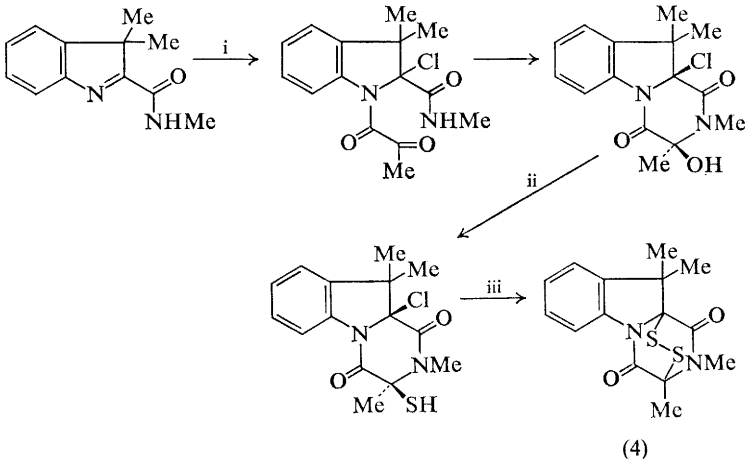


(1)



(2) R^1 = Et, R^2 = *p*-$MeOC_6H_4$
(3) R^1 = H, R^2 = *p*-$MeOC_6H_4$

(3s, 6s) configuration.[4] The dithioacetal (2) has been the subject of an *X*-ray crystallographic investigation.[5] It was prepared by a regiospecific metallation and alkylation of (3), the key intermediate in the elegant syntheses of gliotoxin and sporidesmin B reported last year. The specificity of the metallation is known to be unrelated to the nature of the substituent at the dithioacetal residue. It was hoped that the molecular conformation might shed further light on the reasons for the regiospecificity. This has not proved conclusive and a final solution must await further investigations.



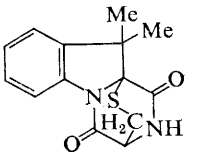
(4)

Reagents: i, MeCOCOCl; ii, H_2S–$ZnCl_2$; iii, O_2.

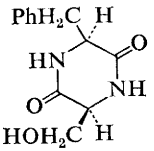
Scheme 1

A simple three-step synthesis for gliotoxin analogues has been outlined[6] in preliminary form (Scheme 1) as has the procedure used to prepare (4) which appears to be a potent antiviral agent. The same group has also described other approaches to this type of compound,[7] as well as the synthesis[8] of the desthiomethylene analogue (5). Surprisingly, the latter was devoid of both antiviral and antibacterial activity.

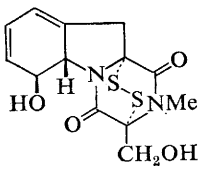
[4] G. M. Strunz, M. Kakushima, and M. A. Stillwell, *Canad. J. Chem.*, 1975, **53**, 295.
[5] K. Sasaki, T. Fukuyama, S. Nakatsuka, and Y. Kishi, *J.C.S. Chem. Comm.*, 1975, 542.
[6] H. C. J. Ottenheijm, G. P. C. Kerkhoff, J. W. H. A. Bijen, and T. F. Spande, *J.C.S. Chem. Comm.*, 1975, 768.
[7] H. C. J. Ottenheijm, A. D. Potman, and T. van Vroonhoven, *Rec. Trav. chim.*, 1975, **94**, 135.
[8] H. C. J. Ottenheijm, J. A. M. Hulshof, and R. J. F. Nivard, *J. Org. Chem.*, 1975, **40**, 2147.



(5)



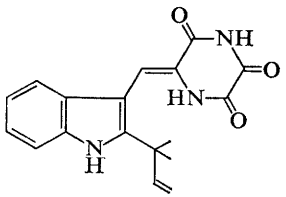
(6)



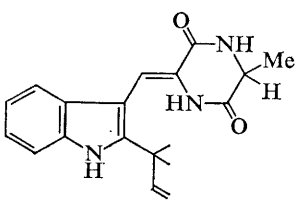
(7)

The biosynthetic evidence, to date, seems to indicate that the epidithiodioxopiperazine antibiotics are formed by modification of the corresponding cyclic dipeptide. A recent claim [9] that the dioxopiperazine (6) is not incorporated into gliotoxin (7) by *Penicillium terlikowskii* has been challenged,[10] on the grounds that the levels of (6) supplied to the organism were in excess of the amounts of (7) produced. The same workers have also reported [10] that (6) is an efficient precursor of (7) in *Trichoderma viride.*

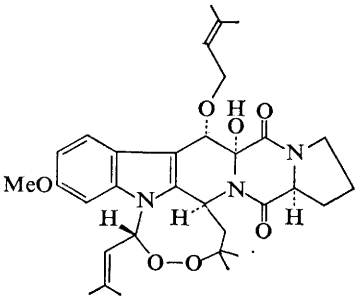
Cryptoechinuline C (8), a new minor metabolite from an undefined *Aspergillus*,[11] belongs to the ever-increasing group of prenylated cyclic dipeptides



(8)



(10)



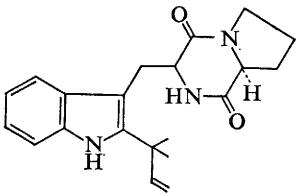
(9)

containing a dehydrotryptophan. Also in this class is fumitremorgin A (9), a potent tremorgenic metabolite, the structure and relative stereochemistry of which have been determined by two groups.[12, 13] Once again the biosynthetic

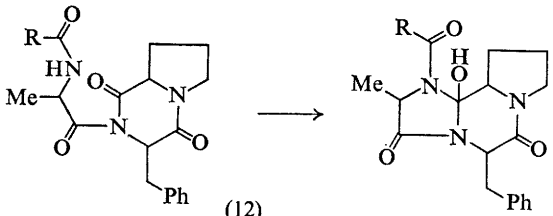
[9] J. C. MacDonald and G. P. Slater, *Canad. J. Biochem.*, 1975, **53**, 475.
[10] J. D. Bu'lock and C. Leigh, *J.C.S. Chem. Comm.*, 1975, 628.
[11] R. Cardillo, G. Fuganti, D. Ghiringhelli, and P. Grasselli, *J.C.S. Chem. Comm.*, 1975, 778.
[12] N. Eickman, J. Clardy, R. J. Cole, and J. N. Kirksey, *Tetrahedron Letters*, 1975, 1052.
[13] M. Yamazaki, H. Fujimoto, and T. Kawasaki, *Tetrahedron Letters*, 1975, 1241.

evidence strongly suggests that the structural modifications for this class of compounds occur on a preformed cyclic dipeptide. Doubly labelled *cyclo*-L-alanyl-L-tryptophan is efficiently incorporated [14] as a unit into the neoechinulines (10) and the same cyclic dipeptide, together with *cyclo*-L-prolyl-L-tryptophan, can be mono-isoprenylated [15] using an enzyme system isolated from *Aspergillus amstelodami* and 3-methyl-2-butenyl pyrophosphate. The oxidation at the β-position of the tryphophan residue in cryptoechinuline has been shown to proceed with the removal of the *pro-S* hydrogen atom.[11] The same stereochemical course had been observed with oxidation of the L-tyrosine in mycelianamide.

A total synthesis of deoxybrevianamide E (11), following an already developed route, has been reported.[16] The reactions of α-keto-acids and proline derivatives have been widely exploited for the formation of cyclic α-hydroxydipeptides. These have subsequently been employed to prepare dehydro-[17] and epidithiodioxopiperazines [18] and as intermediates in a general amino-acid synthesis.[19]



(11)

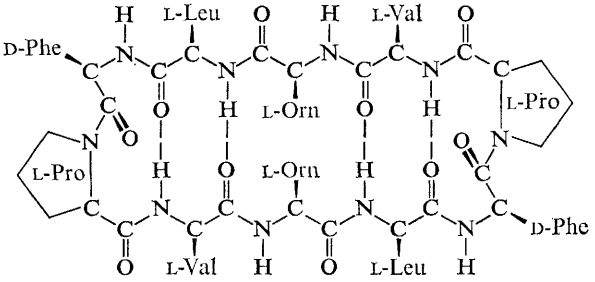


(12) (13)

The configurational stability of *cyclo*-L-prolyl-L-proline under strongly basic conditions has been investigated.[20] The data indicate that racemization proceeds *via* the intermediacy of the *trans-cyclo*-L-prolyl-D-proline. Mass spectrometric studies [21, 22] on natural and synthetic ergot-peptide alkaloids have provided useful documentation of the principal fragmentation processes for cyclol peptides. The cyclization of *N*-acyl cyclic dipeptides (12) to azacyclols (13) is dependent on the geometry of the amide bond.[23]

[14] R. Marchelli, A. Dossena, and G. Casnati, *J.C.S. Chem. Comm.*, 1975, 779.
[15] C. L. Deyrup and C. M. Allen, *Phytochemistry*, 1975, **14**, 971.
[16] R. Ritchie and J. E. Saxton, *J.C.S. Chem. Comm.*, 1975, 611.
[17] E. Öhler and U. Schmidt, *Chem. Ber.*, 1975, **108**, 2907.
[18] H. Poisel and U. Schmidt, *Chem. Ber.*, 1975, **108**, 2917.
[19] B. W. Bycroft and G. R. Lee, *J.C.S. Chem. Comm.*, 1975, 988.
[20] U. Schmidt and A. Nikiforov, *Monatsh.*, 1975, **106**, 313.
[21] D. Groeger, U. Syring, and S. Johne, *Pharmazie*, 1975, **30**, 440.
[22] J. Vokoun and Z. Řeháček, *Coll. Czech. Chem. Comm.*, 1975, **40**, 1731.
[23] G. Lucente, A. Romeo, and G. Zanotti, *Experientia*, 1975, **31**, 17.

Gramicidins and Related Peptides.—Two reviews on gramicidins which concentrate on topochemical aspects have appeared.[24, 25] In addition the gramicidins have been covered in an important general article on peptide antibiotics.[26] Gramicidin S continues to be a favourite testing ground of new techniques for conformational studies. The preferred conformation of gramicidin S in polar solvents is the well established antiparallel pleated sheet structure (14). The



(14)

NH groups of leucine and valine are internally hydrogen-bonded to amide carbonyls, while those of phenylalanine and ornithine are exposed to the solvent. N.m.r. solvent-saturation techniques offer an alternative method of detecting solvent-exposed protons.[27]

Saturation of the methanol signal of a methanolic solution of gramicidin S results in a decrease in the NH signal of the phenylalanine residue by approximately a quarter. This transfer of saturation results from rapid proton exchange between the solvent and the amide group. No significant change in the intensities of the other resonances were observed. Unfortunately the $C^{\alpha}H$ chemical shift of the ornithine residue is the same as that of the methanol and consequently it was not possible to observe nuclear Overhauser effects for its NH group. The use of this technique in other solvents yields information on side-chain–solvent interactions.

Further ^{13}C n.m.r. data, including spin–lattice relaxation times,[28] and natural abundance ^{15}N spectra [29] on gramicidin S, have been reported. Proton double-resonance difference spectroscopy offers a convenient method of simplifying the complex analysis of the amino-acid residues in peptides and has been successfully applied to tyrocidine A.[30]

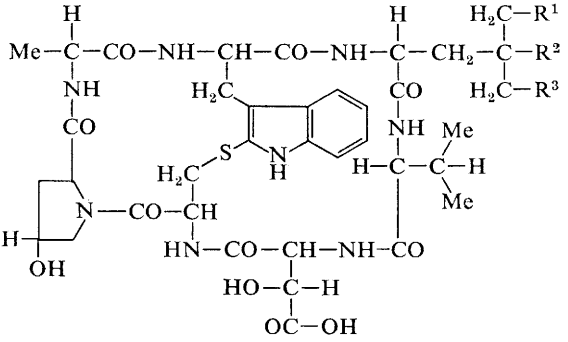
The importance of the secondary structure (14) which is controlled by the intramolecular bonds to the biological activity has been seriously challenged.

[24] N. Izumiya, *Yuki Gosei Kagaku Kyokai Shi*, 1974, **32**, 833.
[25] L. K. Ramachandran, *J. Sci. Ind. Res., India*, 1975, **234**, 249.
[26] C. H. Hassall, in 'Peptides; Chemistry, Structure, and Biology', ed. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, Ann Arbor, 1975, p. 891.
[27] T. P. Pitner, J. D. Glickson, R. Rowan, J. Dadok, and A. A. Bothner-By, *J. Amer. Chem. Soc.*, 1975, **97**, 5917.
[28] R. A. Komoroski, I. R. Peat, and G. C. Levy, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 272.
[29] G. E. Hawkes, E. W. Randall, and C. H. Bradley, *Nature*, 1975, **257**, 767.
[30] W. A. Gibbons, C. F. Beyer, J. Dadok, R. F. Sprecher, and H. R. Wyssbrod, *Biochemistry*, 1975, **14**, 420.

The mono- and di-*N*-methyl-leucine derivatives of gramicidin must lack respectively one and two transannular hydrogen bonds. However, their biological activity is essentially the same as that of the parent antibiotic. A detailed n.m.r. investigation on both derivatives [31, 32] has led to proposed conformations which are very different from that of gramicidin itself.

Further syntheses of new gramicidin S analogues [33, 34] (see Table 1, p. 321) and depsipeptide analogues,[35] employing standard procedures, have been designed to explore further the relationship between topochemistry and biological activity.

Cyclic Peptides from *Amanita phalloides*.—A minor peptide from *Amanita phalloides*, originally designated as phallacidin, has been separated into three compounds which differ only in the degree of hydroxylation of the leucine side-chain.[36] Phallacidin (15) possesses a $\gamma\delta$-dihydroxyleucine residue, whereas



(15) $R^1 = H, R^2 = OH, R^3 = OH$
(16) $R^1 = H, R^2 = OH, R^3 = H$
(17) $R^1 = OH, R^2 = OH, R^3 = OH$

phallacin and phallisacin are the mono- and tri-hydroxy-derivatives (16) and (17). Removal of the phenolic hydroxy-group in α-amanitin by hydrogenation of the tetrazolyl ether affords the deoxy-derivative which possesses similar toxicity to the parent compound.[37] Detailed empirical energy calculations on di- and tri-peptide fragments of antamanide have been used in constructing minimum-energy hydrogen-bonded conformations of the molecule.[38]

Viomycin and Related Peptides.—A limited conformational study of the tuberactinomycins using n.m.r. techniques concluded that the principal conformational

[31] N. G. Kumar, N. Izumiya, M. Miyoshi, H. Sugano, and D. W. Urry, *Biochemistry*, 1975, **14**, 2197.
[32] N. G. Kumar, N. Izumiya, M. Miyoshi, H. Sugano, and D. W. Urry, *J. Amer. Chem. Soc.*, 1975, **97**, 4104.
[33] L. G. Snezhkova, E. N. Shepel, I. D. Ryabova, A. I. Miroshnikov, V. T. Ivanov, and Yu. A. Ovchinnikov, *Bioorg. Khim.*, 1975, **1**, 347.
[34] S. Sofuku, I. Muramatsu, K. Okada, and A. Hagitani, *Bull. Chem. Soc. Japan*, 1975, **48**, 2888.
[35] N. A. Krit, A. L. Zhuze, T. M. Andronova, M. P. Filatova, G. A. Ravdel, and L. A. Shchukina, *Bioorg. Khim.*, 1975, **1**, 247.
[36] H. Faulstich, O. Brodner, S. Walch, and Th. Wieland, *Annalen*, 1975, 2324.
[37] A. Buku and Th. Wieland, *Annalen*, 1975, 1956.
[38] V. Z. Pleutnev, E. P. Gromov, and E. M. Popov, *Bioorg. Khim.*, 1975, **1**, 328.

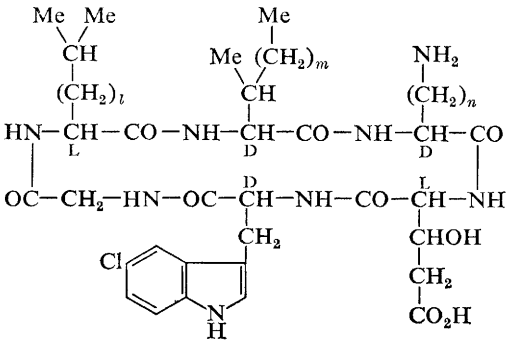
features are the same as those observed in the crystal lattice.[39] A further structure–activity investigation on viomycin suggests that the amino-groups of the β-lysine residue are important factors in relation to the antibacterial activity.[40] On the other hand it is claimed that tuberactinamine N, which lacks the γ-hydroxy-β-lysine residue, showed activity comparable with the parent antibiotic.[41]

Other Naturally Occurring Cyclic Peptides.—A preliminary communication of a full chemical and spectroscopic investigation on alamethicin proposes that the generally accepted cyclic structure for this molecule is incorrect. Alamethicin is now considered as a linear peptide (18) composed of 18 amino-acids with the sequence previously proposed. A β-phenylalaninol is attached to the γ-carboxy-group of glutamic acid-17, and proline-1 is blocked by an *N*-acetyl methylalanine residue. The ion-transporting properties of the molecule through biological and artificial lipid membranes are discussed in the light of the revised structure.[42]

Pro-Mea-Ala-Mea-Ala-Gln-Mea-Val-Mea-Gly-Leu-Mea-Pro-Val-Mea-Mea-Glu-Gln

(18) Mea = methylalanine

Longicatenamycin, the name given to the major component of a new series of closely related peptide antibiotics isolated from a *Streptomyces*, has been assigned the structure (19).[43] The antibiotic contains a number of novel amino-acids, including D-5-chlorotryptophan.[44]

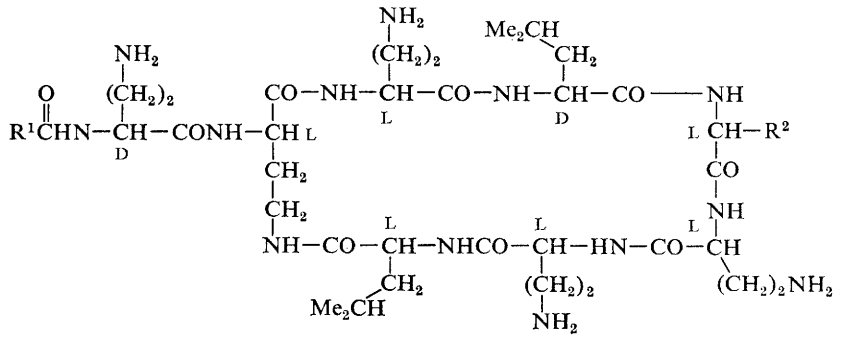


(19)

EM49 also describes a family of closely related peptide antibiotics of general structure (20), which differ from one another in the nature of the β-hydroxy fatty-acid side-chain.[45] These peptides are remarkable in that they contain five 2,4-diaminobutyric acid residues.

An extremely detailed and painstaking analysis of the ^{1}H n.m.r. data on tentoxin, the phytotoxin produced by the fungus *Alternaria tenius*, permitted full

[39] T. Wakamiya and T. Shiba, *Bull. Chem. Soc. Japan*, 1975, **48**, 2502.
[40] T. Kitagawa, T. Miura, M. Takaishi, and H. Taniyama, *Chem. and Pharm. Bull. (Japan)*, 1975, 2123.
[41] T. Wakamiya and T. Shiba, *J. Antibiotics*, 1975, **28**, 292.
[42] D. R. Martin and R. J. P. Williams, *Biochem. Soc. Trans.*, 1975, **3**, 166.
[43] T. Shiba and Y. Mukunoki, *J. Antibiotics*, 1975, **28**, 561.
[44] T. Shiba, Y. Mukunoki, and H. Akiyama, *Bull. Chem. Soc. Japan*, 1975, **48**, 1902.
[45] W. L. Parker and M. L. Rathnum, *J. Antibiotics*, 1975, **28**, 379.



(20)

sequence and conformational assignments for the molecule.[46] These were subsequently shown to be correct by an *X*-ray analysis of the dihydro-derivative.

Conformational Studies.—A further important review from the Russian School on the conformational states and biological activity of cyclic peptides has appeared.[47] A critical analysis of the conformations deduced from n.m.r. data and those obtained from *X*-ray analysis emphasizes the caution that is necessary in correlating the information from these two valuable techniques.[48]

N.m.r. methods still dominate as the most exploited method for the determination of solution conformation. Naturally occurring cyclic peptides of well-established solution conformation, such as gramicidin S and tyrocidine A, offer convenient models for the development of new techniques.[27, 29, 30]

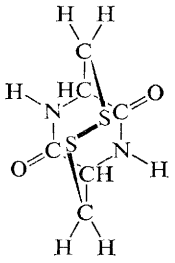
The possibility of employing an empirically derived angular dependence relationship for the vicinal ^{13}C–$C^{\alpha}H$ and HC^{α}–^{13}CN coupling constants has been explored further and initial observations are considered promising.[49] A parallel investigation of C^{β}–N three-bond coupling which would be a possible indicator of the angle ψ in peptide systems has shown that the coupling constants are smaller than 1 Hz in models with defined dihedral angles.[50] Consequently its utility in conformational studies is likely to be limited. The use of lanthanide ions in the determination of conformation has been reviewed.[51]

The molecular structures and conformational states of cyclic dipeptides are now fairly clearly defined and are known to be dependent on the nature of the side-chains. ^{13}C Spin–lattice relaxation times of a series of glycine-containing cyclic dipeptides indicate that the α-carbon of the glycyl residue has enhanced intramolecular motions with respect to those of the α-carbon of the second residue.[52] Analysis of the optical properties of *cyclo*-(alanyl-*p*-hydroxyphenylglycine) indicates that conformations which are characterized by a non-planar

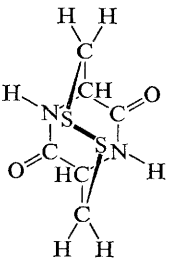
[46] W. L. Meyer, G. E. Templeton, C. L. Grable, R. Jones, L. F. Kuyper, R. B. Lewis, C. W. Sigel, and S. H. Woodhead, *J. Amer. Chem. Soc.*, 1975, **97**, 3802.
[47] Yu. A. Ovchinnikov and V. T. Ivanov, *Tetrahedron*, 1975, **31**, 2177.
[48] F. A. Bovey, in 'Peptides, Polypeptides, and Proteins', ed. E. R. Blout, F. A. Bovey, and M. Goodman, Wiley, New York, 1975, p. 248.
[49] V. F. Bystrov, Yu. D. Gavrilov, and V. N. Solkan, *J. Magn. Resonance*, 1975, **19**, 123.
[50] R. Di Blasi and K. D. Kopple, *J.C.S. Chem. Comm.*, 1975, 33.
[51] B. A. Levine and R. J. P. Williams, *Proc. Roy. Soc.*, 1975, **A345**, 5.
[52] R. Deslauriers, Z. Grzonka, K. Schaunburg, T. Shiba, and R. Walter, *J. Amer. Chem. Soc.*, 1975, **97**, 5093.

state of the dioxopiperazine ring and with the phenolic side-chain orientated at a dihedral angle χ_1 of 120—180° are highly populated.[53]

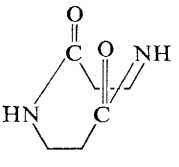
The temperature-induced change in helicity of the inherently dissymmetric disulphide linkage in cyclocystine had been followed using c.d. and ^{13}C spectroscopy. The *P*-helical form (21) predominates below 40 °C, while above this temperature the *M*-helical form (22) increases. The lower limit of $\Delta G^{\ddagger}$ for the change in conformation was estimated to be *ca.* 16.0 kcal mol^{-1}.[54]



(21)



(22)



(23)

cyclo-Di-*β*-alanyl adopts a twist-boat conformation (23) in the crystal lattice,[55] and empirical force-field calculations indicate this is also the preferred conformation in the gas phase.[56] The full crystallographic data from the analysis of the first cyclic tripeptide, *cyclo*-(L-Pro-L-Pro-L-Hyp) have been published.[57] All the peptide units are *cis* and approximately planar. Extended studies, in both the solid and solution states, on cyclic peptides from sarcosine of the general structure *cyclo*-(Sar)$_n$ (n = 2—8) have shown that the rings are conformationally very homogeneous and contain both *cis* and *trans* amide bonds.[58] The crystal conformation of *cyclo*-(Sar)$_7$ hydrate is shown in Figure 1. The conformation about the peptide bonds is *cis*, *cis*, *cis*, *cis*, *trans*, *trans*, *trans*, and the water forms hydrogen-bond bridges linking the peptide molecules.[59] The *cis–trans* pattern of the amide groups was somewhat unexpected. Also one of the *trans* amides deviates considerably from planarity.

The steps in the conformational interconversion processes for the cyclic tri-, tetra-, and penta-peptides containing both Gly and Sar have been described.[60] The crystal structures of the cyclic tetrapeptides *cyclo*-(Gly-Sar$_3$), *cyclo*-(Gly-Sar)$_2$, and *cyclo*-(DL-Ala-Sar$_3$) have been reported separately.[61] The ring conformations are identical for all three peptides, corresponding to the amide sequence *cis–trans–cis–trans*. N.m.r. evidence suggests that these molecules retain the crystal conformation in solution.[60] Solvent-induced shifts have been employed for the analysis of the n.m.r. spectra of *cyclo*-(Sar)$_4$ and *cyclo*-(Pro-Sar-Gly)$_2$.[62] Benzene

[53] J. W. Snow and T. M. Hooker, jun., *J. Amer. Chem. Soc.*, 1975, **97**, 3506.
[54] M. Ottnad, P. Hartter, and G. Jung, *Z. physiol. Chem.*, 1975, **356**, 1011.
[55] D. N. J. White and J. D. Dunitz, *Israel J. Chem.*, 1972, **10**, 249.
[56] D. N. J. White and M. H. P. Guy, *J.C.S. Perkin II*, 1975, 43.
[57] G. Kartha and G. Ambady, *Acta Cryst.*, 1975, **B31**, 2035.
[58] K. Titlestad, *Acta Chem. Scand.* (*B*), 1975, **29**, 153.
[59] P. Groth, *Acta Chem. Scand.* (*A*), 1975, **29**, 38.
[60] J. Dale and K. Titlestad, *Acta Chem. Scand.* (*B*), 1975, **29**, 353.
[61] J. P. Declerq, G. Germain, M. van Meerssche, T. Debaerdemaeker, J. Dale, and K. Titlestad, *Bull. Soc. chim. belges*, 1975, **84**, 275.
[62] T. Sugihara, Y. Imanishi, and T. Higashimura, *Biopolymers*, 1975, **14**, 733.

interacts preferentially with the *trans* peptide bonds, and the analysis confirmed the previously proposed conformation for the former and led to the proposal that *cyclo*-(Pro-Sar-Gly)$_2$ possesses C_2 symmetry with internally hydrogen-bonded glycine residues and all *trans* peptide bonds.

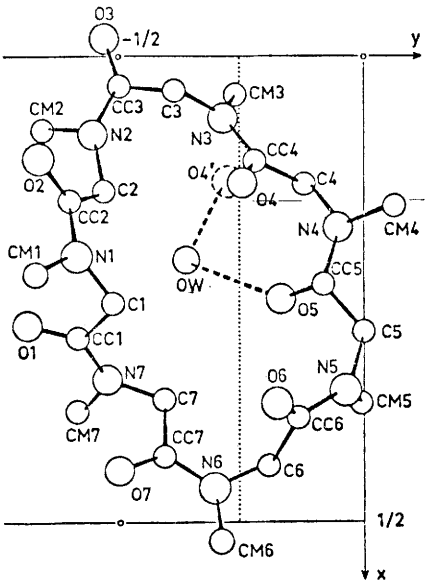


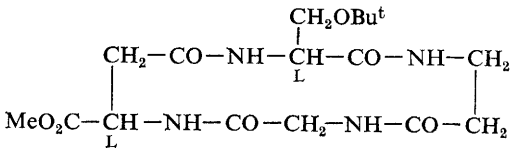
Figure 1 *Crystal conformation of* cyclo-(*Sar*)$_7$ *hydrate, viewed along* [001] (Reproduced by permission from *Acta Chem. Scand.* (*A*), 1975, **29**, 38)

^{1}H and ^{13}C n.m.r. data on *cyclo*-(Gly)$_4$ support the conclusion that in solution the molecule occurs predominantly in an S_4-symmetrical conformation with the principal symmetry axis perpendicular to the ring. In this structure it is proposed that the peptide groups are transoid and non-planar.[63] It is noteworthy that this differs from that of *cyclo*-(Sar)$_4$ which contains two *cis* and two *trans* peptide bonds in both the solution and crystalline states. An interesting correlation between the lowest energy conformer of *cyclo*-(Sar)$_4$, generated by empirical force field calculations, and the known solution and solid conformation has been made.[64]

The cyclic tetrapeptide (24), which contains two β-amino-residues and is analogous to the natural depsipeptide serratamolide, has been prepared as part of a programme aimed at the synthesis of cylindrical peptides. The crystal data demonstrate that in the 14-membered ring of this molecule all the peptide bonds are *trans*.[65] This contrasts with the 12-membered cyclic tetrapeptides and a depsipeptide[66] in which two of the peptide groups are *cis*.

The conformational effects of the presence of an *N*-methylamino-acid in cyclic hexapeptides have been extensively investigated using predominantly n.m.r.

[63] C. Grathwohl, A. Tun-kyi, A. Bundi, R. Schwyzer, and K. Wuethrich, *Helv. Chim. Acta*, 1975, **58**, 415.
[64] D. N. J. White, *Tetrahedron Letters*, 1975, 2101.
[65] I. L. Karle, B. K. Handa, and C. H. Hassall, *Acta Cryst.*, 1975, **B31**, 555.
[66] J. Konnert and I. L. Karle, *J. Amer. Chem. Soc.*, 1975, **91**, 4888.



(24)

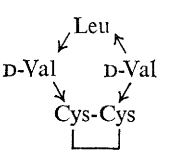
techniques.[67, 68] In addition the complexation of these compounds with alkali metals and the attendant effects on their conformational feature have been described.[69]

Synthesis.—In accord with past policy synthetic studies relating to cyclic hormone peptides and their analogues are not covered in this chapter. Table 1 summarizes the details of cyclic peptides synthesized in 1975. The list includes both those peptides described for the first time and also those which were originally reported in preliminary form and have now been published in full.

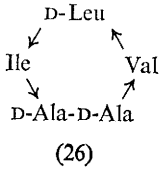
The principal objectives of synthesis continue to be the exploration of structure–activity relationships for analogues of natural peptides, and the provision models for conformational studies. There appears to have been less activity than usual over the past year, and all the syntheses described have followed standard procedures. There have been considerably more synthesis of cyclic depsipeptides recorded and these are described in the next section.

An examination of the cyclization step for a number of linear peptides with the sequence of gramicidin S by the *N*-hydroxysuccinimide, azide, and *p*-nitrophenyl ester methods, concluded that the *N*-hydroxysuccinimide ester method was more favourable than the others.[70] It consistently gave better yields and proved easier to handle experimentally. In contrast, the Russian group have described the synthesis of *enantio*-gramicidin S as well as a number of analogues employing the *p*-nitrophenyl ester method for the cyclization step.[33]

The cyclic pentapeptide *enantio*-[Val^5]-malformin (25), which is the enantiomer of the palindrome peptide [Val^5]-malformin, has been prepared by a stepwise solution synthesis.[71] The ring-closure step was accomplished by the azide method. Like the *retro-enantio* analogues of gramicidin S and of enniatins, the



(25)



(26)

[67] V. T. Ivanov, I. A. Lavrinovich, C. A. Kogan, E. A. Meschcheryakova, S. L. Portnova, M. D. Isabaev, P. V. Kostetskii, and Yu. A. Ovchinnikov, *Khim. prirod. Soedinenii*, 1975, **11**, 202.

[68] V. T. Ivanov, I. A. Lavrinovich, S. L. Portnova, V. V. Lapshin, P. V. Kostetskii, and Yu. A. Ovchinnikov, *Izvest. Akad. Nauk Kazakh. S.S.R., Ser. khim.*, 1974, 2320.

[69] V. T. Ivanov, I. A. Lavrinovich, S. L. Portnova, S. V. Sychov, V. V. Lapshin, P. V. Kostetskii, and Yu. A. Ovchinnikov, *Bioorg. Khim.*, 1975, **1**, 149.

[70] S. Matsuura, H. Takiguchi, M. Waki, and M. Izumiya, *Mem. Fac. Sci. Kyushu Univ.*, 1975, **9**, 277.

[71] M. Bodanszky, G. L. Stahl, and R. W. Curtis, *J. Amer. Chem. Soc.*, 1975, **97**, 2857.

molecule exhibited similar, although somewhat weaker, biological activity to that of the parent compound. Desthiomalformin (26) prepared in a similar manner lacked any biological activity.[72] The cyclization of the corresponding linear pentapeptide is of interest, in that it occurred readily, in high yield, and without accompanying cyclodimerization. This is attributed to the presence of the alternating D and L residues, a feature that results in reverse turns stabilized by intramolecular hydrogen bonds, which facilitates the cyclization.

Table 1 *Syntheses of cyclic peptides reported in* 1975 *(derived from linear analogues unless otherwise stated)*

Peptide	*Bond formed in cyclization step*	*Method for cyclization*	*Ref.*
[5-Gly][10-Gly]-gramicidin S	Val-Gly	*a*	33
enantio-Gramicidin S	Val-Pro	*a*	33
[5-Gly][10-Gly]-*enantio*-gramicidin S	Val-Gly	*a*	33
retro-Gramicidin S	Phe-Pro	*a*	33
[5-Gly][10-Gly]-*retro*-gramicidin S	Phe-Gly	*a*	33
retro-enantio-Gramicidin S	Phe-Pro	*a*	33
[5-Gly][10-Gly]-*retro-enantio*-gramicidin S	Phe-Gly	*a*	33
All-L-gramicidin S	Val-Pro	*a*	33
[4-5,4′-5′-Bis-δ-aminovaleric acid]-gramicidin S	Val-Pro	*b*	34
cyclo-$(Sar)_4$	Sar-Sar	*c*, *d*	58, 74
cyclo-$(Sar)_5$	Sar-Sar	*c*	58
cyclo-$(Sar)_6$	Sar-Sar	*c*	58
cyclo-$(Sar)_7$	Sar-Sar	*c*, *d*	58, 74
cyclo-$(Sar)_8$	Sar-Sar	*c*	58
Desthiomalformin	Ile-Leu	*d*	71
enantio-[Val^5]-malformin	Val-Leu	*d*	72
cyclo-$(Val\text{-}Gly)_3$	Val-Gly	*a*	73
cyclo-$(Nva\text{-}Gly)_3$	Nva-Gly	*a*	73
cyclo-$(Leu\text{-}Gly)_3$	Leu-Gly	*a*	73
cyclo-$(Val\text{-}Gly\text{-}Gly)_2$	Val-Gly	*a*	73
cyclo-$(Nva\text{-}Gly\text{-}Gly)_2$	Nva-Gly	*a*	73
cyclo-$(Val\text{-}Val\text{-}Gly)_2$	Val-Gly	*a*	73
cyclo-$(Nva\text{-}Nva\text{-}Gly)_2$	Nva-Gly	*a*	73
cyclo-(Val-Gly-Val-Sar-Val-Gly)	Val-Gly	*a*	74
cyclo-(Val-Sar-Val-Val-Gly)	Val-Gly	*a*	74
cyclo-$(Val\text{-}Sar)_3$	Val-Sar	*a*	74
cyclo-$(MeVal\text{-}Sar)_3$	prepared by methylation of the above compound		74
cyclo-$(Pro\text{-}Sar\text{-}Gly)_2$	Pro-Gly	*a*	75
cyclo-$(Sar\text{-}Sar\text{-}Gly)_2$	Sar-Gly	*a*	75
cyclo-(Lys-Ala-Arg-Tyr-Gly-Gly)	Lys-Gly	*a*	76

(*a*) *p*-Nitrophenyl ester. (*b*) DCC/*N*-hydroxysuccinimide. (*c*) 2,4,5-Trichlorophenyl ester. (*d*) Azide method.

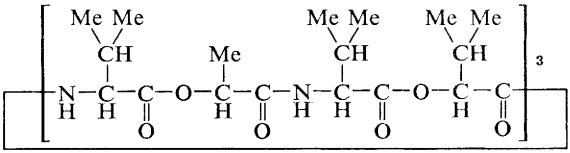
[72] M. Bodanszky and J. B. Henes, *Bioorg. Chem.*, 1975, **4**, 212.
[73] V. T. Ivanov, I. A. Lavrinovich, J. Bernat, P. V. Kostetskii, and Yu. A. Ovchinnikov, *Khim. prirod. Soedinenii*, 1975, **11**, 57.
[74] V. T. Ivanov, I. A. Lavrinovich, V. V. Lapshin, P. V. Kostetskii, and Yu. A. Ovchinnikov, *Khim. prirod. Soedinenii*, 1975, **11**, 63.
[75] T. Sugihara, Y. Imanishi, and T. Higashimura, *Biopolymers*, 1975, **14**, 723.
[76] S. Wiejack and B. Rzeszotarska, *Roczniki Chem.*, 1975, **49**, 1106.

3 Depsipeptides and other Heterodetic Cyclic Peptides

Actinomycin.—An excellent review of the three-dimensional structure of the actinomycins covers the various methods employed to assign the molecular conformation, and also discusses the interaction of the characteristic pseudo C_2-symmetrical structures for all the important actinomycins with nucleic acids.[77] A further analysis of the ^{13}C n.m.r. data for the lactone ring in actinomycins is consistent with the accepted conformation.[78]

A review on the chemistry of the now considerable number of naturally occurring actinomycins has been published.[79] A complex mixture of actinomycins is produced when *Streptomyces parvulus* and *S. chrysomallus* are grown in a medium supplemented with one of the four isomers of isoleucine.[80] It appears that the D-valine and L-*N*-methylvaline are partially replaced by isomers of isoleucine. The full experimental details of the novel thermal degradation of actinomycins to dioxopiperazines, which has implications in terms of peptide sequencing for small peptides, have been reported.[81] 7-Nitro-, 7-amino-, and 7-hydroxyactinomycin D have been synthesized, and chemical and growth-inhibition studies performed. Significant activity against a variety of tumours was indicated.[82]

Valinomycin.—The year has afforded a wealth of crystallographic information on both uncomplexed valinomycin (27) and the potassium complex. Although a



(27)

number of preliminary communications had appeared in the past, no detailed structural information such as atomic co-ordinates or molecular dimensions had been reported. Monoclinic[83] and triclinic forms[83, 84] of uncomplexed valinomycin have been crystallized and their structures determined. An analysis of the conformations of the three observed independent molecules indicates that the gross features of all three are the same. However, minor differences in side-chain conformations were observed. A drawing of one molecule of valinomycin illustrating the observed conformation of the three independent molecules is shown in Figure 2.

[77] H. Lackner, *Angew. Chem. Internat. Edn.*, 1975, **14**, 375.
[78] H. Lackner, *Tetrahedron Letters*, 1975, 1921.
[79] V. S. Kuznetsova, T. I. Orlova, and A. B. Silaev, *Antibiot. Ikh. Produtsenty*, 1975, 40.
[80] T. Yajima, K. T. Mason, and E. Katz, *Antimicrobial Agents Chemotherapy*, 1975, **7**, 773.
[81] A. B. Mauger, *J.C.S. Perkin I*, 1975, 1320.
[82] S. K. Sengupta, S. K. Tinter, H. Lazarus, B. L. Brown, and E. J. Modest, *J. Medicin. Chem.*, 1975, **18**, 1175.
[83] G. D. Smith, W. L. Duax, D. A. Langs, G. T. DeTitta, J. W. Edmonds, D. C. Rohrer, and C. M. Weeks, *J. Amer. Chem. Soc.*, 1975, **97**, 7242.
[84] I. L. Karle, *J. Amer. Chem. Soc.*, 1975, **97**, 4379.

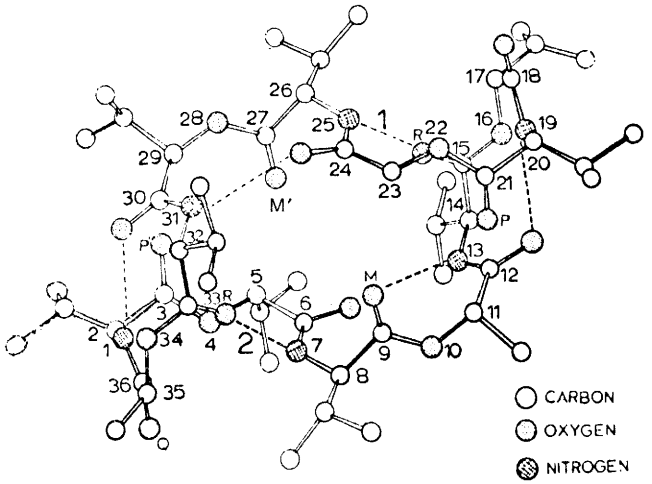


Figure 2 *Structure of uncomplexed valinomycin*
(Reproduced by permission from *J. Amer. Chem. Soc.*, 1975, **97**, 7242)

Two different kinds of hydrogen bond are found in all three molecules. Each molecule has four hydrogen bonds involved in β-turns. Each molecule also has two previously undescribed hydrogen bonds in which the amino-acid is hydrogen-bonded to a carbonyl group of an amino-acid residue four groups away.

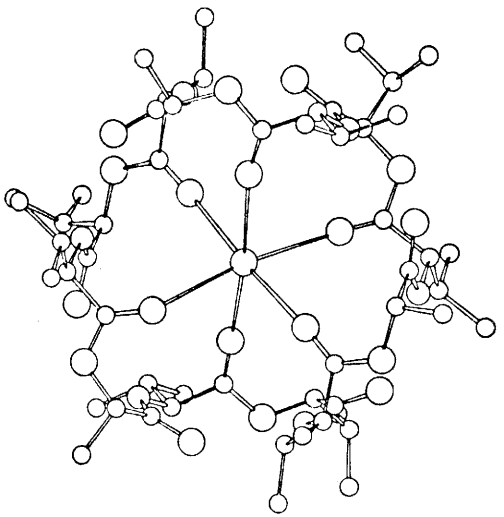


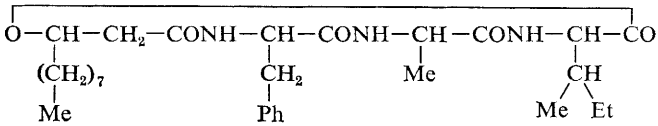
Figure 3 *Structure of the potassium ion complex of valinomycin*
(Reproduced by permission from *Helv. Chim. Acta*, 1975, **50**, 432)

The crystal structure of the potassium complex confirms that the potassium is co-ordinated octahedrally by six carbonyl oxygen atoms (Figure 3).[85] The macrocyclic ring skeleton adopts non-crystallographic S_6-symmetry and is in accord with that previously reported in preliminary form [86] and predicted from studies by i.r., o.r.d., c.d., and n.m.r. techniques (see ref. 47).

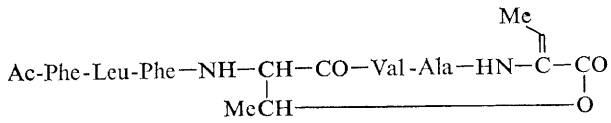
Further n.m.r. studies on valinomycin complexes with ammonium [87] and alkali-metal ions together with the effect of the counterion [88] have been investigated. Lanthanide-induced shifts are observed only for the D-valyl and D-hydroxyiso-valeryl fragments of uncomplexed valinomycin in chloroform solution. A comparison of the observed and calculated induced shifts has provided information on the conformation of these residues.[89]

In an impressive synthetic programme designed to explore the characteristics of the complexing properties associated with the valinomycin molecule, a wide variety of ring, constitutional, and configurational analogues have been prepared and their conformations and complexing properties investigated using mainly n.m.r. techniques [90–94] (see also ref. 47).

Other Naturally Occurring Cyclic Depsipeptides.—Two new depsipeptides, beauvellide (28) [95] and TL-119 (29),[96] isolated from micro-organisms and characterized by physical methods, have been described. The presence of the $\alpha\beta$-



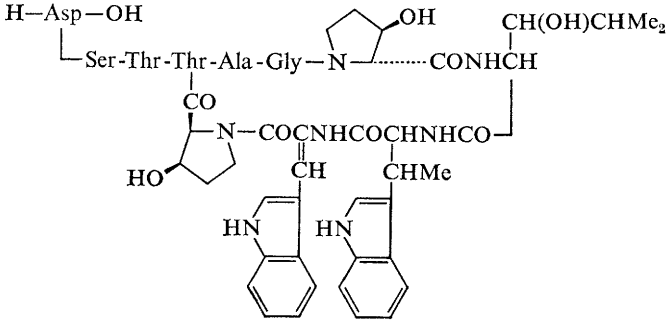
(28)



(29)

[85] K. Neupert-Laves and M. Dobler, *Helv. Chim. Acta*, 1975, **58**, 432.
[86] M. Pinkerton, L. K. Steinrauf, and P. Dawkins, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 512.
[87] D. G. Davis, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 786.
[88] D. G. Davis and D. C. Tosteson, *Biochemistry*, 1975, **15**, 3962.
[89] K. L. Servis and D. J. Patel, *Tetrahedron*, 1975, **31**, 1359.
[90] V. T. Ivanov, A. A. Sanasaryan, I. I. Chervin, G. I. Yakovlev, L. A. Fonina, L. B. Senyavina, S. V. Sychev, E. I. Vinogradova, and Yu. A. Ovchinnikov, *Izvest. Akad. Nauk Kazakh. S.S.R., Ser. khim.*, 1974, 2316.
[91] V. T. Ivanov, L. A. Fonina, L. B. Senyavina, E. I. Vinogradova, Yu. A. Ovchinnikov, I. I. Chervin, and G. I. Yakovlev, *Bioorg. Khim.*, 1975, **1**, 5.
[92] V. T. Ivanov, A. A. Sanasaryan, L. A. Fonina, L. B. Senyavina, E. I. Vinogradova, Yu. A. Ovchinnikov, I. I. Chervin, and G. I. Yakovlev, *Bioorg. Khim.*, 1975, **1**, 214.
[93] V. T. Ivanov, A. A. Sanasaryan, L. B. Senyavina, E. I. Vinogradova, Yu. A. Ovchinnikov, I. I. Chervin, and G. I. Yakovlev, *Bioorg. Khim.*, 1975, **1**, 226.
[94] V. T. Ivanov, L. A. Fonina, L. B. Senyavina, E. I. Vinogradova, Yu. A. Ovchinnikov, I. I. Chervin, and G. I. Yakovlev, *Bioorg. Khim.*, 1975, **1**, 869.
[95] F. Frappier, P. Ferron, and M. Pais, *Phytochemistry*, 1975, **14**, 2703.
[96] Y. Nakagawa, T. Nakazawa, and J. Shoji, *J. Antibiotics*, 1975, **28**, 1005.

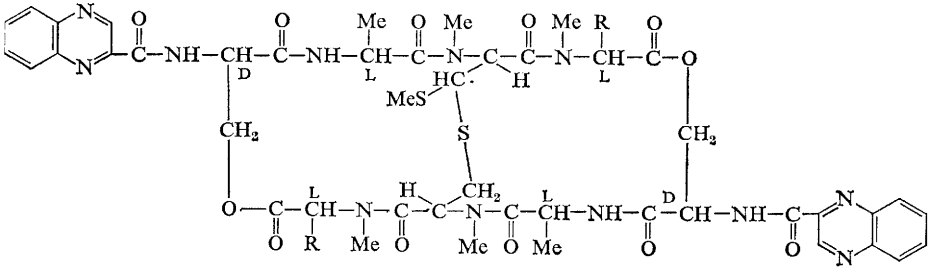
dehydroaminobutyric acid residue in TL-119 is a further example of an oxidized or dehydrated amino-acid residue in a microbial peptide. A dehydrotryptophan unit has also been observed in a new antibiotic (30) related to the telomycin



(30)

group.[97] Conformational studies on the molecule using o.r.d. and c.d. suggest that the dehydrotryptophan and β-methyltryptophan residues are in the same plane.[98]

The full spectral investigation, mentioned briefly last year, which led to the revision of the structure for echinomycin (31) has appeared.[99] In addition an independent investigation along similar lines has reported[100] the revised structures for quinomycin A and C, (31) and (32) respectively. Quinomycin A is

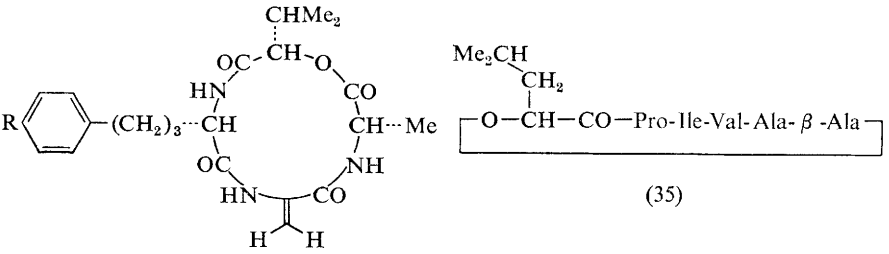


(31) R = $CHMe_2$
(32) R = $CHMeCHMe_2$

identical in formulation with echinomycin but no direct comparison has been reported. The two closely related host-specific phytotoxic metabolites, AM-toxin I and II, which cause leaf-spot disease of apples, have been assigned[101] the structures (33) and (34).

[97] I. G. Smirnova, G. S. Katnikha, and A. B. Silaev, *Khim. prirod. Soedinenii*, 1974, **10**, 632.
[98] V. V. Romanov, I. G. Smirnova, V. E. Minaev, A. B. Silaev, and G. S. Katrakha, *Khim. prirod. Soedinenii*, 1974, **10**, 640.
[99] A. Dell, D. H. Williams, H. R. Morris, G. A. Smith, J. Feeney, and G. C. K. Roberts, *J. Amer. Chem. Soc.*, 1975, **97**, 2497.
[100] D. G. Martin, S. A. Mizsak, C. Biles, J. C. Stewart, L. Baczynsky, and P. A. Meulman, *J. Antibiotics*, 1975, **28**, 332.
[101] T. Ueno, T. Nakashimo, Y. Hayashi, and H. Fukami, *Agric. and Biol. Chem.* (*Japan*), 1975, **39**, 1115.

A synthesis of protodestruxin, the insecticidal cyclohexadepsipeptide (35), employed the *N*-hydroxysuccinimide method for the cyclization step.[102] Various linear precursors were tried, but the best yields were obtained when forming the



(35)

(33) R = OMe
(34) R = H

Ile-Pro bond. The distribution of the iron-complexing metabolites, ferricrocin, ferrichrome, and sideramine, in *Aspergillus* species has been studied.[103]

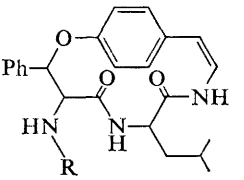
Other Synthetic and Conformational Studies on Cyclic Depsipeptides.—A number of miscellaneous studies which are conveniently included under this heading have accumulated during the past year. Depsipeptides can be prepared in high yield by 1-hydroxybenzotriazole-catalysed alcoholysis of N-protected amino-acid esters. 6-Chloro-1-*p*-chlorobenzenesulphonyloxybenzotriazole is also of general use in the preparation of carboxylic acid esters.[104] Cyclic tetradepsipeptides have been the subject of a number of investigations. A simple model system for echinomycin has been prepared,[105] and the conformations of a number of synthetic compounds have been studied.[106–108] Depsipeptide analogues of gramicidin S lack the biological activity of the parent compound.[109]

4 Peptide Alkaloids

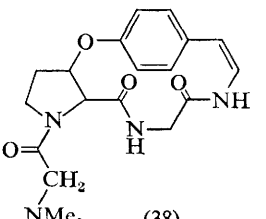
A review covering the occurrence, isolation, and spectroscopic properties of the cyclopeptide alkaloids has been published.[110]

Three more cyclopeptide alkaloids (36), (37), and (38) have been isolated from *Zizyphus nummularia* and identified.[111] Nummularine-E has *NN*-dimethylthreonine and nummularine-F has *NN*-dimethylglycine as terminal amino-acid, neither of which has been found in such alkaloids before.

[102] H. Diekmann and E. Kerzdorn, *Arch. Microbiol.*, 1975, **106**, 191.
[103] S. Lee, N. Izumiya, A. Suzuki, and S. Tamura, *Tetrahedron Letters*, 1975, 883.
[104] Y. S. Klausner and M. Chorev, *J.C.S. Chem. Comm.*, 1975, 973.
[105] W.-Y. Chen, M.-L. Hsu, and R. K. Olsen, *J. Org. Chem.*, 1975, **40**, 3110.
[106] G. N. Tishchenko, Z. Karimov, and V. V. Borisov, *Bioorg. Khim.*, 1975, **1**, 378.
[107] G. N. Tishchenko, N. V. Nazimov, V. I. Andrianov, and Z. Karimov, *Bioorg. Khim.*, 1975, **1**, 386.
[108] G. N. Tishchenko, Z. Karimov, and N. V. Nazimov, *Bioorg. Khim.*, 1975, **1**, 375.
[109] N. A. Krit, A. L. Zhuze, T. M. Andronova, M. P. Filatova, G. A. Ravdel, and L. A. Shchukina, *Bioorg. Khim.*, 1975, **1**, 247.
[110] R. Tschesche and E. U. Kaussmann, in 'The Alkaloids', Academic Press, New York, 1975, Vol. 15, p. 165.
[111] R. Tschesche, M. Elgamal, G. A. Miana, and G. Eckhardt, *Tetrahedron*, 1975, **31**, 2944.

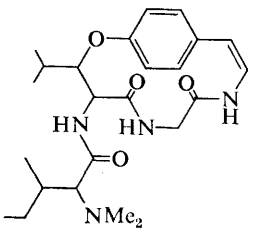


(36) R = —COCH(CHEtMe)NHMe
(37) R = —COCH(CHOHMe)NMe$_2$

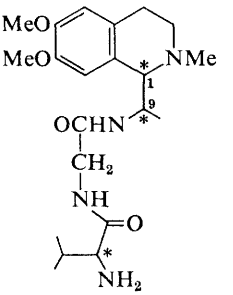


(38)

A detailed study of the conformation of the alkaloid frangulanine (39) has been undertaken,[112] and a preliminary communication of this work was reported last year. In an attempt to elucidate the conformation of frangulanine in solution, several amino-acid derivatives and non-cyclic peptides were produced synthetically, including Ile-Hyleu-Leu-Tyr, the hypothetical intermediate in the biosynthetic pathway to frangulanine. The ^{1}H n.m.r. spectra of these compounds were compared with that of the alkaloid. From the various shielding effects



(39)



(40)

observed on protons in the vicinity of the aromatic ring, it was concluded that frangulanine has the same conformation in solution as in a crystal of its tri-*N*-methyl methiodide derivative.

The structure of the isoquinoline alkaloid amphibine-I (40), which was reported last year, has been confirmed by synthesis, and the configuration at each of the three chiral centres has been determined.[113] The terminal valine residue has been assigned the L-configuration. By comparison of the c.d. spectrum of amphibine-I with the compound produced synthetically, it was deduced that the natural alkaloid occurs as an enantiomeric pair, either with C-1 (S), C-9 (R), or with C-1 (*R*), C-9 (*S*).

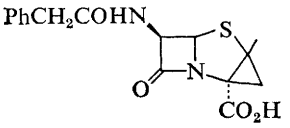
5 Penicillins and Cephalosporins

There is great interest in this field and no attempt will be made to give complete coverage of the whole topic. Two extremely comprehensive reviews have appeared this year, one on the multitude of rearrangements undergone by penicillanic acid

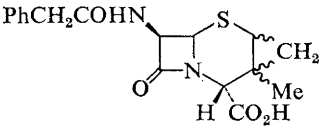
[112] M. Takai, Y. Ogihara, Y. Iitaka, and S. Shibata, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2556.
[113] R. Tschesche, J. Moch, and C. Spilles, *Chem. Ber.*, 1975, **108**, 2247.

derivatives,[114] and the other on the reactions of natural and synthetic β-lactams.[115] D-Penicillamine has been an important molecule in the degradation and synthesis of penicillins, and its production and properties have been reviewed.[116]

There has been continued effort in the search for modified penicillins and cephalosporins which possess antibacterial activity. Several attempts, with emphasis on the alteration of the basic bicyclic ring system, have been reviewed.[117] There are two reports of the synthesis of β-lactam antibiotics containing a third ring, in each case a cyclopropane. One of these [118] was of a penam compound (41) and the other [119] of two cepham derivatives (42) and (43). All of these compounds were found to have biological activity.

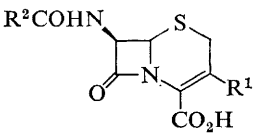


(41)

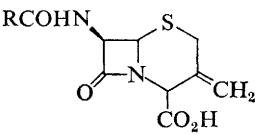


(42) α-Me, β $\diagdown\!\!\diagup CH_2$

(43) β-Me, α $\diagdown\!\!\diagup CH_2$



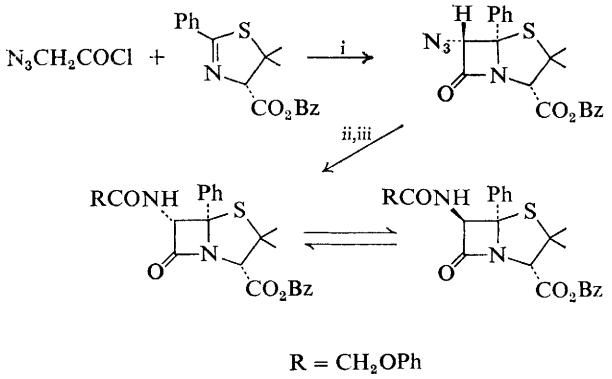
(44) R^1 = OMe, Cl, or Br



(45)

A group of compounds, some of which have been shown to possess potent activity, are the 3-methoxy- and 3-halogeno-3-cephems (44), which are made from cephalosporins *via* the 3-methylene-substituted cephams (45).[120] Another modification to the penicillin and cephalosporin nucleus is the replacement of the β-lactam oxygen with sulphur, to give the β-thionolactam.[121] This gave analogues which were much less effective as antibiotics compared to the oxygen compounds. Little work has been done to alter the 5-position of the penicillin nucleus, but the substitution of the 5-proton for a phenyl ring has been described.[122] This was produced by a route that involved the coupling of α-azaacetic acid with a phenyl-substituted thiazoline (see Scheme 2). The product was reduced and then acylated to give the 5-substituted 6-*epi*-penicillin V ester. Equilibration with base gave the compound with the natural configuration at C-6.

[114] R. J. Stoodley, *Tetrahedron*, 1975, **31**, 2321.
[115] A. K. Mukerjee and A. K. Singh, *Synthesis*, 1975, 547.
[116] W. M. Weigert, H. Offermanns, and P. Sherberich, *Angew. Chem.*, 1975, **14**, 330.
[117] G. Lowe, *Chem. and Ind.*, 1975, 459.
[118] T. Kamiya, T. Teraji, M. Hashimoto, O. Nakguchi, and T. Oku, *J. Amer. Chem. Soc.*, 1975, **97**, 5020.
[119] S. R. Lammert and S. Kukolja, *J. Amer. Chem. Soc.*, 1975, **97**, 5583.
[120] R. R. Chauvette and P. A. Pennington, *J. Medicin. Chem.*, 1975, **18**, 403.
[121] P. W. Wojtkowski, J. E. Dolfini, O. Kocy, and C. M. Cimarusti, *J. Amer. Chem. Soc.*, 1975, **97**, 5628.
[122] H. Vanderhaeghe and J. Thomis, *J. Medicin. Chem.*, 1975, **18**, 486.



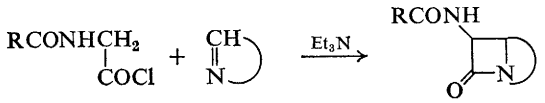
R = CH_2OPh

Reagents: i, Et_3N; ii, Pd–H_2; iii, RCOCl

Scheme 2

This is a route that could be used to provide several different modifications to the penicillin and the cephalosporin nuclei.

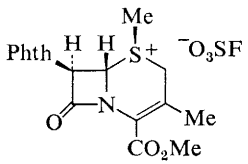
An alternative route has been reported in which the acylamido side-chain is incorporated directly into the β-lactam compound by use of the reaction between an α-acylamido derivative of acetyl chloride and a cyclic imine (Scheme 3).[123]



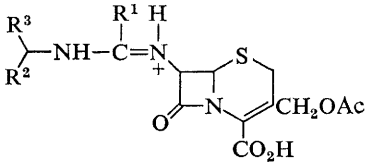
R = OCH_2Ph

Scheme 3

Two accounts of the synthesis of cephalosporin analogues possessing a positive charge have appeared. Treatment of a cephalosporin ester with methylfluorosulphonate gives an *S*-methylated derivative (46).[124] The mechanism of the reaction was thought to involve cleavage of the 1,6-bond, giving the C-6 epimer. A range of zwitterionic 7-*N*-amidinocephalosporins (47) was made, but all of these exhibited low biological activity.[125] The 6-substituted penam and 7-substituted cephem derivatives are still arousing a lot of interest. The formation



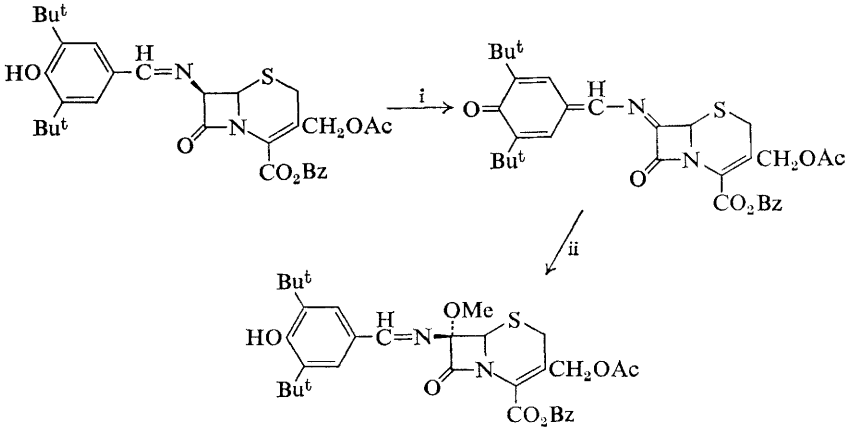
(46)



(47)

[123] A. K. Bose, M. S. Manhas, H. P. S. Chawla, and B. Dayal, *J.C.S. Perkin I*, 1975, 1880.
[124] D. K. Herron, *Tetrahedron Letters*, 1975, 2145.
[125] J. Altman, E. Karoly, and N. Maoz, *J. Medicin. Chem.*, 1975, **18**, 627.

of 6α-methoxy and 6α-cyano derivatives of penicillin V by addition across the penicillin 6-imine has been described.[126] The novelty in the method was that the imine was produced by condensation of a nitrogen Wittig with 6-oxopenicillanic acid.

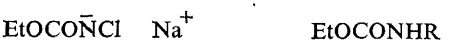


Reagents: i, PbO_2; ii, MeOH

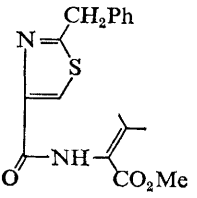
Scheme 4

A very neat way of producing 7α-methoxycephalosporins is outlined in Scheme 4.[127] The Schiff base is formed by condensation of a *p*-hydroxybenzaldehyde derivative with 7-aminocephalosporanic acid. After oxidation to the corresponding quinoid, the compound is readily attacked by methanol to give the 7α-methoxy derivative.

Reaction of *N*-chloro-*N*-sodio-urethane (48) with penicillinate esters and their sulphoxides has been shown to provide a route to 6-substituted penicillins and 7-substituted cephalosporins.[128, 129] The reaction of (48) with penicillins and with penicillin-(*S*)-sulphoxide involves the replacement of the 6-proton with the



(48) (49)



(50)

[126] Y. S. Lo and J. C. Sheehan, *J. Org. Chem.*, 1975, **40**, 191.
[127] H. Yanagisawa, M. Fukushima, A. Ando, and H. Nakao, *Tetrahedron Letters*, 1975, 2705.
[128] M. M. Campbell and G. Johnson, *J.C.S. Chem. Comm.*, 1975, 479.
[129] D. H. Bremner, M. M. Campbell, and G. Johnson, *Tetrahedron Letters*, 1975, 2955.

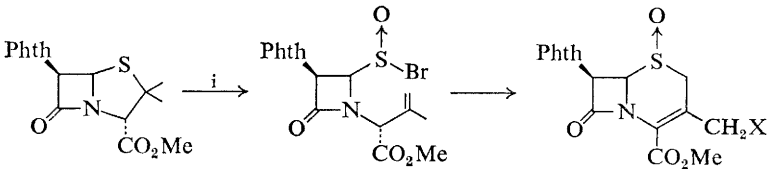
ethoxyformamido-group (49). In the case of penicillin-(*R*)-sulphoxide, the major product is one in which both the 6-proton and the 6-acylamino group have been replaced with ethoxyformamido groups. It was shown that these substituted sulphoxides could be converted into the corresponding cephem derivatives.

Owing to the labile nature of β-lactams, the attachment and removal of protecting groups to penicillins and cephalosporins have been a problem. This is illustrated by an attempt to hydrolyse penicillin G methyl ester using lithium t-butylmercaptide, a compound which has been found to displace carboxylate anions from methyl esters efficiently. On reaction with one equivalent of the base, penicillin G methyl ester gave in 80% yield the thiazole derivative (50).[130]

The diphenylmethyl group has been used widely as a protecting group for carboxylic acids as it is removed by relatively mild hydrolysis. The reagent used to form this ester, diphenyldiazomethane, is dangerous and costly. However, a simple procedure for the attachment of this group, by reaction of the acid with benzophenone hydrazone in the presence of peracetic acid, has now been reported.[131] This method has the disadvantage that in derivatizing penicillin or cephalosporin compounds, the product is the ester sulphoxide. The phthaloyl group is frequently used for the protection of the amino-group in the synthesis of penicillins and cephalosporins, but harsh conditions have been required for its removal. A method has now been described that is mild enough to remove the protecting group selectively, though it suffers from the drawback that three steps are involved.[132] A method for the acylation of secondary amides under neutral conditions has been reported.[133]

Several groups have been working on the conversion of penicillins into cephalosporins. One approach has involved the formation of the 2-halogen derivatives of penicillins, which are subsequently isomerized and dehydrohalogenated to give the corresponding desacetoxycephalosporins. Two groups have adopted this approach, each using an acyl halide in the presence of a base to form the 2-halogenopenam from the penam sulphoxide.[134, 135] Both groups found difficulties in distinguishing the various halogenated penam and cepham compounds, and these were the subject of additional work in which ^{1}H and ^{13}C n.m.r. were used.[136, 137] Another route used to convert penam into cepham compounds is *via* trapped sulphenic acids. Reaction of phthalimido penicillanic acid methyl ester sulphoxide with *N*-bromosuccinimide (NBS) gave the sulphenyl bromide derivative, which was isolated (Scheme 5).[138] This was converted in 47% yield into the cephem sulphoxide (51; X = H) and subsequently treated with NBS to

[130] S. Wolfe, J.-B. Ducep, and J. D. Greenhorn, *Canad. J. Chem.*, 1975, **53**, 3435.
[131] R. Bywood, G. Gallagher, G. K. Sharma, and D. Walker, *J.C.S. Perkin I*, 1975, 2019.
[132] S. Kukolja and S. R. Lammert, *J. Amer. Chem. Soc.*, 1975, **97**, 5582.
[133] L. M. Weinstock, S. Karady, F. E. Roberts, A. M. Hoinowski, G. S. Brenner, T. B. K. Lee, W. C. Lumma, and M. Sletzinger, *Tetrahedron Letters*, 1975, 3979.
[134] S. Kukolja, S. R. Lammert, M. R. Gleissner, and A. I. Ellis, *J. Amer. Chem. Soc.*, 1975, **97**, 3192.
[135] H. Tanida, T. Tsuji, T. Tsushima, H. Ishitobi, T. Irie, T. Yano, H. Matsumura, and K. Tori, *Tetrahedron Letters*, 1975, 3303.
[136] S. Kukolja, N. D. Jones, M. O. Chaney, T. K. Elzey, M. R. Gleissner, J. W. Paschal, and D. E. Dorman, *J. Org. Chem.*, 1975, **40**, 2388.
[137] K. Tori, T. Tsushima, Y. Tamura, H. Shigemoto, T. Tsuji, H. Ishitobi, and H. Tanida, *Tetrahedron Letters*, 1975, 3307.
[138] T. Ishimaru and T. Imamoto, *Bull. Chem. Soc. Japan*, 1975, **48**, 2989.



(51)

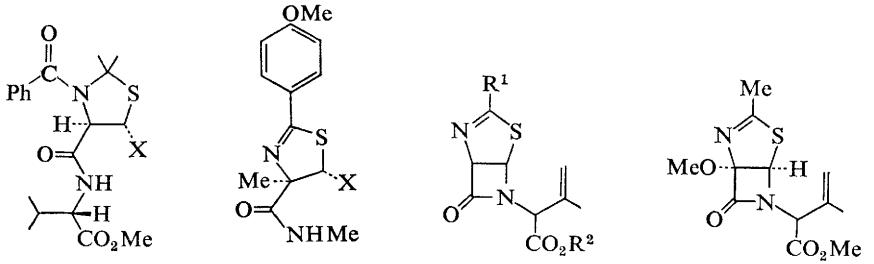
Reagent: i, NBS

Scheme 5

give the brominated derivative (51; X = Br). Reaction of this derivative with silver acetate gave the corresponding acetoxy compound (51; X = OCOMe).

The single-step conversion of penicillin G sulphoxide into the cephem derivative in greater than 80% yield has been reported.[139] The method involved protection of the carboxyl function as the trimethylsilyl ester, and rearrangement using HBr in the presence of a weak base.

There are several accounts of cyclization reactions that are possibly similar to the ways these cyclizations occur naturally. The thiazolidine derivative (52; X = H) of L-cysteinyl-L-valine can be converted *via* the benzoate derivative (52; X = OCOPh) into the chloro compound (52; X = Cl). This readily cyclizes in the presence of base to form the β-lactam.[140] The thiazoline compound (53; X = H) can be similarly treated with NBS to give the bromo derivative (53; X = Br), which on treatment with base forms the β-lactam system.[141]



(52) (53) (54) (55)

Fused β-lactam-thiazoline compounds (54) have previously been converted into penam and cephem ring systems, and have been suggested as possible biosynthetic intermediates. Two syntheses of this type of compound from penicillin sulphoxides have been performed by the same group.[142, 143] There is also a report of the total synthesis of compound (55), the sulphoxide of which was converted into the 6- or 7-methoxy derivatives of penicillin or cephalosporin, respectively.[144]

[139] J. J. de Koning, H. J. Kooreman, H. S. Tau, and J. Verweij, *J. Org. Chem.*, 1975, **40**, 1346.
[140] J. E. Baldwin, A. Au, M. Christie, S. B. Haber, and D. Hesson, *J. Amer. Chem. Soc.*, 1975, **97**, 5957.
[141] S. Nakatsuka, H. Tanino, and Y. Kishi, *J. Amer. Chem. Soc.*, 1975, **97**, 5010.
[142] H. Tanida, R. Muneyuki, and T. Tsushima, *Tetrahedron Letters*, 1975, 3063.
[143] H. Tanida, R. Muneyuki, and T. Tsushima, *Bull. Chem. Soc. Japan*, 1975, **48**, 3429.
[144] S. Nakatsuka, H. Tanino, and Y. Kishi, *J. Amer. Chem. Soc.*, 1975, **97**, 5008.

Reactions of penicillins with mercury(II) acetate [145, 146] and with chloramine T [147–149] continue to be investigated as possible routes to modified penicillins.

There were several reports during the year of studies in which specifically labelled amino-acids were incorporated into penicillin G by *Penicillum chrysogenum*. Two of these accounts [150, 151] describe syntheses in which cysteine (or cystine) was stereospecifically labelled with tritium at the β-centre to give the 3*R*- and 3*S*-forms. It was found in both cases that the 3*R*-form was incorporated into penicillin G whereas the 3*S*-form was not, indicating that the β-lactam ring is formed with rentention of configuration at the β-carbon atom of cysteine. Non-stereospecifically labelled L-[3,3′-$^3H_2$; U-^{14}C]cystine has also been fed with the expected result that approximately half of the tritium was incorporated into penicillin V.[152] The feeding of α-labelled cystine and its incorporation into penicillin G confirm that an αβ-dehydrocysteine residue is not involved in the biosynthetic pathway.[153] There are two reports that D-[^{14}C]valine is incorporated into penicillins as readily as the L-isomer.[152, 154] These papers both go on to describe experiments in which neither D- nor L-α-labelled valine isomers are incorporated into penicillins, thus ruling out the possibility that the D-valine α-centre is incorporated into penicillins without intermediate alteration. DL-[3-^{3}H]Valine has also been fed,[152] and from its negligible incorporation into penicillin one can eliminate certain possible mechanisms for formation of the thiazolidine ring system.

Doubly-labelled L-valine, with ^{15}N and with $^2H_3$ in one of the methyl groups, was fed to *Cephalosporium acremonium*.[155] By mass spectroscopy of the resulting penicillin N and cephalosporin C, it was shown that the valine skeleton had been incorporated intact. On feeding L-[2,3-^{3}H]-valine to starved cells of *C. acremonium*, the tripeptide δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine was found to be labelled only in the C-3 position of the valine residue, demonstrating that the D-centre of this residue is not formed *via* an αβ-dehydrovaline derivative.[155] The tripeptide mentioned above, possessing the D-valine isomer, has now been shown to occur in both mycelial extracts and culture filtrates of *P. chrysogenum*, as determined by its retention on an ion-exchange column.[156]

The final stage of the biosynthesis of penicillin G, *i.e.* the coupling of phenylacetic acid to 6-aminopenicillanic acid or an amide exchange reaction of isopenicillin N, has been investigated using penicillins labelled with tritium in the

[145] D. F. Corbett and R. J. Stoodley, *J.C.S. Perkin I*, 1975, 432.
[146] R. J. Stoodley and N. S. Watson, *J.C.S. Perkin I*, 1975, 883.
[147] M. M. Campbell, G. Johnson, A. F. Cameron, and I. R. Cameron, *J.C.S. Perkin I*, 1975, 1208.
[148] M. M. Campbell and G. Johnson, *J.C.S. Perkin I*, 1975, 1212.
[149] M. M. Campbell and G. Johnson, *J.C.S. Perkin I*, 1975, 1932.
[150] D. J. Morecombe and D. W. Young, *J.C.S. Chem. Comm.*, 1975, 198.
[151] D. J. Aberhart, L. J. Lin, and J. Y.-R. Chu, *J.C.S. Perkin I*, 1975, 2517.
[152] P. Adriaens, H. Vanderhaeghe, B. Meesschaert, and H. Eyssen, *Antimicrobial Agents and Chemotherapy*, 1975, **8**, 15.
[153] B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 123.
[154] B. W. Bycroft, C. M. Wels, K. Corbett, A. P. Maloney, and D. A. Lowe, *J.C.S. Chem. Comm.*, 1975, 923.
[155] F.-C. Huang, J. A. Chan, C. J. Sih, P. Fawcett, and E. P. Abraham, *J. Amer. Chem. Soc.*, 1975, **97**, 3858.
[156] P. Adriaens, B. Meesschaert, W. Wuyts, H. Vanderhaeghe, and H. Eyssen, *Antimicrobial Agents and Chemotherapy*, 1975, **8**, 638.

2β-methyl group.[157, 158] This labelling was achieved by an exchange reaction with ^3H_2O on an ester of penicillin V sulphoxide, which was subsequently converted into 6-aminopenicillanic acid and isopenicillin N. It was found that both of these compounds were incorporated into penicillin G by protein extracts of *P. chrysogenum.*

6 Peptides Linked to Carbohydrates

As in previous years, the emphasis in this section will be on the chemistry and the biosynthesis of the peptide constituents of cell walls.

Glycopeptides from Bacterial Cell Walls.—The biosynthesis of the nucleotide precursor of cell wall peptidoglycan of *Corynebacterium poinsettiae* (56) and of

UDP-*N*Ac-muramyl-Gly-D-Glu$^\gamma$-(X)-D-Ala-D-Ala

(56) X = L-Hse
(57) X = L-Abu

C. insidiosum (57) has been studied using soluble extracts of these organisms.[159] The peptide chains are different from those found in most bacterial species as they possess a glycine as the first amino-acid as opposed to the more usual L-alanine. The enzyme specificities at each of the different steps varied greatly. For each organism, the coupling of glycine to UDP-*N*Ac-muramic acid was found to occur very readily, but L-alanine was found to couple much less readily. It was also found that the addition of the particular third amino-acid was highly specific for each organism.

Spin-labelled UDP-*N*Ac-muramyl pentapeptides have been used to study the initial reactions in peptidoglycan synthesis and the effects that certain antibiotics have on this process.[160]

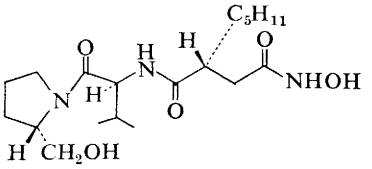
The role of penicillins in the inhibition of cell wall synthesis has aroused considerable interest over the past few years. It has now been shown that enzymes which are implicated in the final steps of cell wall synthesis, namely cross-linking, are able to degrade penicillin G. Both the soluble DD-carboxypeptidase-transpeptidase from *Streptomyces* R61 and R39,[161] and the insoluble D-alanine carboxypeptidase from *Bacillus stearothermophilus*[162] were found to convert penicillin G into *N*-phenacetylglycine. In the latter study, it was shown that the glycine is derived from the 6- and 7-carbon atoms of the penicillin molecule, and the degradation thus involved cleavage of the β-lactam and the C-5—C-6 bonds. Possible mechanisms and their implications on the cross-linking reactions were discussed. The purification of soluble D-alanine carboxypeptidase from *E. coli* has been performed by affinity chromatography on Sepharose to which *p*-aminobenzylpenicillin was covalently bonded.[163] The bound enzyme was removed from the gel by elution with hydroxylamine.

[157] J. J. Usher, B. Loder, and E. P. Abraham, *Biochem. J.*, 1975, **151**, 729.
[158] P. A. Fawcett, J. J. Usher, and E. P. Abraham, *Biochem. J.*, 1975, **151**, 741.
[159] A. W. Wyke and H. R. Perkins, *J. Gen. Microbiol.*, 1975, **88**, 159.
[160] L. S. Johnson and F. C. Neuhaus, *Biochemistry*, 1975, **14**, 2754.
[161] J.-M. Frère, J.-M. Ghuysen, J. Degelaen, A. Loffet, and H. R. Perkins, *Nature*, 1975, **258**, 168.
[162] S. Hammarström and J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3463.
[163] M. Gorecki, A. Bar-Eli, Y. Burstein, A. Patchornik, and E. B. Chain, *Biochem. J.*, 1975, **147**, 131.

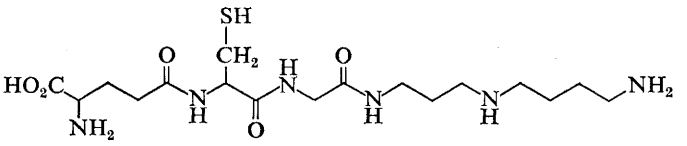
Extracts possessing antitumour properties have been obtained by enzymic digestion of cells of *Lactobacillus bulgaricus* and fractionation of the resulting glycopeptides.[164] The structures of the compounds present in each of the fractions were determined.

7 Linear Peptides containing Unusual Structural Features

A series of papers has appeared that describes the structure determination of the antibiotic actinonin (58),[165] the synthesis of actinonin and many of its analogues,[166–170] the mass spectra of actinonin and related compounds,[171] and a study of the structure–activity relationship of compounds of this type.[172]



(58)



(59)

L-Prolinol and the hydroxamic acid of the type present in actinonin have not previously been found to occur naturally.

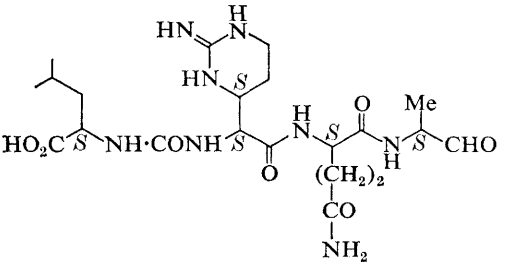
The isolation and characterization of glutathionyl spermidine (59) from *E. coli* have been described.[173] It was suggested that this compound might have a role in the control of growth and nucleic acid metabolism in *E. coli*. Two new antibiotics, cerexins A and B, have been isolated[174] from *Bacillus cereus* and characterized.[175] Both of these compounds are fatty acyl decapeptides containing several D-amino-acids, and a new amino-acid, L-*threo*-γ-hydroxylysine. The

[164] I. G. Bogdanov, P. G. Dalev, A. I. Gurevich, M. N. Kolosov, V. P. Mal'kova, L. A. Plemyannikova, and I. B. Sorokina, *F.E.B.S. Letters*, 1975, **57**, 259.
[165] J. J. Gordon, J. P. Devlin, A. J. East, W. D. Ollis, I. O. Sutherland, D. E. Wright, and L. Ninet, *J.C.S. Perkin I*, 1975, 819.
[166] N. H. Anderson, W. D. Ollis, J. E. Thorpe, and A. D. Ward, *J.C.S. Perkin I*, 1975, 825.
[167] J. P. Devlin, W. D. Ollis, J. E. Thorpe, R. J. Wood, B. J. Broughton, P. J. Warren, K. R. H. Wooldridge, and D. E. Wright, *J.C.S. Perkin I*, 1975, 830.
[168] B. J. Broughton, P. J. Warren, K. R. H. Wooldridge, D. E. Wright, W. D. Ollis, and R. J. Wood, *J.C.S. Perkin I*, 1975, 842.
[169] J. P. Devlin, W. D. Ollis, and J. E. Thorpe, *J.C.S. Perkin I*, 1975, 846.
[170] J. P. Devlin, W. D. Ollis, J. E. Thorpe, and D. E. Wright, *J.C.S. Perkin I*, 1975, 848.
[171] N. H. Anderson, J. P. Devlin, S. Jones, W. D. Ollis, and J. E. Thorpe, *J.C.S. Perkin I*, 1975, 852.
[172] B. J. Broughton, P. Chaplen, W. A. Freeman, P. J. Warren, K. R. H. Wooldridge, and D. E. Wright, *J.C.S. Perkin I*, 1975, 857.
[173] H. Tabor and C. W. Tabor, *J. Biol. Chem.*, 1975, **250**, 2648.
[174] J. Shoji, H. Hinoo, Y. Wakisaka, K. Koizumi, M. Mayama, S. Matsuura, and K. Matsumoto, *J. Antibiotics*, 1975, **28**, 56.
[175] J. Shoji and H. Hinoo, *J. Antibiotics*, 1975, **28**, 60.

amino-acid sequence of cerexin A was established (60).[176] Cerexin B differs from cerexin A in that a serine and valine residue are replaced by a glycine and a phenylalanine residue. The structure of the antibiotic althiomycin, which was reported by two groups last year, has been confirmed by yet another group.[177]

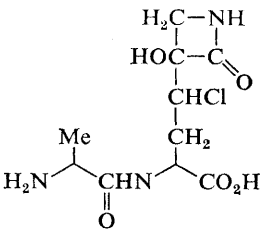
Fatty-acyl-Asn-D-Val-D-Val-Asp(NH_2)$^{\alpha \text{ or } \beta}$-Asn-Lys(5-OH)-D-aThr-Ser-D-Trp-D-aIle

(60)

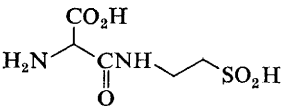


(61)

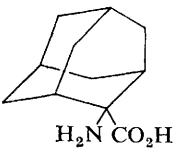
The structure of elastinal (61), an elastase inhibitor produced by various species of actinomycetes, has been reported.[178] The amino-terminal of this unusual peptide is masked by ureide formation. A dipeptide antibiotic (62) has been isolated from the culture filtrate of a *Streptomyces* species.[179] This compound has a very similar structure to the wildfire toxin, tabtoxin, produced by several *Pseudomonas* species, and is among the few compounds found in nature that possess a β-lactam ring. A new metabolite isolated from culture filtrates of *Emericellopsis salmosynnemata* has been identified as *N*-acetyl-L-phenylalanyl-L-phenylalaninol.[180] A dipeptide fish attractant, arcamine (63) (hypotauryl-2-carboxyglycine), has been isolated.[181] The dipeptide D-Ala-D-Ala was found in



(62)



(63)



(64)

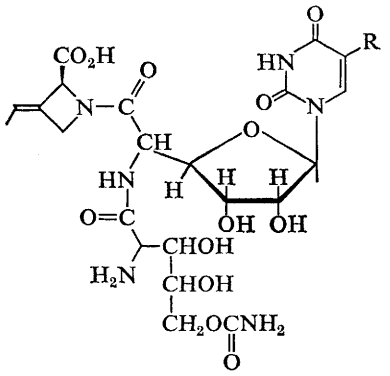
[176] J. Shoji and T. Kato, *J. Antibiotics*, 1975, **28**, 764.
[177] H. A. Kirst, E. F. Szymanski, D. E. Dorman, J. L. Occolowitz, N. D. Jones, M. O. Chaney, R. L. Hamill, and M. M. Hoehn, *J. Antibiotics*, 1975, **28**, 286.
[178] A. Okura, H. Morishima, T. Takita, T. Aoyagi, T. Takeuchi, and H. Umezawa, *J. Antibiotics*, 1975, **28**, 339.
[179] J. P. Scannell, D. L. Pruess, J. F. Blount, H. A. Ax, M. Kellett, F. Weiss, T. C. Demny, T. H. Williams, and A. Stempel, *J. Antibiotics*, 1975, **28**, 1.
[180] A. D. Argoudelis, S. A. Mizsak, and L. Baczynskyj, *J. Antibiotics*, 1975, **28**, 233.
[181] A. W. Sangster, S. E. Thomas, and N. L. Tingling, *Tetrahedron*, 1975, **31**, 1135.

pasture grass, *Phalaris tuberosa* L.[182] It was suggested that this peptide and D-alanine are absorbed and concentrated from the soil where they occur in traces as a result of bacterial activity.

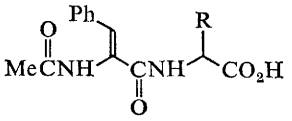
The tripeptide L-Arg-D-*allo*-Thr-L-Phe has been shown to be active against fungi. The variation of activity with structure has been studied, including alteration of the central amino-acid and methylation of the nitrogen of this amino-acid.[183–185] In the search for antitumour drugs, dipeptides containing the tricyclic amino-acid adamantanine (64) have been made.[186]

8 Miscellaneous

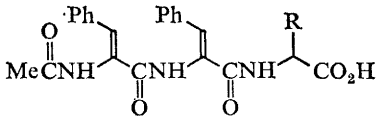
The polyoxins (65) are a group of very interesting antibiotics containing unique amino-acids. A study on the origin of the 3-ethylidine-L-azetidine-2-carboxylic acid (polyoximic acid) portion has been reported.[187] By feeding several specifically labelled amino-acids, this ring system was shown to be derived entirely from isoleucine.



(65)



(66)



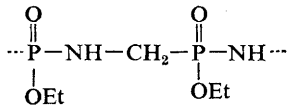
(67)

An investigation has been undertaken into the chiroptical properties and secondary structure of several dehydropeptides with structures (66) and (67).[188] From the data it was suggested that the doubly-unsaturated peptides have a rigid conformation in solution, stabilized by intramolecular hydrogen-bonding.

The synthesis of peptide analogues containing aminomethylphosphoric acid residue (68) has been described.[189] Compounds of this type have been found to occur naturally.

Other peptide analogues have been made using α-aza-amino-acids (69).[190] These amino-acids differ only slightly in their polarity and size from amino-acids possessing an α-CH group.

[182] J. L. Frahn and R. J. Illman, *Phytochemistry*, 1975, **14**, 1464.
[183] K. Eisele, *Z. Naturforsch.*, 1975, **30c**, 541.
[184] K. Eisele, *Experientia*, 1975, **31**, 764.
[185] A. Eisele and K. Eisele, *F.E.B.S. Letters*, 1975, **55**, 153.
[186] H. T. Nagasawa, J. A. Elberling, and F. N. Shirota, *J. Medicin. Chem.*, 1975, **18**, 826.
[187] K. Isono, S. Funayama, and R. J. Suhadolnik, *Biochemistry*, 1975, **14**, 2992.
[188] O. Pieroni, G. Montagnoli, A. Fissi, S. Merlino, and F. Gardelli, *J. Amer. Chem. Soc.*, 1975, **97**, 6820.
[189] K. Yamauchi, Y. Mitsuda, and M. Kinoshita, *Bull. Chem. Soc. Japan*, 1975, **48**, 3285.
[190] A. S. Dutta and J. S. Morley, *J.C.S. Perkin I*, 1975, 1712.

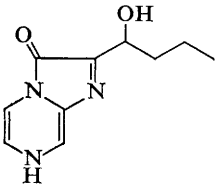


(68)

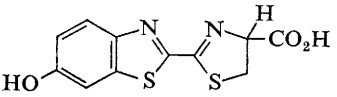


(69)

There has been continued interest in luciferins over the past year. A short review of bacterial and other luciferins has been published.[191] A new, very unstable luciferin has been isolated from the deep-sea shrimp *Oplophorus gracilorostris* and shown to have the imidazo[1,2-*a*]pyrazin-3-one structure (70).[192] This structure is similar to that of the *Cypridina* luciferin reported last year.



(70)



(71)

A biomimetic route to the benzothiazole ring system of firefly luciferin (71) was designed and executed, working on the possibility that the biosynthetic precursors of this compound are one molecule of benzoquinone and two molecules of cysteine.[193]

[191] F. H. Johnson and O. Shimomura, *Bioscience*, 1975, **25**, 718.
[192] I. Yamaguchi, *Biochem. J.*, 1975, **151**, 9.
[193] F. McCapra and Z. Razavi, *J.C.S. Chem. Comm.*, 1975, 42.

5
Chemical Structure and Biological Activity

BY D. J. SCHAFER AND M. SZELKE

1 Introduction

This chapter reviews work published during both 1974 and 1975 which throws some light on the relationship between the chemical structure and biological activity of peptide hormones and related compounds (a term which covers a multitude of sins!). Coverage of the two-year period is necessarily not comprehensive and reflects to some extent our own particular interests. The initial source of references, as in previous years, has been 'Current Contents, Life Sciences' (Institute for Scientific Information, Philadelphia, U.S.A.).

The two-year coverage gives a better overall impression of progress in the field, and the signs are hopeful that progress is indeed steadily being made. The end products of the exercise are slowly finding practical application. The recent clinical reports (see p. 385) on one such product, desamino-[8-D-arginine] vasopressin (DDAVP) bear witness to the success of an earlier exercise in modifying the biological activity of a natural hormone. DDAVP is probably as good an example as one could hope for, in its limited application in diabetes insipidus, of the advantages of peptides as drugs. It is potent, apparently without significant toxicity, and completely specific for its intended use. The problem of metabolic instability generally expected of peptides is overcome in the modified structure, and administration (by intranasal tube) is simple enough in practice.

The impact of the knowledge of the three-dimensional structure of the larger peptide hormones, notably insulin, is evident. One of the most exciting developments here is the recent description (see p. 391) of a clearly defined receptor binding region in the insulin structure which hopefully will lead to the design of much smaller molecules retaining the biological activity. While much useful work has been aimed at elucidating the three-dimensional structure of smaller peptide hormones in solution, and an impressive range of physical techniques brought to bear on the problem, the impact on our knowledge of the biological action has been disappointing. Clearly the conformation of smaller peptides in solution (which may nor may not resemble that at the biological receptor) is by no means the only factor determining their biological efficacy. Indeed the concept of a single solution conformation may be inappropriate in many cases. A recent study with LH-RH, using c.d., o.r.d., and the effects of pH, temperature, and guanidine concentration on fluorescence spectra, has concluded that the decapeptide behaves essentially as a random coil in aqueous solution.[1]

Fundamental knowledge of the actions of peptide hormones is increasing greatly. The interpretation of *in vivo* hormone action in terms of a single molecular

[1] S. Mabrey and M. Klotz, *Biochemistry*, 1976, **15**, 234.

species, as discussed previously,[2] is giving way to a concept where all of the metabolic entities from 'prohormones' to 'degradation products' may potentially contribute to the biological action. At the level of the cell, there has been impressive progress in understanding the molecular nature of biological receptors (located in the cell membrane) which not many years ago were as hypothetical as the unicorn. Again it is in the insulin field where progress has been most evident (see p. 393). However, a degree of caution against an oversimple approach is perhaps necessary. It is clear that the hormone–receptor interaction is a dynamic process and the biological action can itself modify the properties and numbers of receptors.[3,4] It is as well to bear in mind what is known in the case of a typical neurotransmitter, acetylcholine, acting at its receptor, where various proposed biochemical changes including binding, activation of adenyl cyclase, and further enzyme changes lead to a structural modification in proteins and a resultant opening of an ion channel. This complex chain of events takes place in no more than 10^{-3} s compared with minutes or even hours with some hormone actions. Release of hormones seems generally to occur in a pulsatile fashion,[5–7] presumably reflecting an exocytosis step. This can lead to problems in measuring a hormonal response if assays are not carried out sufficiently rapidly.[7]

A great deal of current interest centres on peptides acting on the nervous system and work in this area has been collected together separately in Section 9.

A number of books and reviews covering the area of this review have appeared, including the proceedings of "Endocrinology 1975" published as a special supplement of *Clinical Endocrinology*,[8] books on peptide hormones,[9] hormone chemistry,[10] endocrine physiology,[11] structure–activity relationships of protein and polypeptide hormones.[12] The proceedings of the Fourth American Peptide Symposium[13] have reached us too late to be covered in detail. Others include

[2] R. Camble and A. S. Dutta in this series, Vol. 6, 1975, p. 419.
[3] P. Hinkle and A. Tashjian, *Biochemistry*, 1975, **14**, 3845.
[4] J. R. Tata, *Nature*, 1975, **257**, 740; M. Raff, *ibid.*, 1976, **259**, 265.
[5] C. H. Mortimer, G. M. Besser, J. Hook, and A. S. McNeilly, *Clin. Endocrinol.*, 1974, **3**, 19.
[6] J. P. Martin, L. P. Renaud, and P. Brazeau, *Science*, 1974, **186**, 538.
[7] T. Chard, *Clin. Endocrinol.*, 1975, **4**, 89.
[8] I. MacIntyre and A. G. E. Pearse, eds., *Clin. Endocrinol.*, 1976, **5** (Supplement); (*a*) G. Fink, p. 245S; (*b*) W. Vale, C. Rivier, M. Brown, J. Leppaluoto, N. Ling, M. Monahan, and J. Rivier, p. 261S; (*c*) L. Ferland, F. Labrie, D. H. Coy, E. J. Coy, and A. V. Schally, p. 279S; (*d*) A. S. Dutta, B. J. A. Furr, M. B. Giles, and J. S. Morley, p. 291S; (*e*) D. Sarantakis, W. A. McKinley, I. Jaunakais, D. Clark, and N. H. Grant, p. 275S; (*f*) J. R. J. Baker, H. P. J. Bennett, A. M. Hudson, C. McMartin, and G. E. E. Purdon, p. 61S; (*g*) V. Pliska and J. Rudinger, p. 73S; (*h*) J. E. Gerich, V. S. Schneider, M. Lorenzi, E. Tsalikian, D. M. Bier, J. H. Karam, and P. H. Forsham, p. 299S; (*i*) M. I. Grossman, pre-publication abstracts, p. 10; (*j*) J. H. Rehfeld, L. A. Christiansen, J. Malmström, T. Schwartz, and F. Stadil, p. 185S; (*k*) V. Mutt, p. 175S; M. Bodanszky, M. L. Fink, K. W. Funk, and S. I. Said, p. 195S; (*l*) A. G. E. Pearse and T. Takor Takor, p. 229S; (*m*) S. I. Said, p. 201S; (*n*) S. R. Bloom and J. M. Polak, p. 223S; (*o*) J. T. Potts, p. 307S; (*p*) R. Maier, B. Kamber, B. Riniker, and W. Rittel, p. 327S; (*q*) H. B. Brewer, T. Fairwell, and R. Ronan, pre-publication abstracts, p. 20.
[9] S. A. Berson and R. S. Yalow, 'Peptide Hormones', North Holland, Amsterdam, 1973.
[10] W. R. Butt, 'Hormone Chemistry. Vol. 1. Protein, Polypeptide, and Peptide Hormones', John Wiley, New York, 1975.
[11] MTP International Review of Science, Physiology Series One, Vol. 5, 'Endocrine Physiology', ed. S. M. McCann, Butterworth, London, 1973.
[12] H. C. Cutler, 'Structure–Activity Relationships of Protein and Polypeptide Hormones', ed. M. Margoulies and F. C. Greenwood, Excerpta Medica, Amsterdam, 1972.
[13] 'Peptides: Chemistry, Structure, and Biology', Proceedings of the Fourth American Peptide Symposium, ed. R. Walter and J. Meienhofer, Ann Arbor Science Publishers, Ann Arbor, 1976.

further volumes giving details of the 1973 and 1974 Laurentian Hormone Conferences,[14, 15] a text on the fundamentals of clinical endocrinology,[16] some relevant chapters in 'Methods in Enzymology',[17, 18] a collection of current topics in experimental endocrinology,[19] and a further volume in Weinstein's series.[20] Other topics reviewed have included recent developments in peptide hormone assay and action,[21] the biochemistry of hormones,[22] and the affinity labelling of hormone binding sites,[23] among others.[24–26]

2 Hypothalamic Hormones

The compounds covered here are largely concerned with the regulation of the release of anterior pituitary hormones, but their extrapituitary actions and presence in other parts of the brain (Section 9), gastrointestinal tract (p. 399), and even in frog skin,[27] are becoming increasingly evident. The development of the releasing factor concept [8a] and the pharmacology of hypothalamic regulatory peptides (including recent structure–activity studies) [8b] have been reviewed. Other reviews discuss the hypothalamus as an endocrine organ,[28] the pituitary gland and its neuroendocrine control,[29] and the structure, synthesis, and biological activity of hypothalamic hormones.[30] The hormones are also reviewed in a book on current topics in experimental endocrinology,[19a] and in the proceedings of a

[14] 'Recent Progress in Hormone Research', ed. R. O. Greep, Academic Press, New York, 1974, Vol. 30; (*a*) J. J. Van Wyk, L. E. Underwood, R. L. Hintz, D. R. Clemmons, S. J. Voina, and R. P. Weaver, p. 259; (*b*) R. A. Bradshaw, R. A. Hogue-Angeletti, and W. A. Frazier, p. 575; (*c*) R. S. Yalow, p. 597; (*d*) E. Reiss and J. M. Canterbury, p. 391; (*e*) S. Cohen and J. M. Taylor, p. 533; S. Cohen and C. R. Savage, p. 551; (*f*) R. A. Bradshaw, R. A. Hogue-Angeletti, and W. A. Frazier, p. 575.

[15] 'Recent Progress in Hormone Research', ed. R. O. Greep, Academic Press, New York, 1975, Vol. 31.

[16] 'Fundamentals of Clinical Endocrinology', ed. R. Hall, J. Anderson, G. A. Smart, and G. M. Besser, Pitman Medical, London, 1974.

[17] 'Methods in Enzymology. Vol. 39. Hormone Action part D. Isolated Cells, Tissues, and Organ Systems', ed. J. G. Hardman and B. W. O'Malley, Academic Press, New York, 1975.

[18] 'Methods in Enzymology. Vol. 38. Hormone Action part B. Peptide Hormones', ed. B. W. O'Malley and J. G. Hardman, Academic Press, New York, 1975.

[19] 'Current Topics in Experimental Endocrinology. Vol. 2', ed. V. H. T. James and L. Martini, Academic Press, New York, 1974; (*a*) G. F. Grant and W. Vale, pp. 38, 62; (*b*) W. G. Blackard and S. S. Andrews, p. 130; (*c*) K. Hall and J. J. Van Wyk, p. 156; (*d*) I. MacIntyre, p. 179; (*e*) M. Jutisz and C. Tertrin-Clary, p. 197.

[20] 'Chemistry and Biochemistry of Amino-acids, Peptides, and Proteins. A Survey of Recent Developments', ed. B. Weinstein, Marcel Dekker, New York, Vols. 2 and 3, 1974.

[21] 'Recent Developments in Peptide Hormone Assay and Action', ed. D. T. Kreiger, Intercontinental, New York, 1973.

[22] MTP international Review of Science, Biochemistry Series One, Vol. 8, 'Biochemistry of Hormones', ed. H. V. Rickenberg, Butterworth, London, 1974.

[23] J. A. Katzenellenbogen, *Ann. Reports Medicin. Chem.*, 1974, **9**, 222.

[24] H. S. Tager and D. F. Steiner, *Ann. Rev. Biochem.*, 1974, **43**, 509.

[25] W. Lutz and L. Kiyszejko-Stefanowicz, *Postepy Biochem.*, 1974, **20**, 35.

[26] 'Comprehensive Biochemistry. Vol. 25. Mechanisms of Hormone Action', ed. M. Florkin and E. H. Stotz, Elsevier, Amsterdam, 1975.

[27] T. Yasuhara and T. Nakajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 3301.

[28] G. M. Besser, *Brit. Med. J.*, 1974, iii, 560, 613.

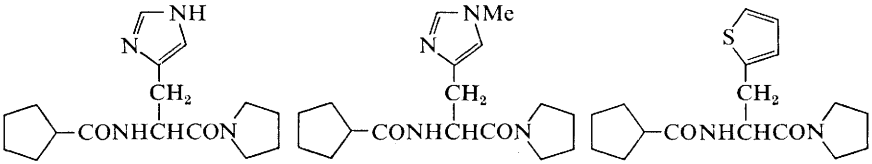
[29] 'Handbook of Physiology. Section 7. Endocrinology. Vol. 4. The Pituitary Gland and its Neuroendocrine Control', ed. R. O. Greep and E. B. Astwood, Williams and Wilkins, Baltimore, 1974.

[30] 'Hypothalamic Hormones – Structure, Synthesis, and Biological Activity', ed. D. Gupta and W. Voelter, Verlag Chemie, Weinheim, 1975.

workshop conference on psychoneuroendocrinology[31] and the October 1974 Serono Foundation symposium.[32] A number of other reviews have appeared.[33–41] Much work continues on the biological and clinical study of the hormones themselves, and this work, the subject of many of the above reviews, will not be covered comprehensively here.

Thyrotropin Releasing Hormone (TRH, thyroliberin).—Table 1 gives structure–activity results for the endocrine activities of TRH analogues reported during the period of the review. The central nervous actions of TRH analogues and related compounds are listed in Table 11 (p. 424) and discussed in Section 9.

The first TRH analogue (1) reported to have inhibitory activity towards TRH[42, 43] has been studied further both *in vitro* using rat pituitaries and *in vivo* in the rhesus monkey.[44] Inhibitory activity was again found but not consistently



(1) (2) (3)

Table 1 *Analogues of TRH*

Compound number	*Structure* [c]	*Biological activity:*[a] *Release of: TSH*	*PRL*	*Binding affinity*	*Test* [b]	*Ref.*
(4)	cCys-His-Pro-NH_2					45
(5)	cSer-His-Pro-NH_2					45
			+++		C	46 [d]
				+++	D	46

[31] 'Psychoneuroendocrinology. Workshop Conference of the International Society of Psychoneuroendocrinology, Mieken, 1973', ed. N. Hatotani, S. Karger, Basel, 1974; (*a*) M. Fukushima, K. Kushima, and K. Okuyama, p. 170; (*b*) M. Igarashi, K. Taya, and J. Ishikawa, p. 178; (*c*) N. Yanaihara, C. Yanaihara, T. Hashimoto, N. Sakura, K. Miyazeki, and T. Kaneko, p. 187; (*d*) T. Kaneko, H. Oka, M. Munemura, S. Saito, and N. Yanaihara, p. 267; (*e*) M. Sakeda and T. Kusaka, p. 221; (*f*) N. Marks and F. Stern, p. 276.

[32] 'Hypothalamic Hormones. Chemistry, Physiology, Pharmacology, and Clinical Uses. Proceedings of the Serono Symposia, Volume 6', ed. M. Motta, P. G. Crosignani, and L. Martini, Academic Press, New York, 1975.

[33] S. A. Kaplan, *Amer. J. Diseases Childhood*, 1974, **128**, 451.

[34] B. A. Sachs and R. Guillemin, *J. Amer. Med. Assoc.*, 1975, **232**, 1217.

[35] P. G. Smelik, *Ned. Tijdschr. Geneesk.*, 1975, **119**, 1240.

[36] J. Meienhofer, *Ann. Reports Medicin. Chem.*, 1976, **10**, 202.

[37] 'Hypothalamic Hormones', ed. E. S. E. Hafez and J. R. Reel, John Wiley, New York, 1975.

[38] R. O. Studer, *Wiener Klinische Wochenschrift*, 1974, **86**, 57.

[39] G. M. Besser and C. H. Mortimer, *J. Clin. Pathol.*, 1974, **27**, 173.

[40] W. Vale and C. Rivier, in 'Handbook of Psychopharmacology. Section I, Vol. 5', ed. L. L. Iversen, S. D. Iversen, and S. H. Snyder, Plenum Press, New York, 1975, p. 195.

[41] J. M. Hershman, *New Eng. J. Med.*, 1974, **290**, 886.

[42] D. J. Schafer, B.P. 1 374 317.

[43] H. Lybeck, J. Leppaluoto, P. Virkkunen, D. J. Schafer, L. Carlsson, and J. Mulder, *Neuroendocrinology*, 1973, **12**, 366.

[44] H. Sievertsson, S. Castensson, K. Andersson, S. Bjorkman, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1401.

[45] M. Fujino, T. Fukuda, and C. Hatanaka, Ger. Offen. 2 408 324.

[46] P. M. Hinkle, E. L. Woroch, and A. J. Tashjian, jun., *J. Biol. Chem.*, 1974, **249**, 3085.

Table 1 (*cont.*)

Compound number	Structure [c]	Biological activity:[a] Release of: TSH	PRL	Binding affinity	Test [b]	Ref.
(6)	cThr-His-Pro-NH_2					45
			+++		C	46
				+++	D	46
(7)	HCO-Pro-His-Pro-NH_2	++				8*b*
(8)	Gln-His-Pro-NH_2 [e]		(+++)		C	46
				(+)	D	46
(9)	Glu(OMe)-His-Pro-NH_2 [e]		(+++)		C	46
				(+++)	D	46
(10)	Pro-His-Pro-NH_2		+		C	46
				++	D	46
(11)	D-Glp-His-Pro-NH_2		−		C	46
				−	D	46
(12)	cThr-His(N^{τ}Me)-Pro-NH_2					45
(1)	cpc-His-pyr	−			B*	44
(3)	cpc-Thi-pyr	−			B*	44
(2)	cpc-His(N^{τ}Me)-pyr	−			B	44
(13)	D-Glp-D-His-D-Pro-NH_2		−		C	46
				−	D	46
(14)	Glp-Arg-Pro-NH_2		−		C	46
				−	D	46
(15)	Glp-Gly-His-Pro-NH_2	−			A	47
			−		E	47
(16)	Glp-D-His-Pro-NH_2		++		C	46
				++	D	46
		+++			A	48, 49
(17)	Glp-Leu-Pro-NH_2	++			A	50
		++			B	50
(18)	Glp-Lys-Pro-NH_2		−		C	46
				−	D	46
(19)	Glp-His(N^{τ}Me)-Pro-NH_2		++++		C	46
				++++	D	46
(20)	Glp-MeHis-Pro-NH_2	++++				51
(21)	Glp-Tyr(Me)-Pro-NH_2	−			A	48, 52
(22)	Glp-Phe-Pro-NH_2	+++			A	48, 53, 54
(23)	Glp-Pyr(1)ala-Pro-NH_2	++++			A	55

[47] H. Sievertsson, S. Castensson, O. Lindgren, and C. Y. Bowers, *Acta Pharm. Suecica*, 1974, **11**, 67.
[48] S. Fuchs, P. Göbel, H. Horn, W. Klinger, G. Kölle, F. Seif, W. Voelter, and K. Zech, *Acta Endocrinol.*, 1975, **78** (*Suppl.* 193), 2.
[49] W. Voelter, S. Fuchs, and K. Zech, *Tetrahedron Letters*, 1974, 3975.
[50] H. Sievertsson, S. Castensson, and C. Y. Bowers, *F.E.B.S. Letters*, 1974, **42**, 340.
[51] B. Donzel, M. Goodman, J. Rivier, N. Ling, and W. Vale, *Nature*, 1975, **256**, 750.
[52] W. Voelter and H. Horn, *Z. physiol. Chem.*, 1974, **355**, 1466.
[53] P. Goebel, W. Klinger, H. Horn, and W. Voelter, *Klin. Wochensch.*, 1974, **52**, 1128.
[54] K. Zech, H. Horn, and W. Voelter, *Chem.-Ztg.*, 1974, **98**, 209.
[55] D. H. Coy, Y. Hirotsu, T. W. Redding, E. J. Coy, and A. V. Schally, *J. Medicin. Chem.*, 1975, **18**, 948.

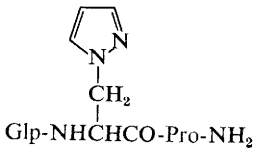
Table 1 (*cont.*)

Compound number	*Structure* [c]	*Biological activity:*[a] *Release of: TSH*	*PRL*	*Binding affinity*	*Test* [b]	*Ref.*
(24)	Glp-Thi-Pro-NH_2	+++			A	50
		+++			B	50
(25)	Glp-His(Bzl)-Pro-Gly-NH_2	−			A	47
(26)	Glp-Leu-Pro-OMe	−			B	50
(27)	Glp-His(N^{τ}Me)-Pro-NH-$(CH_2)_6$-NH_2 (= X-NH_2)	+++				8*b*
(28)	X-NHCO$(CH_2)_2$Ph-(4-OH)	+++				8*b*
(29)	X-dextran	active				8*b*
(30)	Glp-Thi-Pro-OMe	−			B	50
(31)	Glp-His-Gly-Pro-NH_2 [f]	−	−		A E	47 47
(32)	Glp-His-MeAla-NH_2	++				51
(33)	Glp-His-D-Pro-NH_2		−		C	46
				−	D	46
(34)	Glp-His-Pro-NHMe		++		C	46
				++	D	46
(35)	Glp-His-Pro-NHEt					56
			++		C	46
				++	D	46
(36)	Glp-His-Pro-NMe_2		−		C	46
				−	D	46
(37)	Glp-His-Pro		−		C	46
				+	D	46
(38)	Glp-His-Pro-Gly-NH_2 [f]	+++			A	47
			+++		E	47
(39)	Glp-His-X′ [g]		−		C	46
				−	D	46

[a] Activity range −, below the test limit or < 0·1%; +, 0·1—0·99%; ++, 1·0—9·99%; +++, 10·0—99·9%; ++++, 100—999%. [b] A, McKensie assay; B, pituitary tissue *in vitro*; C, prolactin release from GH_3 cells; D, binding affinity to GH_3 cells; E, prolactin release *in vivo* (monkeys); * indicates an inhibitor. [c] cCys = $\overline{CH_2 S \cdot CO \cdot HN \cdot CH \cdot CO}$-; cSer = $\overline{CH_2 \cdot O \cdot CO \cdot NH \cdot CH \cdot CO}$-; cThr = $CH_3 \cdot \overline{CH \cdot O \cdot CO \cdot NH \cdot CH \cdot CO}$-; cpc = cyclopentylcarbonyl; pyr = pyrrolidino; Thi = L-β-(thienyl-2) alanine; Pyr(1)ala = L-β-(pyrazolyl-1) alanine. [d] Most of the compounds in ref. 46 have been reported earlier. [e] Liable to cyclize to TRH. [f] Inactive in releasing LH or FSH. [g] X′ = NH_2, Val-NH_2, Phe-NH_2, Leu-NH_2, Tyr-NH_2, Met-NH_2, Trp-NH_2, Ser-NH_2, Pro-Ala-NH_2.

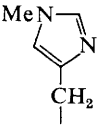
and only at very high analogue : TRH ratios, in contrast to the earlier reports. The *im-*N^{τ}-methyl derivative (2) showed no inhibitory activity, but the [L-β-(thienyl-2) alanine[2]] analogue (3) showed a dose-related inhibition of TRH at an analogue : TRH ratio of 10 000 : 1 or higher. The activity of [L-β-(thienyl-2) alanine[2]] TRH itself is quite high (30% in the McKensie assay *in vivo* and 77% *in vitro*).[50]

The [L-β-(pyrazolyl-1) alanine[2]] analogue (23) of TRH has surprisingly high activity in the McKensie assay – some 50% more than that of TRH and the only



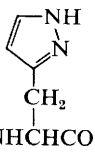
Glp-NHCHCO-Pro-NH$_2$

(23)



Glp-NHCHCO-Pro-NH(CH$_2$)$_6$NH$_2$

(27)



Glp-NHCHCO-Pro-NH$_2$

(40)

analogue other than [*im*-N^{τ}-methylhistidine^2] TRH reported to have substantially higher activity on the thyroid axis than TRH itself.[55] The closely related [L-β-(pyrazolyl-3) alanine^2] analogue (40) was previously found to have only *ca.* 5% of the activity of TRH.

The analogue (27) has *ca.* 45% of the activity of TRH and can be attached to dextran to give a high molecular weight derivative which retains substantial biological activity. The related compound (28) in which this is linked to 3-(*p*-hydroxyphenyl)butyric acid can be used to prepare iodinated derivatives.[8b]

Surprisingly high activities have been reported for [Phe^2] TRH (22) [48, 53, 54, 57] and [D-His^2] TRH (16).[48, 49, 57] The results may possibly reflect the use of rather high doses of compounds in the McKensie assay. The [*N*-formylproline^1] analogue (7) of TRH, which has *ca.* 8% activity, shows resistance to plasma degradation.[8b]

The conformation of [Leu^2] TRH (17), which has *ca.* 2% of the activity of TRH, has been reported (on n.m.r. evidence) to be closely similar to that of TRH,[58] suggesting perhaps not surprisingly that adoption of an appropriate conformation alone is insufficient for biological activity. The α-NH of histidine is not involved in a crucial hydrogen-bond during binding to the biological receptor, since the N^{α}-methylhistidine analogue (20) is fully active. The [N^{α}-methylalanine^3] analogue (32) has 4.5% activity.[51]

The pH dependence of the ^{13}C chemical shifts of histidine in TRH has been studied and compared with that of the *im*-N^{π}-methyl- and *im*-N^{τ}-methyl-histidine analogues. The results point to protonation predominantly at the *im*-N^{τ} position of TRH which is consistent with the higher biological activity of the N^{τ}-methyl analogue.[59] Other work on the conformation of TRH has included interpretations of n.m.r. spectra of TRH analogues modified in position 3,[60] and a study of the conformation of the proline residue based on the ^{13}C n.m.r. vicinal coupling

[56] H. Kalbacher, W. A. König, and W. Voelter, *Z. physiol. Chem.*, 1975, **356**, 1827.
[57] K. von Puttkamer, W. Klemm, D. Gupta, K. Zech, S. Fuchs, and W. Voelter, *J. Endocrinol.*, 1975, **65**, 16P.
[58] A. M. Bellocq, M. Dubien, and E. Dupart, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 1393.
[59] R. Deslauriers, W. H. McGregor, D. Sarantakis, and I. C. P. Smith, *Biochemistry*, 1974, **13**, 3443.
[60] B. Donzel and M. Goodman, *Abstr. Papers Amer. Chem. Soc. Meeting*, 1974, (168), MEDI 45.

constants of the ^{13}C-enriched hormone. A mixture of *cis* (20%) and *trans* (80%) forms is suggested,[61] confirming earlier work based on ^{13}C pulse Fourier transform n.m.r.[62]

The receptor binding affinities of a range of TRH analogues have been measured using the GH_3 (prolactin producing) pituitary cell line in culture, and compared with the prolactin releasing activities measured in the same system, and also the published TSH releasing effects (see Table 1).[46] All three effects run broadly parallel, suggesting similar or identical receptors in each case. The number of receptors on GH_3 cells appears to be decreased by stimulation with TRH, though in a matter of hours rather than the minutes required for binding to be effected.[3]

The *in vivo* prolactin- and TSH-releasing potencies of the five analogues shown in Table 2 have been measured in the rat. The remarkable similarity of the activities in the two tests points again to a close similarity in receptors.[63]

Table 2 In vivo *TSH- and prolactin-releasing potencies of TRH analogues (from ref.* 63)

Compound number	*Structure*	*Potency* [a] *Prolactin*	*TSH*
(41)	Ac-Gly-His-Pro-NH_2	0.3	0.15
(42)	Glp-Met-Pro-NH_2	0.69	0.60
(43)	Glp-His-azetidine	2.0	1.4
(44)	Glp-His-Pro-Ala-NH_2	0.45	0.43
(38)	Glp-His-Pro-Gly-NH_2	50	40

[a] % Activity; TRH = 100%.

The effects of TRH and of [*im-N*$^{\pi}$-methylhistidine^2] TRH on the calcium action potentials of GH_3 cells have been studied. Unlike the analogue, TRH is a potent stimulator, suggesting perhaps that transport of Ca^{2+} across the membrane is an important step in the biological activity.[64] It would be interesting to see the study extended to other TRH analogues.

The characteristics of the disappearance of TRH from plasma have been studied.[65]

Luteinizing Hormone Releasing Hormone (LH-RH, gonadoliberin).—A number of the reviews mentioned earlier deal with LH-RH, and several [31c, d, 66a, c] deal specifically with it. The synthesis of LH-RH analogues continues unabated (Table 3).

Three general modifications to the LH-RH molecule lead to particularly interesting changes in biological activity and have been explored in some detail;

[61] W. Haar, S. Fermandjian, J. Vicar, K. Blaha, and P. Fromageot, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4948.
[62] W. Voelter, O. Oster, and K. Zech, *Angew. Chem. Internat. Edn.*, 1974, **13**, 131.
[63] C. Rivier and W. Vale, *Endocrinology*, 1974, **95**, 978.
[64] Y. Kidokoro, *Nature*, 1975, **258**, 741.
[65] A. Dupont, F. Labrie, L. Levasseur, and A. V. Schally, *Canad. J. Physiol. Pharmacol.*, 1974, **52**, 1012.
[66] 'Recent Progress in Reproductive Endocrinology', Academic Press, New York, 1974; (*a*) L. Martini, p. 295; (*b*) M. Jutisz and B. Kerdelhue, p. 323; (*c*) G. Giustina, p. 407; (*d*) J. B. Josimovich, p. 445.

the replacement of *C*-terminal glycineamide by ethylamido and related groups leads in many cases to increased activity (see Vol. 6, p. 430), the introduction of D-amino-acids in position 6 leads also to dramatically increased activity, and modification (or elimination) of histidine in position 2 generally gives inactive analogues, many of which inhibit the action of LH-RH. Many of the new analogues explore these three changes further or incorporate them with other modifications.

The combination of the changes $GlyNH_2^{10} \rightarrow NHEt$ with $Gly^6 \rightarrow$ D-Ala or D-Leu leads to particularly active derivatives.[67–73] While the initial response in

Table 3 *Analogues of LH-RH*

Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2
1 2 3 4 5 6 7 8 9 10

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(45)	[D-Glp^1]		++	H	69, 74
		++		B	75
(46)	[Pro^1]		−	H	76
		−		B	75
(47)	[Ac-Ala^1]	+		C	75
(48)	[Ac-Gly^1]	+		C	75
(49)	[Glp-Gly^1] acid	−		E	77
(50)	Ac-(2-10)NH_2	+		A	78
			+		78
(51)	[HCO-Gly^1]		++	H	79
(52)	[HCO-Sar^1]		+++	H	79
(53)	[MeGlp^1]		+++	H	79
(54)	[Chl^1, Leu^{2,3}, D-Ala^6]	*			80
(55)	[Leu^1]-(1—9)NHEt		*	H, K	81

[67] A. Arimura, J. A. Vilchez-Martinez, D. H. Coy, E. J. Coy, Y. Hirotsu, and A. V. Schally, *Endocrinology*, 1974, **95**, 1174.

[68] D. H. Coy, E. J. Coy, A. V. Schally, J. Vilchez-Martinez, Y. Hirotsu, and A. Arimura, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 335.

[69] D. H. Coy, J. A. Vilchez-Martinez, E. J. Coy, Y. Hirotsu, N. Nishi, and A. V. Schally, *Endocrinology*, 1974, **94**, (*Suppl.*), A79.

[70] A. de la Cruz, K. G. de la Cruz, A. Arimura, D. H. Coy, J. A. Vilchez-Martinez, E. J. Coy, and A. V. Schally, *Fertility and Sterility*, 1975, **26**, 894.

[71] D. Gonzalez-Barcena, A. J. Kastin, M. C. Miller, D. S. Schalch, D. H. Coy, A. V. Schally, and A. Escalante-Herrera, *Lancet*, 1975, **ii**, 1126.

[72] J. Soria, A. Zarate, E. S. Canales, A. Ayala, A. V. Schally, D. H. Coy, E. J. Coy, and A. J. Kastin, *Amer. J. Obstet. Gynecol.*, 1975, **123**, 145.

[73] J. A. Vilchez-Martinez, D. H. Coy, A. Arimura, E. J. Coy, Y. Hirotsu, and A. V. Schally, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 1226.

[74] Y. Hirotsu, D. H. Coy, E. J. Coy, and A. V. Schally, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 277.

[75] W. Arnold, G. Flouret, R. Morgan, R. Rippel, and W. White, *J. Medicin. Chem.*, 1974, **17**, 314.

[76] H. Yajima, M. Kurobe, I. Yo, N. Fujii, and Y. Baba, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 1622.

[77] J. Humphries, G. Fisher, Y.-P. Wan, K. Folkers, and C. Y. Bowers, *J. Medicin. Chem.*, 1974, **17**, 569.

[78] J. Rivier, M. S. Amoss, C. Rivier, and W. Vale, *J. Medicin. Chem.*, 1974, **17**, 230.

[79] Y. Okada, H. Horikoshi, and Y. Baba, *Chem. and Pharm. Bull.* (*Japan*), 1974, **22**, 721.

[80] C. Y. Bowers, Y.-P. Wan, J. Humphries, and K. Folkers, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 698.

[81] J. A. Vilchez-Martinez, D. H. Coy, E. J. Coy, A. V. Schally, and A. Arimura, *Fertility and Sterility*, 1975, **26**, 554.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(56)	(2—9)NHEt		−	H	81
(57)	des-His^2-[D-Glp^1]-(1—9)NHEt		*	H	81
(58)	[D-His^2]	++		A	82
			++	H	74
(59)	[Arg^2]	−		A	82
				C	75
(60)	des-His^2, des-Pro^9	−		C	75
(61)	des-His^2-[Sar^6]	−		C	75
(62)	[Ile^2]	−		E	77
(63)	[Leu^{2,3}]	−		E	77
(64)	[His-Gly^2] acid	−		E	83
(65)	Ac-(3—10)NH_2	−		A	78
			−		78
(66)	des-His^2	−		A	78
			−		78
		*		S	8*b*, 8*c*
		−*		A	82
(67)	des-His^2-(1—9)NHEt		*		84
			−	H	85
			*	N	85
		d		S	8*c*
			d		8*c*
			*	H, K	81
			*	N	86
(68)	[Pyr(3)ala^2]		+++	H	87
(69)	[Phe^2]	++		A	19*a*
(70)	[Met^2]	++		A	19*a*
(71)	[Tyr^2]	+++		A	19*a*
		++		A	82
(72)	[Trp^2]	+++		A	19*a*
		+++		A	82
(73)	[Gly^2]	−*		A	19*a*
(74)	[Ala^2]	−*		A	19*a*
(75)	[D-Ala^2]	−*		A	19*a*
(76)	[Cys^2]	−*		A	19*a*
(77)	[Asp^2]	−*		A	19*a*
(78)	[D-Trp^2]	−*		A	82
		**		S	8*b*
(79)	[D-Phe^2]	−**		A	82
			−*	H	87
		*		S	8*b*, 8*c*
(80)	[D-Arg^2]	+		A	82

[82] R. W. A. Rees, T. J. Foell, S.-Y. Chai, and N. Grant, *J. Medicin. Chem.*, 1974, **17**, 1016.
[83] J. Humphries, Y.-P. Wan, G. Fisher, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 675.
[84] J. A. Vilchez-Martinez, A. V. Schally, D. H. Coy, E. J. Coy, and A. Arimura, *Endocrinology*, 1974, **95**, 213.
[85] D. H. Coy, E. J. Coy, A. V. Schally, J. A. Vilchez-Martinez, L. Debeljuk, W. H. Carter, and A. Arimura, *Biochemistry*, 1974, **13**, 323.
[86] G. Fink, D. Hudson, I. MacIntyre, A. Pickering, and M. Szelke, *J. Endocrinol.*, 1975, **67**, 39P.
[87] C. W. Beattie, A. Corbin, T. J. Foell, V. Garsky, W. A. McKinley, R. W. A. Rees, D. Sarantakis, and J. P. Yardley, *J. Medicin. Chem.*, 1975, **18**, 1247.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(81)	[Leu^2]	+		A	82
(82)	[D-Leu^2]	−		A	82
(83)	[Thr^2]	+		A	82
(84)	[D-Val^2]	++		A	82
(85)	[D-Phe^2, D-Ala^6]	−		A	88
		**		S	88, 8*b*
			**	P	88, 87
			**		89
			+	H	87
(86)	des-His^2-[D-Ala^6]-(1—9)NHEt		*	H, K	81
		*	*		90
		*		S	8*b*
			*	N	86
(87)	des-His^2-[D-Leu^6]	**			8*c*
		*	**		90
(88)	des-His^2-[D-Ala^6]	**	**		90
			d	H	81
		*		S	8*b*
(89)	[D-Phe^2, D-Leu^6]		−	H	87
			*	P	87
			**		90
			*	Q	91
		**		S	8*c*
(90)	[D-Phe^2, D-Ala^6]-(1—9)NHEt	−		A	88
		**		S	88
			d	P	88
(91)	[Pyr(1)ala^2]		++	H	55
(92)	[D-Phe^2, D-Ser^6]		*d*	P	87
(93)	[D-Phe^2, D-Arg^6]		−	H	87
			*	P	87
(94)	[D-Phe^2, D-Pgl^6]		**	P	87
(95)	[D-Phe^{2,6}]	**		S	8*c*
			*	P	87
(96)	[D-Phe^2, D-Lys^6]		*d*	P	87
(97)	[D-Phe(4-F)^2, D-Ala^6]		**	P	87
(98)	[D-Phe(4-Cl)^2, D-Ala^6]		*d*	P	87
(99)	[D-Phe^2, Spi^6]		−	H	87
			**	P	87
(100)	des-His^2-[Nap(2)ala^3]	−*		E	92
(101)	des-His^2-[Nap(2)ala^3, D-Ala^6]	−*		E	92
(102)	des-His^2-[Orn(N^{δ}Pr^{i})^8]	+*		E	92
(103)	[Pro^2]		−	H	76
(104)	des-His^2-[Leu^3]-(1−9)NHEt		*	H, K	81

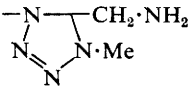
[88] J. P. Yardley, T. J. Foell, C. W. Beattie, and N. H. Grant, *J. Medicin. Chem.*, **18**, 1244.
[89] A. Corbin and C. W. Beattie, *Endocrinology*, 1976, **98**, 247.
[90] L. Ferland, F. Labrie, D. H. Coy, E. J. Coy, and A. V. Schally, *Fertility and Sterility*, 1975, **26**, 889.
[91] A. de la Cruz, D. H. Coy, A. V. Schally, E. J. Coy, K. G. de la Cruz, and A. Arimura, *Proc. Soc. Exp. Biol. Med.*, 1975, **149**, 576.
[92] R. W. Roeske, K. U. Prasad, and C. Y. Bowers, *Abstr. Papers Amer. Chem. Soc. Meeting*, 1975, (170), BIOL 181; K. U. Prasad, R. W. Roeske, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 336.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(105)	[Gly^2, Leu^3]-(1—9)NHEt		*	H	81
			d	K	81
(106)	des-His^2-[Leu^3, D-Ala^6]-(1—9)NHEt		*	H, K	81
(107)	[Spi^2]		*d*	H	81
(108)	des-His^2-[Azagly^6]		−	K	8*d*, 93
			−*d*	G	8*d*, 93
(109)	des-His^2-[Azaala^6]		−	K	8*d*, 93
			−*d*	G	8*d*, 93
(110)	des-His^2-[D-Ala^6, MeLeu^7]	**		S	8*b*
(111)	[D-Phe^2, D-Ala^6,-MeLeu^7]	**		S	8*b*
(112)	[D-Phe^2, D-Trp^6]	***		S	8*b*
(113)	des-His^2-[D-Phe^6]	**		S	8*c*
(114)	des-His^2-[D-Lys^6]	*d*		S	8*c*
(115)	des-His^2-(1—9)	+		A	8*c*
	$NH\cdot CH_2\cdot CF_2\cdot CF_3$	*d*		S	8*c*
(116)	[D-Phe^2, D-Glu^6]	*		S	8*c*
(117)	[D-Phe^2, D-Leu^6]-(1—9)NHEt	*d*		S	8*c*
(118)	des-His^2-(1—9)NHPr^n	*		S	8*c*
(119)	[D-Phe^{2,6,7}]	**		S	8*c*
(120)	[D-Phe^{2,6}, Phe^5]	***		S	8*c*
(121)	[D-Phe^{2,6}, Phe^3]	**		S	8*c*
(122)	[Leu^{2,3}, D-Ala^6]	−**			94
(123)	[Val^2, Leu^3, D-Ala^6]	−**			94
(124)	[Leu^2, Val^3, D-Ala^6]	−*			94
(125)	[Val^{2,3}, D-Ala^6]	−*			94
(126)	[D-Trp^3]		+	H	74
(127)	[Tyr(Me)^3]	++		C	75
(128)	[Tyr(Me)^{3,5}]	+		B, C	75
(129)	[Tyr^3, Trp^5]	−		E	77
			−	H	31*c*
(130)	[Tyr^3, Trp^5] acid	−		E	83
(131)	Ac-(4—10)NH_2	−		A	78
			−		78
(132)	(4—10)NH_2	−*		F	95, 96
(133)	des-Trp^3	−		A	78
			−		78
(134)	des-Trp^3-(1—9)NHEt		−	H	85
(135)	[Phe^3]	++		A	19*a*
(136)	[Gly^3]	−*		A	19*a*
(137)	[Ala^3]	−*		A	19*a*
(138)	[Phe(Me_5)^3]		+++	H	97

[93] A. S. Dutta and J. S. Morley, *J.C.S. Perkin I*, 1975, 1712.
[94] Y.-P. Wan, J. Humphries, G. Fisher, K. Folkers, and C. Y. Bowers, *J. Medicin. Chem.*, 1976, **19**, 199.
[95] D. J. Schafer, A. D. Black, J. D. Bower, and D. B. Crighton, *J. Medicin. Chem.*, 1975, **18**, 613; A. D. Black, J. D. Bower, D. J. Schafer, and D. B. Crighton, Chemical Society Peptide and Protein Group Meeting, London, 1974.
[96] D. B. Crighton and D. J. Schafer, *J. Endocrinol.*, 1974, **61**, xi.
[97] D. H. Coy, E. J. Coy, Y. Hirotsu, J. A. Vilchez-Martinez, A. V. Schally, J. W. van Nispen, and G. I. Tesser, *Biochemistry*, 1974, **13**, 3550.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(139)	[Trp(5-F)^3]		++	H	97
(140)	[Phe(4-NO_2)^3]		−	H	97
(141)	[Phe(4-NH_2)^3]		+	H	97
(142)	[His^3]		−	H	97
(143)	[Pro^3]		−	H	76
(144)	[Leu^3]		*	H	81
(145)	[Leu^3]-(1—9)NHEt		*	H	81
(146)	[Leu^3, D-Ala^6]-		+	H	81
	(1—9)NHEt		*d*	H, K	81
(147)	[Phe^3, Ala^4]-	++		C	98
	(1—9)NHEt		++	M	98
(148)	[Phe^3, Gly^4]-	++		C	98
	(1—9)NHEt		++	M	98
(149)	[Phe^3, Ala^4, Lys^8]-	+		C	98
	(1—9)NHEt		+	M	98
(150)	[Phe^3, Gly^4, Lys^8]-	−		C	98
	(1—9)NHEt				
(151)	[Phe^3, Gly^4, Har^8]-	−		C	98
	(1—9)NHEt				
(152)	[Phe^3, Ala^4, Phe^7]-	++		C	98
	(1—9)NHEt		++	M	98
(153)	[Tyr^3, Ala^4, Phe^5]-	+		C	98
	(1—9)NHEt		+	M	98
(154)	[Phe^3, Ala^4, D-Ala^6]-	+++		C	98
	(1—9)NHEt		+++	M	98
(155)	[Phe^3, D-Ala^4]-	+		C	98
	(1—9)-NHEt		+	M	98
(156)	[Phe^3, Gly^4]-(1—9)-	++		C	98
			+	M	98
(157)	Ac-(5—10)NH_2	−		A	78
			−		78
(158)	des-Ser^4	−		A	78
			−		78
(159)	[Gly^4]	++		A	19*a*
(160)	[Ala^4]		++	M	99
(161)	[Ala^4, Phe^5]		++	H	31*c*
			++	M	99
(162)	[Ala^4, Phe^5, Lys^8]		++	M	99
(163)	[Thr^4]-(1—9)NHEt	+++		C	100
(164)	[Ser(Bu^t)^4]	−		F	95
(165)	[Leu^4]	−		F	95
(166)	(1—3)OH	(++)		F	95, 96
(167)	[Pro^4]		−	H	76
(168)	[D-Tyr^5]		+	H	74

[98] C. R. Beddell, P. J. Fraser, D. Gilbert, P. J. Goodford, L. A. Lowe, and S. Wilkinson, *J. Medicin. Chem.*, 1975, **18**, 417.

[99] H. Immer, V. R. Nelson, C. Revesz, K. Sestanj, and M. Gotz, *J. Medicin. Chem.*, 1974, **17**, 1060.

[100] S. Kobayashi, S. Shinagawa, M. Obayashi, and T. Fukuda, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 2033.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:*[b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(169)	[Ser^5]	+		B, C	75
(170)	des-Tyr^5-[Sar^6]	−		C	75
(171)	[Phe^5]	+ + +		C	101
			+ + +	H	101
			+ + +	J	102
			+ + +	M	99
(172)	[Phe^5, D-Ala^6]-(1—9)NHEt	+ + + +		C	103
			+ + + + +	G	103
(173)	[Phe^5, D-Leu^6]-(1—9)NHEt	+ + + +		B	104
			+ + + + +	G	104
(174)	[Phe^5, D-Phe^6]-(1—9)NHEt	+ + + +		B	104
			+ + + + +	G	104
(175)	[Ile^5, D-Ala^6]-(1—9)NHEt	+ + + +		C	103
			+ + + + +	G	103
(176)	[Ile^5, D-Leu^6]-(1—9)NHEt	+ + +		B	104
			+ + + + +	G	104
(177)	Ac-(6—10)NH_2	−		A	78
			−		78
(178)	des-Tyr^5	−		A	78
			−		78
(179)	[Gly^5]	+		C	101
			+	H	101
		−		A	19*a*
(180)	[Tyr(Me)^5]	+ +		C	101
			+ +	H	101
(181)	[Cha^5]	+ + +		C	101
			+ + +	H	101
(182)	[Ala^5]		+ +	M	99
(183)	[Leu^5]		+ +	H	31*c*
(184)	[Phe^5]-(1—9)NHEt	+ + + +		C	100
(185)	[Phe^5, Ile^7]-(1—9)NHEt	+ + + +		C	100
(186)	[Pro^5]		−	H	76
(187)	[Tyr(^{125}I)^5]	+ + + +		A	105
(188)	des-Gly^6	+ +		B, C	75
		+	−	A	78
(189)	[Sar^6]	+ +		C	75
			+ + +	H	31*c*
(190)	[Ala^6]-(1—9)NHEt	+ + +		C	103
			+ + +	G	103
(191)	[D-Ala^6]-(1—9)NHEt	+ + + +		C	103
			+ + + + +	G	103
		+ + + + +		A	8*b*, 106

[a] H. Künzi, D. Gillessen, A. Trzeciak, R. O. Studer, B. Kerdelhué, M. Jutisz, and W. Lotz, *Helv. Chim. Acta*, 1974, **57**, 2131.

[b] A. J. Kastin, A. V. Schally, D. Gonzalez-Barcena, D. H. Coy, M. C. Miller, H. Porias, and D. S. Schalch, *J. Clin. Endocrinol. Metab.*, 1974, **38**, 801.

[c] M. Fujino, I. Yamazaki, S. Kobayashi, T. Fukuda, S. Shinagawa, R. Nakayama, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Comm.*, 1974, **57**, 1248.

[d] M. Fujino, T. Fukuda, S. Shinagawa, S. Kobayashi, I. Yamazaki, R. Nakayama, J. H. Seely, W. F. White, and R. H. Rippel, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 406.

[e] J. C. Marshall and W. D. Odell, *Proc. Soc. Exp. Biol. Med.*, 1975, **149**, 351.

[f] D. H. Coy, F. Labrie, M. Savary, E. J. Coy, and A. V. Schally, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 576.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
			+++++	K	67, 68, 73, 107, 109
			+++++		70
			+++++		69
			++++	J	72, 109
(192)	[Aib^6]	++++		C	103
			+++++	G	103
(193)	[D-Ala^6]	++++		C	103
			+++++	G	103
			++++	H	31*c*
		++++		A	8*b*, 19*a*, 106
			++++	K	67, 68
			++++	J	109
(194)	Ac-(7—10)NH_2	−		A	78
			−		78
(195)	[D-Leu^6]	++++		A	8*b*, 106
			++++	K	73
		++++		B	104
			+++++	G	104
(196)	[D-Leu^6]-(1—9)NHEt		+++++	K	70
			++++	K	73
		+++++		A	8*b*, 71, 106, 110
		++++		B	104
			+++++	G	104
(197)	[D-Leu^6]-(1−9)NHCH(CH$_2$CH$_2$)		(++++)		111
(198)	[Ala^6]	++		A	19*a*
(199)	[D-Ile^6]	+++		A	19*a*
(200)	[D-Val^6]	+++		A	19*a*
(201)	[D-Lys^6]	++++		A	8*b*
			++++	K	112
(202)	[(-Glu-)^n / D-Lys]		++++	K	112
(203)	[β-Ala^6]		+++	H	31*c*
(204)	[D-Abu^6]-(1—9)NHEt	++++		B	104
			+++++	G	104

[107] A. J. Kastin, D. Gonzalez-Barcena, N. Nishi, L. Lee, A. Gonzalez-Sanchez, L. Cuevas-Lara, F. Gutierrez-Barrientos, and F. Hidalgo, *Endocrinology*, 1974, **94** (Suppl.), A79.

[108] D. H. Coy, J. A. Vilchez-Martinez, E. J. Coy, N. Nishi, A. Arimura, and A. V. Schally, *Biochemistry*, 1975, **14**, 1848.

[109] A. J. Kastin, A. Arimura, D. Gonzalez-Barcena, D. H. Coy, E. J. Coy, M. C. Miller, N. Nishi, L. Lee, H. Duron-Huerta, D. S. Schalch, and A. V. Schally, *Internat. J. Fertility*, 1975, **19**, 202.

[110] J. Zanartu, J. M. Rosner, E. Guiloff, A. A. Ibarra-polo, H. D. Croxatto, H. B. Croxatto, E. Aguilera, D. H. Coy, and A. V. Schally, *Brit. Med. J.*, 1975, **ii**, 527.

[111] Ger. Offen. 2 438 352.

[112] M. S. Amoss, M. W. Monahan, and M. S. Verlander, *J. Clin. Endocrinol. Metab.*, 1974, **39**, 203.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(205)	[D-Nva^6]-(1—9)NHEt	++++		B	104
			+++++	G	104
(206)	[D-Ser^6]-(1—9)NHEt	++++		B	104
			+++++	G	104
(207)	[D-Phe^6]	+++++		A	106
			+++++	H	106
(208)	[D-Phe^6]-(1—9)NHEt	+++++		A	106
			+++++	H	106
(209)	[D-Trp^6]	+++++		A	8*b*, 106
			+++++	H	106
(210)	[D-Trp^6]-(1—9)NHEt	++++++		A	8*b*
		+++++		A	106
			+++++	H	106
(211)	[D-Ala^6, MeLeu^7]	++++		A	8*b*, 113
(212)	[Pro^6]		−	H	76
(213)	[D-Ala^6]-(1—9)NHCH$_2$CF$_3$		+++++	K	108
(214)	[D-Ala^6]-(1—9)-NHCH$_2$CF$_2$CF$_3$		++++	K	108
(215)	[—NHCH$_2$*CH*$_2$—^{6}]-(1—9)NHEt	+		B	114
			−	H	114
(216)	[—NH*N*(*CH*$_3$)CO—^{6}]		(+++)	G	8*d*, 93
			(+++)	K	8*d*, 93
(217)	[—NH*NH*CO—^{6}]		(+++)	G	8*d*, 93
			(+++)	K	8*d*, 93
(218)	[Lys(lauryl)^6]	++++		A	8*b*
(219)	[Lys(dextran)^6]	+++		A	8*b*
(220)	[D-Arg^6]	++++		A	8*b*
(221)	[D-Met^6]	++++		A	8*b*
(222)	[D-Tyr^6]	+++++		A	8*b*
(223)	[D-Lys^6]-(1—9)NHEt	+++++		A	8*b*
(224)	[D-Tyr^6]-(1—9)NHEt	+++++		A	8*b*
(225)	[D-Ala^6, MeLeu^7]-(1—9)NHEt	+++++		A	8*b*
(226)	[D-Leu^7]		+	H	74
(227)	Ac-(8—10)NH$_2$	−		A	78
			−		78
(228)	des-Leu^7	−		A	78
			−		78
(229)	[Gly^7]	+		A	19*a*
(230)	[Ser^7]		+++	M	99
(231)	[Ile^7]		++++	H	31*c*
(232)	[Val^7]		++	H	31*c*
(233)	[Nle^7]-(1—9)NHEt	++++		C	100
(234)	[Met^7]-(1—9)NHEt	++++		C	100
(235)	[MeLeu^7]	++++		A	113
(236)	[Pro^7]		−	H	76
(237)	[D-Arg^8]		+	H	74
(238)	Ac-(9—10)NH$_2$	−		A	78
			−		78

[113] N. Ling and W. Vale, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 801.

[114] D. Hudson, R. Sharpe, A. Pickering, J. McLoughlin, E. Matthews, G. Fink, I. MacIntyre, and M. Szelke. Reported at the Meeting of the Chemical and Biochemical Societies Peptide and Protein Group, Exeter, April 2nd, 1976.

Table 3 (*cont.*)

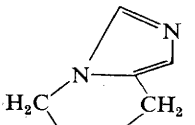
Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(239)	[Gly^8]	+		A	19*a*
(240)	[Har^8]		+++	H	115, 116
			+++	L	117
(241)	[Lys^8]		++	H	115, 116
			+++	M	99
(242)	[Orn^8]		++	H	115, 116
(243)	[Nva^8]		+	H	115
(244)	[Gln^8]		++	H	115
(245)	[Leu^8]		+	H	115
(246)	[His^8]		++	H	115
(247)	[*nor*-Arg^8]		+++	H	116
(248)	[Dab^8]		++	H	116
(249)	[Cit^8]		++	H	116
			+++	L	117
(250)	[Orn(Ac)^8]		++	H	116
(251)	[Orn(Bz)^8]		++	H	116
(252)	[Glu^8]		++	M	99
(253)	[Orn^8]-(1—9)NHEt	+++		C	100
(254)	[Lys^8]-(1—9)NHEt	+++		C	100
(255)	[Pro^8]		−	H	76
(256)	des-Pro^9	++		B, C	75
		+		A	78
			−		78
(257)	[Gly^9]	+		A	19*a*
(258)	[Gly^9]-(1—9)NHEt	++		C	100
(259)	[MeAla^9]	(+++)			118
(260)	(1—8)NHEt		+	H	119
(261)	(1—8)NHBu^n		+	H	119
(262)	(1—9)NH_2	++		B, C	75
		+++		A	78
			+++		78
(263)	(1—9)NHEt		++++	K	108, 120
		++++		B	75
		++++		C	75, 103, 121
			++++	J	102, 109, 122
			++++	G	103, 121, 123
(264)	(1—9)NHPr^n		++++	K	108, 120
			++++	J	109
(265)	[β-Ala^{10}]		++	H	31*c*
(266)	(1—9)$NHCH_2CH{=}CH_2$		++++	K	119

[115] R. Geiger, W. König, J. Sandow, and A. V. Schally, *Z. physiol. Chem.*, 1974, **355**, 1526.
[116] Y. Yabe, K. Kitamura, C. Miura, and Y. Baba, *Chem. and Pharm. Bull.* (*Japan*), 1974, **22**, 2557.
[117] J. Borvendég, S. Bajusz, I. Hermann, and A. Turán, *F.E.B.S. Letters*, 1974, **44**, 233.
[118] W. A. McKinley and W. H. McGregor, U.S.P. 3 813 382.
[119] D. H. Coy, E. J. Coy, A. V. Schally, and J. A. Vilchez-Martinez, *J. Medicin. Chem.*, 1975, **18**, 275.
[120] A. Arimura, J. A. Vilchez-Martinez, and A. V. Schally, *Proc. Soc. Exp. Biol. Med.*, 1974, **146**, 17.
[121] K. Taya and M. Igarashi, *Endocrinol. Japonica*, 1974, **21**, 521.
[122] R. Nakano, H. Takekida, F. Kotsuji, Y. Miyazaki, and S. Tojo, *J. Clin. Endocrinol. Metab.*, 1974, **39**, 802.
[123] S. Shinagawa and M. Fujino, *Chem. and Pharm. Bull.* (*Japan*), 1975, **23**, 229.

Table 3 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *in vitro*	*in vivo*	*Test* [c]	*Ref.*
(267)	(1—9)$NHCH_2C{\equiv}CH$		+++	K	119
(268)	(1—9)$NH(CH_2)_2NH_2$		++	(H)	119
(269)	(1—9)$NHNH_2$		+++	H	119
(270)	[Pro^{10}]		−	H	76
(271)	(1—9)$NHCH_2CF_3$		++++	K	108
(272)	(1—9)$NHCH_2CF_2CF_3$		++++	K	108
(273)	(1—9)$NH\underline{NH}CONH_2$		+++	K	8*d*, 93
			+++	G	8*d*, 93
(274)	(1—10)OMe	++		C	75
(275)	(1—10)NHMe	++		C	75
(276)	(1—10)OH	+		B	75
			−	H	31*c*
(277)	(1—10)-Tyr-NH_2	−		C	75
(278)	(1—10)$NH(CH_2)_4NH_2$		−	M	99
(279)	(1—6)NH_2	++		C	75
(280)	(1—7)OH		−	H	31*c*
(281)	des-Arg^8	−		A	78
			−		78

[a] Chl = Chlorambucil; Pyr(3)ala = L-β-(pyrazolyl-3) alanine; Pyr(1)ala = L-β-(pyrazolyl-1) alanine; Pgl = α-phenylglycine; Nap(2)ala = L-β-(naphthyl-2) alanine; Spi =



spinacin, $-N-CH-CO-$; Azagly = $-NHNHCO-$; Azaala = $-NHN(CH_3)CO-$; Cha = β-cyclohexylalanine. [b] LH release only; the symbols for potency are used as in Table 1; ++++++ ≥1000%; *, **, and *** indicate inhibition (of increasing potency). [c] The following assay systems are referred to: *In vitro*: A, radioimmunoassayable LH release from rat pituitary cell culture; B, bioassayable LH release from rat hemipituitaries; C, radioimmunoassayable LH release from rat hemipituitaries; D, radioimmunoassayable LH release from rat pituitary fragment cell culture; E, radioimmunoassayable LH release from whole rat pituitaries; F, bioassayable LH release from sheep pituitary slices. *In vivo*: G, induction of ovulation in dioestrus rats; H, radioimmunoassayable LH release in ovariectomized, oestrogen/progesterone pretreated (OEP) rats; J, radioimmunoassayable LH release in man; K, LH release in immature rats; L, bioassayable LH release in OEP rats; M, induction of ovulation in Syrian hamsters. Inhibitory: N, antagonism of LH release by LH-RH in OEP rats; P, ovulation inhibition in the proestrus rat; Q, blockade of the pre-ovulatory LH surge in hamsters; R, antagonism of LH release by LH-RH in proestrus rats; S, antagonism of LH release by LH-RH in cell culture. [d] Not antagonistic.

LH and FSH released by (191) is only about twice that of LH-RH, the effect is prolonged and the total response over 6 h (integrating the gonadotropin *versus* time curve) is much higher, some 31 times LH-RH for the effect on LH.[67] Both (191) and (196) are active by subcutaneous, intravaginal, and oral routes in rats,[70] and (196) is active orally in man.[71] It is not yet clear to what extent resistance to enzymic breakdown is responsible for the higher activity. Although the compounds are clearly of high activity, the method of quantifying the response by integrating the time curve may rather overstate the activity since this response will also depend on the half-life of the gonadotropin in serum. It should incidentally be noted that longer duration of action is not necessarily shown by a delayed position of the peak if this is combined with a greater total response, since the

position of the peak can vary (in the case of TRH) in response to different dose levels of the same hormone.[124] The analogue (196) has shown promising preliminary results as an agent for programming ovulation.[110]

The highly active analogue (263) (see Vol. 6, p. 430) has been studied further in the rat [121] and human.[122, 125] Details of its synthesis have also been reported.[123]

The CH_2CF_3 group is a particularly effective *N*-terminal substituent for LH-RH (1—9). The analogue (271) has some 900% activity compared with LH-RH (bearing in mind the reservations expressed above); the ethylamido derivative has *ca.* 550% activity. However, the combination of this change with D-Ala[6] results in no additional increase in activity.[69, 108] In contrast, the analogue (272) with an $NH{-}CH_2CF_2CF_3$ *C*-terminus has much less activity (160% compared with 550%) than the corresponding n-propyl analogue.[69, 108] The LH-releasing potency of LH-RH (1—9) NHR derivatives falls with decreasing lipophilicity in the series $CH_2CH_2CH_3 > CH_2CH{=}CH_2 > CH_2C{\equiv}CH > CH_2CH_2NH_2$. The two analogues (266) and (267) showed a similar time course compared with LH-RH and no sign of prolongation. The ethylamido (260) and n-butylamido (261) derivatives of sequence 1—8 had very low activities (*ca.* 0.1—0.2%).[119] The ethylamido (263) and n-propylamido (264) derivatives have been compared with LH-RH *in vivo*. While both are more active, (264) but not (263) showed a prolonged effect.[120]

The effects of the four analogues (263), (264), (191), and (193) have been compared in man.[107] All were highly potent (20—30 times LH-RH) at releasing LH, but of the four only (191) at a dose of 25 μg had an effect prolonged substantially beyond 4 h. Injection of 500 μg of (171) gave a response similar to that due to 250 μg of LH-RH in man.[102] At these relatively high doses, however, a lower intrinsic activity of the analogue might be missed.

Many analogues have been prepared with D-amino-acids in position 6 (refs 8*b*, 87, 103, 104, 106) and the limit does not appear to have been reached for increasing their potency. The D-tyrosine analogue (222) had 14 times the potency of LH-RH *in vitro* and the D-tryptophan analogue (209) 36 times. Combining these changes with replacement of *C*-terminal glycineamide by NHEt gave a D-tyrosine analogue (224) with 68 times the activity of LH-RH, while the D-tryptophan analogue (210) had no less than 144 times its activity.[8b]

The analogue (201) with a basic D-lysine in position 6 also has a greater *in vivo* activity than LH-RH (variously quoted between 4 and 10 times).[8b, 112] If (201) is coupled to a high molecular weight polyglutamate polymer, the resulting derivative (202), with a 1 : 25 peptide to polymer ratio, has significant and prolonged activity. While both the polymer (202) and LH-RH released FSH, (201) apparently did not, and this interesting observation is being studied further. In view of the rapid initial rise in LH caused by the polymer, a direct effect at the receptor rather than a breakdown to give another active species seems the likely source of its activity.[112] The N^{ε}-lauryl derivative (218) still retains substantial (160%) activity, and the polymeric derivative (219) in which (201) is attached to dextran retains 15% of the activity of LH-RH.[8b]

[124] J. B. Martin and S. Reichlin, *Endocrinology*, 1972, **90**, 1079.
[125] R. Nakano, H. Takekida, F. Kotsuji, and S. Tojo, *Obstet. Gynecol.*, 1975, **45**, 263.

The analogue (235) with *N*-methyl-leucine in position 7 has full activity, and this additional change to (191) gives an active analogue (with 5—6 times the activity of LH-RH)[113] particularly resistant to biological degradation.[8b]

There seems to be a qualitative difference between the part played by the *C*-terminal heptapeptide portion of LH-RH and that played by the *N*-terminal tripeptide. There have been several reports (see Vol. 6, p. 430 and refs. 95 and 96) of LH-RH-like activity, albeit low and erratic, in the tripeptide acid (166) and amide (282). In one study the latter showed signs of inhibition towards LH-RH.[126]

Glp-His-Trp	Glp-His-Trp-NH_2
(166)	(282)

On the other hand, modification of the *N*-terminal portion of LH-RH or its elimination gives rise to compounds including the (4—10) heptapeptide sequence (132),[95] the acetyl octapeptide (65),[78] and of course the well-explored His[2]-modified analogues, with inhibitory activity. Binding studies with pituitary receptors, comparing LH-RH and the des-His[2] antagonist, have shown an equal binding constant for both suggesting that histidine does not contribute significantly to the free energy of binding.[127] Two populations of receptor were identified, with affinity constants of 2×10^{-8} and 2×10^{-7} M, but one must have some reservations about whether these are the true biological receptors (*cf.* ref. 128).

Des-His[2]-LH-RH (1—9) NHEt (67) is a potent inhibitor of LH-RH *in vivo*,[84, 85] capable of blocking ovulation under certain conditions.[84] In one study,[129] the *C*-terminal modification in (67) did not increase the inhibitory potency of des-His[2]-LH-RH (66). On the other hand, in a study[125] of *in vivo* anti-LH-RH activity (using a 2 h infusion of analogue prior to i.v. injection of LH-RH), while (66) had no effect, [Leu[3]] LH-RH (144) and (134) showed limited signs of inhibition and (67) inhibited the effect of LH-RH significantly. The introduction of D-alanine into both (66) and (67) increases inhibitory potency as effectively as it increases the agonist actions of, for example, LH-RH,[129] and this is confirmed by another *in vivo* study in which (86) was a more potent antagonist of LH-RH-induced LH release than (67), although neither was found to block ovulation.[86]

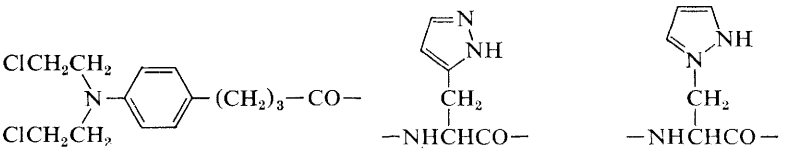
The introduction of further modifications has allowed the development of still more potent inhibitors.[8c, 82, 88, 90] The [D-Phe[2]] analogue (79) is three times more potent as an inhibitor than (66), neither analogue having agonist activity. The introduction of D-leucine in position 6 increases inhibition six-fold (being some four times more effective than the D-alanine modification) and with D-phenylalanine in position 6 the ratio is 50 times. Interestingly, the [D-Lys[6]] derivative (114) is not inhibitory and in fact shows very slight (0.003%) agonist activity. Combining the two most effective single modifications gave (95), 120 times as potent as (66) but with slight (0.003%) agonist activity. Other potent inhibitors had phenylalanine in place of trytophan in position 3 or tyrosine in position 5, for example (120) which had no agonist activity. The more potent analogues

[126] J. A. Vilchez-Martinez, A. V. Schally, L. Debeljuk, D. H. Coy, A. Arimura, and N. Yanahara, *Neuroendocrinology*, 1974, **14**, 121.
[127] J. Spona, *F.E.B.S. Letters*, 1974, **48**, 89.
[128] P. Cuatrecasas, G. P. E. Tell, V. Sica, I. Parikh, and K.-J. Chang, *Nature*, 1974, **247**, 92.
[129] M. S. Amoss, M. W. Monahan, J. Rivier, and R. Guillemin, *Endocrinology*, 1974, **94** (*Suppl.*), A78.

inhibited both the spontaneous LH surge and ovulation *in vivo* and showed signs of a prolonged effect; with (120) the effect was still detectable some 5 h after injection and with some further increase in potency and duration of action these developments could lead to an effective contraceptive method. The two analogues (90)[88] and (117)[8c] were found to have lost the inhibitory effect, the former *in vivo* though not *in vitro*.

The inhibitory activities of a series of LH-RH analogues have been compared and the analogues broadly grouped at three levels of potency: (106), (86), (57), (104) > (145), (105) > (88), (55), (56).[69]

Other analogues reported to have inhibitory activity include [Leu^{2,3}] LH-RH (63) and its [D-Ala^{6}] derivative (122); the latter has no agonist activity. In an attempt to produce an irreversible inhibitor, a chlorambucil (283) group has been



(283) (284) (285)

introduced into position 1 of (122).[80] The resulting analogue (54) surprisingly has slight agonist activity *in vitro*. A series of three incubations with (54), four pre-incubations, and two incubations with LH-RH resulted in an impaired response in both of the latter, suggesting a prolonged inhibitory effect.

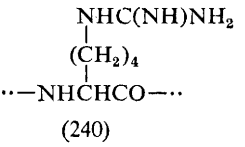
It is now traditional to explore the possible role of the acid–base properties of the imidazole ring of histidine in biologically active peptides by Hoffmann's replacement with L-β-(pyrazolyl-3) alanine (284). In the case of LH-RH, this replacement in position 2 gives an analogue (68) with *ca.* 19% of the LH releasing activity of LH-RH *in vivo*.[87] There are clearly a number of other variants which can be made on the histidine imidazole ring, and the effect of introducing an L-β-(pyrazolyl-1) alanine (285) substituent in TRH is to give a surprisingly active analogue (see p. 344). In the case of LH-RH the difference is notable and an analogue (91) with rather low (*ca.* 1%) activity results.[55]

The basicity of the guanidino group of arginine in LH-RH is not crucial for the activity. The 8-citrulline analogue (249)[116, 117] and the 8-N^{δ}-acetylornithine analogue (250)[116] both retain significant activity. The length of side-chain has some effect and the homoarginine analogue (240)[115, 117] and the norarginine analogue (247)[115] both have substantial but none the less diminished activity.

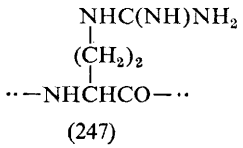
The metabolic fate of LH-RH has been studied and the results used in the design of analogues with peptide bonds modified by isosteric replacement, for example (215).[86, 114]

Charge-transfer complexes have been suggested as important features determining the activity of hormones acting at their receptors. In bradykinin, the high activity of the [β-cyclohexylalanine^{5,8}] analogue[130] rules out any role for this form of complex. The recent discovery (see Vol. 7, p. 289) that replacement of trytophan in LH-RH by pentamethylphenylalanine gives an analogue 10 times

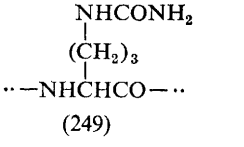
[130] D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc.* (*C*), 1971, 46.



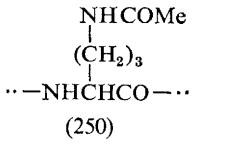
(240)



(247)



(249)



(250)

more active than when 5-fluorotryptophan is the replacement has been interpreted as supporting a crucial role for a charge-transfer complex.[97] However, the electronic exchange which gives rise to the characteristic spectral properties of a charge-transfer complex is not itself the major source of binding free energy, and it is difficult to envisage a necessary functional role for the transfer of an electron in such a pairing. It would be interesting to see the effect of other amino-acids with large hydrophobic side-chains substituted in this position. The [β-cyclohexylalanine^5] analogue (181) has substantial biological activity,[101] ruling out charge transfer involving the amino-acid in position 5.

Replacement of the α-CH of amino-acids by N gives 'aza' analogues (286) and in the case of LH-RH this replacement of glycine (R = H) in positions 6 and 10



(286)

or the substitution of aza-alanine (R = Me) in position 6 gives analogues fully active in the ovulation induction assay.[8d]

Analogues of LH-RH in which pyroglutamic acid is replaced by *N*-formylsarcosine (52) or *N*-methylpyroglutamic acid (53) retain substantial activity (*ca.* 50%) *in vivo*, while the [*N*-formylglycine^1] analogue (51) has *ca.* 1.5% activity.[79]

The *in vitro* LH- and FSH-releasing activities of 21 analogues of LH-RH have been reported.[75] Des-Gly^6-LH-RH (188) had surprisingly high activity, although less activity has been found in other studies.[78]

A series of analogues has been prepared making use of the idea of 'pseudo-symmetry' (*cf.* p. 414) in LH-RH, *i.e.* that one part of the molecule resembles the other taken in reverse sequence.[98]

The effects on cyclic AMP stimulation of a series of LH-RH analogues have been studied.[131] The stimulation parallels the release of LH and FSH, suggesting a functional role, and the high activity of (263) in this system suggests that its high *in vivo* activity is at least partly intrinsic and not merely due to stability to peripheral enzymolysis.

[131] P. Borgeat, F. Labrie, J. Côté, F. Ruel, A. V. Schally, D. H. Coy, E. J. Coy, and N. Yanaihara, *Mol. Cell. Endocrinol.*, 1974, **1**, 7.

Replacement of serine in position 4 by leucine or *O*-t-butylserine gives analogues with less than 0.1% activity,[95] in contrast to the substitution of alanine. The importance of optical integrity to biological activity in LH-RH is shown by a systematic replacement study leading generally to analogues of low activity. Exceptions were [D-Glp^1] and [D-His^2] LH-RH with 8 and 9% activity respectively, and [D-Leu^7] LH-RH with 1% activity.[74]

Cleavage of [(^{3}H)Glp^1] LH-RH by enzymes in hypothalamic tissue has been reported to occur largely between amino-acids 6 and 7.[132] Inactivation by brain homogenates is also due to endopeptidase action, probably between Tyr^5-Gly^6 and Gly^6-Leu^7, and is followed by further degradation of the primary cleavage products by amino- and carboxypeptidases. In LH-RH analogues containing D-Ala at position 6 the enzymatic release of internal amino-acid residues was greatly diminished suggesting, once more, that the Gly^6-Leu^7 bond is the site of primary cleavage.[133]

Another enzyme in brain tissue (possibly the one that is also responsible for the inactivation of oxytocin[134, 135]) removes $GlyNH_2$, Pro, and Arg from the *C*-terminus of LH-RH but not from analogues having ProNHEt as the *C*-terminus.[31f, 133, 136, 137] Thus the increased potency and duration of action found in LH-RH analogues containing D-amino-acids in position 6 and/or ProNHEt at position 9 may be explained, at least in part, by greater resistance to enzymatic breakdown.

The primary cleavage of [(^{3}H)Trp^3][(^{14}C)Pro^9] LH-RH by homogenates of liver and kidney was shown [114] to take place predominantly between Tyr^5-Gly^6.

The immunochemical and chromatographic properties of rat, rabbit, and chicken LH-RH appear to be similar to those of the synthetic decapeptide.[138] A study of the transport capacity of the median eminence for tritiated LH-RH has shown the presence of a specific transport system from the cerebrospinal fluid with receptor binding characteristics similar to those of anterior pituitary receptors.[139] Other studies have included the binding of [(^{3}H)Tyr^5] LH-RH to pituitary plasma membranes [140] and of ^{125}I-labelled LH-RH to the anterior pituitary gland,[141] the preparation of ^{125}I-labelled LH-RH,[105] a study of the specificity of the binding of three different anti-LH-RH antisera,[142] and a study of the metabolism of tritiated LH-RH.[143]

[132] Y. Koch, T. Baram, P. Chobsieng, and M. Fridkin, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 95.
[133] N. Marks and F. Stern, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 1458.
[134] K. C. Hooper and C. R. N. Hopkinson, *Acta Endocrinol.*, 1974, **75**, 636.
[135] E. C. Griffiths and K. C. Hooper, *Acta Endocrinol.*, 1974, **75**, 435.
[136] E. C. Griffiths, K. C. Hooper, S. L. Jeffcoate, and D. T. Holland, *Acta Endocrinol.*, 1974, **77**, 435.
[137] E. C. Griffiths, K. C. Hooper, S. L. Jeffcoate, and D. T. Holland, *Brain Res.*, 1975, **85**, 161.
[138] S. Jeffcoate, P. J. Sharp, H. M. Fraser, D. T. Holland, and A. Gunn, *J. Endocrinol.*, 1974, **62**, 85.
[139] S. S. Vaala and K. M. Knigge, *Neuroendocrinology*, 1974, **15**, 147.
[140] M. Jutisz, A. Berault, and M. Théoleyre, 'Endocrinology 1975' (*cf.* ref. 8), pre-publication abstracts, p. 32.
[141] K. R. Park and B. B. Saxena, 'Endocrinology 1975' (*cf.* ref. 8), pre-publication abstracts, p. 35.
[142] A. Arimura, H. Sato, D. H. Coy, R. B. Worobec, A. V. Schally, N. Yanaihara, T. Hashimoto, C. Yanaihara, and N. Sukura, *Acta Endocrinol.*, 1975, **78**, 222.
[143] A. Dupont, F. Labrie, G. Pelletier, R. Puviani, D. H. Coy, E. J. Coy, and A. V. Schally, *Neuroendocrinology*, 1974, **16**, 65.

Growth Hormone Release Inhibiting Hormone (GH-RIH, Somatostatin).—There have been a number of useful recent reviews [144–150] and several recent syntheses have been reported.[151–153] Full details of the isolation of somatostatin from ovine hypothalamic tissue have now been published.[154]

The hormone has been found to have a surprisingly wide range of actions, inhibiting not only the release of GH from the pituitary [155–157] and of TSH (but not prolactin) in response to TRH,[155, 158] but also the pancreatic release of insulin and glucagon,[159–162] and the gastric release of acid, pepsin, and gastrin.[163] It also inhibits the output of insulin and glucagon from endocrine tumours which release them.[164] The light some of these studies are helping to cast on diabetes and its development is discussed on p. 388.

Perhaps not surprisingly in view of this varied spectrum of activities (and in line with the 'APUD' concept – see p. 399) the peptide, or at least a peptide, which binds antibodies directed towards somatostatin, is found outside the hypothalamus in both the pancreatic *D* cells [165] and in the rat stomach and pancreas.[166] Immunoreactive somatostatin has also been found in the endocrine cells of the human foetal pancrease,[167] in addition to the endocrine pancreas itself.[168, 169]

144 P. Brazeau and R. Guillemin, *New Eng. J. Med.*, 1974, **290**, 963.
145 R. Guillemin and J. E. Gerich, *Ann. Rev. Med.*, 1976, **27**, 379.
146 (Editorial), *Lancet*, 1974, **i**, 1148.
147 (Editorial), *Lancet*, 1975, **i**, 1323.
148 W. Vale, P. Brazeau, C. Rivier, M. Brown, B. Boss, J. Rivier, R. Burgus, N. Ling, and R. Guillemin, *Rec. Progr. Hormone Res.*, 1975, **31**, 365.
149 S. S. C. Yen, B. L. Lasley, C. F. Wang, H. Leblanc, and T. M. Siler, *Rec. Progr. Hormone Res.*, 1975, **31**, 321.
150 G. Benker and D. Reinwein, *Deut. Med. Wochenschrift*, 1975, **100**, 961.
151 N. Fujii and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 1596.
152 J. E. F. Rivier, *J. Amer. Chem. Soc.*, 1974, **96**, 2986.
153 H. U. Immer, K. Sestanj, V. R. Nelson, and M. Götz, *Helv. Chim. Acta*, 1974, **57**, 730.
154 P. Brazeau, W. Vale, R. Burgus, R. Guillemin, *Canad. J. Biochem.*, 1974, **52**, 1067.
155 A. Belanger, F. Labrie, P. Borgeat, M. Savary, J. Côté, J. Drouin, A. V. Schally, D. H. Coy, E. J. Coy, H. Immer, K. Sestanj, V. Nelson, and M. Götz, *Mol. Cell. Endocrinology*, 1974, **1**, 329.
156 G. M. Besser, C. H. Mortimer, A. S. McNeilly, M. O. Thorner, G. A. Batistoni, S. R. Bloom, K. W. Kastrup, K. F. Hanssen, R. Hall, D. H. Coy, A. J. Kastin, and A. V. Schally, *Brit. Med. J.*, 1974, **iv**, 622.
157 P. Brazeau, J. Rivier, W. Vale, and R. Guillemin, *Endocrinology*, 1974, **94**, 184.
158 D. Carr, A. Gomez-Pan, D. R. Weightman, V. C. M. Roy, R. Hall, G. M. Besser, M. O. Thorner, A. S. McNeilly, A. V. Schally, A. J. Kastin, and D. H. Coy, *Brit. Med. J.*, 1975, **iii**, 67.
159 C. H. Mortimer, D. Carr, T. Lind, S. R. Bloom, C. N. Mallinson, A. V. Schally, W. M. G. Tunbridge, L. Yeomans, D. H. Coy, A. Kastin, G. M. Besser, and R. Hall, *Lancet*, 1974, **i**, 697.
160 J. E. Gerich, M. Lorenzi, V. Schneider, J. H. Karam, J. Rivier, R. Guillemin, and P. H. Forsham, *New Eng. J. Med.*, 1974, **291**, 544.
161 F. P. Alford, S. R. Bloom, J. D. N. Nabarro, R. Hall, G. M. Besser, D. H. Coy, A. J. Kastin, and A. V. Schally, *Lancet*, 1974, **i**, 974.
162 D. L. Curry, L. L. Bennett, and C. H. Li, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 885.
163 S. R. Bloom, G. H. Mortimer, M. O. Thorner, G. M. Besser, R. Hall, A. Gomez-Pan, V. M. Roy, R. C. G. Russell, D. H. Coy, A. J. Kastin, and A. V. Schally, *Lancet*, 1974, **i**, 1106.
164 S. E. Christensen, Aa. P. Hansen, K. Lundbaek, H. Ørskov, and K. Seyer-Hansen, *Lancet*, 1975, **i**, 1426.
165 J. Polak, A. G. E. Pearse, L. Grimelius, S. R. Bloom, and A. Arimura, *Lancet*, 1975, **i**, 1220.
166 A. Arimura, H. Sato, A. Dupont, N. Nishi, and A. V. Schally, *Science*, 1975, **189**, 1007.
167 P. M. Dubois, C. Paulin, R. Assan, and M. P. Dubois, *Nature*, 1975, **256**, 731.
168 M. P. Dubois, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1340.
169 M. P. Dubois, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 2883.

The activities of somatostatin raise possibilities of clinical use either through lowering growth hormone levels or through lowering the levels of other hormones, particularly glucagon which may be involved in diabetes. To be clinically useful a somatostatin-like substance would need to be long-acting, but in addition the wide range of actions itself presents problems, and one effect in particular, the *in vivo* inhibition of platelet aggregation which has been studied in rabbits and baboons [170] has raised doubts.[34, 147] Somatostatin ostensibly lacks toxicity in man however,[171] and there are recent signs that it may well proceed to further clinical studies.

Table 4 lists analogues of somatostatin whose activities have been reported. The high *in vitro* activity of the reduced form of somatostatin (311, somatostateine) has been confirmed,[172] but the strong dependence of its *in vivo* activity on the route of administration and its somewhat prolonged action, perhaps upport the view that oxidation to the cyclic form is the principal cause of its activity. The low but significant activities of (321) and (322) [173, 174] (*cf.* ref. 175) suggest that a cyclic form is not absolutely essential for activity but that any intrinsic activity in (311) may be low compared with the cyclic form.

Table 4 *Analogues of somatostatin*

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Compound	*Structure*	*Biological activity against: Growth hormone release: in vitro*	*in vivo*	*Insulin release*	*Glucagon release*	*Ref.*
A. Oxidized form						
(287)	Tyr-somatostatin					176
(288)	Gly_2Tyr-somatostatin	++(+)				8*e*
(289)	[Tyr[1]]	++++				8*b*
(290)	(3—14)	+++	+++(+)			8*b*, 174, 177
(291)	Ac-(3—14)	+++	++++			174, 177
(292)	desamino-(3—14)	++++				148, 174
(293)	des-Lys[4]-(3—14)	++				8*e*
(294)	des-Asn[5]-(3—14)	++				8*e*

[170] T. Chiang, W. C. Duckworth, E. H. Beachey, and A. H. Kang, *Endocrinology*, 1975, **79**, 753; D. Koerker, L. A. Harker, and C. J. Goodner, *New Eng. J. Med.*, 1975, **293**, 476.
[171] C. H. Mielke, J. E. Gerich, M. Lorenzi, E. Tsalikian, R. Rodvien, and P. H. Forsham, *New Eng. J. Med.*, 1975, **293**, 480.
[172] P. Brazeau, W. Vale, J. Rivier, and R. Guillemin, *Life Sci.*, 1974, **15**, 351.
[173] J. Rivier, P. Brazeau, W. Vale, and R. Guillemin, *Abstr. Papers Amer. Chem. Soc. Meeting*, 1974, (168), MEDI 44.
[174] J. Rivier, P. Brazeau, W. Vale, and R. Guillemin, *J. Medicin. Chem.*, 1975, **18**, 123.
[175] D. Sarantakis, W. A. McKinley, and N. H. Grant, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 538.
[176] N. Fujii and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2446.
[177] P. Brazeau, W. Vale, J. Rivier, and R. Guillemin, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 1202.

Table 4 (*cont.*)

Compound	Structure	Biological activity [a] against: Growth hormone release: in vitro	in vivo	Insulin release	Glucagon release	Ref.
(295)	[Ala^{2}]	++++		++++	++++	8*b*
(296)	des-Asn^{5}	++(+)				8*e*
(297)	[Ala^{5}]	++++		++++	++++	8*b*
(298)	[Ala^{6}]	++		− [b]	− [b]	8*b*
(299)	[Ala^{7}]	++				8*b*
(300)	[Ala^{8}]	−		− [b]	− [b]	8*b*
(301)	[D-Ala^{8}]	+				178
(302)	[D-Trp^{8}]	++++				178
		++++		++++	++++	8*b*
(303)	[D-Lys^{9}]	−				8*e*
(304)	[Nle^{9}]	−				8*e*
(305)	[Ala^{10}]	+++		+++	− [b]	8*b*
(306)	[Ala^{11}]	++				8*b*
(307)	[Tyr^{11}]	+++				8*b*
(308)	[Ala^{12}]	++				8*b*
(309)	[Ala^{13}]	++				8*b*
(310)	des-Ser^{13}	++(+)				8*e*
B. Reduced form (Somatostateine)						
(311)	Somatostateine	+++	+++			172
(312)	(3—14)	+++	+++			174, 177
		(+++)		(+++)	(−)	179
(313)	Ac-(3—14)	+++	+++			174, 177
(314)	Me_3CCO-(3—14)	+++	+++			177
(315)	CH_2=CHCO-(3—14)	+++	+++			177
(316)	PhCO-(3—14)	+++	+++			177
(317)	$Me(CH_2)_4CO$-(3—14)	+++	+++			177
(318)	des-Asn^{5}-(3—14)	(+++)		(+++)	(−)	179
(319)	[Ala^{2}]	− [b]				8*b*
(320)	[Ala^{3}]	−		− [b]	− [b]	8*b*
(321)	[Ala^{8,14}]	+	++			174
(322)	[Cys(Me)^{3,14}]	++	+			148, 174
(323)	[Ala^{14}]	−				8*b*

[a] Symbols for potency are used as in Table 3. [b] < 10%.

The *N*-terminal dipeptide is not essential for activity, and (290) is active *in vitro* on rat monolayer pituitary cell cultures (65% activity) and *in vivo* inhibiting pentobarbital-stimulated GH release (215% activity). The acetyl derivative (291) shows higher activity still by s.c. injection. Activity is retained in the reduced form (312) and a series of acyl derivatives (313)—(317) show significant *in vitro* (up to *ca.* 40%) and *in vivo* (up to *ca.* 60%) activity. The acylated derivatives showed a prolonged activity on s.c. injection.[173, 174, 177, 180] Inconsistencies which

[178] J. Rivier, M. Brown, and W. Vale, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 746.
[179] S. Efendic, R. Luft, and H. Sievertsson, *F.E.B.S. Letters*, 1975, **58**, 302.
[180] P. Brazeau, J. Rivier, and J. B. Martin, *Clin. Res.*, 1974, **22**, 729A.

have been observed in the duration of the effect of these compounds have been interpreted in terms of an effect of both pentobarbital and of the releasing hormone on the central nervous system (and therefore indirectly on GH).[181] In man the acetyl and benzoyl des-Ala[1],Gly[2] peptides were active but failed to show a prolonged effect.[181a]

Ring size is not crucial for somatostatin activity, and the analogues (293), (294), (296), and (310) all retain substantial activity. A basic L-amino-acid is essential at position 9 since (303) and (304) both have less than 0.5% activity.[8e] The analogue (294) is active in lowering growth hormone and insulin levels but is inactive against glucagon, and the possibilities this raises for the separation of activities will clearly be a stimulus to further studies aimed at finding analogues with differentiated actions. The analogues (312) and (318) have also been reported to have similarly selective activity.[179]

The [D-Trp[8]] analogue (302) is six to eight times more potent than somatostatin on pituitary cell cultures.[178] It is not yet clear whether this reflects increased receptor binding or increased enzymic resistance, although the 8–9 bond has been identified as a point of cleavage of somatostatin by brain enzymes.[182]

Somatostatin can inhibit the cyclic AMP response of the exocrine pancreas to secretin, and at very high levels in this system it shows secretin-like activity. It may be competing for the same receptors, possibly involving an intriguing tetrapeptide sequence (324) which is present in both secretin (positions 5—8) and somatostatin (positions 10—13).[183]

Thr-Phe-Thr-Ser

(324)

The effect on growth hormone is probably physiologically important, and in acromegaly the raised growth hormone levels may be dramatically reduced by infusion of somatostatin (suggesting incidentally that acromegaly is commonly a hypothalamic disease) although ending the infusion causes a rapid rise to higher levels[184] as observed earlier. The inhibition of TSH is also probably of physiological importance since injection of sheep anti-somatostatin serum into rats increases basal levels in both TSH and GH as well as potentiating the cold stress TSH response.[185] Studies on the inhibition of cyclic AMP accumulation in anterior pituitary tissue by somatostatin also suggest (since 50% of all cells are inhibited) that virtually all GH- and TSH-releasing cells are affected. The effect on cAMP apparently occurs at a later stage than its formation, since theophylline-stimulated cAMP formation and also N^6-butyryl-cAMP-induced GH and TSH

[181] M. Brown, J. Rivier, W. Vale, and R. Guillemin, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 752.

[181a] D. C. Evered, A. Gomez-Pan, W. M. G. Tunbridge, R. Hall, T. Lind, G. M. Besser, C. H. Mortimer, M. O. Thorner, A. V. Schally, A. J. Kastin, and D. H. Coy, *Lancet*, 1975, **i**, 1250.

[182] N. Marks and F. Stern, *F.E.B.S. Letters*, 1975, **55**, 220.

[183] P. Robberecht, M. Deschodt-Lanckman, P. de Neef, and J. Christophe, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 315.

[184] G. M. Besser, C. H. Mortimer, D. Carr, A. V. Schally, D. H. Coy, D. Evered, A. J. Kastin, W. K. G. Tunbridge, M. O. Thorner, and R. Hall, *Brit. Med. J.*, 1974, **i**, 352.

[185] L. Ferland, F. Labrie, M. Joplin, A. Arimura, and A. V. Schally, *Biochem. Biophys. Res. Comm.*, 1976, **68**, 149.

release are inhibited.[186] The radioimmunoassay of somatostatin has been discussed.[187]

Other Releasing Hormones.—The *C*-terminal tripeptide of oxytocin (Pro-Leu-Gly-NH_2) has generally been found not to alter MSH levels by the usual routes of administration in mammals (see Vol. 6, p. 436), and it will not be considered here as an MSH-inhibiting factor. It is without effect on β-MSH levels in normal men,[188] although in the case of man, neither α- nor β-MSH can be detected in the pituitary.[189] It may well be that previous ideas on the role of melanotropins and the control of their secretion in man and probably in other mammals needs to be revised. The CNS effects reported (see Section 9) for β-MSH and particularly for peptides, for example enkephalin, occurring in the *C*-terminal 31 amino-acid sequence of β-lipotropin perhaps add weight to this suggestion.

The decapeptide LH-RH may be by no means the only hypothalamic hormone controlling gonadotropin release. A partially purified FSH releasing factor has been studied; it also releases LH but is apparently distinct from LH-RH.[190, 191] Two analogues of LH-RH, (63) and (122), which inhibit the release of LH by LH-RH, failed to inhibit the release of FSH and LH by the new factor. Further reports on FSH-releasing [31b] and LH-releasing [31a] factors distinct from the decapeptide have appeared. An LH-release-inhibiting factor (factor C) has been partially purified;[191, 192] it inhibits basal and LH-RH-induced LH release and is distinct from the contraceptive tetrapeptide of embryonic origin (see p. 431).

Work continues on the search for the structure of the hypothalamic corticotropin releasing factor,[193, 194] which appears to be different from the various peptides related to vasopressin which have been found to have this activity. It may require a co-factor.[194] Pressinoic acid appears on further study not to be a potent corticotropin releasing agent.[195]

The search for the growth hormone releasing factor also continues. Purified fractions (which release radioimmunoassayable GH) have been prepared from ovine tissue.[196] A GH-releasing factor (factor A) which releases GH at 100—200 μg doses has been isolated from hypothalamic tissue. Its action is blocked by somatostatin.[197] A second GH-releasing compound (factor B) is active at 15 μg.

[186] P. Borgeat, F. Labrie, J. Drouin, A. Belanger, H. Immer, K. Sestanj, V. Nelson, M. Götz, A. V. Schally, D. H. Coy, and E. J. Coy, *Biochem. Biophys. Res. Comm.*, 1974, **56**, 1052.
[187] A. Arimura, H. Sato, D. H. Coy, and A. V. Schally, *Proc. Soc. Exp. Biol. Med.*, 1975, **148**, 784.
[188] A. J. Thody, S. Shuster, N. A. Plummer, W. Bogie, R. J. Leigh, S. K. Goolamali, and A. G. Smith, *J. Clin. Endocrinol. Metab.*, 1974, **38**, 491.
[189] A. P. Scott and P. J. Lowry, *Biochem. J.*, 1974, **139**, 593.
[190] C. Y. Bowers, J. Humphries, Y. P. Wan, B. L. Currie, K. N.-G. Johansson, and K. Folkers, *Endocrinology*, 1974, **94** (*Suppl.*), A81.
[191] K. N.-G. Johansson, T. Greibrokk, B. L. Currie, J. Hansen, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1975, **63**, 62.
[192] D. Chang, T. Greibrokk, R. Knudsen, G. Howard, J. Humphries, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 1208.
[193] B. Gillham, M. T. Jones, E. W. Hillhouse, and J. Burden, *J. Endocrinol.*, 1975, **65**, 12P.
[194] A. F. Pearlmutter, E. Rapino, and M. Saffran, *Endocrinology*, 1975, **97**, 1336.
[195] A. F. Pearlmutter, E. Rapino, and M. Saffran, *Neuroendocrinology*, 1974, **15**, 106.
[196] M. C. Wilson, A. L. Steiner, A. P. Dhariwal, and G. T. Peake, *Neuroendocrinology*, 1974, **15**, 313.
[197] B. L. Currie, K. N.-G. Johansson, T. Greibrokk, and K. Folkers, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 605.

It is also inhibited by somatostatin.[198] Another GH inhibiting factor (factor D), distinct from somatostatin, has been identified.[199] Further work on the putative GH releasing compound (325) has shown that the lack of GH releasing activity

Glp-Ser-Gly

(325)

in the rat (Vol. 6, p. 434) extends also to the human situation.[200] A further synthesis of the tripeptide has been reported.[201]

A prolactin releasing factor different from TRH has been identified in bovine hypothalamic extracts.[202, 203] Further work has been carried out on prolactin inhibiting factor(s).[199, 202, 204] About 100 ng of a material has been isolated from 8000 hypothalami making extensive use of HPLC. The material was active at < 5 ng, and its behaviour on HPLC suggests that it is pure, although there was insufficient material for structural studies.[199, 204] Other isolation work has identified at least four fractions from hypothalamic extracts with PIF activity.[205] Catecholamines may possibly be directly involved in the release of prolactin.[204–206]

3 Anterior Pituitary Hormones

Reviews have appeared covering the glycopeptide hormones of the pituitary,[207] the molecular evolution of pituitary hormones (covering the evolution and structure–activity relationships of anterior and posterior pituitary hormones),[208] and the evolution of vertebrate corticotropin and MSH.[209] Results discussed at recent symposia on growth hormone have been described.[210, 211] Other reviews cover the regulation of growth,[212] diagnostic and therapeutic applications of prolactin physiology,[213] β-MSH,[214] insulin and growth hormone,[215] their receptors,[216] structural and functional inter-relationships of ACTH and melano-

[198] K. N.-G. Johansson, B. L. Currie, and K. Folkers, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 610.
[199] T. Greibrokk, B. L. Currie, K. N.-G. Johansson, J. J. Hansen, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1974, **59**, 704.
[200] D. Gonzalez-Barcena, A. J. Kastin, D. H. Coy, S. Glick, D. S. Schalch, L. A. Lee, J. P. Arzac, and A. V. Schally, *J. Clin. Endocrinol. Metab.*, 1974, **38**, 1134.
[201] K. Zech and W. Voelter, *Z. Naturforsch.*, 1974, **29b**, 818.
[202] R. Dular, F. LaBella, S. Vivian, and L. Eddie, *Endocrinology*, 1974, **94**, 563.
[203] T. Kokubu, S. Sawano, M. Shiraki, M. Yamasaki, and Y. Ishizuka, *Endocrinol. Japonica*, 1975, **22**, 213.
[204] T. Greibrokk, J. Hansen, R. Knudsen, Y.-K. Lam, K. Folkers, and C. Y. Bowers, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 338.
[205] A. V. Schally, H. Arimura, J. Takahara, T. W. Redding, and A. Dupont, *Fed. Proc.*, 1974, **33**, 237.
[206] J. Takamara, A. Arimura, and A. V. Schally, *Endocrinology*, 1974, **95**, 462.
[207] A. Stockell-Hartree, *Biochem. Soc. Trans.*, 1974, **2**, 781; J. G. Pierce, *ibid.*, p. 783; W. R. Butt and S. S. Lynch, *ibid.*, p. 785.
[208] M. Wallis, *Biol. Revs.*, 1975, **50**, 35.
[209] P. J. Lowry and A. P. Scott, *Gen. Comp. Endocrinol.*, 1975, **26**, 16.
[210] M. Wallis, *Nature*, 1975, **257**, 744.
[211] S. Raiti and N. K. MacLaren, *Fed. Proc.*, 1974, **33**, 1682.
[212] W. H. Daughaday, *Ann. Rev. Physiol.*, 1975, **37**, 211.
[213] P. J. R. Fournier, P. D. Desjardins, and H. G. Friesen, *Amer. J. Obstet. Gynecol.*, 1974, **118**, 337.
[214] G. Bloomfield and A. Scott, *Proc. Roy. Soc. Med.*, 1974, **67**, 748.
[215] P. H. Sönksen, *Proc. Roy. Soc. Med.*, 1975, **68**, 707.
[216] J. Roth, C. R. Kahn, M. A. Lesniak, P. Gorden, P. De Meyts, K. Megyesi, D. M. Neville, J. R. Gavín, A. H. Soll, P. Freychet, I. D. Goldfine, R. S. Bar, and J. A. Archer, *Rec. Progr. Hormone Res.*, 1975, **31**, 95.

tropins,[217] human growth hormone,[218–220] the mechanism of action of pituitary growth hormone,[221] and structure–activity relationships in LH and human chorionic gonadotropin.[19e]

Adrenocorticotropin (ACTH).—The sequence (326) of ovine ACTH has been redetermined and the structure found to be identical with that of the bovine hormone.[222] The relevant parts of the bovine, porcine (327), and human (328)

25	26	27	28	29	30	31	32	33	
···-Asn-	Gly-	Ala-	Glu-	Asp-	Glu-	Ser	-Ala-	Gln-···	
					ovine/bovine ACTH				(326)
···-Asn-	Gly-	Ala-	Glu-	Asp-	Glu-	Leu-	Ala-	Glu-···	
					porcine	ACTH			(327)
···-Asn-	Gly-	Ala-	Glu-	Asp-	Glu-	Ser	-Ala-	Glu-···	
					human	ACTH			(328)

sequences are now thought to be as shown. The biological properties of synthetic human ACTH with the corrected sequence have been reported.[223]

Table 5 lists the results of structure–activity studies on ACTH analogues. The importance of tryptophan in position 9 is shown further by a number of the analogues synthesized. The [β-(naphthyl-1) alanine[9]] (339) and [N^{ind}-formyltryptophan[9]] (340) derivatives have reduced but significant ACTH-like activity.[228] While the [Ile[9]] analogue (342), which is inactive, shows inhibitory activity (*vs.* steroidogenesis and cAMP stimulation) in the isolated adrenal cell system,[229] the [Lys[8], Phe[9]] analogue (337) has no *in vivo* inhibitory effects.[228]

Substitution of pentamethylphenylalanine in position 9 in derivatives (332) and (338) leads to a drastic fall in steroidogenic activity (0.2—0.4% activity) in contrast to the effect in LH-RH (see p. 359).[225] This was in spite of a demonstrated ability of the analogues to take part in charge-transfer complexes in solution, where the analogues showed very similar properties to the corresponding tryptophan peptides. On the other hand, full melanotropic activity was retained, unlike the corresponding [Orn[8]] analogue where it was low. The two analogues [Gln[5], Phe[9]]- (336) and [MeTrp[9]] ACTH (1—20)NH_2 (341) bind to bovine adrenal cortical membranes but are inactive and inhibit the action of ACTH on adenyl cyclase activation in this system.[227]

Over a short time-scale, the [D-Ala[1]] analogue (330) shows about the same *in vivo* steroidogenic potency as the [Ala[1]] derivative (329), 153 and 142 U mg^{-1}, respectively. However, (330) was found to have a longer *in vivo* half-life, confirming that the higher activities of analogues with D-amino-acids in position 1 results from increased *in vivo* stability.[224]

[217] J. Ramachandran, *Israel J. Chem.*, 1974, **12**, 675.
[218] C. H. Li, in 'Hormonal Proteins and Peptides. Vol. 3', ed. C. H. Li, Academic Press, New York, 1974.
[219] 'Advances in Human Growth Hormone Research', ed. S. Raiti, D.H.E.W., Washington, 1974.
[220] A. B. Hayles, *Canad. Med. Assoc. J.*, 1975, **112**, 1281.
[221] G. P. Talwar, M. R. Pandian, N. Kumar, S. N. S. Hanjan, R. K. Saxena, R. Krishnaraj, and S. L. Gupta, *Rec. Progr. Hormone Res.*, 1975, **31**, 141.
[222] A. Jöhl, B. Riniker, and L. Schenkel-Hulliger, *F.E.B.S. Letters*, 1974, **45**, 172.
[223] L. Schenkel-Hulliger, R. Maier, P. L. Barthe, P. A. Desaulles, A. Jarret, B. Riniker, W. Rittel, and P. Sieber, *Acta Endocrinol.*, 1974, **75**, 24.
[224] J. Blake and C. H. Li, *J. Medicin. Chem.*, 1974, **17**, 233.

Table 5 *Analogues of ACTH*[a]

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Pro-Val-Lys-Val-Tyr-Pro-Asn-Gly-Ala-Glu-Asp-Glu-Ser-Ala-Gln-Ala-Phe-Pro-
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

Leu-Glu-Phe
37 38 39

Compound number	*Structure*[c]	*Biological activity:*[b] *ACTH-like in vitro*	*ACTH-like in vivo*	*MSH-like*	*Lipolytic*	*Ref.*
(329)	[Ala[1]]-(1—20)NH_2		++++			224
(330)	[D-Ala[1]]-(1—20)NH_2		++++			224
(331)	[(^{14}C)Ala[1]]-(1—20)NH_2		++++			224
(332)	[D-Ser[1], $Phe(Me)_5^9$]-(1—24)	+		+++		225
(333)	(4—23)NH_2	*d*				226
(334)	(5—24)	*d*				226
(335)	(6—24)	*d*				226
(336)	[Gln[5], Phe[9]]-(1—20)NH_2	−*				227
(337)	[Lys[8], Phe[9]]-(1—19)	−		++	−	228
(338)	$[Phe(Me)_5^9]$-(1—24)	+		+++		225
(339)	[Nap(1)ala[9]]-(1—19)	++	++	+++	++	228
(340)	[Trp(N^{ind} HCO)[9]]-(1—19)		++	+++	+++	228
(341)	[MeTrp[9]]-(1—20)NH_2	−*				227
(342)	[Ile[9]]-(1—24)	−*				229
(343)	[($^3H_2$)Tyr[23]]-(1—24)[e]					230

[a] The structure of ovine/bovine ACTH is shown. [b] Symbols for potency are used as in Table 3. [c] Nap(1)ala = L-β-(naphthyl-1)alanine. [d] Data expressed graphically (isolated adrenal cells). [e] Used in binding and metabolic studies.

A comparison of the cAMP activation and corticosterone production of isolated adrenal cortical cells in response to ACTH (1—24), (1—23)NH_2, (5—24), and (6—24) shows a decrease in activity in that order. A switch to partial agonist behaviour is evident as activity decreases, but it occurs with different fragments in each of the tests.[226]

Studies have continued on larger molecular weight precursors of ACTH (see Vol. 6, p. 437).[231, 232] Species in which the predominant circulating hormone is larger than 39 amino-acids (rabbit, rat, mouse) have a higher ratio of cortisone to cortisol than those in which the 39 amino-acid form predominates (man, monkey, sheep, dog, cat, guinea-pig).[233] It would be interesting to see this possible indication of the effect of the different structures explored further with analogues.

[225] J. W. van Nispen and G. I. Tesser, *Internat. J. Peptide and Protein Res.*, 1975, **7**, 57.
[226] G. Sayers, S. Seeling, S. Kumar, G. Karlaganis, R. Schwyzer, and M. Fujino, *Proc. Soc. Exp. Biol. Med.*, 1974, **145**, 176.
[227] K. Hofmann, J. A. Montibeller, and F. M. Finn, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 80.
[228] J. Blake and C. H. Li, *J. Medicin. Chem.*, 1975, **18**, 423.
[229] S. Kumar, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1063.
[230] H. P. J. Bennett, G. Bullock, P. J. Lowry, C. McMartin, and J. Peters, *Biochem. J.*, 1974, **138**, 185.
[231] B. Eipper and R. Mains, *Biochemistry*, 1975, **14**, 3836.
[232] G. Gerwirtz, B. Schneider, D. T. Kreiger, and R. S. Yalow, *J. Clin. Endocrinol. Metab.*, 1974, **38**, 227.
[233] R. Coslovsky and R. S. Yalow, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 1351.

A radiolabelled ACTH (1—24) (343) has been used to study the fate of corticotropins in isolated adrenal cells. No binding to cells was observed at low concentrations which nevertheless elicit 80% of the maximum biological response, and it was therefore estimated that less than 120 receptors per cell (a very small proportion of the total) are occupied under these conditions. Metabolic breakdown, which is strongly diminished by cell purification and seems to be chiefly caused by tryptic enzymes released from damaged cells, is not responsible for certain differences in activity; in this system ACTH (1—24) was consistently five to seven times as potent as the full (1—39) sequence, despite being broken down more rapidly.[230]

Metabolic studies of ACTH (1—24) (tetracosactrin) and [D-Ser^1, Lys^{17,18}] ACTH (1—18) have shown that endopeptidases in rat gut break down both equally well but that, given intravenously, the latter is more stable and causes a more sustained steroidogenic effect.[8f] Clinical and biological studies continue to be reported [234] on the D-serine analogue but are not covered comprehensively here. The metabolism of ACTH (1—24) and [D-Ser^1, Lys^{17,18}] ACTH (1—18) in the rat intestine has been studied.[235] The main breakdown takes place immediately after the peptides leave the stomach; the Phe^7–Arg^8 bond is a principal site of cleavage of both compounds, with a greater susceptibility of tetracosactrin to aminopeptidases. *In vivo* studies in rats indicate that the 1—18 analogue has a higher steroidogenic potency and more prolonged action than either ACTH (1—39) or ACTH (1—24) which are approximately equipotent. Since ACTH (1—24) was found to be metabolized more rapidly than ACTH (1—39), the former must be more potent at the receptor.[236] These *in vivo* findings corroborate the results of *in vitro* studies in isolated adrenal cells.

Crude membrane preparations from human, rat, and ovine adrenals both bind and degrade ^{125}I-ACTH (1—24). Degradation is inhibited by unlabelled ACTH (1—24) and by ACTH (1—10) but not by ACTH (11—24). In contrast, the breakdown of ^{125}I-ACTH (11—24) is inhibited by ACTH (11—24) but not by ACTH (1—10). This suggests the existence of two degradative systems, one for the *N*-terminal and another for the *C*-terminal region. Fragments that inhibit the binding and the degradation of ACTH (1—24) are different, indicating that the two processes are independent.[237]

The increased activity of isolated fat cells in response to ACTH caused by phenoxazones can be explained by the increased number of receptor sites per cell caused by the drug.[238] It is suggested that there are inactive receptors which are stimulated by phenoxazones.

Other work on ACTH has included the preparation of polymer-bound ACTH for affinity chromatography,[239] and of iodinated ACTH (1—24) of full biological activity.[240]

[234] H. Tuchmann-Duplessis and L. Mercier-Parot, *Therapie*, 1974, **29**, 453.
[235] P. J. Lowry and C. McMartin, *Biochem. J.*, 1974, **138**, 87.
[236] C. McMartin and J. Peters, *J. Endocrinol.*, 1975, **67**, 41.
[237] J. Saez, A. Dazord, A. M. Morera, and P. Bataille, *J. Biol. Chem.*, 1975, **250**, 1683.
[238] U. Lang, G. Karlaganis, R. Vogel, and R. Schwyzer, *Biochemistry*, 1974, **13**, 2626.
[239] J. L. Fauchere and G. M. Pelican, *Helv. Chim. Acta*, 1975, **58**, 1984.
[240] P. A. Rae and B. P. Schimmer, *J. Biol. Chem.*, 1974, **249**, 5649.

Melanotropin (MSH) and Lipotropin (LPH).—In mammalian species, the roles of both MSH and lipotropin are obscure. β-MSH (344) occurs as part of the β-lipo-

Asp-Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

(344) porcine β-MSH

tropin molecule (porcine β-MSH corresponds to positions 41—58 of porcine β-lipotropin) which may be a precursor of β-MSH.[208] α-MSH is acetyl-ACTH-(1-13)NH_2 and may derive from ACTH or a related precursor.[241] The ACTH sequence is homologous with the corresponding part of the β-lipotropin sequence. All of the peptides with the characteristic ACTH (4—10) 'message' sequence are lipotropic, and β-lipotropin itself is only weakly so. The two melanotropins are potent skin-darkening agents in amphibians but in mammals their role is obscure. α-MSH has been proposed as being involved in the control of sebaceous gland function, and further studies have been reported on this.[242] In the human pituitary, none of α-MSH, β-MSH, and the corticotropin-like intermediate lobe ('CLIP') peptide could be detected by a variety of techniques, although ACTH and a β-lipotropin-like substance were present.[189] The discovery (see p. 421) of morphine-like CNS action in fragments of β-lipotropin (including enkephalin) may perhaps lead to a new understanding of the role of this family of peptides in mammals.

The behavioural effects of ACTH (4—10) (345) are discussed in Section 9. The lipolytic activities of 23 analogues of (345) have been studied in a rabbit adipose cell system. The differences in activity parallel differences in binding to receptors. Increased activity results from the introduction of basic residues in positions 3 and 5; the lipolytic activity of (346) is 5.6 times that of (345) and that

Met-Glu-His-Phe-Arg-Trp-Gly
4 5 6 7 8 9 10

(345)

Met-Arg-His-Phe-Arg-Trp-Gly

(346)

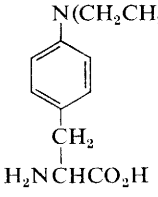
Arg-Met-Arg-His-Phe-Arg-Trp-Gly

(347)

of (347) is 13.7 times that of (345). The results are interpreted in terms of a 'latent anionic locus' in the receptor.[243]

The heptapeptide (345) has also been reported to have effects on mineral metabolism, showing hypocalcaemic and hypophosphataemic effects and inhibiting the osteolytic action of tri-iodothyronine.[244] Analogues of the tetrapeptide sequence (7—10) have been reported with the cytotoxic amino-acid sarcolysine (348) in place of phenylalanine.[245]

[241] D. G. Smyth, *Nature*, 1975, **257**, 89.
[242] A. J. Thody and S. Shuster, *J. Endocrinol.*, 1975, **64**, 503.
[243] M. W. Draper, M. A. Rizack, and R. B. Merrifield, *Biochemistry*, 1975, **14**, 2933.
[244] R. Drouhault, C. Bethous, and P. Blanquet, *Compt. rend.*, 1975, **280**, *D*, 2141.
[245] L. I. Smirnova, N. M. Kashnikova, and E. N. Shkodinskaya, *Zhur. obshchei Khim.*, 1975, **45**, 2082.



(348)

Replacement of the *C*-terminal amide of α-MSH by the hydrazide reduces the melanotropic activity to 25% of that of α-MSH in the *in vitro* frog skin assay and to 50% in the *in vivo* assay using hypophysectomized *Rana pipiens*.[246] Camel β-MSH is found in two forms corresponding to [Gly²] and [Gly², Gln⁸] analogues of the porcine hormone (344) and with 122 and 64% activity (in the frog skin assay *versus* macaque MSH) and 118 and 98% activity (lipolysis *versus* ACTH), respectively.[247] Camel α-MSH is identical with other sequences but, as in the dogfish, is also present in de-acylated form.[247, 248]

For MSH release, the sequence (345) may not be the only 'message' site, since the *C*-terminal tripeptide of α-MSH (Lys-Pro-Val-NH_2) has almost the same melanotropic activity as the ACTH (5—10) hexapeptide. The two 'message' sites have been studied using a large number of analogues.[249, 250]

The effects on biological activity of coupling the carboxy-groups of ACTH and ovine β-lipotropin to taurine and glycine methyl ester (up to 15 residues were incorporated in β-lipotropin by water-soluble di-imide coupling) have been studied.[251]

Several syntheses of relevant sequences have been reported, including porcine CLIP,[252] dogfish MSH,[253] porcine β-MSH,[254] equine β-MSH,[255] and the 42—91 sequence of β-lipotropin[256] (see Vol. 7, p. 297).

Growth Hormone (GH, Somatotropin).—While it seems clear that GH activity may be retained or increased in structures of less than the full 191 amino-acid sequence,[257–260] the biologically important part of the molecule is still not clearly

[246] B. Hemmasi and C. H. Li, *Internat. J. Peptide and Protein Res.*, 1974, **6**, 303.
[247] C. H. Li, W. O. Danho, D. Chung, and A. J. Rao, *Biochemistry*, 1975, **14**, 947.
[248] C. H. Li, D. Yamashiro, and S. Lemaire, *Biochemistry*, 1975, **14**, 953.
[249] A. Eberle and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 1528.
[250] A. Eberle, J.-L. Fauchere, G. I. Tesser, and R. Schwyzer, *Helv. Chim. Acta*, 1975, **58**, 2106.
[251] C. Gilardeau, M. Chrétien, and M. Lis, *Canad. J. Physiol. Pharmacol.*, 1974, **52**, 723.
[252] K. Koyama, Y. Mori, Y. Kiso, S. Hirabayashi, and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2301.
[253] H. Watanabe, M. Kubota, H. Yajima, A. Tanaka, M. Nakamura, and T. Kawabata, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 1889.
[254] H. Watanabe, H. Ogawa, and H. Yajima, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 375.
[255] F. Tamura, Y. Kiso, H. Yajima, A. Tanaka, and M. Nakamura, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2405.
[256] D. Yamashiro and C. H. Li, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 4945.
[257] J. L. Kostyo, *Metabolism*, 1974, **23**, 885.
[258] R. N. P. Singh, B. K. Seavey, V. P. Rice, T. T. Lindsey, and U. J. Lewis, *Endocrinology*, 1974, **94**, 883.
[259] U. J. Lewis, S. J. Pence, R. N. P. Singh, and W. P. Wanderlaan, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 617.
[260] C. R. Reagan, J. B. Mills, J. L. Kostyo, and A. E. Wilhelmi, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1684; C. R. Reagan, J. B. Mills, J. L. Kostyo, and A. E. Wilhelmi, *Endocrinology*, 1975, **96**, 625.

defined. A synthetic triacontapeptide amide corresponding to the [Gln[122]] sequence 95—124 of human GH and its N^{α}-benzyloxycarbonyl derivative have been found to be inactive in the rat tibial epithelial cartilage width assay.[261] Taken with earlier work (see Vol. 6, p. 443) indicating activity in a 95—136 sequence, this could suggest an important role for the sequence 125—136. However, conflicting reports that a synthetic sequence 95—133 is inactive, together with the known unreliability of the tibial width assay, cast doubt on this explanation.

Biological activity has been reported in fragments 96—133 and 151—191 produced by tryptic cleavage of bovine and ovine GH,[262] in fragments 1—34 and 141—191 cleaved from reduced, carbamidomethylated human GH by plasmin[263] (see also ref. 260), and in a 15—125 sequence of similarly modified human GH.[264] Activity has also been reported in the 96—133 sequence of bovine growth hormone,[265] and even in a synthetic (123—131) nonapeptide sequence.[266] In the tibia-width assay (and also in the pigeon crop sac test of prolactin-like activity) many of these fragments have lower activity than GH itself and in some cases log-dose/response lines are not parallel. This, together with the difficult assay, no doubt accounts for some of the discrepancies in reported results.

Seven peptides related to the *N*-terminal sequence of human GH and containing the octapeptide sequence Leu-Ser-Arg-Leu-Phe-Asp-Asn-Ala have been reported to potentiate the action of insulin.[267]

Further work has been reported on the nitration of human chorionic somatomammotropin and human pituitary GH, leading to fully active derivatives.[268]

Growth hormone acts, at least in part, by releasing insulin-like growth-hormone dependent sulphation factors known collectively as somatomedin(s). They have not yet been completely characterized. Recent work[269–272] is not covered in detail here; a number of reviews have appeared.[14a, 19c, 273, 274]

So-called 'super-active' placental lactogen and prolactin have been isolated from hormone linked to Sepharose by cyanogen bromide activation,[275] and are presumably similar in structure to the corresponding insulin derivatives (see p. 394).

[261] R. Camble and R. Cotton, in 'Peptides, 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 17.
[262] L. Graf and C. H. Li, *Biochemistry*, 1974, **13**, 5408.
[263] C. H. Li and L. Graf, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1197.
[264] C. H. Li, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 3878.
[265] N. Yamasaki, J. Shimanaka, and M. Sonenberg, *J. Biol. Chem.*, 1975, **250**, 2510.
[266] S. S. Wang, C. C. Yang, I. D. Kulesha, M. Sonenberg, and R. B. Merrifield, *Internat. J. Peptide and Protein Res.*, 1974, **6**, 103.
[267] F. M. Ng, J. Bornstein, C. Walker, P. Z. Zimmet, and P. Taft, *Diabetes*, 1974, **23**, 943.
[268] H. Kawauchi and C. H. Li, *Arch. Biochem. Biophys.*, 1974, **165**, 255.
[269] J. L. Van den Brande and M. V. L. Du Caju, *Acta Endocrinol.*, 1974, **75**, 233.
[270] J. L. Van den Brande, F. Kootte, R. Tielenburg, M. Van der Wilk, and T. Huyser, *Acta Endocrinol.*, 1974, **75**, 243.
[271] J. P. Liberti, *Biochem. Biophys. Res. Comm.*, 1975, **67**, 1226.
[272] L. Fryklund, K. Uthne, and H. Sievertsson, *Biochem. Biophys. Res. Comm.*, 1974, **61**, 957.
[273] J.-C. Job and R. Rappaport, *Arch. Franc. Pédiatrie*, 1974, **31**, 333.
[274] M. Camerlain, *L'Union Médicale*, 1974, **103**, 107.
[275] B. K. Vonderhaas and Y. J. Topper, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 1323.

Gonadotropins.—The α- and β-subunits of porcine LH can be linked together covalently by treating with water-soluble carbodi-imide, presumably through adjacent carboxyl and amino groups. The covalently linked dimer retains its biological activity, indicating that dissociation into subunits does not precede biological action.[276]

The effect on biological activity of modifying the lysine residues of ovine LH has been explored by methylation, isopropylation, guanidination, and carbamylation.[277] The preparations (none was a single molecular species) had activities varying from 260% on methylation to less than 10% on isopropylation and carbamylation; the guandinated derivative had about half of the activity of native LH.

The role of the carbohydrate in human chorionic gonadotropin (hCG) has been investigated by sequential removal of sialic acid, galactose, *N*-acetylglucosamine, and mannose, leading to decreasing activity (towards stimulation of testosterone and cAMP synthesis). The inactive derivatives were potent inhibitors of (^{125}I)hCG binding to Leydig cells, and a functional ('effector') role has been proposed for the carbohydrate moiety.[278]

Pregnant mare serum gonadotropin has both LH- and FSH-like activity. Recombination of the dissociated α- and β-subunits restores both activities, while combination of the α-subunit with the β-subunit of LH, FSH, or hCG restores the activity appropriate to the subunit, thus suggesting that the activities of the mare serum gonadotropin are both specified in the β-subunit.[279]

Other studies have been reported on the inhibitory activity of hormones (LH, GH, ACTH, lipotropin, calcitonin, and PTH) modified by acetylation or by diazo-coupling (*e.g.* to procainamide),[280] and on the synthesis and immunological evaluation of an eicosapeptide related to the *C*-terminus of the hCG subunit.[281] A highly immunoreactive peptide fragment of human LH has been identified using a sequence-specific radioimmunoassay.[282]

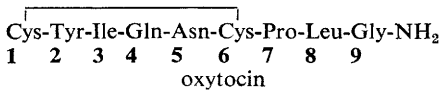
4 Posterior Pituitary Hormones

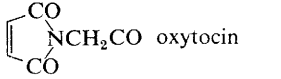
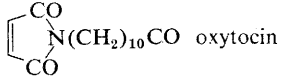
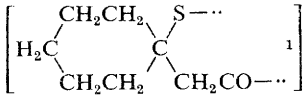
Several reviews have appeared.[7, 208, 283, 284] Table 6 lists oxytocin and vasopressin analogues studied.

The α-amino-group of oxytocin is unnecessary for biological activity (though the reverse is true for binding to neurophysin) and analogues in which it is deleted

[276] Y. Combernous and G. Hennen, *F.E.B.S. Letters*, 1974, **44**, 224.
[277] P. De la Llosa, M. Durosay, C. Tertrin-Clary, and M. Jutisz, *Biochim. Biophys. Acta*, 1974, **342**, 97.
[278] W. R. Moyle, O. P. Bahl, and L. März, *J. Biol. Chem.*, 1975, **250**, 9163.
[279] H. Papkoff, *Biochem. Biophys. Res. Comm.*, 1974, **58**, 397.
[280] V. M. Dilman, M. N. Ostronmova, N. V. Krylova, B. N. Sofronov, L. M. Bernstein, V. N. Golubev, I. G. Kovaleva, L. I. Stekolnikov, L. N. Bulovskaya, A. S. Vishnevsky, Y. F. Bobrov, and S. V. Patokin, *Endocrinol. Exper.*, 1974, **8**, 245.
[281] C. H. Schneider, K. Blaser, C. Pfeuti, and E. Gruden, *F.E.B.S. Letters*, 1975, **50**, 272.
[282] A. Parlow and B. Shome, *J. Clin. Endocrinol. Metab.*, 1975, **41**, 195.
[283] 'Handbook of Physiology', ed. E. Knobil and W. H. Sawyer, American Physiological Soc., 1974, Vol. 4, Section 7.
[284] J. N. Hayward, *Ann. Rev. Physiol.*, 1975, **37**, 191.

Table 6 *Analogues of oxytocin and vasopressin*



Compound number	*Structure* [c]	*Biological activity:* [b] A	B	C	D	E	*Ref.*
A. Oxytocin							
(349)	 oxytocin				(+) [d]		285
(350)	 oxytocin				− [d]		285
(351)	[($^2H_3$)Cys^1]	+++		+++			286
(352)	[D-HOCH(CH_2S)CO—···^{1}]	++					287
(353)	[···—$SCEt_2CH_2CO$—···^{1}]	*	*			*	288
(354)		*	*			*	289
(355)	N^{α}-acyl-[Tyr(Me)^2]	*				*	290
(356)	[···—$(CH_2)_3CO$—···^{1}, Tyr(Me)^2]	++		+++	+		291
(357)	[···—$(CH_2)_3CO$—···^{1}, Phe^2]	+++	+++		+		291

[285] D. H. Rich, P. D. Gesellchen, A. Tong, A. Cheung, and C. K. Buckner, *J. Medicin. Chem.*, 1975, **18**, 1004.
[286] A. F. Spatola, D. A. Cornelius, V. J. Hruby, and A. T. Blomquist, *J. Org. Chem.*, 1974, **39**, 2207.
[287] M. Wälti and D. B. Hope, *J.C.S. Perkin I*, 1974, 202.
[288] D. F. Dyckes, J. J. Nestor, M. F. Ferger, V. du Vigneaud, and W. Y. Chan, *J. Medicin. Chem.*, 1974, **17**, 969.
[289] J. J. Nestor, M. F. Ferger, and V. du Vigneaud, *J. Medicin, Chem.*, 1975, **18**, 284.
[290] M. Krojidlo, T. Barth, L. Servitova, K. Dobrovsky, K. Jost, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1975, **40**, 2708.
[291] I. Fric, M. Kodicek, Z. Prochazka, K. Jost, and K. Blaha, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1290.

Table 6 (*cont.*)

Compound number	*Structure* [c]	*Biological activity:* [b] A	B	C	D	E	*Ref.*
(358)	[···—$(CH_2)_3$CO—··[1], Ile[2]]	+++	+++	+++	+		291
(359)	desamino-[Tyr(4-F)[2]]	+++					292
(360)	desamino-[Tyr(3,5-Br_2)[2]]	*	*			−	293
(361)	[···—$SCEt_2CH_2$CO—··[1], Tyr(3,5-Br)[2]]	*	*			−	293*a*
(362)	des-Cys[1]-[cystine[6]]	−		−			294
(363)	[Asu[1,6], Phe[3]]	++				−	295
(364)	desamino-[Nle[3], Arg[8]]	++	++		+++	+++	296
(365)	desamino-[Val[3], Arg[8]]	++	+++		++	++	296
(366)	desamino-[Pro[3], Arg[8]]	−	−		+	−	296
(367)	desamino-[Glu[4]]	++		++			297
(368)	desamino-[Glu(*O*-Sepharose)[4]]	−		−			297
(369)	[Pen[1], Leu[4]]	*	*		−	−	298
(370)	[···—$SCEt_2CH_2$CO—··[1], Leu[4]]	*	*		−	*	288
(371)	[Asu[1,6], Val[4]]	++				−	295
(372)	desamino-[Ser[4]]-(1—6)OH	(*)	(*)			−	299
(373)	desamino-[Ser[4]]-(1—6)NH_2	*	*			−	299
(374)	[Dsu[1,6]]	+				−	300, 301
(375)	[Cys(S—Hg—S)Cys[1,6]]	++					302
(376)	[Cys(S—Hg^+)[1,6]]	−					302
(377)	[Asu[1,6], Gly[7]]	+++	+	+++	−	−	303
		+++	+		−	*e*	295
(378)	desaminotocinol		−		−		304
(379)	[Tyr(2-I)[2]]	*			slight	−	305
(380)	[Tyr(2-Me)[2]]	*				−	305
(381)	[Phe(4-F)[2]]	+++					292
(382)	[D-Ala[3]]	(*)	(*)		−	−	306
(383)	[Pro[3]]	−	−		−	−	306
(384)	[Glu(OMe)[4]]						8*g*
(385)	[His[4]]	++				−	307
(386)	[D-Ala[4]]	−	−		−	−	306
(387)	[D-Leu[4]]	−	−		−	−	306
(388)	[Phe[4]]	+	−		*[f]	−	308

(389)	[Met^4]						309
(390)	[Ser^4]-(1—6)OH	(*)	(*)	−		−	299
(391)	[Ser^4]-(1—6)NH_2	*	*	−		−	299
(392)	[($^2H_3$)Cys^6]	+++		+++			286
(393)	[Nva^7]						310
(394)	[D-Arg^8]	+++	++		+++	−	296
(395)	[Lys(HCO)^8]	+++	+++		++	++	296
(396)	[Cit^8]	+++	+++		++	++	296
(397)	[Lam^8]						310
(398)	[Lys^8]-(1—9)NHMe	++	*		+	−	311
(399)	[Leu^9]		*				311
(400)	[Glu^9]	−	*		−		311
(401)	[Pro^9]	−	−		−		311
(402)	[Met^9]		*				311
(403)	Retro-D-[D-aIle^3, Gly^7] desaminotocinamide	−	−				312

[292] P. Marbach and J. Rudinger, *Helv. Chim. Acta*, 1974, **57**, 403.
[293] E. O. Lundell and M. F. Ferger, *J. Medicin. Chem.*, 1975, **18**, 1045.
[293a] E. O. Lundell, C. W. Smith, and M. F. Ferger, *J. Medicin. Chem.*, 1975, **18**, 1262.
[294] B. M. Ferrier, J. M. Hendrie, and L. A. Branda, *Canad. J. Biochem.*, 1974, **52**, 60.
[295] T. Yamanaka and S. Sakakibara, *Bull. Chem. Soc. Japan*, 1974, **47**, 1228.
[296] D. Surovec, P. L. Hoffman, and R. Walter, *Experientia*, 1974, **30**, 823.
[297] B. M. Ferrier and L. A. Branda, *Canad. J. Biochem.*, 1975, **53**, 21.
[298] M. F. Ferger and W. Y. Chen, *J. Medicin. Chem.*, 1975, **18**, 1020.
[299] C. W. Smith, V. J. Hruby, and M. F. Ferger, *J. Medicin. Chem.*, 1974, **17**, 873.
[300] O. Keller and J. Rudinger, *Helv. Chim. Acta*, 1974, **57**, 1253.
[301] G. Jutz, V. Pliška, O. Keller, and J. Rudinger, *Experientia*, 1974, **30**, 696.
[302] R. Sperling and M. Gorecki, *Biochemistry*, 1974, **13**, 2347.
[303] R. Walter, T. Yamanaka, and S. Sakakibara, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1901.
[304] H. W. Bossert and P.-A. Jaquenoud, in 'Peptides, 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974,' ed. Y. Wolman, John Wiley, New York, 1975, p. 233.
[305] P. Marbach and J. Rudinger, *Experientia*, 1974, **30**, 696.
[306] J. Roy, R. T. Havran, I. L. Schwartz, and R. Walter, *Internat. J. Peptide and Protein Res.*, 1975, **7**, 171.
[307] M. A. Samartsev, T. K. Lozhkina, and V. F. Martynov, *Khim. prirod. Soedinenii*, 1975, 498.
[308] J. J. Nestor, F. M. Ferger, and W. Y. Chan, *J. Medicin. Chem.*, 1975, **18**, 1022.
[309] T. K. Lozhkina, Z. P. Auna, M. A. Samartsev, and V. F. Martynov, *Khim. prirod. Soedinenii*, 1973, 809.
[310] O. A. Kaurov, Yu. V. Natochin, O. A. Popernatskii, and E. I. Shakhmatova, *Zhur. obshchei Khim.*, 1973, 2521.
[311] Z. Grzonka, J. D. Glass, I. L. Schwartz, and R. Walter, *J. Medicin. Chem.*, 1974, **17**, 1294.
[312] O. Hechter, T. Kato, S. H. Nakagawa, F. Yang, and G. Flouret, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 563.

Table 6 (*cont.*)

Compound number	*Structure* [c]	*Biological activity:* [b] A	B	C	D	E	*Ref.*
(404)	Retro-D-[D-aIle^3] desaminotocinamide	–	–				312
(405)	*N*-formyl-(403)	–	–				312
(406)	*N*-formyl-(404)	–	–				312
B. Vasopressin							
(407)	Gly-VP				*g*		313
					++(4.3) [h]	+(3.2) [h]	314
(408)	Ala-VP					++(2.2) [h]	314
(409)	Leu-VP				+++(1.1) [h]	+++(1.0) [h]	314
(410)	Phe-VP				++(3.2) [h]	++(2.6) [h]	314
(411)	Tyr-VP				+++(1.1) [h]	++(2.2) [h]	314
(412)	Lys-VP				++(2.1) [h]	++(4.9) [h]	314
(413)	Pro-VP				+(2.9) [h]	+(4.7) [h]	314
(414)	Sar-VP				+(1.4) [h]	–*	314
(415)	Gly-Gly-VP				+(3.9) [h]	+(3.9) [h]	314
(416)	Leu-Leu-VP					+++(1.0) [h]	314
(417)	Gly-Pro-VP				++(1.2) [h]	+++(1.2) [h]	314
(418)	Sar-Gly-VP					–(3.2) [h]	314
(419)	Gly-Gly-Gly-VP				+(4.9) [h]	+(5.1) [h]	314
(420)	[···—$SCEt_2CH_2CO$—···[1]]	*	*		*g*	*	313 315
(421)	[···—SCH_2CO—···[1]]	+	*			+	316
(422)	[···—$S(CH_2)_3CO$—···[1]]	*	*			*	316
(423)	[L-HOCH(CH_2S)CO-···[1]]	++				++++	317
(424)	[D-HOCH(CH_2S)CO—···[1]]	–				+	317
(425)	desamino-[Tyr(3,5-Br_2)^2]	*	*			–	318
(426)	desamino-[Ile^3, Leu^4, D-Arg^8]	–			(++++) [i]	–	319
(427)	desamino-[Val^4, Arg^8]				++++	+++	320, 321, 322
(428)	desamino-[Val^4, D-Arg^8]				++++	–	320, 321. 322

(429)	desamino-[—NHCH(CH_2CH_2)-CO—^{6}, Arg^8]	+++		++++	+++	323
				g		324
(430)	desamino-[—NHCH(CH_2CH_2)-CO—^{6}, D-Arg^8]	+		++++(+)	+	323
				g		324
(431)	desamino-[—NHCH(CH_2CH_2)-CO—^{6}, Orn^8]	++		++++(+)	+++	323
				g		324
(432)	desamino-[Har^8]	++		++++	+++	325
		++		+++(+)	+++	326
(433)	desamino-[D-Har^8]	+++		+++	−	326
(434)	desamino-[Hls^8]			++++	+++	325
(435)	desamino-[D-Hls^8]			+++	−	327
(436)	[Phe^2, Orn^8]				*j*	328
(437)	[Phe^2, Ile^3, Orn^8]				*j*	328
(438)	[Thi^3]	++	+++	++++	+++	329
(439)	[Ile^3, Leu^4, D-Arg^8]	−		(++++)[i]	−	319
(440)	[Ile^3, Orn^8]				*j*	328
(441)	[Val^4, Arg^8]			++++	+++	320
(442)	[Val^4, D-Arg^8]			++++	−	320
(443)	[Orn^8]				*j*	328

[313] T. Barth, C. Roy, R. Rajerison, and S. Jard, *Mol. Cell. Endocrinol.*, 1975, **2**, 69.
[314] J. Kynčl, K. Řežábek, E. Kasafírek, V. Pliška, and J. Rudinger, *Eur. J. Pharmacol.*, 1974, **28**, 294.
[315] D. F. Dyckes, J. J. Nestor, M. F. Ferger, and V. du Vigneaud, *J. Medicin. Chem.*, 1974, **17**, 250.
[316] D. F. Dyckes, C. W. Smith, M. F. Ferger, and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1974, **96**, 7546.
[317] M. Wälti and D. B. Hope, *J.C.S. Perkin I*, 1975, 1691.
[318] E. O. Lundell and M. F. Ferger, *Bio-organic Chem.*, 1975, **4**, 377.
[319] M. Zaoral, I. Laine, and F. Brtnik, *Coll. Czech. Chem. Comm.*, 1974, **39**, 2975.
[320] M. Manning, L. Balaspiri, J. Judd, M. Acosta, and W. H. Sawyer, *F.E.B.S. Letters*, 1974, **44**, 229.
[321] W. H. Sawyer, M. Acosta, L. Balaspiri, J. Judd, and M. Manning, *Endocrinology*, 1974, **94**, 1106.
[322] W. H. Sawyer, M. Acosta, and M. Manning, *Endocrinology*, 1974, **95**, 140.
[323] K. Jošt, Z. Procházka, J. H. Cort, T. Barth, J. Skopkova, Z. Prusik, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1974, **39**, 2835.
[324] T. Barth, M. R. Rajerison, C. Roy, and S. Jard, *Mol. Cell. Endocrinol.*, 1975, **2**, 81.
[325] G. Lindeberg, M. Bodanszky, M. Acosta, and W. H. Sawyer, *J. Medicin. Chem.*, 1974, **17**, 781.
[326] M. Zaoral and F. Brtnik, *Coll. Czech. Chem. Comm.*, 1975, **40**, 905.
[327] E. G. G. Lindeberg, *Internat. J. Peptide, and Protein Res.*, 1975, **7**, 395.
[328] B. M. Altura, *J. Pharmacol. Exptl. Ther.*, 1975, **193**, 413.

Table 6 (*cont.*)

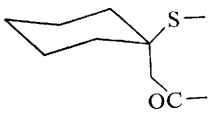
Compound number	*Structure* [c]	*Biological activity:* [b] A	B	C	D	E	*Ref.*
(444)	[Har[8]]	++			++++	+++	325
(445)	[Hls[8]]				+++	++++	325
(446)	[D-Hls[8]]				+++	+	327
(447)	[hNle[8]]				++	++	330
(448)	[Nle(ε-OH)[8]]				+++	+++	331
(449)	(1—7)	−			+	−	332
(450)	(1—8)	+			++	+	332

[a] Structures are stated in relation to [Lys[8]] vasopressin, although [Arg[8]] is the commonest form in mammals. [b] The following test systems are indicated: A, rat uterus *in vitro* (oxytocic); B, avian vasodepressor; C, milk-ejecting; D, antidiuretic; E, rat pressor; symbols for potency are used as in Table 3 (% activities are calculated against the more potent of oxytocin or vasopressin in each case); inhibitory activity is indicated * without distinguishing potency. [c] Asu[1,6] = [(CH$_2$)$_5$CO-···[1],···-NHCHCO-···[6]]; Pen = penicillamine, HSCMe$_2$CH(NH$_2$)CO$_2$H Dsu[1,6] = [(CH$_2$)$_4$CH(NH$_2$)CO-···[1],···-NHCHCO-···[6]]; Lam = laminine, NH$_2$CH([CH$_2$]$_4$NMe$_3^+$)CO$_2$H; Hls = homolysine, NH$_2$CH([CH$_2$]$_5$NH$_2$)CO$_2$H; hNle = homonorleucine, NH$_2$CH([CH$_2$]$_4$Me)CO$_2$H. [d] Toad bladder. [e] Weak depressor. [f] Natriuretic at high doses. [g] Activation of renal adenyl cyclase studied. [h] Index of persistence (see text). [i] Variable. [j] Comparison of receptor affinities and activity on three (aorta, arteriole, venule) vascular tissues.

329 C. W. Smith, M. F. Ferger, and W. Y. Chan, *J. Medicin. Chem.*, 1975, **18**, 822.
330 M. L. T. Fink, *Diss. Abs.*, 1975, **B35**, 4363B.
331 P. Dreyfuss, *J. Medicin. Chem.*, 1974, **17**, 252.
332 T. Barth and I. Kluh, *Coll. Czech. Chem. Comm.*, 1974, **39**, 506.

or replaced by a hydroxy-group[333] are highly active. An amino-group in the D-configuration in position 1 does, however, drastically reduce activity and the α-hydroxy-derivative in the D-configuration has now been prepared and shown also to have drastically reduced activity (27 U mg^{-1} oxytocic activity compared with 1607 U mg^{-1} for the L-diastereoisomer).[287]

A number of further studies have been carried out on analogues with ββ-dialkyl β-mercaptopropionic acid and other substituents related to penicillamine in position 1 which have inhibitory properties. Two of the more potent inhibitors, (353) and [penicillamine[1]] oxytocin, have been studied in an *in vivo* inhibition of the oxytocin-induced uterine contraction test in the rat, which is a model of one potential clinical application of these compounds, *i.e.* their possible use in threatened abortion and premature labour. The more potent inhibitor in other tests (353), suppressed more or less completely the oxytocin-induced contractions in every case. The penicillamine analogue also inhibited but was less effective; wide variations in sensitivity prevented quantitative comparison of results.[334] A more potent inhibitor than (353) results by the introduction of a cyclohexane ring in place of $C(Et)_2$.[289] The analogue (354) has no oxytocic, avian vaso-



(354)

depressor (AVD), or rat pressor activity but is a potent inhibitor of oxytocin in the first two tests. It also inhibits weakly the pressor effect of vasopressin.

Analogues which are natriuretic and inhibit the antidiuretic action of vasopression result when lipophilic amino-acids, particularly leucine, replace glutamine in position 4 of oxytocin (see Vol. 6, p. 445). The unusual anti-antidiuretic action of [Leu[4]] oxytocin is not enhanced by introducing penicillamine into position 1. The resulting analogue (369) shares the anti-oxytocic and anti-AVD properties of [penicillamine[1]] oxytocin, though at lower potency, but is devoid of natriuretic, anti-antidiuretic, or other actions on the kidney.[298] Similarly, when β-mercapto-ββ-diethylpropionic acid is introduced to give (370), the analogue retains its antihormonal activity but at lower potency.[288] The inhibition of antidiuresis, although often not found in analogues modified in position 4, is enhanced by a phenylalanine in that position. The analogue (388) has slight oxytocic activity (0.15% of oxytocin) and no pressor, AVD, or antidiuretic action. While natriuretic at only relatively high doses, it is an effective inhibitor of [Arg[8]] vasopressin, showing marked diuresis in an anti-antidiuretic assay.[308]

The tyrosine-substituted analogue (360) is a potent inhibitor of the oxytocic and AVD actions of oxytocin, with a potency comparable to that of [penicillamine[1]] oxytocin, but it does not inhibit the pressor effect of vasopressin.[293] It was originally synthesized as a possible crystalline analogue for *X*-ray studies.

[333] D. B. Hope and M. Wälti, *Proc. Roy. Soc. Med.*, 1974, **67**, 40.
[334] W. Y. Chan, J. J. Nestor, M. F. Ferger, and V. du Vigneaud, *Proc. Soc. Exp. Biol. Med.*, 1974, **146**, 364.

The *o*-iodotyrosine analogue (379), prepared both by iodination and total synthesis, has no pressor activity, slight antidiuretic activity, and similarly inhibits the uterine action of oxytocin.[305] The *o*-methyl derivative is also an inhibitor, suggesting that the inhibitory properties of these tyrosine-substituted analogues (and presumably also the methyl ether) arise through steric effects rather than through modification of the pK_a of the phenol. Again combination of 3,5-dibromotyrosine in position 2 with diethylmercaptopropionic acid in position 1 gives an analogue (361) less potent than (353).[293a]

Analogues (349) and (350) have been prepared as potential irreversible inhibitors of the vasopressin-stimulated loss of water from isolated toad bladder, based on the irreversible inhibitory action of N^{α}-bromoacetyloxytocin in this system. They would be expected to show selectivity for thiol nucleophiles and their lack of any specific inhibitory effect [285] could mean either that they are sterically not positioned to react with nucleophile or that a thiol nucleophile is not involved.

The [*p*-fluoro-L-phenylalanine^2] analogues of oxytocin (381) and desamino-oxytocin (359) have now been prepared;[292] previous attempts to obtain them were thwarted by synthetic difficulties. Both have high oxytocic activity (97 and 57 U mg^{-1}, respectively), some two to three times the activity of the [Phe^2] derivatives, and the highest yet found for 2-substituted oxytocins. The lower activity of the desamino derivatives is also found in the [Phe^2] case and is in contrast to the effect of substitutions in other parts of molecule. As with other 2-substituted analogues, (359) was a partial agonist under the assay conditions used. The ^{19}F n.m.r. spectra of the analogues were virtually identical with those of acyclic (S-substituted) precursors, suggesting no dramatic change in conformation involving the aromatic ring on forming the disulphide bond. It seems likely therefore that the proposed conformation for oxytocin in which tyrosine in position 2 is folded back over the 20-membered ring (see Vol. 5, p. 411) is not of major importance in solution for biological activity.

Desamino-[Glu^4] oxytocin (367) has somewhat higher oxytocic and milk-ejecting activities (13 and 34 U mg^{-1}, respectively) than [Glu^4] oxytocin.[297] The carboxyl thus introduced in position 4 has been used to attach the molecule to Sepharose by water-soluble di-imide coupling. The polymeric derivative (368) was inactive in the same tests.

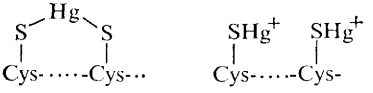
Earlier work on 'carba' analogues of oxytocin generally involved desamino derivatives for synthetic convenience. The 1,6-dicarba derivative of oxytocin (374) containing an L-α-amino-group has now been synthesized and its oxytocic (*ca.* 5 U mg^{-1}) and pressor (<0.1 U mg^{-1}) activities briefly noted.[300] The time course of the actions of a series of oxytocin analogues modified in the cystine portion ('carba' analogues, desamino-oxytocin, and the L-α-hydroxy analogue) has been studied *in vitro* in the K$^+$-depolarized, oil-immersed intact rat uterus. The results appear to support theories of disposition of the compounds involving multiple 'compartments' rather than on simple enzymic inactivation.[301] The [Glu(OMe)^4] analogue (384) has now been studied in the same system, and its breakdown in rat serum (which is fairly rapid) has also been studied. The results apparently confirm that the action of the analogue, which is prolonged as with desamino-oxytocin, is explained by the multiple compartment model.[8g] Other work on 'carba' analogues includes a study of the prolonged action of such

derivatives of oxytocin on the rat uterus *in vivo*,[335] and their hydro-osmotic activity on frog bladder.[336]

Replacement of glycine in position 9 by other amino-acids gives analogues (399)—(402) with very low oxytocic, antidiuretic, and AVD activities. Compounds (399), (401), and (402) are inhibitory in the AVD assay, and (400) and (401), as well as (398), are resistant to the toad bladder enzyme which cleaves aminoacyl–GlyNH_2 bonds.[311]

Three analogues (363), (371), and (377) of desaminodicarba-oxytocin have been studied in the search for a specific oxytocic with high uterotonic but minimal pressor and antidiuretic effects. The [Gly^7] analogue (377) was an improvement over the unsubstituted form but was still not entirely specific.[295]

Interaction of mercuric ions (under the conditions of potentiometric titration) with oxytoceine leads to the mercury mercaptide derivatives (375) and (376).[302]

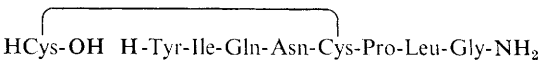


(375) (376)

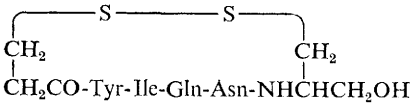
The open-chain derivatives (376) has less than 0.1% oxytocic activity, while (375) has *ca.* 1% activity, though this may perhaps not be intrinsic to the structure in view of the possibility of oxidation back to oxytocin.

Attempts at finding active compounds by reserving the peptide backbone, in the form of 'retro-all-D' analogues, have not been particularly successful with peptide hormones, although refinements in structural detail introduced to make the analogy as close as possible are showing increasing ingenuity (see p. 397). In the oxytocin case, four such analogues (403)—(406) of desaminotocinamide were completely inactive.[312]

The first cleavage of oxytocin by plasma oxytocinase is between the amino-acids in positions 1 and 2. The product (362) of this cleavage has now been synthesized and found to be virtually inactive.[294] Although it inhibited the action of cystine di-β-naphthylamide as a substrate for the enzyme, it had practically no effect on the enzyme's degradation of oxytocin. Desaminotocinol (378) has



(362)



(378)

[335] T. Barth, I. Krejčí, J. Vaněčková, K. Jošt, and I. Rychlík, *European J. Pharmacol.*, 1974, **25**, 67.

[336] T. Barth, N.-C. Le Bars, C. Roy, and S. Jard, *European J. Pharmacol.*, 1975, **32**, 214.

been found (even after purification by counter-current distribution) in preparations of desamino-oxytocin. It has no detectable AVD and very low (0.15 U mg^{-1}) antidiuretic activity.[304]

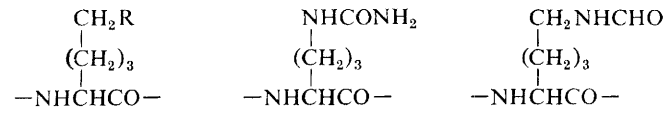
Binding studies with [(^{3}H)Tyr^2] oxytocin at uterine receptors have shown that the increased response resulting from pretreatment with oestrogens is accompanied by both an increased binding affinity (detectable after 6 h) and by an increase in the number of binding sites per uterus (detectable after 12 h and doubled after 24 h).[337]

Preparations of [Ser^4] tocinoic acid and amide, with the ring structure common to isotocin and glumitocin, and the corresponding desamino derivatives, have been described.[299] Other analogues reported include [His^4] oxytocin (385).[307]

The highly active analogue of oxytocin with an L-α-hydroxy-group in place of the α-amino-group shows a greater increase in pressor activity (10-fold) than in uterotonic activity (three-fold) compared with oxytocin. Deletion of the α-amino-group of vasopressin, on the other hand, leads to a fall in pressor activity. The L (423) and D (424) α-hydroxy analogues of vasopressin have now been synthesized, and (423) shows greater pressor activity than vasopressin.[317] As in the oxytocin case, (424) has very low activity.

The β-mercapto-ββ-diethylpropionic acid group, which in the case of oxytocin leads to an inhibitory analogue, has been introduced into the 1 position of vasopressin. The resulting analogue (420) has no agonist activity but inhibits the oxytocic and AVD effects of oxytocin with a potency about one-half to one-third of (353), similar to that of [penicillamine^1] oxytocin. However, it is about eight times as potent as (353) as an inhibitor of the pressor effects of vasopressin.[315] Desamino-[3,5-dibromotyrosine^2] vasopressin (425) has about one-sixth the anti-oxytocic and one-third the anti-AVD activity of (360), but like the latter it has no anti-pressor activity.[318]

The change in side-chain from leucine to lysine (or arginine) has to account for much of the difference in activities between oxytocin and vasopressin. A number of analogues have been prepared with side-chains intermediate between the two and it seems that a basic group is not crucial for antidiuretic activity; the ε-hydroxynorleucine analogue (448) retains high antidiuretic activity (75 U mg^{-1}).[331] Arguments based only on hydrogen-bonding in this case fail to



(447) R = Me
(448) R = OH

(451)

(452)

explain the much lower activity of the [citrulline^8] (451) and [ε-formyl-lysine^8] (452) analogues, so that there must be important structural requirements also. Significant pressor and antidiuretic activity is found in the homonorleucine analogue (447)[330] and some is retained of course in oxytocin itself. The homoarginine analogue (444) has somewhat higher antidiuretic and lower pressor

[337] M. S. Soloff, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 205.

activity than [Arg^8] vasopressin. In contrast, its desamino derivative (432) has less than half the antidiuretic activity of desamino-[Arg^8] vasopressin, though still more than either [Arg^8] vasopressin or (444). Its pressor activity is also much lower than that of any of the other three.[325]

The size of the normally 20-membered ring is important in determining the activity of compounds related to oxytocin or desamino-oxytocin (see Vol. 5, p. 416). Two analogues (421) and (422) of desaminovasopressin have now been reported with ring sizes of 19 and 21 atoms, respectively. The effect on pressor activity (<1% of the activity of the corresponding 20-membered ring peptide) is dramatic. The mercaptoacetic acid analogue (421) had slight oxytocin activity (3.3 U mg^{-1}) but inhibited the AVD activity of oxytocin. The larger ring peptide (422) was an inhibitor of both activities with a potency approaching that of desamino-[penicillamine^1] oxytocin.[316]

Replacement of the disulphide bridge of desaminovasopressin by a thio-ether link (with the CH_2 replacing the sulphur of cysteine 6 – '6-carba') has given analogues (429)—(431) with extremely high antidiuretic activity.[423] The antidiuretic : pressor ratio of (430) was very high, and in one assay (anaesthetized rat) the antidiuretic activity was claimed to be too high to be measured.

One of the most encouraging results arising from peptide chemistry in recent years has been the development and use in medicine of desamino-[D-Arg^8] vasopressin (DDAVP, Desmopressin). The analogue is used in diabetes insipidus as a replacement for vasopressin when the absence of the latter is the cause of this (relatively rare) disease in which failure of re-absorption at the kidney results in large quantities of urine being passed. DDAVP has the advantages over vasopressin of a long duration of action with a very high antidiuretic : pressor ratio. Following its synthesis[338] and initial clinical study,[339] the drug has been undergoing further clinical study during the past few years and the results of these studies are now being published.[340–345] It is clearly of much longer duration than vasopressin, and a convenient intranasal administration twice daily has proved generally satisfactory, with no side-effects observed.[340, 341]

The effects of DDAVP, (429), (430), and (431) on the activation of rat kidney adenyl cyclase have been studied.[324] Several of the analogues showed less activity in this system than might have been expected from their *in vivo* activities, presumably reflecting their increased metabolic stability, which is well established for DDAVP.[340] Similar studies have been carried out on five aminoacylated vasopressin and oxytocin derivatives, the absence of increased activity on incubation with renal plasma membranes pointing, it is claimed, to a direct effect of the analogues rather than breakdown to the parent hormone being responsible for their activities.[313]

[338] M. Zaoral, J. Kolc, and F. Šorm, *Coll. Czech. Chem. Comm.*, 1967, **32**, 1250.
[339] I. Vàvra, A. Machová, V. Holeček, J. H. Cort, M. Zaoral, and F. Šorm, *Lancet*, 1968, **i**, 948.
[340] C. R. W. Edwards, M. J. Kitau, T. Chard, and G. M. Besser, *Brit. Med. J.*, 1973, **iii**, 375.
[341] M. K. Ward and T. Russell Fraser, *Brit. Med. J.*, 1974, **iii**, 86.
[342] K.-E. Andersson, B. Arner, E. Fürst, and P. Hedner, *Acta Med. Scand.*, 1974, **195**, 17.
[343] V. Némethová and B. Lichardus, *Endokrinologie*, 1974, **63**, 137.
[344] A. S. Aronson, K.-E. Andersson, C. G. Bergstrand, and J. L. Mulder, *Acta Paediat. Scand.*, 1973, **62**, 133.
[345] W. Borner and E. R. Froesch, *Schweiz. Med. Woch.*, 1974, **104**, 579.

Introduction of valine in position 4 of [Arg^8] vasopressin leads to an increase in the antidiuretic : pressor ratio. Analogues (427), (428), and (442), in which this change is combined with deletion of the α-amino-group and introduction of D-arginine in position 8, have also been reported.[320–322] The effects on the ratio of each structural change appear to be roughly additive, and the analogue (428) combining all three changes is reported to have the highest antidiuretic : pressor ratio so far observed, although at these low levels of pressor activity quantitative comparisons are open to question, and clearly the change to D-arginine is the most crucial factor. In the case of arginine vasotocin, the [leucine^4] analogue (439) and its desamino derivative (426) both have a highly specific antidiuretic effect (the antidiuretic activity though high was erratic and is being studied further). In this series, introduction of leucine in position 4 decreases the antidiuretic : pressor ratio.[319] Desamino-[D-homoarginine^8] vasopressin has rather lower antidiuretic activity (5% of that of DDAVP), though the antidiuretic : pressor ratio is still high.[326]

The thiophen ring is less aromatic than phenyl, so that replacement of phenylalanine by L-β-(thienyl-2) alanine might be expected to give a vasopressin analogue whose activities are intermediate between those of vasopressin and vasotocin. The analogue (438) has greater oxytocic (19 U mg^{-1}) and AVD (87 U mg^{-1}) activity than vasopressin, but unlike [Ile^3] vasopressin retains high pressor and antidiuretic activity.[329]

The activities and receptor-binding affinities of four analogues, (443), (436), (437), and (440), on three different vascular tissues, aorta, arteriole, and venule, have been compared.[328] The results (*cf.* Vol. 6, p. 451) support the existence of receptors of different specificity in each tissue, though other interpretations based on metabolism or 'compartments' are not ruled out. Analogues have also been compared in the rat pressor test and on rat arteries.[346]

The relationship between structure, potency, and the time course of pressor and antidiuretic activities of a range of hormonogen (*cf.* ref. 347) analogues of vasopressin (407)—(419) has been studied further.[314] The properties *in vivo* of compounds of this kind are clearly difficult to explain fully and quantitatively, a result no doubt reflecting also the natural situations which the analogues are designed to mimic. The activities in Table 6 are accompanied by an 'index of persistence', which is defined as the exponential constant of the time course of elimination divided by the corresponding constant for vasopressin. Different combinations of time course and potency are found, for example (419) has about five times the duration of action of (409) but only 1% of its potency. Sarcosylvasopressin (414), which has high antidiuretic but no rat pressor action, has been found to inhibit the pressor response to vasopressin and is being studied further.

The pharmacological properties of vasopressin sequences (449) and (450), shortened at the *C*-terminus, have been studied.[332] The derivatives, prepared by limited proteolysis with trypsin followed by carboxypeptidase B, are of interest in view of the reported protective action of (450) against puromycin memory blocking.[348] The octapeptide (450) has slight antidiuretic (3 U mg^{-1}) and pressor

[346] B. M. Altura, *Proc. Soc. Exp. Biol. Med.*, 1974, **146**, 1054.
[347] J. Rudinger, in 'Drug Design. Vol. 2', ed. E. J. Ariens, Academic Press, New York, 1971, p. 394.
[348] S. Lande, J. B. Flexner, and L. B. Flexner, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 558.

(1 U mg^{-1}) activity, the values for the heptapeptide (449) being about one-tenth of these.

A study of the action of vasopressin on the toad bladder luminal membrane by freeze-fracture electron microscopy has revealed a remarkable ordering of protein particles in the membrane in response to the hormone.[349] This ordering into linear arrays involves quite large numbers of protein particles, and since the degree of ordering is quantitatively linked with vasopressin-induced osmotic water flow it is likely to be functionally significant. The effect of vasopressin on the amphibian urinary bladder is limited to the apical membrane of the epithelial cells. Vasopressin has now been shown to enhance the mucosal release of membrane-bound hydrolytic enzymes from these cells which are rich in intracellular secretion granules, and the effect is suggested as a possible cause of the increased membrane permeability and fluidity characterizing the response.[350]

Amino-acids in positions 3, 4, 7, and 8 occupy corners of the β-turns in the proposed conformation of oxytocin, and as such might be expected to be available for intermolecular interactions in receptor binding, rather than being partly involved in intramolecular interaction.[303] The expectation arising from this, that changes in these positions will be associated with major and selective changes in activities, is borne out by evolutionary changes which occur in positions 3, 4, and 8. Synthetic modifications in these positions lead in many cases to inactive analogues,[296, 306] and it has been argued that enzyme attack will take place principally outside the 20-membered ring and that analogues resistant to enzymic cleavage are therefore likely to result from modification of the *C*-terminal region.[303, 351]

Recent studies on the conformation of oxytocin include those based on the proton n.m.r. of ^{2}H- and ^{15}N-substituted molecules[352] on the ^{13}C n.m.r. of ^{13}C-enriched molecules,[353] among others,[354, 355] and the c.d. spectra of a series of vasopressin analogues have been studied and some tentative conclusions drawn about conformations in solution.[291, 356, 357]

The pig kidney receptor binding and adenyl cyclase activation properties of a series of some 21 oxytocin analogues were found to parallel one another closely, supporting a common mechanism,[358] although this was not the case with a series of vasopressin analogues.[359]

Effects of structural changes on the binding of oxytocin and vasopressin to bovine neurophysin have been reported.[360] Modifications in positions 2 and 3 and deletion of the *C*-terminal tripeptide leave the binding unaffected. This clearly

[349] W. Kachadorian, J. Wade, and V. DiScala, *Science*, 1975, **190**, 67.
[350] R. J. Pietras, B. J. Seeler, and C. M. Szego, *Nature*, 1975, **257**, 493.
[351] R. Walter and P. L. Hoffman, *Biochim. Biophys. Acta*, 1974, **336**, 294.
[352] A. F. Bradbury, A. S. V. Burgen, J. Feeney, G. C. K. Roberts, and D. G. Smyth, *F.E.B.S. Letters*, 1974, **42**, 179.
[353] J. H. Griffin, R. Alazard, C. Dibello, E. Sala, R. Mermet-Bonvier, and P. Cohen, *F.E.B.S. Letters*, 1975, **50**, 168.
[354] R. Deslauriers, I. C. P. Smith, and R. Walter, *J. Amer. Chem. Soc.*, 1974, **96**, 2289.
[355] R. Deslauriers, R. Walter, and I. C. P. Smith, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 265.
[356] I. Frič, M. Kodiček, K. Jošt, and K. Bláha, *Coll. Czech. Chem. Comm.*, 1974, **39**, 1271.
[357] I. Frič, M. Kodicek, M. Flegel, and M. Zaoral, *European J. Biochem.*, 1975, **56**, 493.
[358] C. Roy, T. Barth, and S. Jard, *J. Biol. Chem.*, 1975, **250**, 3157.
[359] C. Roy, T. Barth, and S. Jard, *J. Biol. Chem.*, 1975, **250**, 3149.
[360] E. Sedláková, I. Kluh, T. Barth, and K. Jošt, *Coll. Czech. Chem. Comm.*, 1974, **39**, 2998.

demonstrates a generally much lower specificity for neurophysin binding compared with biological receptor binding, although the situation is reversed with respect to the α-amino-group[317] (see Vol. 6, p. 453). The interaction between oxytocin and bovine neurophysin has been studied by ^{1}H n.m.r.[361]

Other analogues studied include [D- and L-homolysine[8]] vasopressins and their desamino derivatives[325, 327] and fully active oxytocins deuteriated in the cysteine 1 and 6 positions.[286] The dimer of [*o*-methyltyrosine[2]] oxytocin has been described.[362]

5 Pancreatic Hormones

Reviews have appeared on insulin receptors,[363] the mechanism of action of insulin,[364] the endocrine function of the pancreas,[365] nerve growth factor and insulin,[14b] diabetes mellitus,[366] insulin, glucagon, and growth hormone,[19b] insulin and growth hormone receptors,[216] and on the biological activity of structural variants of insulin.[367] Proceedings have been published of symposia on endocrinology and diabetes,[368] and on the synthesis, release, interaction with receptors, and mechanism of action of insulin,[369] including the mechanism of its release,[369a] its biosynthesis and secretion,[369b] interaction with membranes,[369c] and the relationship between insulin receptors and insulin resistance.[369d] The biosynthesis of insulin has been reviewed with particular emphasis on the proinsulin → insulin conversion.[370] The text of the Bakerian lecture on the chemistry and biochemistry of insulin has been published.[371] The total synthesis of human insulin was discussed in Vol. 7 (p. 292).

Insulin.—The concept of diabetes mellitus as a single hormone (insulin) deficiency has lost ground to a more general multiple-deficiency concept with insulin in a central though not solitary role. In particular, growth hormone and glucagon have been implicated.[19b, 372] The discovery that somatostatin lowers glucagon and insulin[372a] as well as growth hormone levels has provided a tool to investigate particularly the role of glucagon in diabetes, and a number of reports have suggested that it is important in diabetes,[160, 373–376] particularly in the develop-

[361] J. H. Griffin, J. S. Cohen, P. Cohen, and M. Camier, *J. Pharm. Sci.*, 1975, **64**, 507.
[362] M. Flegel, T. Barth, I. Frič, K. Bláha, and K. Jošt, *Coll. Czech. Chem. Comm.*, 1975, **40**, 2700.
[363] P. Cuatrecasas, M. D. Hollenberg, K.-J. Chang, and V. Bennett, *Rec. Progr. Hormone Res.*, 1975, **31**, 37.
[364] S. J. Pilkis and C. R. Park, *Ann. Rev. Pharmacol.*, 1974, **14**, 365.
[365] M. E. Krahl, *Ann. Rev. Physiol.*, 1974, **36**, 331.
[366] A. Y. Chang, *Ann. Reports Medicin. Chem.*, 1974, **9**, 182.
[367] D. G. Smyth, in 'Amino-acids, Peptides, and Related Compounds', MTP International Reviews of Science, Organic Chemistry, Series One, Vol. 6, ed. D. H. Hey and D. I. John, Butterworths, London, 1973, p. 109.
[368] 'Endocrinology and Diabetes. Proceedings of the 13th International Symposium, Philadelphia, 1973', ed. L. J. Kryston and R. A. Shaw, Grune and Stratton, New York, 1975.
[369] P. Cuatrecasas (Symposium Chairman), *Fed. Proc.*, 1975, **34**; (*a*) G. W. G. Sharp, C. Wollheim, W. A. Muller, A. Gutzeit, P. A. Trueheart, B. Blondel, L. Orci, and A. E. Renold, p. 1537; (*b*) M. A. Permutt and D. M. Kipnis, p. 1549; (*c*) M. D. Hollenberg and P. Cuatrecasas, p. 1556; (*d*) D. H. Lockwood, J. N. Livingston, and J. M. Amatruda, p. 1564.
[370] D. F. Steiner, W. Kemmler, H. S. Tager, and J. D. Peterson, *Fed. Proc.*, 1974, **33**, 2105.
[371] D. C. Hodgkin, *Proc. Roy. Soc.*, 1974, **186**, 191.
[372] K. Johansen, J. S. Soeldner, and R. E. Gleason, *Metabolism*, 1974, **23**, 1185.
[372a] G. W. De Vane, T. M. Silver, and S. S. C. Yen, *J. Clin. Endocrinol. Metab.*, 1974, **38**, 913.
[373] R. H. Unger and L. Orci, *Lancet*, 1975, **i**, 14.
[374] R. Guillemin, *Compt. rend.*, 1975, **280**, *D*, 2353.
[375] R. P. Eaton, *Diabetes*, 1975, **24**, 523.
[376] T. H. Maugh, *Science*, 1975, **188**, 920.

ment of diabetic ketoacidosis,[8h, 377] although contrary views have been put forward.[378–380]

Table 7 lists analogues of insulin prepared by partial/semi-synthesis or modification, and their associated biological activities. 'Immunoreactivity' studies are not detailed; these generally have been measured with pre-formed antibodies and do not necessarily give a guide to any inherent tendency to produce antibodies *in vivo*.

Table 7 *Analogues of insulin*

Compound number	*Structure* [a]	*Species* [b]	*Biological activity* [c]	*Test* [d]	*Ref.*
(453)	[Ala^{A1}]	p	++(+)		381
(454)	[D-Ala^{A1}]	p	+++(+)		381
(455)	[Glu^{A1}]	b	++	C	382
(456)	[Leu^{A1}]	b	+++	C	382
(457)	[Lys^{A1}]	b	++	C	382
(458)	[Pro^{A1}]	b	++	C	382
(459)	[Val^{A1}]	b	++	C	382
(460)	[Boc-Val^{A1}]	b			382
(461)	[Sar^{A1}]	o	+++	A	383
(462)	des-Gly^{A1}	o	+	C	384
		b	++	C, D	385
			+++	B	385
(463)	des-Gly^{A1}, des-Phe^{B1}		++	C [e]	386
(464)	$(Met)_3$A1, B1, B29		+++	C [e]	386
(465)	$(Ac)_2$A1, B29		*f*		387
(466)	$(Oxalyl)_2$A1, B29		*f*		387
(467)	$(Dodecoyl)_2$A1, B29		*f*		387
(468)	$CO(Met)_2$A1, B29	b	*g*		388, 389
(469)	$SO_2(Eoc)_2$A1, B29	b	*g*		390
(470)	[Glu^{A5}, Ala^{A12}, Ala^{A18}, Ala^{A21}]	o	+++	A	391, 392
(471)	[Hcy^{A7,A20}]	o	−	A	393
(472)	[Tyr(I)^{A14}] [h]	b	+++	A, B	394
(473)	[D-Asn^{A21}]	o	+++	A	395

[377] K. G. M. M. Alberti, N. J. Christensen, J. Iversen, and H. Ørskov, *Lancet*, 1975, **i**, 1307.
[378] A. J. Barnes, A. Bloom, M. F. Crowley, J. W. Tuttlebee, S. R. Bloom, K. G. M. M. Alberti, P. Smythe, and D. Turnell, *Lancet*, 1975, **ii**, 734.
[379] A. J. Barnes and S. R. Bloom, *Lancet*, 1976, **i**, 219.
[380] K. Lundbaek, S. E. Christensen, Aa. P. Hansen, J. Iversen, H. Ørskov, K. Seyer-Hansen, K. G. M. M. Alberti, and R. Whitefoot, *Lancet*, 1976, **i**, 215.
[381] R. Geiger, K. Geisen, H.-D. Summ, and D. Langer, *Z. physiol. Chem.*, 1975, **356**, 1635.
[382] G. Krail, D. Brandenburg, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 981.
[383] Y. Okada and P. G. Katsoyannis, *J. Amer. Chem. Soc.*, 1975, **97**, 4366.
[384] H. Berndt, H. G. Gattner, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 1469.
[385] D. Brandenburg, M. Biela, L. Herbertz, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 961.
[386] K. Rager and U. Buss, *Z. physiol. Chem.*, 1975, **356**, 647.
[387] C. Tompkins, P. H. Sönksen, and R. H. Jones, *J. Endocrinol.*, 1975, **65**, 59P.
[388] W.-D. Busse and F. H. Carpenter, *J. Amer. Chem. Soc.*, 1974, **96**, 5947.
[389] W.-D. Busse, S. R. Hansen, and F. H. Carpenter, *J. Amer. Chem. Soc.*, 1974, **96**, 5949.
[390] R. Obermeier and R. Geiger, *Z. physiol. Chem.*, 1974, **356**, 1631.
[391] U. Weber and M. André, in 'Peptides 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 153.
[392] U. Weber and M. André, *Z. physiol. Chem.*, 1975, **356**, 701.
[393] A. Cosmatos and P. Katsoyannis, *J. Biol. Chem.*, 1975, **250**, 5315.
[394] J. L. Hamlin and E. R. Arquilla, *J. Biol. Chem.*, 1974, **249**, 21.
[395] A. Cosmatos, S. Johnson, B. Breier, and P. G. Katsoyannis, *J.C.S. Perkin I*, 1975, 2157.

Table 7 (*cont.*)

Compound number	*Structure* [a]	*Species* [b]	*Biological activity* [c]	*Test* [d]	*Ref.*
(474)	des-Asn^{A21}, des-Ala^{B30}		++	C [e]	386
(475)	[(^{3}H)Phe^{B1}]	p	+++	B	396
(476)	des-Phe^{B1}	cam	+++	D	397
		b	+++	*i*	398
(477)	des-Phe^{B1}, Val^{B2}	b	+++	*i*	398
(478)	des-(Phe^{B1}-Asn^{B3})[Glp^4]	b	+++	*i*	398
(479)	des-(Phe^{B1}-Glu^{B4})	b	+++	*j*	398
(480)	A-B(2—25)	cam	+++	B	397
(481)	[Lys^{B22}]	h	+++	A	399
(482)	des-(B23—B30)		+	C [e]	387
	(= R)	p	−	A	400, 401
(483)	R-Phe	p	−	A	400, 401
(484)	R-Ala-Phe	p	−	A	400, 401
(485)	R-Val-Phe	p	−	A	400, 401
(486)	R-Phe-Phe	p	−	A	400, 401
(487)	R-Asn-Phe	p	++	A	400, 401
(488)	R-Ser-Phe	p	++	A	400, 401
(489)	R-D-Ala-Phe	p	+++	A	400, 401
(490)	R-D-Val-Phe	p	+++	A	400, 401
(491)	R-D-Phe-Phe	p	+++	A	400, 401
(492)	R-D-Ser-Phe	p	+++	A	400, 401
(493)	des-(B24—B30) (= R′)	p	−	A	400, 401
(494)	R′-Arg	p	−	A	400, 401
(495)	R′-Glu	p	−	A	400, 401
(496)	R′-Ser	p	−	A	400, 401
(497)	R′-Tyr	p	+++	A	400, 401
(498)	R′-Gly-Phe	p	+++	A	400, 401
(499)	R′-Gly-D-Phe	p	−	A	400, 401
(500)	R′-NHBzl	p	+++	A	400, 401
(501)	des-(B26—B30)	p	+++	A	402
		b	+++	B, C	403
		cam	+++	D	397
(503)	des-(B29—B30)	b	+++		404
		p	+++		404
(504)	des-Ala^{B30}	b	+++	A	405

[a] $SO_2(Eoc)_2$ = $SO_2(CH_2CH_2OCO—)_2$; Hcy = homocysteine. [b] Human = h, Bovine = b, Porcine = p, Ovine = o, Camel = cam. [c] Symbols for potency are used as in Table 3. [d] The following tests are indicated: A, mouse convulsion assay; B, *in vivo* blood glucose assay; C, isolated fat cell assay; D, *in vitro* glucose oxidation. [e] Similar activity was found in five other tests. [f] Effect on glucose metabolism studied. [g] Cross-linking agent. [h] An estimated 76% substituted on A14. [i] Unspecified *in vitro* and *in vivo* tests. [j] Unspecified *in vivo* test.

396 P. A. Halban and R. E. Offord, *Biochem. J.*, 1975, **151**, 219.
397 W. O. Danho, H.-G. Gattner, D. Nissen, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 1405.
398 R. Geiger and D. Langner, in 'Peptides, 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 159.
399 P. G. Katsoyannis, J. Ginos, A. Cosmatos, and G. P. Schwartz, *J.C.S. Perkin I*, 1975, 464.
400 S.-C. Chu, K.-T. Li, C.-P. Tsao, Y.-S. Chang, and T.-H. Lu, *Scientia Sinica*, 1973, **16**, 71.
401 The Shanghai Insulin Research Group, *Scientia Sinica*, 1973, **16**, 61.
402 P. G. Katsoyannis, J. Ginos, G. P. Schwartz, and A. Cosmatos, *J.C.S. Perkin I*, 1974, 1311.
403 H. G. Gattner, *Z. physiol. Chem.*, 1975, **356**, 1397.
404 H. Gregory, *F.E.B.S. Letters*, 1975, **51**, 201.
405 S. P. Krasnoshchekova, V. F. Krivtsov, E. N. Voluiskaya, A. I. Ivanova, V. V. Abramova, V. P. Fedetov, and Y. P. Shvachkin, *Zhur. obschei Khim.*, 1974, **44**, 2341.

The methods of synthesis established for insulin are now providing a steady flow of semi-synthetic analogues for structure–activity studies. The procedure of total synthesis of one (modified) chain and recombination with the second from natural insulin has been used to provide a variety of analogues. An analogue (473) in which asparagine is replaced by D-asparagine has *ca.* 32% of the activity of insulin.[395] The result is thought to be due to interference with a salt bridge between the *C*-terminal carboxy-group (at A21) and the B22 arginine, with a consequent change in conformation, a suggestion supported by c.d. and o.r.d. studies. The [$AsnNH_2^{A21}$] analogue is being prepared to study this further, although the activity found in (473) must still be considered quite high.

An analogue of sheep insulin has been prepared in which the two interchain disulphide bridges are elongated by a CH_2 residue (replacing cysteine in positions A7 and A20 by homocysteine). The analogue, (471), was biologically inactive ($<5\%$) in the mouse convulsion assay. The result demonstrates the crucial importance of the correct spacing of the two chains, possibly because of a magnified effect at the active site of a small (*ca.* 1.5 Å) movement at the disulphide bridge.[393] The interpretation requires the assumption that the two chains were recombined with the correct alignment, which appears likely to be the case.[393]

A semi-synthetic analogue (481) of human insulin with lysine in place of arginine at B22 has been prepared by total synthesis of the modified B chain and recombination with porcine (identical with human) A chain.[399] Some 56% activity was retained.

The determination of the three-dimensional structure of insulin has proved to be an important stimulus. It has led to the design and synthesis of molecules, *e.g.* (468) and (469), which share with proinsulin the ability to adopt the correct arrangement of cross-links and therefore the prospect of improved methods of synthesis[388–390, 406] (see Vol. 7, p. 290), it is being invoked to explain the molecular evolution of insulin,[407] and perhaps the most exciting application is the hope it raises of designing a small, more easily synthesized structure with the features necessary for biological activity. As a first step attempts have been made to bring together the results of *X*-ray, c.d., receptor binding and biological activity–structure studies in order to define the biologically important part of the molecule, and a possible binding region has been described (Figure 1).[408, 409] The Chinese insulin research groups, who have determined the three-dimensional structure to 1.8 Å,[410] have also commented on this problem,[411] suggesting different regions for binding and transmission of the message at the receptor.

The four insulin derivatives (463), (464), (474), and (482), which have been described earlier, have been studied in a range of *in vitro* and *in vivo* systems (including labelled glucose incorporation in a variety of tissues and anti-lipolysis) with results broadly expected from earlier work. The loss of activity can be

[406] A. Wollmer, D. Brandenburg, H.-P. Vogt, and W. Schermutzki, *Z. physiol. Chem.*, 1974, **355**, 1471.
[407] T. L. Blundell and S. P. Wood, *Nature*, 1975, **257**, 197.
[408] R. A. Pullen, D. G. Lindsay, S. P. Wood, I. J. Tickle, T. L. Blundell, A. Wollmer, G. Krail, D. Brandenburg, H. Zahn, J. Gliemann, and S. Gammeltoft, *Nature*, 1976, **259**, 369.
[409] R. A. Pullen, J. A. Jenkins, I. J. Tickle, S. P. Wood, and T. L. Blundell, *Mol. Cell. Biochem.*, 1975, **8**, 5.
[410] The Peking Insulin Structure Research Group, *Scientia Sinica*, 1973, **16**, 136; 1974, **17**, 752.
[411] The Peking and Shanghai Insulin Research Groups, *Scientia Sinica*, 1974, **17**, 779.

correlated with changes in the conformation of the molecules, the least active differing most from the native structure.[387]

A series of insulin analogues, (482)—(500), shortened at the *C*-terminal part of the B chain, have been prepared using (482) prepared enzymically from porcine insulin.[400, 401] The biological activities, which have been compared in the results

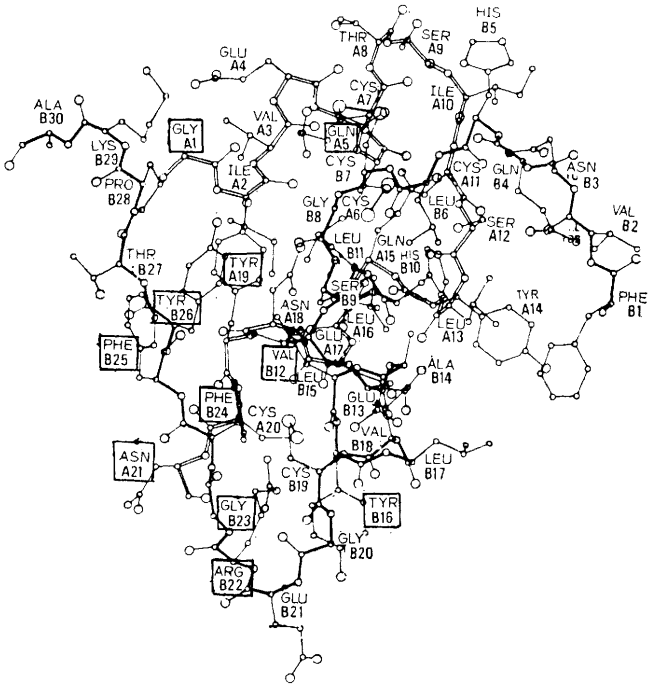


Figure 1 *The proposed receptor-binding region of insulin; the residues involved are indicated by boxes*
(Reproduced by permission from *Nature*, 1976, **259**, 369)

given in Table 7 with that of insulin resynthesized under the same conditions (5—10% as active as pure insulin before further purification), indicate that an aromatic amino-acid is required at B24 or B25 for activity. Replacement of glycine at B23 by a number of L-amino-acids led to inactive analogues, while in contrast (and with intriguing similarity to the case of Gly^6 in LH-RH) D-amino-acid replacements at B23 gave active analogues. Otherwise, the *C*-terminal region is relatively unimportant for the biological activity, as has been found in other studies.[381, 402, 405, 412] The shortened B-chain insulin analogues (476), (480), and (501) prepared from camel insulin have high biological activity, similar to that found in the corresponding bovine analogues, except that the des-Phe^{B1} analogue (476) has somewhat lower activity (60% *in vitro*) than might have been expected.[397]

The des-[B chain (29—30) dipeptide] derivatives (503) of porcine and bovine insulins have been prepared from the native hormones by the action of an enzyme

[412] L. Kerp, S. Steinhilber, H. Kasemir, J. Han, H. R. Henrichs, and R. Geiger, *Diabetes*, 1974, **23**, 651.

(from the fungus *Armillaria mellea*) which specifically cleaves peptide bonds of Lys-X sequence.[404] Both derivatives have full biological activity.

In contrast to the *C*-terminal part of the B chain, the A1 glycine is very important for biological activity, as shown by the semi-synthesis of des-Gly^{A1}-ovine insulin (462), which is virtually inactive (0.4—0.6%) in the fat cell assay.[384] The bovine derivative, prepared by partial synthesis, had 1—2% activity in the fat cell assay, although showing rather higher *in vivo* hypoglycaemic activity.[385] Replacement of A1 glycine by other amino-acids has generally led to a sharp fall in activity. Of six such analogues of bovine insulin, (455)—(460), the [Leu^{A1}] derivative (456) had the highest activity, some 16% of insulin in the *in vitro* fat cell assay.[382] On the other hand, while [Ala^{A1}] porcine insulin was also of diminished activity, the [D-Ala^{A1}] analogue (454) was as active as insulin towards elevated blood glucose and slightly more active towards glucose uptake into rat diaphragm.[381] Since the specific binding to rat liver receptors was less for (454) than for insulin (though still more than the L-alanine derivative), increased metabolic stability may be a factor. Replacement by sarcosine at A1 gives a highly active analogue.[383]

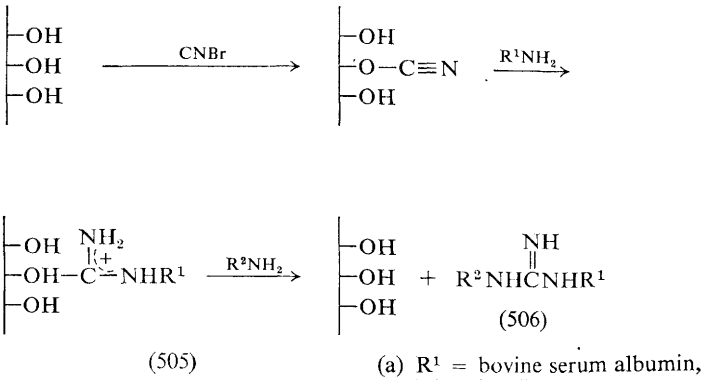
The analogue (470) has been prepared by fragment condensation on solid phase of the A chain and combination with natural B chain. This approach gave a more active product than that involving stepwise synthesis of the A chain.[391, 392]

Cuatrecasas has reviewed recent studies on the insulin receptor.[363, 369c] The pitfalls in this sort of work are many and include for example the difficulty of distinguishing specific enzymes present in receptor cells from the biological receptor itself.[128] Nevertheless progress in this area has been dramatic in recent years and the prospect of an explanation of hormone action in molecular terms now seems closer. In his more recent interpretations, Cuatrecasas is moving away from the concept of a single receptor–adenyl cyclase enzyme molecule, and proposes instead that the receptor and enzyme can move around in the plane of the fluid lipid bilayer, the hormone receptor complex but not the unoccupied receptor being able to interact with the enzyme and to activate it, essentially a reaction mechanism in two dimensions.[413] As with other hormones, stimulation with insulin can regulate the receptor concentrations as has been demonstrated directly in cell culture.[414] An insulin-induced reduction of membrane receptor concentrations, which occurs at higher levels of insulin than those which would normally saturate the receptors and which is independent of cell damage and blocking of protein synthesis, has been interpreted in terms of inherent proteolytic enzyme activity in the insulin molecule.[415] In a comparison of receptor binding with *in vitro* and *in vivo* biological assays for a range of modified insulins, the activities in each system changed in parallel.[416]

Differences between the *in vitro* and *in vivo* potencies of cross-linked insulins and those of insulin have been explained by the slower rate of metabolism of the former.[417]

[413] P. Cuatrecasas, *Biochem. Pharmacol.*, 1974, **23**, 2353.
[414] J. R. Gavin, J. Roth, D. M. Neville, P. de Meyts, and D. N. Buell, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 84.
[415] D. Huang and P. Cuatrecasas, *J. Biol. Chem.*, 1975, **250**, 8251.
[416] P. Freychet, D. Brandenburg, and A. Wollmer, *Diabetologia*, 1974, **10**, 1.
[417] R. H. Jones, D. I. Dron, M. J. Ellis, and P. H. Sönksen, *J. Endocrinol.*, 1975, **65**, 58P.

An explanation has been proposed for the mysterious 'superactive' insulin produced as a soluble by-product of insulin coupled by cyanogen bromide activation to Sepharose.[418–420] The substance has greater activity than insulin particularly in certain situations (for example with species of genetically obese mice) where insulin has a diminished effect.[419, 420] The mechanism shown in Scheme 1 was proposed for cyanogen bromide activation, the resulting *O*-



Scheme 1

glycosyl-N-substituted isourea (505) being stable towards buffers free of amines but reacting with amines (for example bovine serum albumin) to give the soluble active substance (506a).[418] Whether this is the superactive derivative has been put in some doubt more recently, because removal with ammonium bicarbonate also gives a highly active derivative.[421] Surprisingly, guanidination of lysine appears not to be involved and histidine and tyrosine are suggested as possible points of attachment. What is perhaps most surprising is how little was known of the chemical nature of the link formed using cyanogen bromide activation, despite widespread use of the method.

A monoiodinated bovine insulin has been purified and the iodine shown to be principally (76%) on tyrosine A14. The derivative is fully active.[394] The ^{125}I and ^{127}I derivatives have also been prepared.[422]

A study of the n.m.r. spectra of ^{13}C-enriched carbamylated insulins has given information on the surrounding structure and particularly on the relative mobility of the location (A1, B1, and B29 substitution was involved) of the parts of the molecule to which the carbamyl groups were attached.[423]

[418] M. Wilchek, T. Oka, and Y. J. Topper, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1055.
[419] T. Oka and Y. J. Topper, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1630.
[420] T. Oka and Y. J. Topper, *Science*, 1975, **188**, 1317.
[421] Y. J. Topper, T. Oka, B. K. Vonderhaar, and M. Wilchek, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 793.
[422] J. C. Sodoyez, F. Sodoyez-Goffaux, M. M. Goff, A. E. Zimmerman, and E. R. Arquilla, *J. Biol. Chem.*, 1975, **250**, 4268.
[423] J. J. Led, D. M. Grant, W. J. Horton, F. Sundby, and K. Vilhelmsen, *J. Amer. Chem. Soc.*, 1975, **97**, 5997.

Insulin and a range of related structures have been studied as substrates of the enzyme glutathione-insulin transhydrogenase. While a range of compounds were all effective, insulin appeared to be the preferred substrate.[424]

Other reported studies include the effect of insulin, proinsulin and three analogues substituted at A1, B29 on glucose metabolism,[387] the preparation of semi-synthetic tritiated (Phe^{B1}) porcine insulin (475) with up to 20 Ci $mmol^{-1}$ specific activity and full biological activity,[396] the preparation of methylsulphonyl-ethyloxycarbonyl substituted insulins,[425] and the immunological properties of trifluoroacetyl insulins.[426]

Recent studies [427–430] on the proinsulin C-peptide or connecting peptide are not discussed in detail here.

Insulin-like activity has been reported in tetrapeptides related to (507) which contains arginine and forms part of the insulin sequence,[431, 432] though it is not

Arg-Gly-Phe-Phe
(507) (insulin B22–B25)

clear whether the activity is related to the latter or to the former feature (arginine itself having certain insulin-like effects).

Glucagon.—The three-dimensional structure of glucagon has now been accurately determined by *X*-ray diffraction. Unlike other protein structures derived by crystallography there is no hydrophobic core. Instead it is suggested that while there is probably not a single conformation in solution, the particular conformation in the trimeric crystal form is stabilized by hydrophobic interactions between the monomers, with the hydrophilic parts of the molecules exposed to the solvent.[433] This makes the glucagon structure more flexible than that of insulin.[409] A further intriguing suggestion is that a similar process takes place at the hydrophobic receptor site and that the hydrophobic region in this conformation of the monomer therefore represents the biologically important structure.[433] The conformation in solution has been predicted by Chou and Fasman using their new rules.[434] The predictions include the interesting observation that there should be two stable conformations, one containing only β-secondary structure and the second (argued from past structure–activity studies to be the biologically inactive form) with some α-helix and some β-structure. They go on to suggest that small modifications in the sequence, for example Phe^{22}-Val^{23} → Glu-Glu, would cause a switch from the β-structure to the inactive α-helical form.

[424] P. T. Varadani, M. A. Nafz, and M. L. Chandler, *Biochemistry*, 1975, **14**, 2107, 2115.
[425] R. Geiger, R. Obermeier, and G. I. Tesser, *Chem. Ber.*, 1975, **108**, 2758.
[426] R. A. Paselk and D. Levy, *Biochim. Biophys. Acta*, 1974, **359**, 215.
[427] N. Yanaihara, N. Sakura, C. Yanaihara, T. Hashimoto, A. H. Rubenstein, and D. F. Steiner, *Nature*, 1975, **258**, 365.
[428] V. K. Naithani, M. Dechesne, J. Markussen, and L. G. Heding, *Z. physiol. Chem.*, 1975, **356**, 997.
[429] V. K. Naithani, M. Dechesne, J. Markussen, L. G. Heding, and U. D. Larsen, *Z. physiol. Chem.*, 1975, **356**, 1305.
[430] A. Wollmer, H.-P. Vogt, V. K. Naithani, and H. Zahn, *Z. physiol. Chem.*, 1975, **356**, 1655.
[431] K. Eisele, *Z. physiol. Chem.*, 1975, **356**, 845.
[432] G. Weitzel, K. Eisele, and W. Stock, *Z. physiol. Chem.*, 1975, **356**, 583.
[433] K. Sasaki, S. Dockerill, D. A. Adamiak, I. J. Tickle, and T. Blundell, *Nature*, 1975, **257**, 751.
[434] P. Y. Chou and G. D. Fasman, *Biochemistry*, 1975, **14**, 2536.

The reactions of glucagon with the nucleophiles taurine, ethylenediamine, and glycineamide (using a water-soluble carbodi-imide) have been studied. The number of carboxy-groups modified was found to increase with the concentration of guanidine in the reaction mixture, pointing perhaps to a specific folded conformation in solutions of low ionic strength. The derivative with taurine attached to the *C*-terminus had *ca.* 10% activity towards rat liver membrane adenyl cyclase activation.[435]

The biological activity of three glucagon analogues, produced by modification of the native hormone, has been studied further. Mono-iodoglucagon is as active as glucagon in activating hepatic adenyl cyclase and in binding to hepatic plasma membrane sites. The des-(Asn^{28}-Thr^{29})-[homoserine^{27}] glucagon produced by cyanogen bromide cleavage has *ca.* 2% activity but this could all be explained by contaminating unchanged glucagon. The des-His^{1} derivative, formed by Edman degradation, has *ca.* 2% activity in activating hepatic adenyl cyclase and *ca.* 7% activity in binding to membrane sites. It behaves as a partial weak agonist with a maximal effect some 70% that of glucagon.[436]

A range of iodine-substituted glucagons has been prepared and found to be more active than glucagon towards rat liver cell membrane binding and adenyl cyclase activity.[437] The maximum increase was five-fold in the adenyl cyclase test (with an average substitution of 4 mol of iodine) and two-fold in the membrane binding system (with an average substitution of 3 mol of iodine). The effects are probably caused by a substantial (2—20-fold) decrease in plasma and membrane inactivation rates. Beyond 4 mol of iodine there is a decrease in biological activity. A similar study has been carried out with acetylated glucagons.[438]

Other reported work includes the effect of inhibitors of the enzymic breakdown of glucagon (including ACTH, secretin, and angiotensinogen sequences) on its activation of adenyl cyclase in liver cell membranes,[439] the effect of trinitrophenylation of α- and ε-amino-groups in glucagon on its biological activity and conformation,[440] and structure–antigenicity relationships of glucagon and related peptides.[441] An anglerfish proglucagon has been characterized.[442]

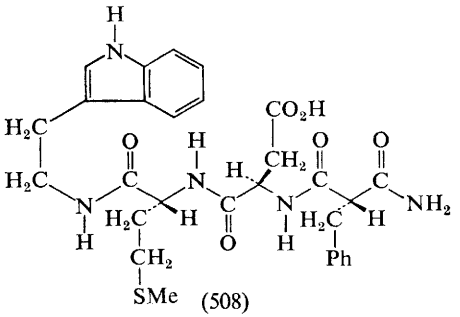
6 Gastrointestinal Hormones

The text of the 1973 Bayliss-Starling lecture has now been published [443] and provides a useful and very readable account of recent advances in studies on GI hormones. Other reviews have dealt with gastrin,[444, 445] the endocrinology of the

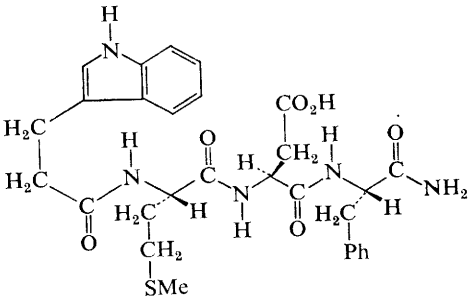
[435] G. E. Wheeler, R. M. Epand, and D. Barrett, *Biochim. Biophys. Acta*, 1974, **372**, 440.
[436] M. C. Lin, D. E. Wright, V. J. Hruby, and M. Rodbell, *Biochemistry*, 1975, **14**, 1559.
[437] B. Desbuquois, *European J. Biochem.*, 1975, **53**, 569.
[438] B. Desbuquois, *European J. Biochem.*, 1975, **60**, 335.
[439] B. Desbuquois, F. Krug, and P. Cuatrecasas, *Biochim. Biophys. Acta*, 1975, **343**, 101.
[440] R. M. Epand and G. E. Wheeler, *Biochim. Biophys. Acta*, 1975, **393**, 236.
[441] A. Vinik and A. Hardcastle, *Hormone Metab. Res.*, 1974, **6**, 396.
[442] A. C. Trakatellis, K. Tada, K. Yamaji, and P. Gardiki-Kouidou, *Biochemistry*, 1975, **14**, 1508.
[443] R. A. Gregory, *J. Physiol.*, 1974, **241**, 1.
[444] J. H. Walsh and M. I. Grossman, *New Eng. J. Med.*, 1975, **292**, 1324, 1377.
[445] J. E. McGuigan, *Vitamins and Hormones*, 1974, **32**, 47.

gut,[446] gastric inhibitory peptide (GIP),[447] gastrointestinal hormones,[448–451] hormone-secreting tumours,[451a] and gastrointestinal motility.[452]

Gastrin.—The importance of the direction of the peptide chain in gastrin has been demonstrated by the synthesis of the 'retro-D' isomer (508).[453] Normally in such analogues, although the configuration of the side-chains is similar to the native



(508)



(509)

structure, both the direction of the peptide bonds and the identity of the end-groups (amino or carboxy) differ. In the present case, the desamino derivative (509) is fully active and by introducing a malonic acid derivative as shown, the structure (508) was prepared which essentially differs from (509) only in the direction of the bonds. Despite the closeness in structure, (508) was completely inactive (< 0.1% activity) and showed no inhibitory effects.

[446] M. Guiducci, in 'Endocrinology of the Gut', ed. W. Y. Chey and F. P. Brooks, C. B. Slack Inc., New Jersey, 1974, p. 103.

[447] J. C. Brown, J. R. Dryburgh, S. A. Ross, and J. Dupré, *Rec. Progr. Hormone Res.*, 1975, **31**, 487.

[448] F. P. Brooks, *J. Amer. Med. Assoc.*, 1975, **232**, 357.

[449] E. Wünsch, *Helv. Med. Acta*, 1974, **37**, 307.

[450] 'Handbook of Experimental Pharmacology', ed. J. E. Jorpes and V. Mutt, Springer-Verlag, Berlin, 1973.

[451] S. R. Bloom, *Proc. Roy. Soc. Med.*, 1975, **68**, 710.

[451a] L. H. Rees, *J. Endocrinol.*, 1975, **67**, 143.

[452] 'Proceedings of the 4th International Symposium on Gastrointestinal Motility', ed. E. E. Daniel, Mitchell Press, Vancouver, 1974.

[453] C. F. Hayward and J. S. Morley, in 'Peptides, 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 287.

Four acylated derivatives (510)—(513) of the gastrin *C*-terminal pentapeptide have been prepared for photolabelling studies.[454] As a model experiment, the

R-Gly-Trp-Met-Asp-Phe-NH_2

(510) R = Ph(4-N_3)CO—

(511) R = Ph(1-NO_2,4-N_3)CO—

(512) R = Ph(4-$COCH_3$)CO—

(513) R = Ph(4-COPh)CO—

covalent binding to bovine serum albumin (BSA) and lysozyme has been studied. The gastrin pentapeptide is known to bind more effectively to BSA than to lysozyme, and under similar conditions 50% of the photolabelled peptide becomes attached to BSA while only 8% becomes attached to lysozyme. Studies using hormone receptors are now under way.

Iodogastrins have been prepared by iodination of synthetic human gastrin I.[455] The mono-iodo derivative is fully active in a rat gastric acid secretion test, and binds to gastrin antibodies as effectively as gastrin. The di-iodo derivative has 30—40% biological activity but still binds effectively (90—100%) to the antibodies.

The situation found with many hormones that metabolic breakdown products of 'the hormone', or precursors, have biological effects, is particularly evident with gastrin. Clearly the biological role can only be understood fully by considering the totality of molecular species which are both present and active, since the hormone as initially identified may simply be the one species which was first isolated. With gastrin, species identified in addition to gastrin itself include 'big big gastrin', 'big gastrin', and 'little gastrin',[8i, 443, 445, 456–459] and there may be others.[8j] The stability, as well as the inherent biological activity, is important in determining the role of such species. The larger gastrin precursors are more stable than the smaller species (for example 'big big gastrin' t_{50} = 90 min, 'big gastrin' t_{50} = 9 min, gastrin t_{50} = 3 min).[460]

The peptide (514) has been reported to show slight inhibition of gastrin-mediated gastric secretion, but no inhibition of total acid secretion. Its effect is suggested to be on gastric volume secretion.[461]

Z-Trp-Met-Gly-Phe-NH_2

(514)

Intestinal Hormones.—Tissue from intestinal wall has proved in the past to be a rich source of biologically active peptides and judging by recent reports,[8k] the supply is by no means exhausted. Not all of the peptides can be identified as hormones but they are grouped together here for convenience.

[454] R. E. Galardy, L. C. Craig, J. D. Jamieson, and M. P. Prinz, *J. Biol. Chem.*, 1974, **249**, 3510.
[455] J. P. Girma, J. L. Morgat, P. Fromageot, M. Dubrasquet, J. P. Accary, J. Vatier, and S. Bonfils, *Biochimie*, 1974, **56**, 763.
[456] R. Gregory and H. Tracy, *Gut*, 1974, **15**, 683.
[457] J. H. Walsh, *Ann. Rev. Physiol.*, 1975, **37**, 211.
[458] J. F. Rehfeld, *Digestion*, 1975, **11**, 397.
[459] H. T. Debas, J. H. Walsh, and M. I. Grossman, *Gut*, 1974, **15**, 686.
[460] E. Straus and R. S. Yalow, *Gastroenterology*, 1974, **66**, 936.
[461] C. T. Wang, I. D. Kulesha, P. L. Stefko, and S. S. Wang, *Internat. J. Peptide and Protein Res.*, 1974, **6**, 59.

Evidence on the secondary structure of secretin has been re-examined; the α-helical region is apparently near the *C*-terminus as was earlier thought. Replacement of glutamic acid in position 9 by glutamine or of aspartic acid in position 15 by asparagine in the *C*-terminal tricosapeptide leads to a fall in helical character, both changes together having a more pronounced effect. On smooth muscle preparations, the decrease in biological activity is paralleled by these changes in secondary structure.[8e]

A new role for secretin has been suggested by an observed rise in its level during starvation and it may be that its lipolytic effects under these conditions have an important physiological role.[462]

Anaphylactoid skin reactions have been observed after intradermal administration of secretin, and it has been suggested that there is a factor conditioning this response, present particularly in patients with duodenal ulcer.[463]

It has been suggested that vertebrate cells capable of secreting peptides have a common embryological origin in the neural crest, cells of which are proposed to migrate to a variety of hormone-releasing sites, and that they belong to a common ('APUD') type.[8l] While this origin is disputed in the case of peptide-secreting cells in pancreatic and gastrointestinal tissue, peptides characteristic of such cells are being found to be more widespread than previously thought and the use of immunohistochemical methods is providing an increasing number of such results. The presence of substance P in both neural and intestinal tissue has been a pointer towards such a unifying theory for some time. Further evidence comes from hormone-secreting tumours (for a recent review see ref. 451*a*) which are found in a variety of tissues of neural crest origin, for example those recently found to secrete the vasoactive intestinal peptide (VIP)[8m, 8n, 463a] and which appear to be involved in the Verner–Morrison (watery diarrhoea) syndrome. More recently, immunoreactive somatostatin has been found in pancreatic D cells whose secretory products have been a mystery since the original identification of the cells in 1931,[165] and immunoreactive gastrin has been identified in the vertebrate cortex.[464]

Replacement of methionine in position 17 of the VIP (14—28) pentadecapeptide by norleucine leads to an equally active analogue. Both peptides have *ca.* 2% of the activity of VIP itself on rat stomach strip, guinea-pig trachea and gall bladder, and chick rectum *in vitro*.[465]

The o.r.d. spectrum of VIP in water points to a low α-helical content. The addition of small quantities of organic solvent brings about a pronounced increase in helical character, and the hypothesis has been tentatively advanced that this bears some similarity to the binding at the biological receptor. The o.r.d. spectra of a range of fragments of VIP were measured and compared with their known biological activity (see Vol. 6, p. 462). The extent of the change in spectrum on adding organic solvent was found to correlate with the biological activities.[466]

[462] R. W. Henry, R. W. J. Flanagan, and K. D. Buchanan, *Lancet*, 1975, **ii**, 202.
[463] H. W. Baenkler, W. Domschke, S. Domschke, L. Demling, E. Wünsch, and E. Jaeger, *Brit. Med. J.*, 1975, **ii**, 747.
[463a] S. Said and G. Faloona, *New Eng. J. Med.*, 1975, **293**, 155.
[464] J. J. Vanderhaeghen, J. C. Signeau, and W. Gepts, *Nature*, 1975, **257**, 604.
[465] M. Bodanszky, C. Y. Lin, and S. I. Said, *Bio-organic Chem.*, 1974, **3**, 320.
[466] M. Bodanszky, A. Bodanszky, Y. S. Klausner, and S. I. Said, *Bio-organic Chem.*, 1974, **3**, 133.

Details of a recent synthesis of fully active VIP have been published.[467] The structure of chicken intestine VIP has been reported.[468]

Synthesis of the 43 amino-acid sequence (515) of porcine gastric inhibitory peptide (GIP) has been described.[469] It inhibited the gastrin-stimulated gastric

Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-Arg-Gln-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Gln-Lys-Gly-Lys-Lys-Ser-Asp-Trp-
20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37

Lys-His-Asn-Ile-Thr-Gln
38 39 40 41 42 43

(515) porcine GIP

acid secretion of the dog Heidenhein pouch preparation and caused release of insulin in the rat.

A synthesis of porcine motilin (516) has been described. The synthetic docosapeptide on i.v. infusion in dogs caused contractile activity of stomach and duo-

Phe-Val-Pro-Ile-Phe-Thr-Tyr-Gly-Glu-Leu-Gln-Arg-Met-Gln-Glu-Lys-Glu-Arg-
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Asn-Lys-Gly-Gln
19 20 21 22

(516) porcine motilin

denum similar to their characteristic 'burst' activity.[470, 471] A radioimmunoassay of motilin has been described.[472] A second synthesis has also been reported.[473] The peptide behaved similarly to the natural material in a variety of biological tests including the *in vitro* contractile effect on rabbit duodenum, jejunum, and colon.

The *C*-terminal octapeptide of cholecystokinin (CCK) is highly active see Vol. 6, p. 461), and a number of further biological studies of it have been reported.[474–477]

Other reported studies include the action of [Nle^{13}] motilin on the GI tract,[478] the effects of bombesin and bombesin-like peptides on gastrointestinal mycoelectric activity,[479] and the synthesis of secretin.[480]

[467] M. Bodanszky, Y. S. Klausner, C. Y. Lin, V. Mutt, and S. I. Said, *J. Amer. Chem. Soc.*, 1974, **96**, 4973.
[468] A. Nilsson, *F.E.B.S. Letters*, 1975, **60**, 322.
[469] H. Yajima, H. Ogawa, M. Kobuta, T. Tobe, M. Fujimura, K. Henmi, K. Torizuka, H. Adachi, H. Imura, and T. Taminato, *J. Amer. Chem. Soc.*, 1975, **97**, 5593.
[470] H. Yajima, Y. Kai, and H. Kawatani, *J.C.S. Chem. Comm.*, 1975, 159.
[471] Y. Kai, H. Kawatani, H. Yajima, and Z. Itoh, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 2346.
[472] J. R. Dryburgh and J. C. Brown, *Gastroenterology*, 1975, **68**, 1169.
[473] S. Yamada, N. Ikota, T. Shioiri, and S. Tachibana, *J. Amer. Chem. Soc.*, 1975, **97**, 7174.
[474] K.-E. Andersson, P. Hedner, and C. G. A. Persson, *Acta Physiol. Scand.*, 1974, **90**, 657.
[475] J. A. Levant, T. L. Kun, J. Jachna, R. A. L. Sturdevant, and J. I. Isenberg, *Amer. J. Digestive Diseases*, 1974, **19**, 207.
[476] J. A. Levant and R. A. L. Sturdevant, *Amer. J. Roentgenol.*, 1974, **121**, 380.
[477] W. M. Yau, G. M. Makhlouf, L. E. Edwards, and J. T. Farrar, *Canad. J. Physiol. Pharmacol.*, 1974, **52**, 298.
[478] W. Domschke, *Naturwiss.*, 1974, **61**, 370.
[479] R. Caprilli, P. Melchiori, G. Improta, P. Vernia, and G. Frieri, *Gastroenterology*, 1975, **68**, 1228.
[480] G. Jaeger, W. König, H. Wissmann, and R. Geiger, *Chem. Ber.*, 1974, **107**, 215.

Bovine trypsinogen activation peptide (516*a*) has been reported to inhibit gastric secretion. The peptide has been compared with the gastrin fragment (517) which is also supposed to inhibit. The hexapeptide (516), its methyl ester and amide, the [Thr^1], [Pro^1], and [Ile^1] analogues (corresponding to sequences found in other species) and the [Ala^1], [D-Val^1], and des-Val^1 derivatives, in addition to (517), have now been synthesized.[481] The compounds, tested on both the Heidenhein pouch and gastric fistula preparations, showed some slight inhibitory effects, but these were too weak to be considered physiologically significant.

Val-Asp$_4$-Val (516*a*) Glu$_5$-Ala-Tyr-Gly (517)

Further studies on the insulin releasing peptide (IRP) and glucose inhibiting peptide have been reported.[482] The substances augment the repose of the pancreas towards oral glucose, and a physiological role has been suggested.

7 Thyroid and Parathyroid Hormones

Recent reviews have dealt with parathyroid hormone (PTH), calcitonin and vitamin D,[19d] the chemistry and physiology of parathyroid hormones,[80, 483] calcitonin,[484] and calcium and phosphorus metabolism.[485]

Calcitonin.—Over the past few years, calcitonin has proved to be the treatment of choice in Paget's disease, which is a painful condition resulting from an increased metabolic turnover of bone constituents. Porcine calcitonin and the highly potent synthetic salmon hormone (518) have been used (see *e.g.* ref. 486), but their effectiveness in a significant proportion of patients is impaired by the formation of antibodies and development of resistance.[487] Synthetic human calcitonin appears to be free from these disadvantages and, although less potent than the salmon hormone, has been shown to be safe and effective over long periods.[488] The reasons for the difference in activity between human calcitonin (519) and (518) have been explored by the synthesis of a number of analogues. The [Ser^2, Val^8] analogue of human calcitonin (with the *N*-terminal decapeptide of salmon calcitonin) has about the same hypocalcemic activity, 80—120 MRC units, as the human hormone.[489] The [Ser^{29}, Val^{31}] human calcitonin, on the other hand, had some five times the activity of the human hormone and longer

[481] J. M. Bower, C. F. Hayward, I. S. Morley, and J. S. Morley, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 820.
[482] D. S. Turner, L. Etheridge, J. Jones, V. Marks, B. Meldrum, S. R. Bloom, and J. C. Brown, *Clin. Endocrinol.*, 1974, **3**, 489.
[483] H. B. Brewer, T. Fairwell, W. Rittel, T. Littledike, and C. D. Arnaud, *Amer. J. Med.*, 1974, **56**, 759.
[484] S. F. Queener and N. H. Bell, *Metabolism*, 1975, **24**, 555.
[485] A. B. Borle, *Ann. Rev. Physiol.*, 1975, **37**, 361.
[486] C. R. Hamilton, *Amer. J. Med.*, 1974, **56**, 315.
[487] J. DeRose, F. R. Singer, A. Avramides, A. Flores, R. Dziadiw, R. K. Baker, and S. Wallach, *Amer. J. Med.*, 1974, **56**, 858.
[488] P. B. Greenberg, F. H. Doyle, M. T. Fisher, C. J. Hillyard, G. F. Joplin, J. Pennock, and I. MacIntyre, *Amer. J. Med.*, 1974, **56**, 867; F. H. Doyle, J. Pennock, P. B. Greenberg, G. F. Joplin, and I. MacIntyre, *Brit. J. Radiol.*, 1974, **47**, 1; F. H. Doyle, N. J. Woodhouse, A. C. A. Glen, G. F. Joplin, and I. MacIntyre, *Brit. J. Radiol.*, 1974, **47**, 9.
[489] S. Nozaki and I. Muramatsu, *Chem. Letters*, 1974, 417.

(518) salmon calcitonin Cys-Ser-Asn-Leu-Ser-Thr-Cys-Val-Leu-Gly-Lys-Leu-Ser-
1 2 3 4 5 6 7 8 9 10 11 12 13

(519) human calcitonin Cys-Gly-Asn-Leu-Ser-Thr-Cys-Met-Leu-Gly-Thr-Tyr-Thr-
1 2 3 4 5 6 7 8 9 10 11 12 13

(518) salmon calcitonin Gln-Glu-Leu-His-Lys-Leu-Gln-Thr-Tyr-Pro-Arg-Thr-Asn-
14 15 16 17 18 19 20 21 22 23 24 25 26

(519) human calcitonin Gln-Asp-Phe-Asn-Lys-Phe-His-Thr-Phe-Pro-Gln-Thr-Ala-
14 15 16 17 18 19 20 21 22 23 24 25 26

(518) salmon calcitonin Thr-Gly-Ser-Gly-Thr-Pro-NH_2
27 28 29 30 31 32

(519) human calcitonin Ile-Gly-Val-Gly-Ala-Pro-NH_2
27 28 29 30 31 32

duration of action, and this region is clearly important for the increased activity of salmon calcitonin but does not account entirely for it.[490] The analogue [Ala^{29}, Val^{31}] human calcitonin had only 10% of the activity of the human hormone; it corresponds to the sequence of a salmon calcitonin isohormone.[490] The importance of the amino-acids at positions 12, 16, and 19 is shown by their replacement with leucine as is found in the salmon hormone. [$Leu^{12, 16, 19}$] human calcitonin had some 10 times the activity of the human hormone. Again the additional change in [$Leu^{12, 16, 19}$, Val^{8}] human calcitonin added no further increase in activity. Desamino-[$Leu^{12, 16, 19}$] human calcitonin was also of the same activity as the [$Leu^{12, 16, 19}$] derivative. The importance of tyrosine at position 22 is shown by the doubling of activity in [$Leu^{12, 16, 19}$, Tyr^{22}] human calcitonin, which is 20 times as active as the human hormone (half as active as salmon calcitonin itself) and with approximately double the duration of action (though still shorter than that of the salmon hormone).[8p]

The effects of salmon, porcine, and human calcitonin on gastric secretion and gastric emptying in rats have been studied. The actions are apparently not mediated by changes in serum calcium and a separation of activities was observed between the different hormones.[491]

Other work reported includes studies on the biosynthesis of calcitonin providing some evidence for a prohormone,[492, 492a] studies on the metabolic fate of human calcitonin in the dog,[493] degradation of porcine calcitonin by a human plasma fraction,[494] and ectopic production of calcitonin by various tumours.[495, 496] A useful short review covers various aspects of calcitonin chemistry and physiology.[484]

[490] R. Maier, B. Riniker, and W. Rittel, *F.E.B.S. Letters*, 1974, **48**, 68.
[491] G. R. Bobalik, R. R. Kleszynski, J. P. Aldred, and J. W. Bastian, *Proc. Soc. Exp. Biol. Med.*, 1974, **147**, 284.
[492] B. A. Roos, K. Okano, and L. J. Deftos, *Biochem. Biophys. Res. Comm.*, 1974, **60**, 1134.
[493] M. B. Clark, C. G. Williams, B. M. Nathanson, R. E. Horton, H. I. Glass, and G. V. Foster, *J. Endocrinol.*, 1974, **61**, 199.
[494] R. K. O'Dor, F. E. Newsome, C. O. Parkes, and D. H. Copp, *Hormone and Metab. Res.*, 1974, **6**, 161.
[495] R. C. Coombes, G. C. Easty, S. I. Detre, C. J. Hillyard, U. Stevens, S. I. Girgis, L. S. Galante, L. Heywood, I. MacIntyre, and A. M. Neville, *Brit. Med. J.*, 1975, **iv**, 197.
[496] C. J. Hillyard, R. C. Coombes, P. B. Greenberg, L. S. Galante, and I. MacIntyre, *Clin. Endocrinol.*, 1976, **5**, 1.

Parathyroid Hormone.—The discrepancy (see Vol. 7, p. 298) between the two proposed amino-terminal sequences of human PTH, that of Brewer *et al.* (520),[497] and that of Niall *et al.* (521),[498] remains unresolved. Re-investigations in both cases [8a, 499] have confirmed the respective original work and some form of iso-hormonal variation appears likely. The sequence (520) appears to contain fewer differences from the porcine and bovine sequences than (521).[500]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
(520)	Ser-	Val-	Ser-	Glu-	Ile	-Gln-	Leu-	Met-	His-	Asn-	Leu-	Gly-	Lys-	His-	Leu-	Asn-	Ser -
(521)	Ser-	Val-	Ser-	Glu-	Ile	-Gln-	Leu-	Met-	His-	Asn-	Leu-	Gly-	Lys-	His-	Leu-	Asn-	Ser -

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
(520)	Met-	Glu-	Arg-	Val-	Gln-	Trp-	Leu-	Arg-	Lys-	Lys-	Lys-	Gln-	Leu-	Val-	His-	Asn-	Phe-
(521)	Met-	Glu-	Arg-	Val-	Glu-	Trp-	Leu-	Arg-	Lys-	Lys-	Leu-	Gln-	Asp-	Val-	His-	Asn-	Phe-

Parathyroid hormone appears to be a good example of the need to consider the totality of molecular species, prohormones and degradation products, related to a 'hormone' before its role can be fully understood, and much work has been carried out on the various species involved.[14d, 501, 502] The *N*-terminal sequence (522) has been found for a bovine pro-PTH,[503] and a 'pre-pro-PTH' has been

1	2						
Lys-	Ser-	Val-	Lys-	Lys-	Arg-	Ala-	Val-...

(522)

isolated as a direct translational product of the appropriate messenger RNA which is so ephemeral as not to be detectable even intracellularly.[504] Biologically active sequence 1—65 of bovine PTH has been isolated for bovine parathyroid glands,[505] and preliminary evidence obtained for a precursor to human PTH.[506] The effects of the heterogeneity of immunoreactive PTH on the interpretation of radioimmunoassay results in man have been considered further.[507]

The four sequences, bovine PTH (3—34) (523), desamino-(1—34) (524), PTH (13—34) (525), and PTH (1—26) (526), have been studied *in vitro* using a rat renal cortical adenyl cyclase assay. While none of the sequences showed agonist activity in this assay, (523) and (524) had a specific dose-related, and reversible

[497] H. B. Brewer, T. Fairwell, R. Ronan, G. W. Sizemore, and C. D. Arnaud, *Proc. Nat. Acad. Sci. U.S.A.*, 1972, **69**, 3585.
[498] H. D. Niall, R. T. Sauer, J. W. Jacobs, H. T. Keutmann, G. V. Segre, J. L. H. O'Riordan, G. D. Aurbach, and J. T. Potts, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 384.
[499] H. T. Keutmann, H. D. Niall, J. L. H. O'Riordan, and J. T. Potts, *Biochemistry*, 1975, **14**, 1842.
[500] R. T. Sauer, H. D. Niall, M. L. Hogan, H. T. Keutmann, J. L. H. O'Riordan, and J. T. Potts, *Biochemistry*, 1974, **13**, 1994.
[501] G. V. Segre, H. D. Niall, J. F. Habener, and J. T. Potts, *Amer. J. Med.*, 1974, **56**, 774.
[502] D. V. Cohn, R. R. MacGregor, L. L. H. Chu, D. W. Y. Huang, C. S. Anast, and J. W. Hamilton, *Amer. J. Med.*, 1974, **56**, 767.
[503] J. W. Hamilton, H. D. Niall, J. W. Jacobs, H. T. Keutmann, J. T. Potts, and D. V. Cohn, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 653.
[504] B. Kemper, J. F. Habener, R. C. Mulligan, J. T. Potts, and A. Rich, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 3731.
[505] T. M. Murray, S. A. Muzaffar, J. A. Parsons, and H. T. Keutmann, *Biochemistry*, 1975, **14**, 2705.
[506] E. T. Wong and A. W. Lindale, *Proc. Soc. Exp. Biol. Med.*, 1975, **148**, 387.
[507] C. A. Arnaud, R. S. Goldsmith, P. J. Bordier, G. W. Sizemore, J. A. Larsen, and J. Gilkinson, *Amer. J. Med.*, 1974, **56**, 785.

inhibitory effect on the action of PTH. The sequences (525) and (526) showed no agonistic effect. The results point to a crucial effector role for the *N*-terminal part of the molecule (Ala-Val- ...) while the (3—27) sequence seems to be the minimum for binding.[508]

A new assay has been developed which measures the receptor binding of PTH but, unlike radioreceptor assays, required no radiolabelled hormone. Instead an ^{125}I-labelled antibody directed towards a part of the molecule not involved in binding to the receptor is used to determine the amount of bound hormone.[509]

Other work reported includes the effect of bovine PTH (1—34) on renal cAMP production and excretion in man,[510] immunological studies on human PTH,[511] the effects of PTH and synthetic human and bovine PTH peptides on chick kidney adenyl cyclase,[512] and the effect of oxidation on the biological and immunological activity of porcine PTH.[513]

8 Vasoactive Peptides

Bradykinin.—Reviews have covered structure–activity relationships [514] and present trends in kinin research,[515] and the proceedings of an international conference on the chemistry and biology of the kallikrein–kinin system have been published.[516]

Table 8 gives biological activities of bradykinin analogues. Subtle modification of the arginine residues of bradykinin has revealed a previously unsuspected specificity required in the two basic residues. While introduction of $N(\omega)$-methyl arginine (558) into positions 1 or 9 gave analogues (530) and (555) with high activity, *ca.* 23 and 60% on the isolated rat uterus, introduction of the still highly basic δ-acetimidoyl-L-ornithine (559) in the same positions gave two analogues (531) and (556) with only *ca.* 1% activity. The result has been interpreted in terms of possible binding with carboxy-groups at the receptor. The analogue (528) with an α-guanidino-group in place of an amino-group shows remarkably high activity (200% on rat uterus and 100% on guinea-pig ileum); the metabolism in lung and the effect of a bradykinin potentiating factor are similar to those of bradykinin itself, suggesting no unusual metabolic stability. Reversal of the α-amino- and ω-guanidino-groups in (529) reduced the activity to that of [Lys^1] bradykinin.[518]

The homoproline analogue (551) has high activity, equal to bradykinin on rat ileum [525] and rat blood pressure.[526] The homophenylalanine analogue (553) has much reduced activity (*ca.* 1—3% against rat blood pressure), but since its action

[508] D. Goltzmann, A. Peytremann, E. Callahan, G. W. Tregear, and J. T. Potts, *J. Biol. Chem.*, 1975, **250**, 3199.
[509] C. H. S. McIntosh and R.-D. Hesch, *Biochem. Biophys. Res. Comm.*, 1975, **64**, 376.
[510] S. Czekalski, N. Loreau, F. Paillard, R. Ardaillou, J.-P. Fillastre, and E. Mallet, *European J. Clin. Invest.*, 1974, **4**, 85.
[511] G. N. Hendy, P. M. Barling, and J. L. H. O'Riordan, *Clin. Sci. Mol. Med.*, 1974, **47**, 567.
[512] T. J. Martin, N. Vakakis, J. A. Eisman, S. J. Livesey, and G. W. Tregear, *J. Endocrinol.*, 1974, **63**, 369.
[513] J. L. H. O'Riordan, J. S. Woodhead, G. N. Hendy, J. A. Parsons, C. J. Robinson, H. T. Keutmann, B. F. Dawson, and J. T. Potts, *J. Endocrinol.*, 1974, **63**, 117.
[514] S. Reissman, I. Paegélow, and H. Arold, *Acta Biol. Med. Germanica*, 1974, **33**, 77.
[515] M. Rocha e Silva, *Life Sci.*, 1974, **15**, 7.
[516] 'Fogarty International Center Proceedings No. 27', ed. J. J. Pisano and K. F. Austen, U.S. Government Printing Office, Washington, 1976.

Table 8 *Analogues of bradykinin*

Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
1 2 3 4 5 6 7 8 9

bradykinin

Compound number	*Structure* [a]	*Biological activity* [b]	*Test* [c]	*Ref.*
(527)	$BrCH_2CO$-bradykinin	+++	A	517
		+++	D	517
(528)	$HN{=}C(NH_2)$-bradykinin	++++	A	518
		++++ [d]	B	518
(529)	[$HN{=}C(NH_2)$-Lys^1]	++	A	518
(530)	[$Arg(Me)^1$]	+++	A	518
(531)	$\left[\begin{array}{c}NH\\ \|\\ Orn(CMe)^1\end{array}\right]$	++	A	518
(532)	[Har^1]	++		519
(533)	$BrCH_2CO$—[$Phe(4\text{-}NHCOCH_2Br)^5$]	−	A	517
		−	D	517
(534)	[Val^1, Thr^6]	−	A	520
		+	B	520
		−	E	520
(535)	$BrCH_2CO$—[$Phe(4\text{-}NHCOCH_2Br)^8$]	+	A	517
		+	D	517
(536)	[$Orn^{1,9}$]	−	A	518
(537)	[$Har^{1,9}$]	++		518
(538)	[$Hypro^2$]	++++	A	521
		++++ [d]	B	521
		+++++	F	521
		+++	D	521
(539)	[$Hypro^3$]	++++ [d]	A	521
		+++	B	521
		+++	F	521
		+++	D	521
(540)	[D-Ala^4]	−	A	522
		−	D	522
(541)	[$Phe(^{\alpha}Me)^5$]	++	A	522
		++	D	522
(542)	[Cha^5]			523
(543)	[$Cha^{5,8}$]			523
(544)	[$Phe(4\text{-}NO_2)^5$, $Tyr(Me)^8$]	+++	B	524
(545)	[$Tyr(Me)^5$, $Phe(4\text{-}NO_2)^8$]	++	B	524
(546)	[Orn^6]	−	A	518
(547)	[$Orn(CH_2CH_2CO_2H)^6$]	−	A	518
(548)	[$Glyc^6$]			523
(549)	[Gly^6, Phl^8]			523

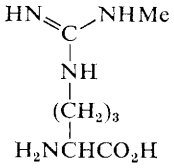
[517] J. Turk, P. Needleman, and G. R. Marshall, *J. Medicin. Chem.*, 1975, **18**, 1135.
[518] T. G. Pinker, G. T. Young, D. F. Elliot, and R. Wade, *J.C.S. Perkin I*, 1976, 220.
[519] M. E. Safdy and P. A. Lyons, *J. Medicin. Chem.*, 1974, **17**, 1227.
[520] S. Reissmann, H. Arold, and I. Paegelow, *J. prakt. Chem.*, 1974, **316**, 87.
[521] J. M. Stewart, J. W. Ryan, and A. H. Brady, *J. Medicin. Chem.*, 1974, **17**, 537.
[522] J. Turk, P. Needleman, and G. R. Marshall, *J. Medicin. Chem.*, 1975, **18**, 1139.
[523] M. P. Filatova, N. A. Krit, G. S. Suchkova, G. A. Ravdel, and V. T. Ivanov, *Bio-org. Khim.*, 1975, **1**, 437.
[524] T. Abiko, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2191.

Table 8 (*cont.*)

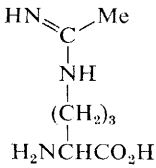
Compound number	*Structure* [a]	*Biological activity* [b]	*Test* [c]	*Ref.*
(550)	[Hypro^7]	++	A	521
		++	B	521
		+++	F	521
		++	D	521
(551)	[Hpr^7]	++++ [d]	C	525
		++++ [d]	D	526
(552)	[Phe($^{\alpha}$Me)^8]	+++	A	522
		++	D	522
(553)	[Hph^8]	++	D	526
(554)	[Cha^8]			523
(555)	[Arg(Me)^9]	+++	A	518
(556)		++	A	518
(557)	[Har^9]	++		519

[a] Hypro = *trans*-4-hydroxy-L-proline, Phe(αMe) = —NHCMe(CH_2Ph)CO—, Cha = L-β-cyclohexylalanine, Glyc = glycollic acid, Phl = phenyl-lactic acid, Hpr = L-α-homoproline (), Hph = L-α-homophenylalanine (—NHCH(CH_2Ph)CH_2CO—).

[b] Symbols for potency are used as in Table 3. [c] The following tests are indicated: *in vitro*: A, rat uterus; B, guinea-pig ileum; C, rat ileum: *in vivo*: D, rat blood pressure; E, rabbit blood pressure; F, guinea-pig vascular permeability. [d] '100%.'



(558)



(559)

is, unlike that of (551), potentiated by converting enzyme inhibitors, (551) may owe some of its *in vivo* activity to metabolic resistance.[526] This may also apply *in vitro*, or perhaps the *cis* configuration of the modified amino-acid in (551) requires less overall alteration in the conformation of the molecule.

The biological activities of analogues with hydroxyproline in place of proline have been reported.[521] The [Hypro^2] analogue (538) is surprisingly highly active, particularly since substitution of alanine in this position gives an analogue of considerably reduced activity, whereas [Ala^3] bradykinin is fully active.

Three analogues, (540), (541), and (552), which have a methyl group in place of C^{α}-H in positions 4, 5, and 8 of bradykinin, have been synthesized. The [C^{α}-methyl-L-phenylalanine^8] analogue (552) showed an interesting selectivity for uterine tissue (with 31% of the activity of bradykinin). It was considerably less

[525] L. Balaspiri, B. Penke, G. Papp, G. Dombi, and K. Kovacs, *Helv. Chim. Acta*, 1975, **58**, 969.

[526] M. A. Ondetti and S. L. Engel, *J. Medicin. Chem.*, 1975, **18**, 761.

readily degraded on passage through the lung than is bradykinin, presumably reflecting resistance to cleavage between residues 7 and 8.[522]

An effective inhibitor of bradykinin remains one of the elusive objectives of structure–activity studies in this field. Following earlier reports that [Tyr(Me)^{5,8}] bradykinin had inhibitory but also agonist activity, two analogues, (544) and (545), have been synthesized hopefully to retain the former and lose the latter activity. Both, however, had surprisingly high agonist activity, and were not tested as antagonists.[524]

Bromoacetyl substitution at the *N*-terminus of oxytocin has given compounds which appear to act as irreversible inhibitors, and the same modification has now been introduced in the *N*-terminus of bradykinin and to the amino-group of a *p*-aminophenylalanine in positions 5 or 8 (with the *N*-terminus also substituted). None of the three analogues, (527), (533), or (535), showed inhibitory activity.[517]

Analogues (532), (537), and (557) of bradykinin, with homoarginine in positions 1 and 9, have been prepared by solid-phase synthesis [519] and have surprisingly low activity in the range 2—6% (*cf.* ref. 527).

Earlier reports that [Val^1, Thr^6] bradykinin (isolated from natural sources) had potent bradykinin-like activity were surprising in view of the known structure–activity relationships. The compound (534) has now been synthesized and shown to have very low activity (0.34, 0.004, and 0.03% of bradykinin on guinea-pig ileum, rat uterus, and rabbit blood pressure, respectively).[520]

Other reported analogues include those with β-cyclohexylalanine and phenyllactic acid in positions 5 and 8.[523] Kinin-like activities have been reported in synthetic fibrinogen peptides.[528]

Angiotensin.—Reviews have covered structure–activity relationships in angiotensin analogues,[529] the pharmacology of angiotensin (including structure–activity studies),[530, 531] the renin–angiotensin system,[532, 533] and the clinical applications of angiotensin analogues,[534] among other topics.[535, 536]

Table 9 lists angiotensin* analogues whose biological activities were discussed in publications appearing during 1974 and 1975, though many of the compounds are not new. One of the more interesting findings to emerge from structure–activity studies on angiotensin has been the group of analogues modified in positions 1 and 8 which are inhibitory, notably analogues with sarcosine in position 1 and alanine ('Saralasin'), isoleucine, or other aliphatic amino-acids in

[527] G. A. Fletcher and G. T. Young, *J.C.S. Perkin I*, 1972, 1867.
[528] A. Takaki, T. Yamaguchi, and K. Ohsato, *Thromb. Diath. Haemorrhag.*, 1974, **32**, 350.
[529] 'Angiotensin', ed. I. H. Page and F. M. Bumpus, Springer-Verlag, Berlin, 1974.
[530] D. Regoli, W. K. Park, and F. Rioux, *Pharmacol. Rev.*, 1974, **26**, 69.
[531] D. Regoli, W. K. Park, and F. Rioux, *Acta Physiol. Lat. Amer.*, 1975, **24**, 525.
[532] S. Oparil and E. Haber, *New Eng. J. Med.*, 1974, **290**, 389, 446.
[533] W. S. Peart, *New Eng. J. Med.*, 1975, **292**, 302.
[534] T. Ogihara, T. Yamamoto, and Y. Kumahara, *Jap. Circ. J.*, 1974, **38**, 997.
[535] J. Fasciolo, *Acta Physiol. Lat. Amer.*, 1975, **24**, 391.
[536] I. H. Page, *Acta Physiol. Lat. Amer.*, 1975, **24**, 395.

* The term angiotensin is used to refer specifically to [Asp^1, Ile^5] angiotensin II; other derivatives are named with reference to this.

Table 9 *Analogues of angiotensin*

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
1 2 3 4 5 6 7 8
angiotensin II

Compound number	*Structure* [a]	*Biological activity:* [b] *Pressor*	*Other*	*Test* [c]	*Ref.*
(560)	(2—8)	+ + +			537
(561)	[Ala^1]	+ + +			538
(562)	[Sar^1]	+ + +			538
(563)	[Pro^1]	+ + +			538
(564)	[Me_2Gly^1]	+ + +			538
		+ + + +			539
(565)	[Bet^1]	+ + +			538
(566)	[D-Ala^1]	+ + + + [d]			538
(567)	(3—8)	+			537
(568)	Ac-(3—8)	+ +			540
(569)	EtCO-(3—8)	+ +			540
(570)	$Me(CH_2)_3CO$-(3—8)	+ +			540
(571)	Ala-(3—8)	+ +			540
(572)	D-Ala-(3—8)	+ + +			540
(573)	Nva-(3—8)	+ +			540
(574)	D-Nva-(3—8)	+ + +			540
(575)	(4—8)	−			537
(576)	(5—8)	−			537
(577)	[Val^5]-(3—8) OC Arg-Gly-NH_2	+ + +			541
(578)	[D-Arg^2, Val^5]-(2—8) OC Gly-OEt	+ +			542
(579)	[D-Arg^2, Val^5]-(2—8) OC Gly-NH_2	+ +			542
(580)	[Leu^8]-(3—8)		−	A, B, C, D	543
(581)	[Asn^1, Pro^3, Val^5]	+ + +			544
(582)	[Suc(OMe)^1, Pro^3, Val^5]	+ +			544
(583)	[Suc(NH_2)^1, Pro^3, Val^5]	+ +			544
(584)	[Suc(NHMe)^1, Pro^3, Val^5]	+ +			544
(585)	[Asn^1, Phe(4-NH_2)^4]	+ + +			545

[537] W. K. Park, C. Choi, F. Rioux, and D. Regoli, *Canad. J. Biochem.*, 1974, **52**, 106.
[538] W. K. Park, C. Choi, F. Rioux, and D. Regoli, *Canad. J. Biochem.*, 1974, **52**, 113.
[539] M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Medicin. Chem.*, 1974, **17**, 1156.
[540] E. C. Jorgensen, I. C. Kiraly-Olah, T. C. Lee, and G. C. Windridge, *J. Medicin. Chem.*, 1974, **17**, 323.
[541] R. E. Vegners and G. I. Cipens, *Zhur. obshchei Khim.*, 1975, **45**, 444.
[542] R. E. Vegners, G. I. Cipens, and V. K. Kibirev, *Khim. prirod. Soedinenii*, 1973, **9**, 511.
[543] T. B. Paiva, G. Goissis, L. Juliano, M. E. Miyamoto, and A. C. M. Paiva, *J. Medicin. Chem.* 1974, **17**, 238.
[544] P. J. Romanovski, Z. P. Auna, and G. I. Cipens, *Bio-org. Khim.*, 1975, **1**, 182.
[545] K. H. Hsieh, *Dissertation Abstr.*, 1975, **B35**, 3847.

Table 9 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *Pressor*	*Other*	*Test* [c]	*Ref.*
(586)	[Asn^1, Phe(4-NO_2)^4]	−			545
(587)	[Boc-Cys(Acm)-Asn^1, Val^5]	+++			546
(588)	[Suc(OMe)^1, Val^5]	++(+)			544, 547
(589)	[Suc(NH_2)^1, Val^5]	+++(+)			544, 547
(590)	[Suc(NHMe)^1, Val^5]	+++			544, 547
(591)	[AspNH_2^{1}, Val^5]	++++			547
(592)	[Suc(NMe_2)^1, Val^5]	+++			547
(593)	[Asp(OMe)^1, Val^5]	+++			547
(594)	[Asp(NHMe)^1, Val^5]	+++			547
(595)	[Me_2Gly^1, Ile^8]	−*	−*	C, E, F	548
(596)	[D-aMeIle^1, Ile^8]	+	*	C	539
(597)	[NH_2CO-Gly^1, Val^5, Ala^8]	−*			549
(598)	[Asn^1, Val^5, Ala^8]	−*			549
(599)	[Asn^1, Pgl^8]	*	*		550
(600)	[Z-Asp^1, Pgl^8]	*	*		550
(601)	[Z-Asn^1, Pgl^8]	*	*		550
(602)	[β-Ala^1, Pgl^8]	*	*		550
(603)	[PhCO-β-Ala^1, Pgl^8]	*	*		550
(604)	[Sar^1, Pgl^8]	*	*		550
(605)	[HOCO(CH_2)$_2$CO—^{1}, Pgl^8]	*	*		550
(606)	[Suc(NH_2)^1, Pgl^8]	*	*		550
(607)	[Pac^1, Pgl^8]	*	*		550
(608)	[Asn^1, Pya^6]	++			545
(609)	[Asn^1, Ima^6]	+			545
(610)	[Asn^1, Phe(4-NH_2)^6]	−			545
(611)	[Asn^1, Phe(4-NO_2)^6]	−			545
(612)	[Sar^1, Hypro^7, MePhe^8]	++*	+	A, B	551
(613)	[Sar^1, Ala^7, MePhe^8]	++*	+	A, B	551
(614)	[Glp^1, Leu^8]	+			538
(615)	[Glp^1, Ala^8]	+	*	C	539
(616)	[Glp^1, Gly^8]	−			538
(617)	[AspOH^1, Leu^8]	+			538
(618)	[Sar^1, Gly^8]	−			538
(619)	[Sar^1, Thr^8]	+	*	C	539
(620)	[Sar^1, Abu^8]	−			538
(621)	[Sar^1, Val^8]	−			538
(622)	[Pro-Sar^1, Ile^8]	−			539
(623)	[Pro^1, Leu^8]	+			538
(624)	[Pro^1, Gly^8]	−			538
(625)	[Sar^1, Ile-Pro^8]	−			539
(626)	[Me_2Gly^1, Leu^8	+			538
(627)	[Bet^1, Leu^8]	+			538

546 H. J. Möschler and R. Schwyzer, *Helv. Chim. Acta*, 1974, **57**, 1576.
547 P. Y. Romanovskii, Z. P. Auna, and G. I. Cipens, *Zhur. obshchei Khim.*, 1974, **44**, 421.
548 R. K. Turker and N. U. Gündogan, *Arch. Pharmacol.*, 1974, **282**, 411.
549 I. K. Romanovska, A. P. Pavar, and G. I. Cipens, *Bio-org. Khim.*, 1975, **1**, 1257.
550 H. Wissman, B. Schoelkens, E. Lindner, and R. Geiger, *Z. physiol. Chem.*, 1974, **355**, 1083.
551 C. Pena, J. M. Stewart, and T. C. Goodfriend, *Life Sci.*, 1974, **14**, 1331.

Table 9 (*cont.*)

Compound number	*Structure* [a]	*Biological activity:* [b] *Pressor*	*Other*	*Test* [c]	*Ref.*
(628)	[$HOCO(CH_2)_2CO$-^{1}, Leu^8]	+*	−	B, C	543
			+	A	543
(629)	des-Asp^1-[Leu^8]	−*	−	A, B, C	543
(630)	des-Asp^1-[Ile^8]	+*			552
(631)	[Pro^1, Ile^8]	+*			552
(632)	[$MePhe^1$, Ile^8]	+*			552
(633)	[Me_2Gly^1, Ile^8]	+*			552
(634)	[$MeAla^1$, Ile^8]	++*			552
(635)	[$MeIle^1$, Ile^8]	+*			552
				C	
(636)	[NH ‖ H_2NC-Gly^1, Ile^8]	+*			552
(637)	[Asn^1, Phe(4-NO_2)^8]	+++			545
(638)	[Asn^1, Phe(4-NH_2)^8]	+++			545
(639)	[Asn^1, Ahp^8]	+++			545
(640)	[Asn^1, Nle^8]	++			545
(641)	[Sar^1, $MePhe^8$]	++*	−	A, B	551
(642)	des-Asp^1-[Ile^8]	*e*	*	G	553
(643)	[Ala^2]	++			538
(644)	[Pro^2]	++			538
(645)	[D-Abu^2]	+			538
(646)	[Ala^3]	+++			538
(647)	[Ala^4]	+			538
(648)	[Gly^4]	+			538
		(−)	(+++)	E	554
(649)	[Leu^4]	+			538
(650)	[Phe^4]	+++			538
(651)	[$Melp^4$]	+			538
(652)	[Ala^4, Ala^6]	−			538
(653)	[Gly^4, Gly^6]	−			538
(654)	[His^4, Tyr^6]	−			538
(655)	[$Leu^{4,8}$]	−			538
(656)	[$Acpc^{4,8}$]	−			538
(657)	[Ala^4, Tyr^8]	−			538
(658)	[Phe^4, Tyr^8]	++			538
(659)	[Ala^5]	++			538
(660)	(1—4)	−			537
(661)	[Ala^6]	+			538
(662)	[Gly^6]	−			538
(663)	[Leu^6]	−			538
(664)	[Tyr^6]	−			538
(665)	[Arg^6, Ala^8]	−			538
(666)	[Ala^7]	++			538
(667)	[Gly^7]	−			538
		(−)	(+++)	E	554
(668)	[Ala^7, $MePhe^8$]	+*	−	A	551
			+	B	551

[552] M. C. Khosla, M. M. Hall, R. R. Smeby, and F. M. Bumpus, *J. Medicin. Chem.*, 1974, **17**, 431.
[553] E. L. Bravo, M. C. Khosla, and F. M. Bumpus, *J. Clin. Endocrinol. Metab.*, 1975, **40**, 530.
[554] A. P. Pavar, I. A. Bite, G. Y. Avotin, and G. I. Cipens, *Zhur. obshchei Khim.*, 1975, **45**, 1632.

Table 9 (*cont.*)

Compound number	*Structure* [a]	*Biological activity*: [b] *Pressor*	*Other*	*Test* [c]	*Ref.*
(669)	(1—7)	−			537
(670)	[Gly^8]	−			538
(671)	[Nva^8]	+			538
(672)	[Nle^8]	+			538
(673)	[Melp^8]	+			538
(674)	[D-Ala^8]	−			538
(675)	[D-Leu^8]	−			538
(676)	[Pgl^8]	*	*		550
(677)	[MePhe^8]	+ + *	−	A	551
			+	B	551
(678)	[Trp^8]	+ + +	*	C	539
(679)	[Thi^8]	+ + +	*	C	539
(680)	angiotensinyl-His-Leu-Leu-Val-Tyr-Ser	+ +			537
(681)	···-His-Leu-Leu-Val-Tyr	+ +			537
(682)	···-His-Leu-Leu-Val	+ +			537
(683)	···-His-Leu-Leu	+			537
(684)	···-His	−			537
(685)	···-Leu	+			537

[a] Bet = betaine, $Me_3\overset{+}{N}CH_2CO-$, Suc = succinyl, $-CO(CH_2)_2CO-$, Pgl = α-phenylglycine, Pac = 2-pyrrolidone-*N*-acetyl, Pya = β-(pyridyl-2) alanine, Ima = β-(imidazolyl-2) alanine, Ahp presumed to be α-amino-n-heptanoic acid, Melp = melphalan, Phe-[4-$N(CH_2CH_2Cl)_2$], Acpc = 1-aminocyclopentane carboxylic acid. [b] Symbols for potency are used as in Table 6. [c] A, rat uterus; B, guinea-pig ileum; C, rabbit aorta; D, rat blood pressure; E, rat colon; F, isolated cat heart; G, aldosterone release. [d] '100%.' [e] Not an inhibitor.

position 8. A wealth of publications (for recent discussions see refs. 529, 555—558) attests to the use of these derivatives in biological research and potentially in medicine. Saralasin for example has promise as a diagnostic agent and as a relatively non-toxic antihypertensive.[556] A single i.v. injection has been proposed as a rapid screening procedure for renin-mediated hypertension (the 'Saralasin bolus test'),[559] although there are transient hypertensive effects (presumably related to the analogue's agonist activity) which could be a problem and an infusion may be preferable.[560] There is also a profound hypotensive effect when the analogue is given in combination with vasodilator drugs.[561] Its short half-life in blood (*ca.* 3 min) makes infusion necessary for any antihypertensive use.

Other reports deal with the effect of [Leu^8] angiotensin on water and sodium transport in toad skin,[562] and on guinea-pig aorta.[563] The effects of [Sar^1, Leu^8] angiotensin on the pressor action of angiotensin[564] and on glycerol-induced

[555] J. O. Davis, R. H. Freeman, J. A. Johnson, and W. S. Spielman, *Circ. Res.*, 1974, **34**, 279.
[556] (Editorial), *Brit. Med. J.*, 1975, **iii**, 59.
[557] D. H. P. Streeten, J. M. Freiberg, G. H. Anderson, and T. G. Dalakos, *Circ. Res.*, 1975, **36**, (*Suppl.* 1), 1.
[558] I. Ishikawa and N. Hollenberg, *Life Sci.*, 1975, **17**, 121.
[559] L. S. Marks, M. H. Maxwell, and J. J. Kaufman, *Lancet*, 1975, **ii**, 784.
[560] G. A. MacGregor and P. M. Dawes, *Lancet*, 1975, **ii**, 923.
[561] W. A. Pettinger and K. Keeton, *Lancet*, 1975, **i**, 1387.
[562] A. Coviello, G. Orce, and J. Causarano, *Acta Physiol. Lat. America*, 1975, **24**, 409.
[563] P. LeMorvan, D. Palaic, and W. K. Park, *Canad. J. Physiol. Pharmacol.*, 1974, **52**, 236.
[564] G. J. O'Connor, R. L. Baranowski, and N. A. Kurzman, *Arch. Int. Pharm. Ther.*, 1974, **209**, 75.

acute renal failure [565] have been studied. It has been suggested that the inhibitory properties of [Sar^1, Ile^8] angiotensin may derive partly from inhibition of converting enzyme, at least *in vivo*.[566]

The inhibitory activities of a series of [Ile^8] analogues with variations in position 1 have been compared with [Sar^1, Ile^8] angiotensin.[552] Substitutions included proline (631), *N*-methylphenylalanine (632), *NN*-dimethylglycine (633), *N*-methylalanine (634), *N*-methylisoleucine (635), and guanidinoacetic acid (636). All showed substantial inhibitory effects, though log dose–response lines were distinctly non-parallel, from which it was suggested that different receptor sites were involved (no doubt there could be other explanations).

A further range of inhibitory analogues with variations in both positions 1 and 8 has been described.[539] It was concluded that pressor and antagonistic activities were both increased by increasing the basicity of the *N*-terminal nitrogen, while antagonism was decreased by adding amino-acids at the *N*-terminus. The [Sar^1, Thr^8] derivative (619) was identified as a useful inhibitor with somewhat reduced initial transient pressor activity compared with the [Ile^8] analogue.[539, 567]

The agonist and antagonist activities of [Leu^8] angiotensin and three derivatives (580), (628), and (629) with a shortened or modified *N*-terminus have been studied. All the peptides except the hexapeptide (580) were inhibitors (with low agonist activity).[543] The [Me_2Gly^1] (595) and [Sar^1] derivatives of [Ile^8] angiotensin have been compared. The inhibitory properties of (595) are similar to the [Sar^1] derivative but its duration of action is shorter; *in vitro* studies suggest that this might reflect increased susceptibility to angiotensinase enzymes.[548] Other inhibitory analogues include those with *N*-methylphenylalanine in position 8,[551] and those with α-phenylglycine in position 8.[550]

As with other peptide hormones, there is more than one biologically active species of angiotensin present under physiological conditions, and the des-Asp^1 heptapeptide has been identified and called 'angiotensin III' [566, 568–571] to reflect its role in this repect.

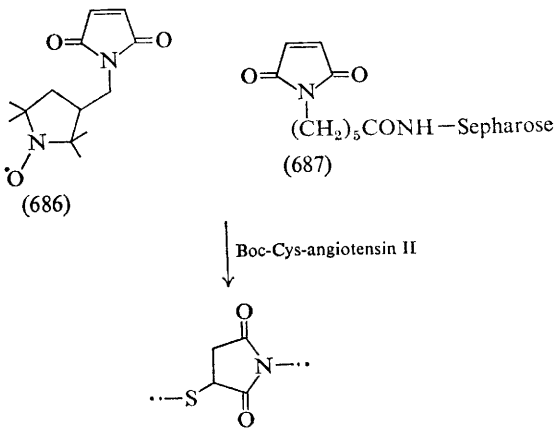
The biosynthesis of aldosterone by rabbit adrenal cells is stimulated by angiotensin and also by the heptapeptide (angiotensin III), as well as by ACTH. [Sar^1, Ile^8] Angiotensin is an effective inhibitor of the action of the angiotensin derivatives (but not of ACTH) on this steroidogenesis.[572] Analogues of the heptapeptide with Ile [553, 573] or other aliphatic amino-acids [567] exhibit an interesting selective inhibitory effect on this steroidogenesis, in that they do not also inhibit the pressor effects of angiotensin.

The removal of amino-terminal arginine from the heptapeptide reduces the activity about 100-fold, and a range of N-substituted hexapeptides has been

[565] R. L. Baranowski, G. J. O'Connor, and N. A. Kurzman, *Arch. Int. Pharm. Ther.*, 1975, **217**, 322.
[566] B.-S. Tsai, M. J. Peach, M. C. Khosla, and F. M. Bumpus, *J. Medicin. Chem.*, 1975, **18**, 1180.
[567] M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Abs. Papers Amer. Chem. Soc. Meeting*, 1975, (169), MEDI 57.
[568] T. L. Goodfriend and M. J. Peach, *Circ. Res.*, 1975, **36**, (*Suppl.* 1), 1.
[569] T. E. Lohmeier, J. O. Davis, and R. H. Freeman, *Proc. Soc. Exp. Biol. Med.*, 1975, **149**, 515.
[570] R. Freeman, J. O. Davis, and T. E. Lohmeier, *Circulation Res.*, 1975, **37**, 30.
[571] T. L. Goodfriend, *Acta Physiol. Lat. America*, 1975, **24**, 520.
[572] A. T. Chiu and M. J. Peach, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 341.
[573] C. A. Sarstedt, E. D. Vaughan, and M. J. Peach, *Circulation Res.*, 1975, **37**, 350.

studied to explore the structural features of the arginine which are necessary for the increased activity, and a semi-quantitative rule of reasonable predictive value proposed.[540]

Work has been described aimed at preparing a biologically active angiotensin II analogue which can be substituted by a spin label or be easily joined to an insoluble support.[546] The approach considered would be to add N^{α}-Boc-cysteine to the *N*-terminus, so that the thiol can react with substituted maleimides, either the spin-labelled 3-maleimidomethyl-2,2,5,5-tetramethyl-1-pyrrolidinyl-oxide (686) or the Sepharose-bound derivative (687) as shown in Scheme 2. So far,



Scheme 2

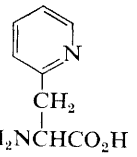
N-Boc, *S*-acetamidomethylcysteinyl-[Asn[1], Val[5]] angiotensin II (587) has been prepared and its reasonably high activity (20—25% pressor, without antagonist effects) suggests that the approach may be feasible.

The pressor activities of a large series of angiotensin analogues have been reported [537, 538] (see Table 9; some of the analogues have been reported earlier). Derivatives lengthened at the *C*-terminus are, as expected, of lowered pressor activity; the order of activity (684) < (685) < (683) confirms that the converting enzyme rather than a carboxypeptidase is important in activating angiotensin I.[537]

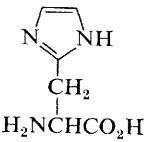
The effects of angiotensin and its [Leu[8]] and [Gly[1], Pro[8]] analogues on a spin-labelled target cell membrane have been studied by e.s.r. The spectra changed less with the (less biologically active) analogues than with angiotensin itself, though the high concentrations of peptide needed for an effect suggest certain deficiencies in the test system.[574]

Analogues of angiotensin have been described with replacements in the 4, 6, and 8 positions.[545] Replacement of phenylalanine by the *p*-nitro (637) or *p*-amino (638) amino-acid gave analogues which both had fairly high activity (15 and 53%, respectively). In position 4 (replacing tyrosine), the *p*-amino derivative (585) had 24% activity but the *p*-nitro derivative (586) was inactive. Two interesting

[574] S. Schreier-Muccillo, G. X. Niculitcheff, M. M. Oliveira, S. Shimuta, and A. C. M. Paiva, *F.E.B.S. Letters*, 1974, **47**, 193.



(688)



(689)

replacements of histidine 6 by L-β-(2-pyridyl) alanine (688) and L-β-(2-imidazolyl) alanine (689) gave analogues (608) and (609) with surprisingly low activities of 3.5 and 0.4%, respectively.

The competitive binding activities of a range of angiotensin analogues have been measured in an adrenal cortex radio-ligand receptor assay.[575] The binding affinities paralleled the biological activities, or in the case of antagonists the inhibitory activities.

While both [Asn[1], Val[5]] angiotensin and the *NN*-dimethylglycine analogue (564) have much decreased biological activity after passage over isolated rabbit aorta, this is not the case with [Sar[1]] angiotensin, which is presumably resistant to whatever metabolic breakdown is involved.[576]

The [Gly[4]] and [Gly[7]] analogues (648) and (667) are reported to be highly active on rat colon but to have little or no pressor activity.[554]

Studies on the effects of angiotensin, and of a series of non-pressor derivatives and fragments, on the inhibition of renin secretion stimulated by isoproterenol and glucagon point to a direct action independent of its vasoconstrictor effects. The structural requirements for both renin release and vasoconstriction appear however to be similar.[577] Studies on the effect of calcium ions on renin release also support an action of angiotensin independent of vasoconstriction.[578]

Studies have been carried out on the effect of pH on the c.d. spectra of truncated angiotensin sequences and related peptides.[579] Other reported work includes the synthesis of a series of analogues designed to explore a suggested link in structure with that of vasopressin, in that the reversed vasopressin is supposed to resemble the *N*-terminal sequence,[541, 542, 544] and several related [Ala[8]] derivatives, *e.g.* (597), have also been prepared.[549] Studies on the effect of sarcosine substitution in position 1 of angiotensin,[580] the inhibition by 8-substituted analogues of angiotensin-induced adrenal catecholamine release,[581] and the inhibition of the effect on cat heart and artery tissue by [Sar[1], Ile[8]] angiotensin[582] have been reported. Aza analogues,[583] [His[2]] analogues,[584] a fluoresc-

[575] S. Saltman, A. Baukal, S. Waters, F. M. Bumpus, and K. J. Catt, *Endocrinology*, 1975, **97**, 275.
[576] R. K. Türker and Z. S. Ercan, *Pharmacology*, 1975, **13**, 155.
[577] R. Van Dongen, W. S. Peart, and G. W. Boyd, *Amer. J. Physiol.*, 1974, **226**, 277.
[578] R. Van Dongen and W. S. Peart, *Brit. J. Pharmacol.*, 1974, **50**, 125.
[579] K. Lintner, S. Fermandjian, P. Fromageot, M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *F.E.B.S. Letters*, 1975, **56**, 366.
[580] M. M. Hall, M. C. Khosla, P. A. Khairallah, and F. M. Bumpus, *J. Pharmacol. Exp. Ther.*, 1974, **188**, 222.
[581] M. J. Peach and M. Ober, *J. Pharmacol. Exp. Ther.*, 1974, **190**, 49.
[582] R. K. Turker, M. M. Hall, and F. M. Bumpus, *J. Pharm. Pharmacol.*, 1974, **26**, 582.
[583] J. Ancans and G. I. Cipens, *Latv. P.S.R. Zinat. Acad. Vestis, Kim. Ser.*, 1974, 241 (*Chem. Abs.*, 1974, **81**, 78 221).
[584] P. Cordopatis and D. Theodoropoulos, *Experientia*, 1975, **31**, 1255.

amine derivative,[585] and polymer-bound angiotensin II for affinity chromatography,[239] have also been described. Other studies on position 1 modification have appeared.[586]

Potentiating Peptides and Enzyme Inhibitors.—The converting enzyme inhibitor (690) (Squibb 20881) continues to be studied [587, 588] (see Vol. 6, p. 478). It may

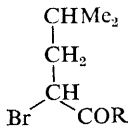
Glp-Trp-Pro-Arg-Pro-Glu-Ile-Pro-Pro
(690)

possibly find medical use in lowering the blood pressure of patients with renovascular hypertension.[556]

A series of peptides has been synthesized with L-α-bromoisocaproic acid in place of leucine in sequences from the angiotensinase substrate (691), and related

····-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser-····
(691)

sequences, as potential renin inhibitors.[589] Three effective inhibitors (692), (693), and (694) were identified in particular. The inhibitory effects of the acids (692) and (693) were strongly pH dependent, unlike those of (694). Corresponding D-α-bromoisocaproic acid derivatives were inactive.



(692) R = Val-Tyr-Ser
(693) R = Leu-Val-Tyr-Ser
(694) R = Val-Tyr-NH_2

A series of analogues of the sequence (691) failed to show inhibition of converting enzyme at physiological pH (*ca.* 7.5) because of solubility problems, and structural changes have been introduced in an attempt to increase solubility. Of these, introduction of prolyl residues at the *N*-terminus led to soluble inhibitors active at pH 7.5, particularly (695).[590]

Pro-His-Pro-Phe-His-Leu-Phe-Val-Tyr
(695)

The synthesis and properties of a series of new bradykinin potentiating peptides with a *C*-terminal Ile-Pro-Pro sequence have been described.[591] The tripeptide Arg-Pro-Pro is a competitive inhibitor of converting enzyme, and since it occurs as part of the bradykinin sequence, a possible natural role has been suggested.[592]

[585] G. Forget, S. Heisler, W. K. Park, P. Sirois, D. Gagnon, and D. Regoli, *J. Pharm. Pharmacol.*, 1975, **27**, 491.
[586] D. Regoli, F. Rioux, W. K. Park, and C. Choi, *Canad. J. Physiol. Pharmacol.*, 1974, **52**, 39.
[587] E. G. Erdos, W. H. Massion, D. R. Downs, and A. Gecse, *Proc. Soc. Exp. Biol. Med.*, 1974, **145**, 948.
[588] E. E. Muirhead, B. Brooks, and K. K. Arora, *Lab. Invest.*, 1974, **30**, 129.
[589] Y. Suketa and T. Inagami, *Biochemistry*, 1975, **14**, 3188.
[590] J. Burton, K. Poulsen, and E. Haber, *Biochemistry*, 1975, **14**, 3892.
[591] M. Tominaga, J. M. Stewart, T. B. Paiva, and A. C. M. Paiva, *J. Medicin. Chem.*, 1975, **18**, 130.
[592] G. Oshima and E. G. Erdos, *Experientia*, 1974, **30**, 733.

Surprisingly potent renin inhibitors have been identified among a range of common proteins and protein fragments. One of the most potent was the 1—55 sequence of horse β-haemoglobin, the inhibition perhaps arising because of the sequence (696) which is similar to that of renin substrate.[593]

-Arg-Leu-Leu-Val-Val-Tyr-
30 31 32 33 34 35

(696)

Hog renal renin has been purified by affinity chromatography using the inhibitor (697) coupled to Sepharose.[594]

His-Pro-Phe-His-Leu-D-Leu-Val-Tyr

(697)

Canine fibrinopeptide A (698) potentiates the bradykinin-induced contraction of rat uterine muscle *in vitro*. The *N*-terminal octapeptide shares the activity, as does the des-Thr[1]-hexadecapeptide:

Thr-Asn-Ser-Lys-Glu-Gly-Glu-Phe-Ile-Ala-Glu-Gly-Gly-Gly-Val-Arg
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

(698)

Shortening the octapeptide from the *C*-terminus destroys the activity, and the hexadecapeptide in which serine is phosphorylated is also inactive.[595]

Tachykinins.—A review has appeared on substance P.[596] Table 10 lists tachykinin sequences and analogues whose biological activity has been reported.

The amphibian skin has proved to be a rich source of new peptides and shows no signs yet of exhaustion. Recent structures include uperolein (739), an endecapeptide closely related to physalaemin,[605] and litorin (740), related to bombesin by the change Leu[8] → Phe[8] and with the *C*-terminal octapeptide sequence of ranatensin, from the skin of the Australian frog *Litoria aurea*.[606] The synthesis of litorin has been described.[607] Another new peptide from this source, xenopsin (471), shows a strong *in vitro* rat fundus contractile effect.[608] Most surprisingly, TRH has been found as a component of amphibian skin.[27]

The role of substance P as a neurotransmitter is discussed in Section 9. The structural specificity for these actions seems quite different from that with smooth muscle[597] (see Table 10).

As with other tachykinins, shortened *C*-terminal sequences of substance P are more active than the full endecapeptide towards the guinea-pig ileum.[598] The maximum activity (264%) is found with the octapeptide amide (708), the nonapeptide (714) being the next most active (172%). The c.d. and i.r. spectra of these compounds have also been reported,[609] and the activity of some of them compared on guinea-pig ileum and rat colon with [Lys[6]] eledoisin (6—11) hexapeptide.[599]

A further synthesis of biologically active substance P has been described. In addition to the expected effects on guinea-pig ileum, blood pressure, and blood

[593] R. J. Workman, M. M. McKown, and R. I. Gregerman, *Biochemistry*, 1974, **13**, 3029.
[594] K. Poulsen, J. Burton, and E. Haber, *Biochim. Biophys. Acta*, 1975, **400**, 258.
[595] A. J. Osbahr and R. W. Colman, *Biochim. Biophys. Acta*, 1974, **336**, 273.
[596] S. E. Leeman and E. A. Mroz, *Life Sci.*, 1974, **15**, 2033.

Table 10 *Analogues of substance P and other tachykinins*

Compound number	*Structure* [a]	*Biological activity:* *g.p.i.* [b]	*b.p.* [c]	*sal.* [d]	*CNS* [e]	*Ref.*
1 2 3 4 5 6 7 8 9 10 11						
Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2 substance P					200	
Glp-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2 physalaemin					1500	
Glp-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2 eledoisin					2000	
(699)	Lys-Phe-Ile-Gly-Leu-Met-NH_2				770	
(700)	Lys-Phe-Tyr-Gly-Leu-Met-NH_2				150	
(701)	$H_2N(CH_2)_5CO$-Phe-Tyr-Gly-Leu-Met-NH_2				130	
(702)	Lys-Phe-Ile-Gly-MeLeu-Met-NH_2				50	597
(703)	Lys-Phe-Ile-Gly-Hic-Met-NH_2				30	
(704)	Lys-Phe-Gly-Gly-Leu-Met-NH_2				2	
(705)	Lys-Phe-Lac-Gly-Leu-Met-NH_2				2	
(706)	Lys-MePhe-Ile-Gly-Leu-Met-NH_2				1	
(707)	Lys-MePhe-Ile-Gly-MeLeu-Met-NH_2				1	
(708)	Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2				15	
(709)	Glp-Phe-Phe-Gly-Leu-Met-NH_2				< 5	
(710)	Phe-Phe-Gly-Leu-Met-NH_2				1.2	
(711)	Phe-Gly-Leu-Met-NH_2				< 0.1	
(712)	Gly-Leu-Met-NH_2				< 0.04	
(710)	Phe-Phe-Gly-Leu-Met-NH_2	3				
(709)	Gln-Phe-Phe-Gly-Leu-Met-NH_2	109				
(713)	Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	127				598
(708)	Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	264				
(714)	Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	172				
(715)	Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	70				

[597] M. Otsuka, S. Konishi, and T. Takahashi, *Fed. Proc.*, 1975, **34**, 1922.

[598] J. Bergmann, M. Bienert, H. Niedrich, B. Mehlis, and P. Oehme, *Experientia*, 1974, **30**, 401.

Table 10 (*cont.*)

Compound number	*Structure* [a]	*Biological activity*: *g.p.i.* [b]	*b.p.* [c]	*sal.* [d]	*CNS* [e]	*Ref.*
(710)	Phe-Phe-Gly-Leu-Met-NH_2	*f*				599
(709)	Gln-Phe-Phe-Gly-Leu-Met-NH_2	*f*				
(708)	Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2	*f*				
(699)	Lys-Phe-Ile-Gly-Leu-Met-NH_2	*f*				
(716)	Phe-Phe-Phe-Gly-Leu-Met-NH_2	(1.9) [g]				600
(717)	Asn-Phe-Phe-Phe-Gly-Leu-Met-NH_2	(7.0) [g]				
(718)	Pro-Asn-Phe-Phe-Phe-Gly-Leu-Met-NH_2	(13.5) [g]				
(719)	Gln-Phe-Phe-Phe-Gly-Leu-Met-NH_2	(3.7) [g]				
(720)	Pro-Gln-Phe-Phe-Phe-Gly-Leu-Met-NH_2	(2.7) [g]				
	[Lys[6]]-eledoisin (6—11) analogues with modified peptide bonds:					
	Lys———Phe———Ile———Gly———Leu———Met—NH_2					
(721)	COO		90			601
(722)	CONMe		10			
(723)	COO		10			
(724)	CONMe		0.1			
(725)	COO		30			
(726)	CONMe		200			
(727)	COO		120			
(728)	CONMe		120			
(729)	COO		0.1			

(730)		CONMe		0.1	
(731)	COO	COO		30	
(732)	CONMe	CONMe		10	
(733)	$H_2NN(Me)CO$-Phe-Ile-Gly-Leu-Met-NH_2		0.6		602
(734)	Ala-$NHN(Me)CO$-Phe-Ile-Gly-Leu-Met-NH_2		0.8		602
(735)	Lys-Asp-$NHN(Me)CO$-Phe-Ile-Gly-Leu-Met-NH_2		4		603
(736)	Asp-$NHN(Me)CO$-Phe-Ile-Gly-Leu-Met-NH_2		17		603
(737)	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Nle-NH_2			100	604
(738)	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Nle-OH			<0.1	604

[a]Hic = α-hydroxyisocaproic acid; Lac = lactic acid. [b] Guinea-pig ileum *in vitro*; substance P = 100%. [c] Rat blood pressure (%). [d] Rat salivary secretion; substance P = 100%. [e] Depolarization of frog spinal motoneurons (L-glutamate = 1). [f] Studied on guinea-pig ileum and rat colon. [g] Bradykinin = 1.

599 J. Bergmann, P. Oehme, M. Bienert, and H. Niedrich, *Experientia*, 1974, **30**, 1314.
600 K. Channabasavaiah and K. M. Sivanandaiah, *Indian J. Chem.*, 1974, **12**, 1203.
601 M. Miyoshi and H. Sugano, in 'Peptides 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 355.
602 H. Niedrich, C. Oehme, and J. Bergmann, *J. prakt. Chem.*, 1974, **316**, 741.
603 H. Niedrich and G. Köller, *J. prakt. Chem.*, 1974, **316**, 729.
604 J. van Rietschoten, G. W. Tregear, S. Leeman, D. Powell, H. Niall, and J. T. Potts, in 'Peptides 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 113.

Glp-Pro-Asp-Pro-Asn-Ala-Phe-Tyr-Gly-Leu-Met-NH_2

(739)

Glp-Gln-Trp-Ala-Val-Gly-His-Phe-Met-NH_2

(740)

Glp-Gly-Lys-Arg-Pro-Trp-Ile-Leu

(741)

flow, the peptide has slight but definite gonadotropin releasing activity, though no effect on growth hormone, prolactin, or thyrotropin. This is explained as a possible interaction with the LH-RH receptor.[610] An alternative explanation might perhaps involve low levels of substance P receptors in the membranes of anterior pituitary gonadotroph cells.

Some of the problems of interpreting data on peptides made by solid-phase synthesis, whose purity may be in some doubt (see for example Vol. 7, p. 293), may be overcome by a new method [604] in which two sequences are synthesized simultaneously on different insoluble supports. Supports are chosen which are easily separated by physical methods (for example differences in density) and the approach makes possible the synthesis of an analogue, and of the native hormone as a control, under virtually identical conditions. The method has been demonstrated in the synthesis of [Nle^{11}] substance P (737) and the corresponding acid (738).

Studies on the cleavage of substance P by a partially purified enzyme from rat brain suggest cleavage either between the Gln^6 and Phe^7 residues, or between Phe^7 and Phe^8, and also between Gly^9 and Leu^{10}.[611]

A series of analogues has been described in which the amide bonds in the *C*-terminal hexapeptide of eledoisin are replaced by —COO— or —CONMe—.[601] The effect varies dramatically, depending on the bond being modified. With the ester group between lysine and phenylalanine (721), 90% of the depressor activity is retained, but with the CONMe substitution (722) it falls to 10%. Between isoleucine and glycine, the ester has 30% activity and the CONMe derivative 200%, while both changes to the leucylmethionine bond lead to a fall to 0.1% in both cases. Taken with c.d. spectra, the results have been used to predict a possible active conformation for the hexapeptide.

The replacement of alanine by an aza-alanine (—NHNMeCO—) residue in the eledoisin hexa- and hepta-peptide sequences (733) and (734),[602] or in the octa- and hepta-peptide sequences (735) and (736),[603] leads to substantial loss of activity. Other reported work includes the synthesis and biological activity (guinea-pig ileum) of a range of *C*-terminal peptide analogues of physalaemin (with similarities also to other tachykinins),[600] the effects of bombesin and related peptides on

[605] A. Anastasi, V. Erspamer, and R. Endean, *Experientia*, 1975, **31**, 394.
[606] A. Anastasi, V. Erspamer, and R. Endean, *Experientia*, 1975, **31**, 510.
[607] F. Angelucci and R. de Castiglione, *Experientia*, 1975, **31**, 507.
[608] K. Araki, S. Tachibana, M. Uchiyama, T. Nakajima, and T. Yasuhara, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 3132.
[609] B. Mehlis, S. Böhm, M. Becker, and M. Bienert, *Biochem. Biophys. Res. Comm.*, 1975, **66**, 1447.
[610] G. H. Fisher, J. Humphries, K. Folkers, B. Pernow, and C. Y. Bowers, *J. Medicin Chem.*, 1974, **17**, 843.
[611] M. Benuck and N. Marks, *Biochem. Biophys. Res. Comm.*, 1975, **65**, 153.

gastrointestinal myo-electric activity,[612] and the mechanism of action of substance P.[613]

9 Peptides acting on the Nervous System

The period under review has witnessed dramatically increased interest in the activities and the role of peptides in nervous tissue. It has been known for some time that certain peptides have CNS activity, and substance P has been for some time thought possibly to be a neurotransmitter. Recent results in a number of fields are pointing, however, to a much more widespread presence and action of peptides than was hitherto believed.

One particularly significant pointer emerged when the long postulated and recently much sought after natural morphine receptor agonist (or at least *a* morphine receptor agonist) was isolated from pig brain by Kosterlitz and his co-workers and shown to be a simple pair of pentapeptides (742) and (743).[614]

Tyr-Gly-Gly-Phe-Met (742) Tyr-Gly-Gly-Phe-Leu (743)

Such a physiological transmitter-like substance has long been postulated to explain the functioning of morphine receptors which clearly are important in the pain process but for which morphine itself is presumably not the usual agonist. The two pentapeptides, methionine enkephalin (742) and leucine enkephalin (743), act as potent agonists on the specific morphine receptors in vas deferens tissue. Their actions are characterized by a rapid onset and short duration and require relatively large amounts of naloxone to be antagonized, presumably reflecting a high binding constant and rapid metabolism. Structurally the most remarkable feature is that the sequence of (742) is identical with the sequence 61—65 of

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Glu-Thr-Pro-Leu-Val-Thr (744)

β-lipotropin. Indeed, it has more recently been shown that large quantities of hexadecapeptide (744) corresponding to positions 61—76 of β-lipotropin (*i.e.* with methionine enkephalin as its *N*-terminus) are present in extracts of porcine hypothalamic tissue and this peptide, called α-endorphin, also has morphine-like properties in classical bioassays and binds to stereospecific synaptosomal morphine receptors in competition with tritiated etorphine.[615]

Given intraventricularly in the rat both (742) and (743) showed analgesic activity, though at doses (100—200 μg per animal) which were higher by a factor of 10 than the analgesic morphine dose.[616]

Smyth on the other hand has found analgesic activity, as determined by intracerebral administration of peptide in the cat, to be absent with (742).[617] Extremely

[612] R. Caprilli, G. Frieri, G. Improta, P. Melchiorri, and P. Vernia, *Brit. J. Pharmacol.*, 1974, **52**, 465P.

[613] P. Stern, S. Catovic, and M. Stern, *Arch. Pharmacol.*, 1974, **281**, 233; (erratum) 1974, **283**, 115.

[614] J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, and H. R. Morris, *Nature*, 1975, **258**, 577.

[615] R. Guillemin, N. Ling, and R. Burgus, *Compt. rend.*, 1976, **282**, *D*, 783.

[616] J. D. Belluzzi, N. Grant, V. Garsky, D. Sarantakis, C. D. Wise, and L. Stein, *Nature*, 1976, **260**, 625.

[617] A. F. Bradbury, D. G. Smyth, C. R. Snell, N. J. M. Birdsall, and E. C. Hulme, *Nature*, 1976, **260**, 793; A. F. Bradbury, D. G. Smyth, and C. R. Snell, *Biochem. Biophys. Res., Comm.*, 1976, **69**, 950.

high analgesic activity (200 × morphine) was, however, found in the *C*-fragment corresponding to positions 61—91 of porcine β-lipotropin and this activity is lost when the *C*-terminal tetrapeptide (88—91) is not present.

The activities of a number of analogues of enkephalin on the vas deferens preparation have been described.[618] Leucine enkaphalin (743) was claimed to have greater (160%) activity than (742), although in the original paper (743) appeared to be less active. The *N*-methyl derivative of (742) had 20% activity and other derivatives (including [Phe^1], des-Tyr^1, [D-Phe^4], and [Tyr(Bu^t)^1, Leu^5]) had low activities of the order of 3% or less.

To complicate the picture still further, salmon calcitonin given by the intracerebral route also has a potent analgesic effect but this is possibly mediated by an action on calcium ions.[619] Interestingly, morphine is antagonized by both β-MSH and ACTH (1—24).[620] Some of the work prior to the determination of the enkephalin structure is described in a recent book,[621] and opiate receptors are the subject of recent reviews.[622, 623]

In recent years there has been much interest in the actions of releasing hormones in the CNS and their possible clinical value. In particular the possible use of TRH in treating depressive illness, or possibly schizophrenia,[624] has led to a number of further studies in man. These studies turned out to be somewhat disappointing and despite some suggestions of the possible value of TRH,[625] perhaps in distinguishing different kinds of depression,[626, 627] reports generally indicated that it was unlikely to be a useful antidepressant drug.[626, 628–631] A clinical value was also proposed for Pro-Leu-Gly-NH_2 (a purported MIF) particularly as an adjunct to L-dopa in parkinsonism. Again, while there are reports that it is valuable in this situation,[632, 633] another report concluded that the observed effect was not statistically significant.[634]

Nevertheless the initial biological work appeared to demonstrate a real CNS activity of hypothalamic peptides, and a number of further studies have

[618] L. Fothergill, J. Hughes, H. Kosterlitz, T. Smith, J. Bower, K. Guest, B. Morgan, and H. Morris, Reported at the Meeting of the Chemical and Biochemical Societies Peptide and Protein Group, Exeter, April 2nd, 1976.
[619] A. Pecile, S. Ferri, P. C. Braga, and V. R. Olgiati, *Experientia*, 1975, **31**, 332.
[620] W. Krivoy, D. Kroeger, A. Newman-Taylor, and E. Zimmermann, *European J. Pharmacol.*, 1974, **27**, 339.
[621] 'Opiate Receptor Mechanisms: Neurochemical and Neurophysical Processes in Opiate Drug Action and Addition', ed. S. H. Snyder and S. Matthysse, MIT Press, Cambridge, Mass., 1975.
[622] S. H. Snyder, *Nature*, 1975, **257**, 185.
[623] A. Goldstein, *Life Sci.*, 1974, **14**, 615.
[624] A. M. Drayson, *Lancet*, 1974, **i**, 312.
[625] W. N. Hutton, *Lancet*, 1974, **ii**, 53.
[626] R. H. Ehrensing, A. J. Kastin, D. S. Schalch, H. C. Friesen, J. R. Vargas, and A. V. Schally, *Amer. J. Psychiatry*, 1974, **131**, 714.
[627] W. Pühringer, A. Wirz-Justice, and G. Hole, *Lancet*, 1975, **i**, 1344.
[628] L. B. Bigelow, J. C. Gillin, C. Semal, and R. J. Wyatt, *Lancet*, 1975, **ii**, 869.
[629] A. Coppen, S. Montgomery, M. Peet, J. Bailey, V. Marks, and P. Woods, *Lancet*, 1974, **ii**, 433.
[630] C. Q. Mountjoy, J. S. Price, M. Weller, P. Hunter, R. Hall, and J. H. Dewar, *Lancet*, 1974, **i**, 958.
[631] C. Q. Mountjoy and R. Hall, *Lancet*, 1974, **ii**, 415.
[632] N. P. Plotnikoff and A. J. Kastin, *Pharmacol. Biochem. Behaviour*, 1974, **2**, 417.
[633] A. Barbeau, *Lancet*, 1975, **ii**, 683.
[634] T. N. Chase, A. C. Woods, M. A. Lipton, and C. E. Morris, *Arch. Neurology*, 1974, **31**, 55.

strengthened and extended this assertion. Some relevant studies include the action of TRH, Pro-Leu-Gly-NH_2, and angiotensin II on the behavioural effects of L-dopa and 5-HT in mice,[635] the tremorogenic effect of TRH in rats (including hypophysectomized or thyroidectomized rats),[636] the inhibition of pentobarbital effects, including toxicity, by TRH,[637–639] the inhibition of strychnine actions by TRH,[637] and its antagonism of ethanol narcosis, tri-iodothyronine being ineffective.[640] Other studies include demonstrations of enhancement of L-dopa activity by TRH [641a] and Pro-Leu-Gly-NH_2 [641b] in thyroidectomized rats, enhancement of cerebral noradrenaline turnover,[642] potentiation of behavioural changes associated with increased accumulation of 5-HT in brain,[643] and an effect on the behaviour of rats pretreated with α-methyltyrosine [644] by TRH. The possible antidepressant effect of Pro-Leu-Gly-NH_2 has also been studied.[645]

Somatostatin also has CNS effects including an inhibition of the action of strychnine.[637] It has been examined in a series of seven tests for CNS activity, showing significant activity (at doses of 1—4 mg kg^{-1} i.p.) in the dopa potentiation test in which antidepressant drugs are active, but was inactive as a dopa antagonist, in potentiating or antagonizing serotonin or as an oxotremorine antagonist. It showed no protective action against audiogenic seizures or in preventing footshock-induced fighting behaviour.[646]

The actions of hypothalamic hormones in the CNS have been rationalized as a pathway for carrying chemical signals from the hypothalamus to higher brain centres. The mechanism suggested would involve a neurosecretory process similar to that releasing the hormones into the portal blood vessels. The evidence, apart from the observed effects of the substances in behavioural and electrophysiological tests and the knowledge that the hypothalamus does exert considerable effect on other brain centres, includes direct studies with antisera directed against the hormones which demonstrate (by immunohistochemical methods) their widespread presence in different areas of brain and particularly in neurons which terminate in extrahypothalamic regions.[647–651] There are also

[635] J. P. Huidobro-Toro, A. Scotti de Carolis, and V. G. Longo, *Pharmacol. Biochem. Behaviour*, 1974, **2**, 105.
[636] L. Schenkel-Hulliger, W. P. Koella, A. Hartmann, and L. Maître, *Experientia*, 1974, **30**, 1168.
[637] M. Brown and W. Vale, *Endocrinology*, 1975, **96**, 1333.
[638] A. J. Prange, G. R. Breese, J. M. Cott, B. R. Martin, B. R. Cooper, I. C. Wilson, and N. P. Plotnikoff, *Life Sci.*, 1974, **14**, 447.
[639] A. J. Prange, G. R. Breese, G. D. Jahnke, B. R. Martin, B. R. Cooper, J. M. Cott, I. C. Wilson, L. B. Alltop, M. A. Lipton, G. Bissette, C. B. Nemeroff, and P. T. Loosen, *Life Sci.*, 1975, **16**, 1907.
[640] G. R. Breese, J. M. Cott, B. R. Cooper, A. J. Prange, and M. A. Lipton, *Life Sci.*, 1974, **14**, 1053.
[641] (*a*) N. P. Plotnikoff, A. J. Prange, G. R. Breese, and I. C. Wilson, *Life Sci.*, 1974, **14**, 1271; (*b*) N. P. Plotnikoff, F. N. Minard, and A. J. Kastin, *Neuroendocrinology*, 1974, **14**, 271.
[642] H. H. Keller, G. Bartholini, and A. Pletscher, *Nature*, 1974, **248**, 528.
[643] A. R. Green and D. G. Grahame-Smith, *Nature*, 1974, **251**, 524.
[644] B. Kulig, *Neuropharmacology*, 1975, **14**, 489.
[645] R. H. Ehrensing and A. J. Kastin, *Arch. Gen. Psychiatry*, 1974, **30**, 63.
[646] N. P. Plotnikoff, A. J. Kastin, and A. V. Schally, *Pharmacol. Biochem. Behaviour*, 1974, **2**, 693.
[647] J. B. Martin, L. P. Renaud, and P. Brazeau, *Lancet*, 1975, **ii**, 393.
[648] A. Winokur and R. D. Utiger, *Science*, 1974, **185**, 265.
[649] M. J. Brownstein, M. Palkovits, J. M. Saavedra, R. M. Bassiri, and R. D. Utiger, *Science*, 1974, **185**, 267.
[650] E. C. Griffiths, K. C. Hooper, and S. L. Jeffcoate, *Lancet*, 1975, **ii**, 554.
[651] G. Setals, *Brain Res.*, 1975, **90**, 352.

enzyme systems in these regions which rapidly inactivate the hormones,[650] and receptors for *e.g.* TRH in brain membranes.[652] The pineal gland has been suggested as the source of material for the extrapituitary effects of releasing hormones.[653]

Many of the tests use dose levels much higher than those required for endocrine effects but still comparable with doses of other pharmacological agents acting in such systems. The high dose levels may reflect difficulty in crossing physiological barriers before reaching target tissue. One CNS action of TRH has been described, however, which is an exception. The administration of doses as low as 3 ng of TRH into the cerebral ventricles of cats causes a fall in body temperature of up to 0.5 °C. The effect is shared by brain amines and some CNS active drugs but at higher levels, and TRH is in fact the most potent known hypothermic agent. In the rabbit the effect is opposite, the resulting hyperthermia again being found with brain amines and some CNS drugs.[654]

Using some of the test systems described, analogues of hypothalamic hormones are now being tested for their CNS actions. Table 11 gives some of the first

Table 11 *TRH analogues and related peptides with central nervous effects*

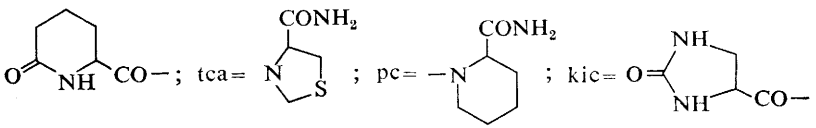
Compound number	*Structure* [b]	*Biological activity:*[a] *TSH*	*PRL*	*CNS*	*Test*	*Ref.*
	TRH			–	A	
	Pro-Leu-Gly-NH_2			30 mg kg^{-1}	A	
(24)	Glp-Thi-Pro-NH_2			8 mg kg^{-1}	A	655
(745)	Glp-Leu-Gly-NH_2			19 mg kg^{-1}	A	
(17)	Glp-Leu-Pro-NH_2			–	A	
(746)	Glp-Gly-Pro-NH_2			–	A	
(35)	Glp-His-Pro-NHEt					
(747)	Glp-His-Pro-$NHPr^n$					
(748)	Glp-His-Pro-$NHBu^i$					656
(749)	Glp-His-Pro-$NH(CH_2)_4Me$					
(750)	Glp-His-Pro-$NH(CH_2)_2Ph$					
(751)	Glp-His-Pro-$NH(CH_2)_5Me$		(+++)		B	656, 657
(752)	Glp-His-Pro-NH-cyclohexyl		(+++)		B	656, 657
				+++	C	657
(753)	Glp-His			++++	C	
(754)	Glp-His-pyr			+++	C	
(37)	Glp-His-Pro			++++	C	
(755)	Glp-His-Trp-NH_2			+++	C	658
(756)	Glp-Phe-Pro-NH_2			+++	C	
(42)	Glp-Met-Pro-NH_2			(++++)	C	
(757)	Glp-Lys-Pro-NH_2			+++	C	
(758)	Glp-His-Pro-$NH(CH_2)_2CO_2H$			+++	D	659

[652] D. Burt and S. Snyder, *Brain Res.*, 1975, **93**, 309.
[653] W. F. White, M. T. Holland, G. F. Weber, R. H. Rippel, E. S. Johnson, and J. F. Wilber, *Endocrinology*, 1974, **94**, 1422.
[654] G. Metcalf, *Nature*, 1974, **352**, 310.
[655] S. Castensson, H. Sievertsson, B. Lindeke, and C. Y. Sum, *F.E.B.S. Letters*, 1974, **44**, 101.
[656] Belg.P. 819 198.
[657] H. Wissmann, R. Geiger, W. König, H.-J. Kruse, and K. Seeger, *Ger. Offen.*, 2 343 035.
[658] W. König, R. Geiger, H. Wissmann, and H.-J. Kruse, *Ger. Offen.*, 2 343 037.
[659] C. B. Nemeroff, A. J. Prange, G. Bissette, G. R. Breese, and M. A. Lipton, *Psychopharmacol. Comm.*, 1975, **1**, 305.

Table 11 (*cont.*)

Compound number	*Structure* [b]	*Biological activity:* [a] *TSH*	*PRL*	*CNS*	*Test*	*Ref.*
(759)	kpc-His-tca-NH_2	100		800	E, F	660
(760)	kpc-His-Pro-NH_2	100		400	E, F	
(761)	DL-kpc-His-Pro-NH_2	20		300	E, F	
(762)	Glp-DL-His-tca-NH_2	20		200	E, F	
(763)	Glp—His-pc—OMe	20		100	E, F	
(764)	kic—His-Pro-NH_2	10		100	E, F	
(765)	Glp—His($^{\tau}CH_2CO_2H$)-Pro-NH_2	10		100	E, F	
(766)	D-kpc-His-Pro-NH_2	10		25	E, F	
(767)	kpc-His-pc-OMe	1.6		100	E, F	
(768)	Glp-DL-His-DL-pc-OMe			50	F	
(769)	Glp-DL-His—pc-OMe			100	F	
(770)	Glp-His-pc-NH_2	100		100	E, F	
(771)	DL-kpc-His-Pro-OMe	2			E	
(772)	kic-His-Pro-OMe	0.1			E	
(773)	cSer-His-Pro-NH_2	0.1			E	
(774)		0.1			E	

[a] A, Dose (mg kg^{-1}) i.p. to block 90—95% of tremor induced by oxotremorine; B, prolactin release in the cow; C, Dopa potentiation test (TRH = 100%); D, potentiation of the anticonvulsant effect of phenobarbital; E, TSH releasing (unspecified, TRH = 100%); F, antidepressant (test unspecified), TRH = 100%. [b] kpc =



results of such studies, partly taken from recent patent literature. The effects on oxotremorine-induced tremor (used as a test for antidepressant activity) of TRH, Pro-Leu-Gly-NH_2, and some related peptides are shown in the Table. A non-peptide antidepressant product candidate was used for comparison and was active at 2.9 mg kg^{-1} i.p. Of the compounds studied, the β-(thienyl-2) alanine analogue (24), active at 8 mg kg^{-1}, was most effective. TRH itself was inactive.[655] In the other studies [657–660] the limited data available suggest a parallelism between CNS effects and prolactin releasing properties in many analogues, but that the effect of TRH on the thyroid axis may be lost in analogues which retain the former activities.

The effects of injection of 0.3—3 mg kg^{-1} TRH i.v. in rats include muscle tremor, excitation, tail lifting, pilo-erection, and cardiovascular effects. The [mono-iodohistidine[2]] and [di-iodohistidine[2]] analogues had similar effects but at much higher doses, representing *ca.* 1% activity in each case.[636] TRH and the analogue (758) with a *C*-terminal β-alanine potentiate the anticonvulsant properties of small doses of phenobarbital, in contrast to the antagonism of the sedation produced by larger doses of phenobarbital.[659]

[660] D. F. Veber and F. W. Holly, *Ger. Offen.*, 2 514 381.

Earlier results on the effect of vasopressin and particularly desglycineamide[9] vasopressin on memory consolidation are supported by a study of rats with hereditary diabetes insipidus which are unable to synthesize vasopressin and which fail also to inhibit passive avoidance behaviour.[661] The effects of vasopressin, [Arg[8]] vasopressin, and the desglycineamide[9] derivative on the consolidation of learned passive avoidance behaviour were studied in such animals. While the absence of vasopressin did not affect learning itself, the memory consolidation was impaired. This was also the case when available vasopressin was blocked by anti-vasopressin antibodies injected into the lateral ventricle.[662]

The effects of vasopressin, oxytocin, and a range of analogues, fragments and related structures, on puromycin-induced amnesia have been studied. A number of vasopressin derivatives, particularly vasopressin itself and triglycylvasopressin, were highly active. Some, including pressinoic acid and [Arg[8]] vasopressin acid were inactive. Oxytocin derivatives were generally inactive with the exception of the *N*-triglycyl derivative. Of the smaller peptides, Pro-Leu-Gly-NH_2 and Z-Pro-Leu-Gly-NH_2 as well as D- or L-Leu-Gly-NH_2 and the leucylglycine dioxopiperazine were highly active.[663] Desglycineamide[9] vasopressin has been shown to facilitate the development of resistance to morphine analgesia.[664]

The ACTH (4—10) sequence (778) has an interesting range of behavioural effects. Some of the work carried out to date has been summarized [665, 666] (see also Vol. 6, p. 441), and some of these results are listed in Table 12. The sequence is common to ACTH and β-MSH, and the effects perhaps take on a new significance when viewed against the recently discovered enkephalin structure lying just eight amino-acids away in the β-lipotropin molecule. The behavioural effects are commonly measured by a test involving an inhibition of a pole-jumping avoidance response and the effects have been shown to be clearly separable from the melanotropic effect. The results indicate a rather less precise dependence of activity on structure, uncharacteristic of peptide hormone responses and there is even a surprising total reversal of the behavioural effect on introduction of D-phenylalanine in position 7. This is accompanied by opposite effects of the two diasterioisomers on the metabolism of biogenic amines in rat brain.[667] It would be interesting to know what sort of fundamental reasons might lead to such differences from the typical hormone response. In a comparison of melanotropic and behavioural (inhibition of pole-jumping avoidance) effects of a series of analogues of ACTH (4—9), the latter activity was considerably increased by the changes: methionine → methionine sulphoxide in position 4, arginine → D-lysine in position 8, and tryptophan → phenylalanine in position 9. The peptide (801) with all three changes had 1000 times the activity of (782), although metabolic studies pointed to resistance towards proteolysis as a factor contributing to the

[661] D. de Wied, B. Bohns, and Tj. B. van Wimersma Greidanus, *Brain Res.*, 1975, **85**, 152.
[662] Tj. B. van Wimersma Greidanus, B. Bohus, and D. de Wied, *J. Endocrinol.*, 1975, **64**, 30P.
[663] R. Walter, P. L. Hoffman, J. B. Flexner, and L. B. Flexner, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **12**, 4180.
[664] W. A. Krivoy, E. Zimmermann, and S. Lande, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 1852.
[665] D. de Wied, A. Witter, and H. M. Greven, *Biochem. Pharmacol.*, 1975, **24**, 1463.
[666] D. de Wied and W. de Jong, *Ann. Rev. Pharmacol.*, 1974, **14**, 389.
[667] B. E. Leonard, *Arch. Internat. Pharm. Ther.*, 1974, **207**, 242.

Table 12 *Inhibition of pole-jumping avoidance response by ACTH sequences and analogues (from refs.* 665 *and* 666)

Compound number	*Structure*	*Response*
	1 2 3 4 5 6 7 8 9 10	
(775)	Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly	1
(776)	Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly	1
(777)	Ser-Met-Glu-His-Phe-Arg-Trp-Gly	1
(778)	Met-Glu-His-Phe-Arg-Trp-Gly	1
(779)	Glu-His-Phe-Arg-Trp-Gly	0.5
(780)	His-Phe-Arg-Trp-Gly	0.1
(781)	Phe-Arg-Trp-Gly	0.1
(782)	Met-Glu-His-Phe-Arg-Trp	1
(783)	Met-Glu-His-Phe-Arg	1
(784)	Met-Glu-His-Phe	1
(785)	Met-Glu-His	0.3
(786)	Ser-Tyr-Ser-Met-Glu-His-D-Phe-Arg-Trp-Gly	(1) [a]
(787)	Met-Glu-His-D-Phe-Arg-Trp-Gly	(1) [a]
(788)	Met-Glu-His-D-Phe	(1) [a]
(789)	D-Phe-Arg-Trp-Gly	(0.1) [a]
(790)	Met-Glu-His-Leu-Lys-Trp	1
(791)	Met-Glu-His-Trp-Arg-Trp-Gly	1.5
(792)	Met-Glu-His-Phe(Me_5)-Arg-Trp-Gly	3
(793)	Met-Glu-His-D-Leu-Lys-Trp	0.3
(794)	Met-Glu-His-D-Trp-Arg-Trp-Gly	0.3
(795)	Met-Glu-His-D-Phe(Me_5)-Arg-Trp-Gly	1.5
(796)	Met-Glu-His-Phe-Lys-Trp	1
(797)	Met-Glu-His-Phe-Lys-Phe	1
(798)	Met(O)-Glu-His-Phe-Arg-Trp-Gly	10
(799)	Met-Glu-His-Phe-D-Lys-Trp	30
(800)	Met-Glu-His-Phe-D-Lys-Phe	100
(801)	Met(O)-Glu-His-Phe-D-Lys-Phe	1000
(802)	Phe-D-Lys-Phe	0.1
(803)	Glp-His-Phe-NH_2	0.3
	Glp-His-Pro-NH_2 (TRH)	0.3
(755)	Glp-His-Trp-NH_2	0.5
	LH-RH	1

[a] With reversal of the effect.

high activity. The melanotropic activity was decreased, in sharp contrast, by some 1000-fold.[668]

Other related work on these ACTH sequences includes the effect of (778) on performance during a serial reaction task,[669] the attenuation of amnesia by (778) and its relationship to plasma corticosterone levels,[670] and the effect of (778) on male rat sexual behaviour and its response to threshold doses of testosterone.[671] Studies on rats with lesions in different areas of the brain point to a different location for the effects of (778) and of vasopressin.[672]

[668] A. Witter, H. M. Greven, and D. de Wied, *J. Pharmacol. Exp. Ther.*, 1975, **193**, 853.
[669] A. W. K. Gaillard and A. F. Sanders, *Psychopharmacologia*, 1975, **42**, 201.
[670] H. Rigter, *Behavioural Biol.*, 1975, **15**, 207.
[671] B. Bohus, H. H. L. Hendrickx, A. A. van Kolfschoten, and T. G. Krediet, *J. Endocrinol.*, 1975, **64**, 37P.
[672] Tj. B. van Wimersma Greidanus, B. Bohus, and D. de Wied, *Neuroendocrinology*, 1974, **14**, 280.

A further activity of (778) and a potential clinical application, is its effect on muscle action potentials. ACTH has been used therapeutically in conditions in which these are defective, for example myasthenia gravis and progressive spinal muscular atropy, but its use is complicated by release of adrenal corticosteroids. The shorter (778) has no significant steroidogenic effect, but has now been shown to give a response in the two conditions described (one example of each) similar to that of ACTH.[673]

Scotophobin is a peptide which is also claimed to have an effect on 'conditioned responses' or 'conditioned memory'.[674–676] The biological results have been the object of some controversy, and recently published chemical data have not helped to resolve the confusion. A peptide of the sequence originally proposed has been found to be inactive in assays using mice, rats, or roach to test conditioned behaviour, although it was active in an assay using goldfish. A peptide of the same sequence synthesized by classical methods was found to be completely inactive, and different from the natural material, which was, however, found to have the claimed activity.[677] Syntheses of [Asn^2, $Gln^{5,11}$]- and [Asp^2, $Gln^{5,11}$]scotophobins (with 10—12% and 100%, respectively, of the activity of the natural material) have been described.[678]

A new peptide has been identified with the claimed ability to transfer, in mice, a tendency not to step down from a platform in response to a painful stimulus. The peptide, 'catabathmophobin', has a molecular weight of *ca.* 1700—1950 and a modulating substance of molecular weight 540—740 has also been identified.[679]

A peptide has been isolated from the brain of mice conditioned not to respond to sound impulses and called 'ameletin'. The hexapeptide structure (804) has

Glp-Ala-Gly-Tyr-Ser-Lys
(804)

been proposed, but the synthetic hexapeptide has less activity than the natural material.[680, 681]

There is a lack of clear identification of the transmitter or transmitters involved in many neurons, for example the primary sensory neurons in the emerging dorsal nerve roots of the spinal cord. Substance P has been suggested as one possible candidate and further evidence has now come from immunohistochemical studies.[682] Using anti-substance P antisera, immunoreactive material is found in the neurons, supporting the idea of a transmitter role for substance P. The immunoreactive material is localized in particular populations of the primary sensory neurons, suggesting that there may be other as yet unidentified transmitters. Further evidence for a neurotransmitter role of substance P comes from

[673] F. L. Strand, H. Stoboy, F. Krivoy, and H. van Riezen, *Lancet*, 1975, **ii**, 919.
[674] D. de Wied, D. Sarantakis, and B. Weinstein, *Neuropharmacology*, 1973, **12**, 1109.
[675] G. Ungar, *Life Sci.*, 1974, **14**, 595.
[676] G. Ungar, *Biochem. Pharmacol.*, 1974, **23**, 1553.
[677] H. N. Guttman, B. Weinstein, R. M. Bartschot, and P. S. Tam., *Experientia*, 1975, **31**, 285.
[678] G. U. Holzer, *Diss. Abs.*, 1973, **B34**, 1424.
[679] H. N. Guttman and R. S. Cooper, *Life Sci.*, 1975, **16**, 915.
[680] H. Lackner and N. Tiemann, *Naturwiss.*, 1974, **61**, 217.
[681] B. Weinstein, R. M. Bartschot, R. M. Cook, P. S. Tam, and H. N. Guttman, *Experientia*, 1975, **31**, 754.
[682] T. Hökfelt, J. O. Kellerth, G. Nilsson, and B. Pernow, *Science*, 1975, **190**, 889.

its activity in depolarizing frog spinal motoneurons[597, 683] (see also Vol. 6, p. 482 and ref. 684). The peptide has also been found to affect the response of the guinea-pig vas deferens to transmural nerve stimulation,[685] and along with (but more effectively than) bradykinin and angiotensin to excite rat cerebral cortical neurons.[686] The possible involvement of substance P in retinal neurotransmission,[687] and its stimulation of adenyl cyclase in different areas of human brain[688] have also been studied.

Peptides related to sleeping and active states and present in the brain,[689] and in cerebrospinal fluid, have been studied.[690]

10 Miscellaneous

There have been reviews on intestinal absorption of peptides,[691, 692] prohormones in tissues and in the circulation,[693] and on different types of peptide with inhibitory activities.[694]

Peptides with new kinds of activity and from hitherto largely unexplored sources are being discovered at an apparently still increasing pace and no doubt many of them will prove to be fruitful subjects of future structure–activity studies. Insect peptide hormones are beginning to appear on the scene for the first time. A peptide (805) of 27 amino-acids is produced in the paragonial gland

Asp-Val*-Pro-Ser-Ala-Asn-Ala-Asn-Ala-Asn-Asn-Gln-Arg-Thr-
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Ala-Ala-Ala-Lys-Pro-Gln-Ala-Asn-Ala-Glu-Ala-Ser-Ser
15 16 17 18 19 20 21 22 23 24 25 26 27

(805) *Val : Leu 70 : 30

Arg-Tyr-Leu-Pro-Thr

(806)

Gly-Val-Ser-Pro-Lys-Leu

(807)

of the male of the fly *Drosophila funebris*; on injection it was found to influence the mating behaviour of virgin female flies.[695] A myotropic pentapeptide neurohormone, proctolin (806), has been isolated from the cockroach *Periplaneta americana*. The peptide has activity on cockroach proctodeal (hindgut) muscle

[683] S. Konishi and M. Otsuka, *Brain Res.*, 1974, **65**, 397.
[684] J. Henry, K. Krnjević, and M. E. Morris, *Canad. J. Physiol. Pharmacol.*, 1975, **53**, 423.
[685] U. S. von Euler and P. Hedqvist, *Acta Physiol. Scand.*, 1974, **90**, 651.
[686] J. W. Phillis and J. J. Limacher, *Exper. Neurol.*, 1974, **43**, 414.
[687] A. Winder and P. Patsalos, *Biochem. Soc. Trans.*, 1975, **2**, 1260.
[688] M. J. Duffy, J. Wong, and D. Powell, *Neuropharmacol.*, 1975, **14**, 615.
[689] H. Nagasaki, M. Iriki, S. Inoué, and K. Uchizono, *Proc. Japan Acad.*, 1974, **50**, 241.
[690] J. R. Pappenheimer, V. Fencl, M. L. Karnovsky, and G. Koski, in 'Brain Dysfunction in Metabolic Disorders', ed. F. Plum, Raven Press, New York, 1974, p. 201.
[691] D. M. Mathews, *Physiol. Revs.*, 1975, **55**, 537.
[692] 'Peptide Transport in Protein Nutrition', ed. D. M. Mathews and J. W. Payne, North Holland, Amsterdam, 1975.
[693] F. Melani, *Hormone Metab. Res.*, 1974, **6**, 1.
[694] J. Ramachandran, *Life Sci.*, 1974, **14**, 993.
[695] H. Baumann, K. J. Wilson, P. S. Chen, and R. E. Humbel, *European J. Biochem.*, 197, **52**, 521.

at concentrations as low as 10^{-9}—10^{-10} mol l^{-1}. The peptide and some analogues (not specified in the abstract) have been prepared.[696] Peptides, *e.g.* (807), with mosquito-attractant properties have been synthesized.[697]

The structure of urogastrone has finally been determined, and has proved to be something of an eye-opener.[698] The peptide exists in two forms of 52 and 53 amino-acids with a *C*-terminal arginine-53 as the only difference, and is closely similar in structure to the epidermal growth factor (EGF) previously isolated from mice. Mouse EGF causes premature opening of the eyes of neonatal mice, and urogastrone has been found to share this effect in addition to its inhibitory effects towards gastric secretion. The two peptides have an interesting *C*-terminal pentapeptide sequence in common but this is not necessary for the biological effects so far studied.[699] As might be expected from these findings, EGF has urogastrone-like properties.[700] The material (isolated from submaxillary glands of male mice) caused near maximal inhibition of gastric secretion in the histamine-stimulated perfused rat stomach or in dog gastric pouches stimulated by histamine, pentagastrin, or methacholine. The doses needed for these effects (which were of long duration – *ca.* 90 min, despite a t_{50} for the peptide in blood of *ca.* 4 min) were less than those needed for epidermal growth effects, and it was suggested that the former effects may be physiologically important, at least in some species.

Some studies have already been carried out on the possible clinical value of urogastrone. In the Zollinger–Ellison syndrome, gastric acid volume and concentration, and basal acid output were all decreased, and ulcer pain diminished 30—60 min after infusion of urogastrone.[701] At the same time, plasma gastrin as well as intrinsic factor and pepsin were increased.

An EGF has been identified,[702] isolated from human urine and characterized.[703] Its amino-acid composition is similar to that of mouse EGF. In view of the urogastrone findings this might be expected to be the same substance; the amino-acid composition looks intriguingly similar but with apparently significant differences, notably in tyrosine content. Recent work on EGF has been reviewed.[146]

Further details have been published of the determination of the structure[704] and the synthesis[705] of the red-pigment concentrating hormone (808) of the

Glp-Leu-Asn-Phe-Ser-Pro-Gly-Trp-NH_2

(808)

[696] A. N. Starratt and B. E. Brown, *Abs. Papers Amer. Chem. Soc. Meeting*, 1975, (170), PEST 98.
[697] L. V. Federova, L. G. Kovalenko, and N. Y. Krasnodrizhy, *Khim. prirod. Soedinenii*, 1975, 41.
[698] H. Gregory, *Nature*, 1975, **257**, 325; H. Gregory and I. R. Willshire, *Z. physiol. Chem.*, 1975, **356**, 1765.
[699] H. Gregory and B. M. Preston, B.P. Application No. 39 599/74; R. Camble, E. L. Gerring, and H. Gregory, Belg.P. 812 973; quoted in ref. 697.
[700] J. M. Bower, R. Camble, H. Gregory, E. L. Gerring, and I. R. Willshire, *Experientia*, 1975, **31**, 825.
[701] J. B. Elder, P. C. Ganguli, I. E. Gillespie, W. I. Delamore, and H. Gregory, *Lancet*, 1975, **ii**, 424.
[702] R. H. Starkey, S. Cohen, and D. N. Orth, *Science*, 1975, **189**, 800.
[703] S. Cohen and G. Carpenter, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1317.
[704] P. Ferlund, *Biochim. Biophys. Acta*, 1974, **371**, 304.
[705] P. Ferlund, *Biochim. Biophys. Acta*, 1974, **371**, 312.

shrimp. This is active in picogram amounts, concentrating pigment in the chromatophore thereby causing blanching.

Work continues on characterizing the FSH-inhibiting substance from bull seminal plasma known as inhibin and postulated as long ago as 1932 as an important controlling factor in spermatogenesis. It may also be involved in the oestrus cycle and in the transition in the female from sexual immaturity to adulthood.[706, 707] Its site of action is at the pituitary, and not through any change in LH-RH release.[708] A testis specific basic protein of 54 amino-acids, associated with spermatogenesis, has been isolated from homogenized rat and human testicles.[709] A tetrapeptide (809) has been isolated which has contraceptive

Thr-Pro-Arg-Lys
(809)

properties.[710] It is produced by the embryo at a very early (two cell or four cell) stage,[711] and is distinguishable from the Factor C LH-IH. Unlike the latter it fails to inhibit LH release.[192]

The cell-mediated immune response directed towards myelin basic protein has been used as a model of degenerative nervous disease. In a common form of the model (experimental allergic encephalomyelitis, EAE) in the guinea-pig, one antigenic region of the basic protein in particular has been implicated, covering residues 113–121 (810),[712] and structure–activity studies have been carried out around this sequence.[713] Other parts of the sequence can also give

Ser-Arg-Phe-Ser-Trp-Gly-Ala-Glu-Gly-Gln-Arg-*Pro-Gly*
111 112 113 114 115 116 117 118 119 120 121 122 123
(810)

rise to the immunological response, for example the *N*-terminal 1—43 region. Unlike the situation in the guinea-pig, the *C*-terminal sequences 153—169 and 153—166 of bovine basic protein induce EAE in the rhesus monkey.[714]

The replacement of glutamic acid 118 in the nonapeptide (113—121) by glycine gives an analogue with high EAE activity.[715] Replacement of glutamine 120 in the decapeptide (112—121) leads to a significant loss of activity.[716] Systematic replacement with glycine suggests that alanine in position 117 is critical, but that serine 115 and glutamic acid 119 are less important.[717]

[706] P. Franchimont, S. Chari, M. T. Hagelstein, and S. Duraiswami, *Nature*, 1975, **257**, 402.
[707] G. Lugaro, M. M. Casellato, G. Mazzola, G. Fachini, and G. Carrea, *Neuroendocrinology* 1974, **15**, 62.
[708] F. J. Bramble, A. L. Houghton, S. S. Eccles, M. A. F. Murray, and H. S. Jacobs, *Clin. Endocrinol.*, 1975, **4**, 443.
[709] W. S. Kistler, C. Noyes, R. Hsu, and R. L. Heinrikson, *J. Biol. Chem.*, 1975, **250**, 1847.
[710] H. A. Kent, *Fertility and Sterility*, 1975, **26**, 833.
[711] H. A. Kent, *Gen. Comp. Endocrinol.*, 1974, **22**, 397.
[712] P. R. Carnegie, *Nature*, 1971, **229**, 25.
[713] F. C. Westall, A. B. Robinson, J. Caccam, J. Jackson, and E. H. Eylar, *Nature*, 1971, **229**, 22.
[714] Y. D. Karhanis, D. J. Carlo, S. W. Brostoff, and E. H. Eylar, *J. Biol. Chem.*, 1975, **250**, 1718.
[715] A. A. Gershkovich, V. K. Kibirev, L. M. Fedorchenko, and S. B. Serebryanyi, *Khim. prirod. Soedinenii*, 1975, 439.
[716] Y. Sasaki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2188.
[717] K. Suzuki and Y. Sasaki, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2181.

Injection of basic protein in animals with established EAE can give clinical improvement,[718] possibly through a delayed-type hypersensitivity response,[719] as opposed to the cell-mediated basis of EAE. Since basic protein can cause EAE its use is problematic, but peptides containing parts of the (113—121) sequence have shown delayed hypersensitivity without EAE,[720, 721] and a tetrameric form of one of these (811) has now been synthesized. On injection it leads to a signifi-

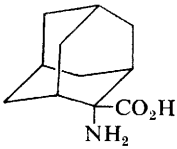
$$(\text{Phe-Ser-Trp-Gln-Lys})_4\text{-Gly}$$
(811)

cant reversal of the clinical signs of EAE and a significant reversal of the EAE response to challenge with basic protein.[721] The sera of animals injected with basic protein is cytotoxic towards nervous cell tissue cultures. By studying fragments the main sequence responsible has been located in the 1—43 *N*-terminal region.[722] Other work in this area includes a study of the effect of basic protein and related peptides on leucocyte migration in Agarose gel.[723]

A series of diastereoisomers of di- and tri-alanine have been studied for their effects on lysosomal enzyme activity, the results being interpreted in terms of transport specificity across the lysosomal membrane.[724] A similar study has been reported on a methionine-requiring auxotroph of *Saccharomyces cerevisiae* with a series of methionine and glycine di- to penta-peptides. The ability of the organism to utilize peptides reflected the membrane-transport process since peptidases from the bacteria cleaved the three peptides which failed to satisfy the nutritional requirements.[725] Structurally, the requirement is not clearly defined but differs from that in *E. coli*.[726] Other studies on peptide transport in bacteria,[727–729] and in the gastrointestinal tract,[730, 731] including human studies,[732–734] have been reported or reviewed.[735]

The concept of using active peptide transport systems to facilitate the distribution of biologically active molecules forming part of the peptide (*cf.* ref. 692) has been applied to adamantamine (812).[736] This inhibits methionine and leucine transport into carcinoma cells but its further study has been hindered by a lack of solubility. The dipeptide derivatives (813)—(816) were synthesized to give

[718] B. F. Driscoll, M. W. Kies, and E. C. Alvord, *J. Immunol.*, 1974, **112**, 392.
[719] G. A. Hashim and R. D. Sharpe, *Immunochemistry*, 1974, **11**, 633.
[720] G. A. Hashim and R. D. Sharpe, *Nature*, 1975, **255**, 484.
[721] G. A. Hashim, *Nature*, 1975, **256**, 593.
[722] O. Berg and H. Bergstrand, *Neurobiology*, 1974, **4**, 191.
[723] H. Bergstrand, B. Kallen, and O. Nilsson, *Acta Neurol. Scand.*, 1974, **50**, 227.
[724] R. Goldman and F. Naider, *Biochim. Biophys. Acta*, 1974, **338**, 224.
[725] F. Naider, J. M. Becker, and E. Katzir-Katchalski, *J. Biol. Chem.*, 1974, **249**, 9.
[726] J. W. Payne, *J. Gen. Microbiol.*, 1974, **80**, 269.
[727] Z. Barak and C. Gilvarg, *J. Biol. Chem.*, 1974, **249**, 143.
[728] J. M. Becker and F. Naider, *J. Bacteriol.*, 1974, **120**, 191.
[729] T. Cascieri and M. F. Mallette, *Appl. Microbiol.*, 1974, **27**, 457.
[730] J. M. Addison, D. M. Matthews, and D. Burston, *Clin. Sci. Mol. Med.*, 1974, **46**, 707.
[731] D. M. Matthews, J. M. Addison, and D. Burston, *Clin. Sci. Mol. Med.*, 1974, **46**, 693.
[732] D. B. A. Silk, P. J. Kumar, D. Perrett, M. L. Clark, and A. M. Dawson, *Gut*, 1974, **15**, 1.
[733] D. B. A. Silk, J. P. W. Webb, A. E. Lane, M. L. Clark, and A. M. Dawson, *Gut*, 1974, **15**, 444.
[734] S. A. Adibi and M. R. Soleimanpour, *J. Clin. Invest.*, 1974, **53**, 1368.
[735] D. B. A. Silk, *Gut*, 1974, **15**, 494.
[736] H. T. Nagasawa, J. A. Elberling, and F. N. Shirota, *J. Medicin. Chem.*, 1975, **18**, 826.



(812) Adam

(813) Gly-Adam
(814) Leu-Adam
(815) Adam-Gly
(816) Adam-Leu

greater water solubility and increased transport rates. Although absorbed, the compounds failed to show useful *in vivo* activity.

The synthesis and antireserpine activity of peptides incorporating L-dopa as one of their amino-acids have been studied. Although some peptides were active, no clear structure–activity relationships emerged.[737]

A peptide sequence (817) unique to the ϵ-chain of the immunoglobin IgE has been synthesized and shown to inhibit the Prausnitz–Keustner reaction and the

Asp-Ser-Asp-Pro-Arg

(817)

Knobun positive skin test reaction, both of which involve, as a crucial link in the chain of events, the binding of IgE to the mast cell or related cells. The sequence is that part of the IgE sequence supposed to be responsible for binding to such cells, though direct evidence for this is lacking. A series of related (but less effective) sequences was also prepared and tested.[738]

A peptide rich in hydrophobic residues and with a bitter flavour has been isolated from Cheddar cheese, and its structure identified with the sequence glutamine 46 to position 67 of β-casein. Since the bitterness is lost when the glutamine is cyclized to a pyroglutamyl residue, hydrophobicity is not the only factor in its taste. A second pyroglutamyl peptide spanning residues 46 to 84 had no bitter flavour.[739] The bitterness in cocoa has been shown to be due to dioxopiperazines acting synergistically with theobromine.[740] A number of dioxopiperazines have been studied for this property and many (particularly those containing D- or L-phenylalanine) were found to have the appropriate flavour. The dioxopiperazine cycloprolyl-leucine has been isolated as a bitter component of aged sake.[741] The structure–taste relationships of peptides released by rennin on caseins have been studied.[742] There has been further work on the structure–taste relationships of compounds related to Asp-Phe-OMe (Aspartame) but this is not covered in detail here. Recent results in this area have been discussed.[743]

[737] A. M. Felix, D. P. Winter, S.-S. Wang, I. D. Kulesha, W. R. Pool, D. L. Hane, and H. Sheppard, *J. Medicin. Chem.*, 1974, **17**, 422.

[738] R. N. Hamburger, *Science*, 1975, **189**, 389.

[739] J. S. Hamilton, R. D. Hill, and H. van Leeuwen, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 375.

[740] W. Pickenhagen, P. Dietrich, B. Keil, J. Polonsky, F. Nouaille, and E. Lederer, *Helv. Chim. Acta*, 1975, **58**, 1078.

[741] K. Takahashi, M. Tadenuma, K. Kitamoto, and S. Sato, *Agric. and Biol. Chem. (Japan)*, 1974, **38**, 927.

[742] J. P. Pelissier, J. C. Mercier, and B. R. Dumas, *Ann. Biol. Anim. Biochim. Biophys.*, 1974, **14**, 343.

[743] M. Goodman and C. Gilon, in 'Peptides 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 271; Y. Ariyoshi, N. Yasuda, and T. Yamatani, *Bull. Chem. Soc. Japan*, 1974, **47**, 326.

Work on the various thymic hormones has been reviewed,[744–746] and their therapeutic potential in cancer chemotherapy has also been discussed.[747] The hormone thymopoietin (previously thymin [748]) is a 49-amino-acid peptide which acts both to induce thymocyte cell differentiation and to block the neuromuscular response in mice.[749] The neuromuscular action is proposed as the causative factor in myasthenia gravis, and this supports the use of thymectomy, which is found to be an effective treatment of the condition. A synthetic tridecapeptide corresponding to the sequence 29—41 has been found to share both activities.[750]

The β-nerve growth factor protein is one of two identical subunits (molecular weight 26 500) with actions on fibre outgrowth from dorsal root ganglia and which forms part of the 7S NGF complex. Recent studies on NGF have been reviewed.[14f, 751, 752] Cross-linking the two subunits with dimethylsuberimidate has given a product with equal activity and ability to form the high molecular weight complex.[753]

A hypotensive peptide, neurotensin (818), has been isolated from bovine hypothalami. Its structure is similar to that of xenopsin (819), isolated earlier from frog skin.[754] A synthesis of (818) has been reported.[755]

Glp-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu
(818)

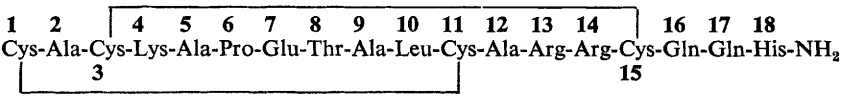
Glp-Gly-Lys-Arg-Pro-Trp-Ile-Leu
(819)

Lipolytic peptides, structurally distinct from the β-lipotropin family (see p. 371), have been isolated in the past from pig posterior pituitary, and an apparently homologous peptide has now been prepared from human posterior pituitary tissue.[756] It is a peptide of molecular weight *ca.* 8000 with a high content of cysteine and called 'peptide S', and it has been separated from associated growth hormone and prolactin-like fractions.

Work on the isolation and study of numerous venom and toxin peptides has continued. The results are not covered comprehensively here; several reviews summarize recent work.[757–759]

[744] A. White, *Ann. New York Acad. Sci.*, 1975, **249**, 523.
[745] G. Goldstein, *Ann. New York Acad. Sci.*, 1975, **249**, 177.
[746] J. A. Hooper, M. C. McDaniel, G. A. Thurman, G. H. Cohen, R. S. Schulof, and A. L. Goldstein, *Ann. New York Acad. Sci.*, 1975, **249**, 523.
[747] (Editorial), *Science*, 1975, **187**, 1183.
[748] G. Goldstein, *Nature*, 1974, **247**, 11.
[749] D. H. Schlesinger and G. Goldstein, *Cell*, 1975, **5**, 367.
[750] G. Goldstein and D. H. Schlesinger, *Lancet*, 1975, **ii**, 256.
[751] L. F. Boyd, *Life Sci.*, 1974, **15**, 1381.
[752] S. Varon, *Exper. Neurol.*, 1975, **48**, 75.
[753] R. W. Stach and E. M. Shooter, *J. Biol. Chem.*, 1974, **249**, 6668.
[754] R. Carraway and S. E. Leeman, *J. Biol. Chem.*, 1975, **250**, 1907.
[755] H. Yajima, K. Kitagawa, T. Segawa, M. Nakano, and K. Kataoka, *Chem. and Pharm. Bull. (Japan)*, 1975, **23**, 3299.
[756] L. Gráf, E. Barát, A. Patthy, and G. Cseh, *Arch. Biochem. Biophys.*, 1974, **161**, 211.
[757] E. Condrea, *Experientia*, 1974, **30**, 121.
[758] C. C. Yang, *Toxicon*, 1974, **12**, 1.
[759] M. L. Peck and R. O'Connor, *J. Agric. Food Chem.*, 1974, **22**, 51.

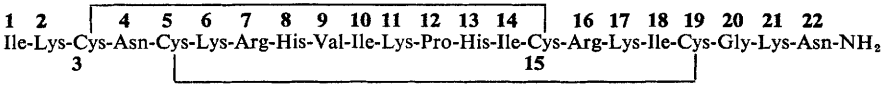
The neurotoxic properties of a series of modified derivatives of apamin (820) have been studied.[760] Guanidination of the lysine in position 4 gives a derivative



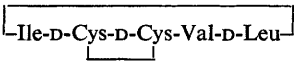
(820)

with the same toxicity as apamin, but in other cases toxicity is reduced. A radiolabelled derivative has been used to explore the site of action which is in the spinal cord. Remarkably for such a large polar molecule, the compound appears to cross the blood–brain barrier.

Oxidative sulphitolysis followed by reduction and reoxidation of apamin and the mast cell degranulating (MCD) peptide (821), (peptide 401), has led to recovery of biologically active products (with 50—80% activity).[761]

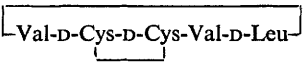


(821)

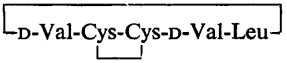


(822)

The structure of malformin has been shown by synthesis to be (822), contradicting that previously proposed. The compound has a potent disruptive effect on plant root growth and possibly some antiviral properties.[762, 763] The derivative (823) with valine in place of isoleucine is probably active since amino-acid compositions of other natural malformins correspond to such a structure. Compound (823) can be called a 'palindromic' peptide since its sequence is unchanged on reversal. Its enantiomeric form as a result is identical, but for the direction



(823)



(824)

of the peptide bonds, with the original and it is therefore a good model on which to study the effect of this change. The appropriate malformin derivative (824)

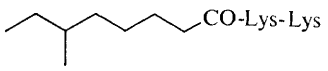
[760] J. P. Vincent, H. Schweitz, and M. Lazdunski, *Biochemistry*, 1975, **14**, 2521.
[761] P. Hartter and U. Weber, *Z. physiol. Chem.*, 1975, **356**, 693.
[762] M. Bodanszky and G. L. Stahl, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 2791.
[763] M. Bodanszky and G. L. Stahl, in 'Peptides 1974. Proceedings of the 13th European Peptide Symposium, Kiryat Anavim, 1974', ed. Y. Wolman, John Wiley, New York, 1975, p. 33.

has been synthesized and found to have about 10% of the activity of malformin.[764, 765] The analogue in which the D-Cys-D-Cys group is replaced by D-Ala-D-Ala ('desthiomalformin') is inactive and compounds in which the disulphide is replaced by a hydrocarbon bridge are planned.[765, 766]

A tripeptide with the structure (825) has 'antibiotic' activity (acting particularly against fungi and moulds), and a range of structural variants has been studied for similar activity. Replacement with many amino-acids in position 2 left the activity undiminished, including replacement with other D-amino-acids, some

1 2 3
Arg-D-aThr-Phe
(825)

DL-amino-acids, and *N*-methylamino-acids.[767–769] On the other hand, replacement of arginine by lysine, glutamine, aspartic acid, or glutamic acid, or of phenylalanine by alanine, leucine, or tyrosine, led to inactive compounds.[768] A series of acyl dilysine and acyl dilysine esters, *e.g.* (826), have been studied as



(826)

antibacterial agents.[770] The derivative (827) is reported to be a particularly non-toxic antibiotic.[771]

$$PO(OH)_2 \cdot (CH_2)_2 \cdot C(NH_2) \cdot CO\text{-Ala-Ala}$$
(827)

Two peptides released by the action of trypsin on hen egg lysozyme, of sequence 1—5 (828) and 68—72 (829) and which were previously reported to inhibit the

1 2 3 4 5	**68 69 70 71 72**	**1 2 3 4 5**
Lys-Val-Phe-Gly-Arg	Thr-Pro-Gly-Ser-Arg	Lys-Val-Tyr-Gly-Arg
(828)	(829)	(830)

action of histamine on isolated guinea-pig ileum, have been synthesized, together with the turkey sequence 1—5 (830). None of the peptides or the *C*-terminal tetrapeptides of (828) or (829) inhibited the action of histamine. There was a slight effect of (828) on barium chloride-induced contraction. Contamination of

[764] M. Bodanszky, G. L. Stahl, and R. W. Curtis, *J. Amer. Chem. Soc.*, 1975, **97**, 2857.
[765] M. Bodanszky, J. B. Henes, S. Natarajan, and G. L. Stahl, *Abs. Papers Amer. Chem. Soc. Meeting*, 1975, (170), POLY 28.
[766] M. Bodanszky, and J. B. Henes, *Bio-organic Chem.*, 1975, **4**, 212.
[767] K. Eisele, *Experientia*, 1975, **31**, 764.
[768] K. Eisele, *Z. Naturforsch.*, 1975, **30c**, 541.
[769] A. Eisele and K. Eisele, *F.E.B.S. Letters*, 1975, **55**, 153.
[770] E. Morier, H. Pacheco, J. Koeberle, and D. Yavordios, *European J. Medicin. Chem.*, 1975, **10**, 221.
[771] T. Niida *et al.*, *Sci. Rep. Meiji Seika Kaisha*, 1973, **13**, 34, 42, 49, 54, 60; quoted in Y. Yagisawa, *Japan Med. Gazz.*, 1975, **12**, 6.

the natural peptides with lysozyme (which has this effect itself) was advanced as a possible explanation.[772]

Chemotaxis is the direct migration of cells along a chemical gradient. Chemotactic factors for eosinophil cells in anaphylaxis have now been identified as two tetrapeptides (831) and (832), which have been synthesized.[773] *N*-Formyl di- and

Val-Gly-Ser-Glu	Ala-Gly-Ser-Glu
(831)	(832)

tri-peptides containing methionine have been found to be strong attractants for leucocytes (for which *N*-formylmethionine but not methionine is chemotactic).[774]

That a broad range of hormone receptors is likely to be present in various mutant cell lines is demonstrated by the range of factors (usually provided by serum) which is necessary for the growth of such cells (GH_3'HeLa, *etc.*). Substitution of serum by tri-iodothyronine, TRH, transferrin, a biologically active parathyroid hormone sequence, and partially purified somatomedin was sufficient to allow growth to continue, but no hormone could be omitted [775] (*cf.* also ref. 776).

The cytochrome *c* haemoctapeptide has been found to protect against the denaturation of tissue caused by oxygen deficiency,[777] and the tissue damage caused by isoproterenol-induced cardiac infarct-like angina.[778]

Other reported studies covered the isolation of a peptide of 36 amino-acids from the chicken pancreas, with a proposed but so far unidentified hormonal role,[779] isolation of a peptide (mol. wt. *ca.* 1000) which inhibits the adenyl cyclase response to a variety of substances including parathyroid hormone, glucagon, and epinephrine,[780] and the isolation of two pairs of hypotensive peptides from the urophysis of the teleost fish *Catostomus commersoni* with urotensin I (long-acting rat hypotensive) and urotensin II (fish smooth muscle stimulating) activities.[781] The complete sequence of ubiquitin from cattle and man has been reported. The substance is a 74-amino-acid peptide stimulating adenyl cyclase and reportedly present universally in living cells.[782, 783] Other studies have been carried out on tuftsin and some analogues,[784] the intensely sweet-tasting proteins thaumatin and monellin from tropical fruits,[785] and on the effects of incorporating proline analogues biosynthetically into collagen.[786]

[772] K. Suzuki, N. Endo, R. Tani, and H. Kikuchi, *Chem. and Pharm. Bull. (Japan)*, 1974, **22**, 2462.
[773] E. J. Goetzl and K. F. Austen, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 4123.
[774] E. Schiffman, B. A. Corcoran, and S. M. Wahl, *Proc. Nat. Acad. Sci. U.S.A.*, 1975, **72**, 1059.
[775] I. Hayashi and G. H. Sato, *Nature*, 1976, **259**, 133.
[776] R. W. Holley, *Nature*, 1975, **258**, 487.
[777] M. Murata, H. Yasuda, and Y. Baba, *J. Pharm. Soc. Japan*, 1975, **95**, 659.
[778] M. Murata, Y. Baba, E. Matsuo, H. Yasuda, T. Okonogi, and H. Watanabe, *J. Pharm. Soc. Japan*, 1974, **94**, 1.
[779] J. R. Kimmel, J. Hayden, and H. G. Pollock, *J. Biol. Chem.*, 1975, **250**, 9369.
[780] G. S. Levey, D. C. Lehotay, J. M. Canterbury, L. A. Bricker, and G. J. Meltz, *J. Biol. Chem.*, 1975, **250**, 5730.
[781] G. Moore, A. Letter, M. Tesanovic, and K. Lederis, *Canad. J. Biochem.*, 1975, **53**, 242.
[782] D. H. Schlesinger, G. Goldstein, and H. D. Niall, *Biochemistry*, 1975, **14**, 2214.
[783] D. H. Schlesinger and G. Goldstein, *Nature*, 1975, **255**, 423.
[784] Z. Spirer, V. Zakuth, A. Golander, N. Bogair, and M. Fridkin, *J. Clin. Invest.*, 1975, **55**, 198.
[785] H. Van der Wel, *Z. physiol. Chem.*, 1974, **355**, 107.
[786] J. Uitto and D. J. Prockop, *Biochim. Biophys. Acta*, 1974, **336**, 234.

6

Further Extracts from the Rules and Tentative Rules of the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature†

In Volume 2, Chapter 5, we reprinted the following extracts from the I.U.P.A.C.–I.U.B. Recommendations relevant to amino-acids, peptides, and proteins: *Tentative Abbreviated Designation of Amino-acid Derivatives and Peptides; Tentative Rules for Naming Synthetic Modifications of Natural Peptides; Tentative Abbreviated Nomenclature of Synthetic Polypeptides (Polymerised Amino-acids).* In Volume 4, Chapter 5, we reprinted a revised version of the first of these recommendations, together with the new recommendation: *Abbreviations and Symbols for the Description of the Conformation of Polypeptide Chains.* These last two recommendations have now been approved and republished by I.U.P.A.C. (*Pure Appl. Chem.*, 1974, **40**, 317, 293), apparently without major revision. We now reprint approved rules for *Nomenclature of Multiple Forms of Enzymes* (from *Pure Appl. Chem.*, 1974, **40**, 311), together with new Recommendations concerning *Nomenclature of Amino-acids* (from *Biochemistry*, 1975, **14**, 449) and *Nomenclature of Peptide Hormones* (from *Biochim. Biophys. Acta*, 1975, **404**, 152).

I Nomenclature of Multiple Forms of Enzymes‡

Rules Approved 1974

Recommendations on the nomenclature of multiple forms of enzymes were made by a subcommittee set up by the International Union of Biochemistry and were published in 1964 in a number of journals.[1] Since that time, there have appeared a large number of publications in this field; some hundreds of enzymes have been shown to exist as heteromorphs. It seemed appropriate therefore to review the earlier recommendations[1] in the light of current knowledge. For that purpose the IUPAC–IUB Commission on Biochemical Nomenclature (CBN) set up a subcommittee consisting of G. Brewer, O. Hoffmann-Ostenhof, R. S. Holmes, P. Karlson (Convenor), B. Keil, G. B. Kitto, C. L. Markert, C. J. Masters,

† Members of the Commission are: O. Hoffmann-Ostenhof (Chairman), W. E. Cohn (Secretary), A. E. Braunstein, B. L. Horecker, W. B. Jakoby, P. Karlson, B. Keil, W. Klyne, C. Liébecq and E. C. Webb.

‡ This document is a revision of proposals published in provisional form as Tentative Nomenclature Appendix No. 22 (June 1972) to *IUPAC Information Bulletin* and in: *Arch. Biochem. Biophys.*, **147**, 1 (1971); *Biochem. J.*, **126**, 769 (1972); *Biochemistry*, **10**, 4825 (1971); *Biochim. Biophys. Acta*, **258**, 1 (1972); *Europ. J. Biochem.*, **24**, 1 (1971); *J. Biol. Chem.*, **246**, 6127 (1971); *Biochimie* (in French), **54**, 123 (1972); *Z. Physiol. Chem.* (in German), **353**, 852 (1972).

Comments on and suggestions for future revisions of these rules should be sent to: Prof. O. Hoffmann-Ostenhof, Institut für Allgemeine Biochemie der Universität Wien, Währingerstrasse 38, A-1090 Wien, Austria.

F. Moyer, J. Scandalios, C. R. Shaw, E. C. Slater, R. Tashian and E. C. Webb. The subcommittee reported to CBN, the report was discussed, and the recommendations were finally adopted.

1. Definition of Isozymes or Isoenzymes

The 1964 Committee recommended[1] that 'multiple enzyme forms' in a single species should be known as isoenzymes (or isozymes). It is known that enzymes catalysing essentially the same reaction may differ in various ways, as shown in *Table* 1. In this table, some prominent examples are also listed.

Table 1. Multiple forms of enzymes. Artifacts occurring during preparation are outside the scope of this document

Group	Reason of multiplicity	Example
1	Genetically independent proteins	Malate dehydrogenase in mitochondria and cytosol
2	Heteropolymers (hybrids) of two or more polypeptide chains, non-covalently bound	Lactate dehydrogenase
3	Genetic variants (allelic)	Glucose-6-phosphate dehydrogenase in man
4	Proteins conjugated with other groups	Phosphorylase *a* and *b*
5	Proteins derived from one polypeptide chain	The family of chymotrypsins arising from chymotrypsinogen
6	Polymers of a single subunit	Glutamate dehydrogenase of mol. wt. 1 000 000 and 250 000
7	Conformationally different forms	All allosteric modifications of enzymes

According to the original definition, which was meant to be purely operational, all of these multiple forms should be termed 'isozymes' or 'isoenzymes'. However, most biochemists feel that the term 'isoenzymes' should be restricted to those forms arising from genetic control of primary protein structure. Genetically determined differences in primary structure are the reason for the multiplicity in Groups 1 to 3 of *Table 1*, but not in Groups 4 to 7. Indeed, scientists working on conjugated or derived enzymes do not use the isoenzyme terminology for characterization of their multiple forms.

It is therefore recommended:

1. The term 'multiple forms of the enzyme...' should be used as a broad term covering all proteins possessing the same enzyme activity and occurring naturally in a single species.

2. The term 'isoenzyme' or 'isozyme' should apply only to those multiple forms of enzymes arising from genetically determined differences in primary structure, and *not* to those derived by modification of the same primary sequence.

Nevertheless, the term 'isozyme' or 'isoenzyme' is being used as an operational term in dealing with enzyme proteins with the same catalytic activities but separable by suitable methods (e.g. electrophoresis) and where knowledge of the

nature of multiplicity is lacking. These terms should not be used in cases in which other multiple forms, e.g. conjugated or derived, are primarily under study.

The present recommendations deal only with isoenzymes and genetic variants.

2. Nomenclature of Isozymes or Isoenzymes

The earlier committee[1] recommended that individual isoenzymes (isozymes) should be distinguished and numbered on the basis of electrophoretic mobility, with the number 1 being assigned to that form having the highest mobility toward the anode.

Of all of the means available to indicate the different properties of isoenzymes (electrophoresis, chromatography, kinetic criteria, chemical structure, etc.), electrophoresis is most widely used for the following reasons:

(a) It is still extremely important in the study of enzyme heterogeneity to avoid any artifactual consequences of the handling or 'purification' of enzymes, and zone electrophoresis is one procedure in which the resolution of individual protein species is not unduly influenced by their application in the original state (i.e. as tissue homogenates, etc.).

(b) The degree of resolution is another factor of prime importance in this field, and resolution by electrophoretic procedures is generally more effective than other methods of protein separation.

(c) Electrophoresis offers the advantages of rapidity and broad applicability.

The recognition of these facts and the consequent wide utilization of electrophoretic procedures by workers in this field has built up a substantial literature on the subject and has facilitated communication and reference.

Other alternatives suggested for the distinction of isoenzyme forms include kinetic criteria and structural data. While kinetic criteria are extremely valuable as an adjunct to investigations of enzyme multiplicity, they cannot provide information on the extent of heterogeneity. With regard to structural criteria, it has long been the hope of many workers in the field that the interrelationships in multiple forms might soon be delineated in chemical terms. However, such information is available only for very few enzyme systems, so that it would not be useful at this stage to make general recommendations about isoenzyme nomenclature based on structural considerations. When structural details are available, a system similar to that used for hemoglobin, in which the polypeptide chains are represented by Greek letters, could be used. [The use of upper-case Roman letters (as have been used for lactate dehydrogenase and aldolase) would be more acceptable.]

Isoenzymes (isozymes) or their subunits should not be labelled on the basis of tissue distribution (e.g. brain type, heart type) since confusion can arise on account of species variation; homologous forms may occur in altogether different tissues in other species.

It is therefore recommended:

3. In naming isozymes (isoenzymes), the normal enzyme name (either systematic or trivial)[2] should be used, followed by a number. The numbers should be allotted consecutively, preferably on the basis of electrophoretic mobility under defined conditions, with the lower numbers given to the forms with the higher mobility towards the anode. In photographs or diagrams of electrophoretic

results, the anode should be oriented to the top or right-hand side of the page.

4. Where complex isoenzyme (isozyme) patterns occur, with major groups each composed of several different electrophoretic zones, the numbers may be used to designate the major groups, with subscript lower-case letters used consecutively for the individual subzones (1_a, 1_b, 1_c, 2_a, 2_b, etc.).

5. For unambiguous identification of particular isozymes (isoenzymes), additional parameters such as molecular weight, stability, or subunit structure should be given where available. Subunits may be denoted by upper case Roman letters or lower case Greek letters, but not by terms based on tissue distribution.

3. Nomenclature of Genetically Variant Enzymes

In the case of genetic variants, a flexible and open system is desirable. The chief consideration is that the investigator recognize that the new variant that he describes will probably not be the last one discovered, and, in the case of some enzymes, a very large number may eventually be found.

A special committee on the nomenclature of glucose-6-phosphate dehydrogenase in man (of which more than 50 genetic variants are known) was convened by the World Health Organization, and the report of its recommendations was published in abbreviated form in several journals;[3] it forms the basis for certain of the recommendations presented here.

This report considers only the naming of the variant enzymes; it does not consider the designation of genotype and phenotype symbols, since these have been standardized by several appropriate genetic groups and differ somewhat among various organisms. The name of the enzyme variant can be readily adapted to the appropriate genetic terminologies.

It is therefore recommended:

6. In naming genetic variants, the normal enzyme name (either systematic or trivial) should be used, followed by a trivial name for the variant. This can be of any sort provided that it is sufficiently adaptable. A suitable system (as has been used for glucose-6-phosphate dehydrogenase) is to use the name of the town, university, country, etc., where the variant was discovered. The use of superscript letters or numbers is considered acceptable initially, but, once it is apparent that more than a few variants are being found, the trivial nomenclature should be initiated, to avoid using the same letter for two different variants.

7. When two variants originally considered as different are subsequently discovered to be identical, the name first used for that variant should then be accepted, the other one being discontinued.

References

[1] Webb, E. C., *Lancet*, **1**, 1110 (1964); *Nature*, **203**, 821 (1964); *Experientia*, **20**, 592 (1964); *Z. Klin. Chem. Klin. Biochem.*, **2**, 160 (1964); *Postepy Biochem.*, **10**, 525 (1964); *Enzymol. Biol. Clin.*, **5**, 124 (1965).

[2] *Enzyme Nomenclature: Recommendations* (1972) of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry on the nomenclature and classification of enzymes, together with their units and the symbols of enzyme kinetics. Elsevier: Amsterdam (1973).

[3] *Biochem. Genet.*, **1**, 198 (1967).

II The Nomenclature of Peptide Hormones*

Recommendations (1974)

In the last two decades, the structures of many peptide hormones have been elucidated and other peptide hormones have been obtained in pure form. However, there are presently no accepted guidelines for nomenclature in this field. Therefore, the IUPAC–IUB Commission on Biochemical Nomenclature (CBN) appointed a subcommittee consisting of R. A. Acher, R. A. Boissonnas, H. B. F. Dixon, R. Guillemin, P. Karlson (Convenor), H. Rasmussen, J. Rudinger and N. A. Yudayev to discuss the question of the nomenclature of peptide hormones and to report to CBN. The International Union of Physiological Sciences nominated, as consultants to the subcommittee, A. Brodish, S. M. McCann and F. Ulrich. The present Recommendations are based on the report of this subcommittee.

1. General Principles

Naturally occurring oligo- and polypeptides are generally referred to by trivial names; their systematic names are so cumbersome that they are of little use. Most of the peptide hormones already have well established trivial names indicating either natural source (e.g. insulin) or physiological action (e.g. relaxin, prolactin). However, some of the trivial names are so long that these hormones are known mainly by abbreviations (e.g. FSH for follicle-stimulating hormone). This is unfortunate, and it was therefore considered advisable to create suitable names for those peptide hormones not already having well established short trivial names. Three principles have been observed: (a) new names for hormones of the adenohypophysis bear the ending "-tropin"; (b) hypothalamic releasing factors (hormones) bear the ending "-liberin"; (c) hypothalamic release-inhibiting factors (hormones) bear the ending "-statin" (see below).

2. Trivial Names

The trivial names proposed for peptide hormones are given in the Appendix. Abbreviations of the new names are not proposed, and the use of currently fashionable abbreviations is discouraged.

3. Species Designation

Since peptide hormones show species variation in their amino-acid sequence, their names are essentially "generic names", and are insufficient to specify a single chemical compound. It is therefore recommended that authors add to the name of each hormone the species from which the hormone was isolated, or at least indicate the biological source(s) where appropriate in each paper.

* Document of the Commission on Biochemical Nomenclature (CBN) of the International Union of Pure and Applied Chemistry (IUPAC) and the International Union of Biochemistry (IUB), approved by CBN, IUPAC, and IUB in 1974 and published by permission of IUPAC and IUB. Comments on and suggestions for future revisions of these Recommendations may be sent to any member of CBN. Reprints of this publication may be obtained from W. E. Cohn, Director, NRC Office of Biochemical Nomenclature, Biology Division, Oak Ridge National Laboratory, Box Y, Oak Ridge, Tenn., U.S.A. 37830.

4. Special Groups of Hormones

(*a*) *Hypothalamic Factors* (*Hormones*)

The hypothalamic "releasing factors" or "releasing hormones" have no well established trivial names. It is recommended that the trivial names given in the Appendix be used for the releasing factors (hormones). They are based on the ending "-liberin" added to the prefix of the pituitary hormone released by the factor. Thus, "thyroliberin" indicates the hypothalamic peptide stimulating the release (and perhaps also the biosynthesis) of thyrotropin, the corresponding tropic hormone, from the pituitary gland. (Note that the ending "-tropin" is no longer retained in the name; it is implied in the definition of "-liberin".

The names of those factors inhibiting the release (and perhaps the synthesis) of pituitary hormones are formed in a similar way with the suffix "-statin".

(*b*) *Pituitary Hormones*

Most of the hormones of the adenohypophysis have acceptable trivial names ending in -tropin.† The committee has created the missing names for follicle-stimulating hormone, "follitropin," and for luteinizing hormone, "lutropin." It is recommended that pituitary hormones discovered in the future also be named with the ending -tropin. This suffix should be restricted to pituitary and similar hormones and should not be used for, e.g. crustacean hormones acting on pigment cells.

Some placental hormones are physiologically very similar to pituitary hormones. They are named accordingly with the prefix "chorio-", e.g. chorio-gonadotropin for chorionic gonadotropin.

(*c*) *Invertebrate Peptide Hormones*

Though some of the invertebrate peptide hormones have been isolated in pure form and their amino-acid compositions have been determined, the field has not yet developed to a stage where a list of names seems warranted.

It is, however, recommended that the suffixes defined above for hypothalamic and pituitary hormones not be used in a different sense in invertebrates. Thus, a crustacean color change hormone acting on, e.g. erythrophores, should not be named "erythrotropin," a hormone causing release of eggs and/or sperm in sea urchins should not be called "gametoliberin."

Appendix

LIST OF PEPTIDE HORMONES [a]

Trivial name	Other names	Current abbreviation [b]
1. Hypothalamic Factors		
Corticoliberin	Corticotropin-releasing factor	CRF
Folliberin	Follicle-stimulating-hormone-releasing factor	FSH-RF
Gonadoliberin [c]	Gonadotropin-releasing factor	(LH/FSH-RF)
Luliberin	Luteinizing hormone-releasing factor	LH-RF (LRF)
Melanoliberin	Melanotropin-releasing factor	MFR
Melanostatin	Melanotropin release-inhibiting factor	MIF

† The committee has re-evaluated the arguments for and against the suffix "-trophin," still used by many anatomists and physiologists. Since the bioassay systems are based mostly on effects other than the trophic one, it was decided to recommend "-tropin" for general usage in biochemistry.

Appendix (*cont.*)

Trivial name	Other names	Current abbreviation [b]
Prolactoliberin	Prolactin-releasing factor	PRF
Prolactostatin	Prolactin release-inhibiting factor	PIF
Somatoliberin	Somatotropin-releasing factor; growth hormone-releasing factor	SRF; GH-RF
Somatostatin	Somatotropin release-inhibiting factor	
Thyroliberin	Thyrotropin-releasing factor	TRF
2. Pituitary and Related Hormones		
Choriogonadotropin [d]	Chorionic gonadotropin	CG
Choriomammotropin	Chorionic somatomammotropin	CS
Corticotropin	Adrenocorticotropic hormone	ACTH
Follitropin	Follicle-stimulating hormone	FSH
Gonadotropin [e]	Gonadotropin hormone	
Glumitocin [f]	[Ser^4, Gln^8]Ocytocin [j]	
Isotocin [g]	[Ser^4, Ile^8]Ocytocin	
Lipotropin	Lipotropic hormone	LPH
Lutropin	Luteinizing hormone; (Interstitial cell-stimulating hormone)	LH (ICSH)
Melanotropin [h]	Melanocyte-stimulating hormone	MSH
Mesotocin [i]	[Ile^8]Ocytocin [j]	
Ocytocin [j] (Oxytocin)		OXT
Prolactin	Mammatropic hormone; mammatropin; lactotropic hormone; lactotropin	PRL
Somatropin	Somatropic hormone; growth hormone	STH; GH
Thyrotropin	Thyrotropic hormone	TSH
Urogonadotropin [k]	(Human) Menopausal gonadotropin	HMG
Vasopressin	Adiuretin; antidiuretic hormone	VP, ADH
Vasotocin	[Arg^8]Ocytocin [j]	
3. Other Peptide Hormones		
Angiotensin	Angiotensin II	
Bradykinin	Kinin-9	
Calcitonin	Thyrocalcitonin	
Erythropoietin		
Gastrin		
Gastrin sulphate	Gastrin II	
Glucagon	Hyperglycemic factor	(HGF)
Insulin		
Kallidin	Kinin-10	
Pancreozymin	Cholecystokinin	
Parathyrin [l]	Parathyroid hormone; Parathormone	
Proangiotensin	Angiotensin I	
Relaxin		
Secretin		
Somatomedin [m]	Sulfation factor	
Thymopoietin [n]	Thymin	

[a] For convenience, some biologically active peptides that may not fulfill all criteria of a hormone are included. [b] Abbreviations, old or new, are not recommended; they are given here for identification purposes only. [c] This name indicates a hypothalamic substance releasing gonadotropin. It may also be used for the decapeptide isolated from pig hypothalami and known as luteinizing hormone/follicle-stimulating-hormone releasing factor, abbreviated LH/FSH-RF,[b] since the peptide induces the release of both lutropin and follitropin in constant proportions and thus

carries the activity of both luliberin and folliberin (see also Footnote *e*). [d] The chorionic gonadotropins have in most species (including man) the action of both follitropin and lutropin and are therefore termed "gonadotropins". [e] Gonadotropin is to be used for hormones having the activity of both follitropin and lutropin, like the gonadotropins of cold-blooded vertebrates. It may also be used for impure preparations containing lutropin and follitropin. [f] In elasmobranch fishes. [g] In bony fishes. [h] Two peptides have been sequenced and designated α-melanotropin and β-melanotropin. [i] In birds and reptiles. [j] The name of this hormone is derived from Greek ωκυτοκοσ (OKYTOKOS = fast birth, prompt delivery), not from the Greek οξυσ (oxys = acid; fast). The spelling ocytocin should therefore be preferred; moreover, it avoids confusion with oxy, meaning "related to oxygen". However, oxytocin is in wide use, especially in the English language. Therefore, both spellings are listed as optional. [k] Most work has been done on the human hormone, known as Human Menopausal Gonadotropin (HMG); it is a pituitary hormone, chemically changed during passage through the kidney. Due to its occurrence in urine, it has been termed "urogonadotropin". [l] Parathyrin is a new name suggested here. The synonym Parathormone is a proprietary name. [m] The name "somatomedin" was suggested by a group working in the field (*Nature* **235**, 107 (1972)). [n] A polypeptide from the thymus. The name proposed was suggested in a letter to *Nature* (**249**, 863 (1974)) to avoid confusion with the earlier "thymine" from nucleic acids. "Thymin" should be abandoned.

III Nomenclature of α-Amino Acids

IUPAC Commission on the Nomenclature of Organic Chemistry[1] and
IUPAC–IUB Commission on Biochemical Nomenclature

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Introduction

The traditional and well-known names of the common α-amino acids were, in general, given to them by their discoverers and bear no relationship to their chemical structures (1, 2). The modification of these names to accommodate derivatives and to designate configuration was first codified by the former IUPAC Commission on the Nomenclature of Biological Chemistry in 1947 (3) and revised in 1960 (4). Further proposals for the revision of the rules for naming α-amino acids with two centres of chirality appeared in 1963 (5). Recommendations for symbols of amino-acid residues in peptide sequences, together with rules for their use, were made by the present IUPAC–IUB Commission on Biochemical Nomenclature (CBN) (6, 7).

The present revision of the α-Amino-acid Rules brings the nomenclature of these substances into conformity with the IUPAC Rules for the Nomenclature of Organic Chemistry (8, 9). The revision was formulated by a committee composed of H. B. Vickery (convenor), K. Bláha, L. Fowden, W. Klyne, P. M. Scopes, and S. Veibel, with assistance from W. E. Cohn, J. S. Fruton, G. W. Kenner, P. O. Larsen, R. C. Sheppard, and G. T. Young. CNOC and CBN are grateful to these individuals for their efforts.

Recommendations

2AA-1. The Common α-Amino Acids

1.1. Trivial Names of the Common α-Amino Acids

The trivial names of the α-amino acids that are commonly found in proteins and are represented in the genetic code, together with their symbols, systematic names (8), and formulas, are given in Table I.

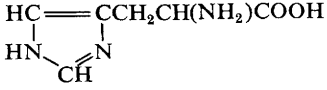
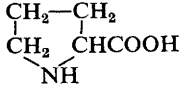
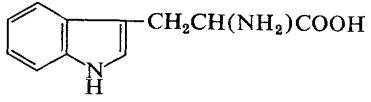
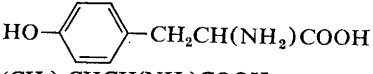
1.2. Coining New Trivial Names

(a) The coining of trivial names for newly discovered α-amino acids should be avoided in the absence of compelling reasons.

(b) A new α-amino acid should be named, wherever possible, as a derivative of a well-known α-amino acid (see Recommendation 2AA-2). The configuration, when known, should be indicated in strict conformity with Recommendation 2AA-3.

(c) Where names so constructed are unduly cumbersome and the substance is of sufficient importance, a new trivial name may be coined. This name should

Table I: α-Amino Acids under Direct Genetic Control

Trivial Name	Symbol	Systematic (and Semisystematic) Name	Formula
Alanine	Ala	2-Aminopropionic acid	$CH_3CH(NH_2)COOH$
Arginine	Arg	2-Amino-5-guanidinovaleric acid	$H_2NC(NH)NH(CH_2)_3CH(NH_2)COOH$
Asparagine	Asn	2-Aminosuccinamic acid	$H_2NCOCH_2CH(NH_2)COOH$
Aspartic acid	Asp	Aminosuccinic acid	$HOCOCH_2CH(NH_2)COOH$
Cysteine	Cys	2-Amino-3-mercaptopropionic acid (3-Mercaptoalanine)	$HSCH_2CH(NH_2)COOH$
Glutamine	Gln	2-Aminoglutaramic acid	$H_2NCO(CH_2)_2CH(NH_2)COOH$
Glutamic acid	Glu	2-Aminoglutaric acid	$HOCO(CH_2)_2CH(NH_2)COOH$
Glycine	Gly	Aminoacetic acid	$CH_2(NH_2)COOH$
Histidine	His	α-Amino-1*H*-imidazole-4-propionic acid (1*H*-Imidazole-4-alanine)	
Isoleucine	Ile	2-Amino-3-methylvaleric acid	$C_2H_5CH(CH_3)CH(NH_2)COOH$
Leucine	Leu	2-Amino-4-methylvaleric acid	$(CH_3)_2CHCH_2CH(NH_2)COOH$
Lysine	Lys	2,6-Diaminohexanoic acid	$H_2N(CH_2)_4CH(NH_2)COOH$
Methionine	Met	2-Amino-4-(methylthio)butyric acid	$CH_3S(CH_2)_2CH(NH_2)COOH$
Phenylalanine [a]	Phe	2-Amino-3-phenylpropionic acid	$C_6H_5CH_2CH(NH_2)COOH$
Proline [a]	Pro	2-Pyrrolidinecarboxylic acid	
Serine	Ser	2-Amino-3-hydroxypropionic acid	$HOCH_2CH(NH_2)COOH$
Threonine	Thr	2-Amino-3-hydroxybutyric acid	$CH_3CH(OH)CH(NH_2)COOH$
Tryptophan [a]	Trp	2-Amino-3-(3-indolyl)propionic acid (α-Amino-1*H*-indole-3-propionic acid)	
Tyrosine [a]	Tyr	2-Amino-3-(4-hydroxyphenyl)propionic acid [3-(4-Hydroxyphenyl)alanine]	
Valine	Val	2-Amino-3-methylbutyric acid	$(CH_3)_2CHCH(NH_2)COOH$

[a] See Sections 2.2–2.4 for numbering.

include either some element of its chemical structure or a reference to its biological origin. Attention should be paid to derivations from Greek or Latin roots (but not both in the same name) and ease of pronunciation. However, where a new trivial name is introduced or used, it is essential that a correctly constructed systematic or semisystematic name be stated at least once in each paper.

A number of existing trivial names are given in Appendixes A and B.

1.3. Ionic Forms

When it is desirable to mention or stress the ionic nature of an α-amino acid, the three kinds of ions possible may be indicated by adding the terms "anion," "cation," or "amphion" (alternatively, "dipolar ion") to the trivial name of the α-amino acid.

Examples:

glycine anion	$NH_2CH_2COO^-$
glycine cation	$NH_3^+CH_2COOH$
glycine amphion or glycine dipolar ion	$NH_3^+CH_2COO^-$

1.4. Names of Radicals and Residues (8)

1.4.1. The *acyl radical* of an α-amino acid is a structure that lacks the hydroxyl of the carboxyl group ($H_2NCHRCO—$). The names of such radicals are formed by replacing the ending "ine" (or "an" in tryptophan) by "yl".

Examples (8): alanyl, arginyl, leucyl, tryptophyl, tyrosyl, valyl, etc. "Cysteinyl" is used instead of "cysteyl," because of potential confusion with the radical of cysteic acid. "Cystyl" is the diacyl radical of cystine and "half-cystyl" is the acyl radical of cysteine lacking also the H of its SH group.

1.4.2. The monoacyl radicals of the dicarboxylic acids, $HOOC(CH_2)_nCHNH_2CO—$ and $—OC(CH_2)_nCHNH_2COOH$, are designated α- and β-aspartyl (α- and γ-glutamyl), respectively (8). The diacyl radicals are aspartoyl and glutamoyl (8). The acyl radicals of asparagine and glutamine are termed asparaginyl and glutaminyl, respectively (8).

1.4.3. α-Amino-acid *residues* are structures that lack a hydrogen atom of the amino group (—NHCHRCOOH) or that lack both a hydrogen atom of the amino group and the hydroxyl moiety of the carboxyl group (—NHCHRCO—); all units of a polypeptide chain except for the amino-terminal *acyl radical* are therefore *amino-acid residues.* Residues are named from the trivial name of the α-amino acid (omitting the word "acid" from aspartic acid and glutamic acid). Examples: glycine residue, glutamic residue.

1.4.4. In many cases, biochemical names (see Appendixes) require that radicals formed at a locus other than the carboxyl group of an amino acid be named in terms of the parent amino acid and appear as prefixes rather than roots of the final names.[2] Such "radical-prefix" names representing radicals derived by loss of hydrogen from a nitrogen atom are formed by substituting o- for the terminal -e in those names ending in -e (by analogy with amine → amino, etc.); *e.g.* alanino, valino, tyrosino. Tryptophan adds the -o directly, and the two dicarboxylic acids become asparto and glutamo. When there is more than one nitrogen atom in the amino acid, a locant of the form N^x must precede the radical name. Examples: N^6-lysino (see Appendix B2); N^ω-arginino; N^5-glutamino; π-histidino (see 2.4.).

1.4.5.[2] Radicals formed by loss of a hydrogen atom from carbon, sulphur, or oxygen atoms (excepting the carboxylic oxygen atoms) are named by substituting

-*x*-yl for the terminal -e, *x* being the locant of the atom from which the hydrogen atom has been lost (examples: norleucin-6-yl, cystein-*S*-yl, threonin-O^3-yl; *re* norleucin-6-yl, see Appendix B2; exception: asparagine forms aspartamid-*x*-yl, glutamine forms glutamid-*x*-yl) or adding -*x*-yl to aspartic, glutamic, and tryptophan (examples: aspartic-2-yl, tryptophan-1-yl; see 2.3).

1.5. Aldehydes and Alcohols[2]

Aldehydes and alcohols obtained by successive stages of reduction of the carboxyl group of α-amino acids are named by replacing the final "e" of a trivial name ending in "ine" (or the "ic acid" of aspartic and glutamic acids) with, respectively, the endings "al" and "ol".

Examples ($RCHNH_2CHO$): alaninal, leucinal, lysinal, serinal, aspart-1-al, glutaminal, etc.
($RCHNH_2CH_2OH$): alaninol, leucinol, lysinol, serinol, aspart-1-ol, glutaminol, etc.

The aldehyde and alcohol derivatives of tryptophan take the names tryptophanal and tryptophanol.

1.6. Amides, Anilides, and Analogous Derivatives ($H_2NCHRCONHR'$)

Amides, anilides, and analogous derivatives of α-amino acids are structures in which the hydroxyl group of the carboxyl has been replaced by an amino, anilino, or analogous group. They may be named by replacing the final "e" of the trivial amino-acid name by the word "amide," "anilide," etc., or by adding these words to the name of the amino acid. Thus, glycinamide, argininamide, leucinanilide, etc.; glycine amide, leucine anilide, etc.

Note that the β-amide of aspartic acid and the γ-amide of glutamic acid have specific trivial names, asparagine and glutamine. Their α-amides are named aspartic α-amide and glutamic α-amide, or isoasparagine and isoglutamine.

1.7. Esters and Salts

The names of esters of amino acids bearing trivial names are derived by replacing "ic acid" or the terminal "e" with "ate" (or adding "ate" to tryptophan) and prefixing these with the radical of the alcohol in the usual fashion.

Examples:

diethyl glutamate[3]	methyl cysteinate
α-ethyl glutamate[3]	methyl prolinate
ethyl glutaminate[3]	methyl tryptophanate

Salts of α-amino acids with bases are named in similar fashion (*e.g.*, sodium tryptophanate, monosodium glutamate). Salts of α-amino acids with acids are named by adding the name of the associated acid to the name of the amino acid (*e.g.*, glycine hydrochloride, histidine hydrochloride, lysine hydrochloride) or by using the " 'ium" form (*e.g.*, glycinium chloride, histidinium chloride, lysinium chloride).

It is permissible and customary to use the "ate" form without naming the cation when discussing the role of amino acids in biochemical reactions or where the co-ion is unimportant, unknown, or a mixture of ions. The "ate" form is preferred when the name of an amino acid is used adjectivally (*e.g.*, "glutamate-dependent reaction" instead of "glutamic-acid-dependent reaction").

1.8. Use of the Prefix "homo"

An α-amino acid that is otherwise similar to a common one (Table I) but that contains one more methylene group in the carbon chain may be named by prefix-

ing "homo" to the name of that common α-amino acid. (Examples are included in Appendix A, alphabetized under "H".)

2AA-2. Formation of Semisystematic Names of α-Amino Acids

Semisystematic names of substituted α-amino acids are formed according to the general principles of organic nomenclature (8), by attaching the name of the substituting radical to the trivial name of the amino acid. The position of substitution is preferably indicated by numerical locants, although Greek letters are frequently used, especially in the older literature.

2.1. Numbering

In the aliphatic α-amino acids, the carbon atom of the carboxyl group adjacent to the carbon atom carrying the amino group is numbered 1. Where Greek letters are used, the carbon atom adjacent to the carbon atom 1 and carrying the amino group is designated alpha (α). Example:

lysine $H_2N\overset{\epsilon}{\overset{6}{C}}H_2\overset{\delta}{\overset{5}{C}}H_2\overset{\gamma}{\overset{4}{C}}H_2\overset{\beta}{\overset{3}{C}}H_2\overset{\alpha}{\overset{2}{C}}H(NH_2)\overset{1}{C}OOH$

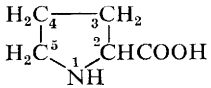
2.2. Proline

The carbon atoms in proline are numbered as in pyrrolidine, the nitrogen atom being numbered 1, and proceeding toward the carboxyl group.

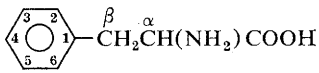
2.3. Aromatic Rings

The carbon atoms in the aromatic rings of phenylalanine, tyrosine, and tryptophan are numbered as in systematic nomenclature, with 1 (or 3, for tryptophan) designating the carbon atom holding the aliphatic chain. The carbon atoms of the latter are designated α (for the carbon atom attached to the amino and carboxylic groups) and β (for the atom attached to the ring system).

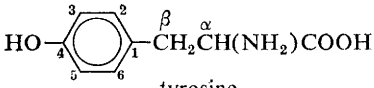
Note: This numbering system should also be used for the decarboxylated products (*e.g.*, for tryptamine).



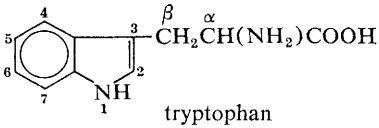
proline



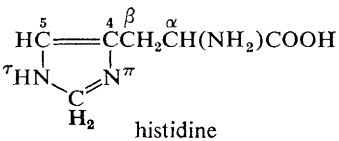
phenylalanine



tyrosine



tryptophan



histidine

2.4. Histidine

The nitrogen atoms of the imidazole ring of histidine are denoted by *pros* ("near," abbreviated π) and *tele* ("far," abbreviated τ) to show their position

relative to that of the side chain.[4] The carbon atom between the two imidazole nitrogen atoms is numbered 2 (as in imidazole), and the carbon atom adjacent to the τ nitrogen atom is numbered 5. The carbon atoms of the aliphatic chain are designated α and β, as in 2.1 and 2.3 above.

Note: this numbering should also be used for the decarboxylation product, histamine, and substituted histidines (*e.g.*, anserine, carnosine, homocarnosine, ophidin, thiolhistidine; see Appendix B) utilizing these trivial names.

2AA-3. Configuration at the α-Carbon Atom

3.1. Use of D *and* L

The absolute configuration at the α-carbon atom of the α-amino acids is designated by the prefixed small capital letters D or L to indicate a formal relationship to D- or L-serine and thus to L- or D-glyceraldehyde. An additional symbol (*i.e.*, a plus or minus sign in parentheses) to denote the direction of rotation is not necessary although it may be inserted where it is essential or desirable to emphasize the direction of rotation under specified conditions. (See 2AA-6.)

Examples: L-leucine, D-valine, L-asparagine, L-canavanine, L(+)-alanine.

3.2. Position of Prefix and Exceptions

In naming α-amino acids as derivatives of substances that have well-known trivial names, the prefix L or D is placed immediately before the trivial name of the parent amino acid and set off by hyphens.

Examples: 4-methyl-L-glutamic acid, *S*-methyl-L-cysteine, 4-hydroxy-L-proline, 3,5-diiodo-L-tyrosine, 5-hydroxy-L-lysine.

Note: Admissible exceptions to this rule are L-phenylalanine, L-hydroxyproline, and L-hydroxylysine, but only in general biochemical writing in a context such that the position of substitution is well understood. Note further that in the names of optically active derivatives of glycine, such as L-2-phenylglycine, the prefix must be placed before the name of the substituent as glycine itself is achiral. In the names of salts, esters and other derivatives (1.7), including peptides (2AA-7), the prefix is placed immediately before the trivial name of the parent acid or its radical.

Examples: L-histidine monohydrochloride monohydrate, cupric L-aspartate, D-ornithine dihydrochloride, *N*-acetyl-L-tryptophan, diethyl D-glutamate, 3-hydroxy-D_8-glutamic acid (see 3.4), N^6-methyl-L-lysine.

Other semisystematic names involving α-amino-acid configurations are treated similarly.

Example: *S*-(D-2-amino-2-carboxyethyl)-D-homocysteine (D-cystathionine).

3.3. Omission of Prefix

The prefix may be omitted where the amino acid is stated to be or is obviously the enantiomer derived from a protein source and is thus assumed to be L. It may also be omitted where the amino acid is synthetic and not resolved and is therefore, save in rare exceptional cases, an equimolecular mixture of the enantiomers; or in a general statement that is true for either enantiomer or for any mixture of these; or in symbolic representations (6, 7).

3.4. Subscripts to D *and* L

Where confusion is possible between the use of the capital letter prefix for the configuration of the α-carbon atom in amino-acid nomenclature and for that of

the highest numbered asymmetric carbon atom in carbohydrate nomenclature (13), a subscript is added to the small capital letter prefix. Where the prefix is used in the amino-acid sense, the subscript s is added; where the prefix is used in the carbohydrate sense, the subscript g is added. These subscripts (lower-case Roman letters) refer, respectively, to serine, the fundamental substance to which α-amino acids that bear structural resemblance to the carbohydrates can be formally related, and to glyceraldehyde, the fundamental substance to which the configuration of the carbohydrates is formally related.

Examples: L$_s$-threonine, for which the synonym in carbohydrate nomenclature is 2-amino-2,4-dideoxy-D$_g$-threonic acid; D$_s$-threonine, for which the synonym is 2-amino-2,4-dideoxy-L$_g$-threonic acid; L$_s$-allothreonine, for which the synonym is 2-amino-2,4-dideoxy-L$_g$-erythronic acid; D$_s$-allothreonine, for which the synonym is 2-amino-2,4-dideoxy-D$_g$-erythronic acid.

Note that the subscripts are essential only in discussions where both amino-acid names and those of carbohydrate derivatives occur. Nevertheless, they are highly desirable in naming α-amino acids that possess more than one centre of chirality. (See 5.2.)

3.5. The Sequence Rule

A more general system of stereochemical designation, which is especially convenient for comparisons with other series of compounds, is the "Sequence Rule" of Cahn, Ingold, and Prelog (9—12). A full discussion of this system (the *RS* system) of naming chiral substances is to be found in the references cited.

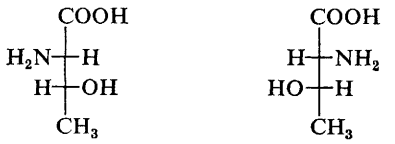
Note that almost all of the enantiomers of the α-amino acids derived from proteins and most of the α-amino acids and their derivatives found in plant or animal tissues have the α-L configuration, which corresponds in almost all cases to *S* in the Sequence Rule convention. The most important exceptions are L-cystine and L-cysteine and their derivatives, which are *R*.[5] A few α-amino acids of the α-D configuration, which generally corresponds to *R* in the Sequence Rule convention (except D-cystine, D-cysteine, and related compounds, which are *S*), are found among the products of hydrolysis of certain antibiotic polypeptides, although rarely elsewhere.

3.6. Amino Acids Derived from Amino Sugars

Amino acids derived from amino sugars containing five or more carbon atoms are named in conformity with the Rules of Carbohydrate Nomenclature (13) or with a recommended trivial name.

Examples: (1) D$_g$-glucosaminic acid for 2-amino-2-deoxy-D$_g$-gluconic acid, the α-carbon atom of which has the configuration of that in D-serine, and in which carbon atom 5, the highest numbered chiral centre, also has the D configuration; (2) D$_g$-mannosaminic acid for 2-amino-2-deoxy-D$_g$-mannonic acid, the α-carbon atom of which has the configuration of that in L-serine, but in which carbon atom 5 has the D configuration. The subscript g may be omitted except where confusion with the use of the small capital letter prefixes in amino-acid nomenclature is possible.

Note: the structures of α-amino acids to show configurational relationships may be drawn in several ways. In Fischer-style formulas, the carbon chain is written vertically with the carboxyl group at the top. With L-amino acids, the amino group is shown at the left, with D-amino acids at the right.



L$_S$-threonine D$_S$-threonine

Alternatively, heavy and dotted lines may be used to represent bonds projecting respectively in front of and behind the plane of the paper.

2AA-4. Optically Inactive α-Amino Acids

4.1. Use of DL *or* ±

The optically inactive mixture or racemic compound of the enantiomers is designated by the prefix DL (no comma) or by the plus and minus sign (±) in parentheses.

Examples: DL-leucine, (±)-leucine.

4.2. Use of meso

The prefix *meso* or its abbreviation *ms* in lower case italic letters is used to denote those α-amino acids and derivatives of α-amino acids that are optically inactive because of internal compensation.

Examples: *meso*-lanthionine, *ms*-cystine.

2AA-5. Configuration at Chiral Centres Other than α-C

5.1. Use of Sequence Rule

In general, the Sequence Rule symbols are used to designate configuration, where known, at centres other than α-C. The configuration at α-C is customarily designated by D or L placed immediately before the trivial name.

Examples: (3*R*)-L$_S$-threonine, (3*S*)-L$_S$-isoleucine, (4*S*)-4-hydroxy-L$_S$-proline (see 5.3).

However, those who prefer not to use two different systems to indicate configuration in the same name may convey the same information as in the following examples: (2*S*,3*R*)-threonine, (2*S*,3*S*)-isoleucine, (2*S*,4*S*)-hydroxyproline.

5.2. Use of Carbohydrate Prefixes

Alternatively, names of α-amino acids having two or more chiral centres and in which a hydrogen atom is attached to each centre may be formed with use of the prefixes of carbohydrate nomenclature[6] (13) to define the configurational relationships. The small capital letter prefixes L$_S$ or D$_S$ are placed immediately before the trivial name of the parent α-amino acid or its radical. The subscripts must invariably be inserted in order to show that the capital letter prefix refers to the configuration of the α-carbon atom (carbon atom 2). The carbohydrate-name prefix is italicized in print and is not capitalized even at the beginning of a sentence.

Examples:

erythro-4-hydroxy-L$_S$-proline (from proteins)
threo-4-hydroxy-L$_S$-proline (otherwise allo-4-hydroxy-L$_S$-proline)
erythro-L$_S$-isoleucine (from proteins)
threo-L$_S$-isoleucine (otherwise allo-L$_S$-isoleucine)
erythro-5-hydroxy-L$_S$-lysine (from proteins)

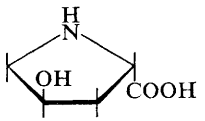
threo-5-hydroxy-L$_S$-lysine (otherwise allohydroxy-L$_S$-lysine)
ribo-3,4-dihydroxy-L$_S$-glutamic acid
xylo-3,4-dihydroxy-L$_S$-glutamic acid
arabino-3,4-dihydroxy-L$_S$-glutamic acid
lyxo-3,4-dihydroxy-L$_S$-glutamic acid

Note that the *ribo* and *xylo* isomers belong to the L$_g$ series, the *arabino* and *lyxo* isomers to the D$_g$ series.

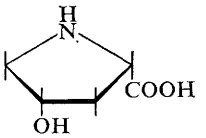
Note further that the relative configuration between the α centre and the second centre of chirality is already implied in the trivial name threonine.

5.3. Use of cis and trans

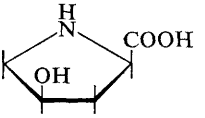
The amino acids 4-hydroxy-L-proline and 3-hydroxy-L-proline and analogous substituted prolines may also be named as follows (*cf.* 3.2, 5.1, 5.2)



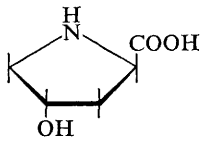
trans-4-hydroxy-L-proline
4-hydroxy-L-proline
erythro-4-hydroxy-L$_S$-proline



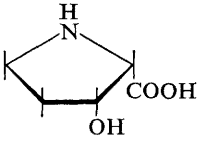
cis-4-hydroxy-L-proline
allo-4-hydroxy-L-proline
threo-4-hydroxy-L$_S$-proline



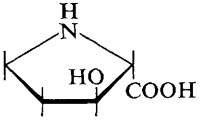
cis-4-hydroxy-D-proline
allo-4-hydroxy-D-proline
threo-4-hydroxy-D$_S$-proline



trans-4-hydroxy-D-proline
4-hydroxy-D-proline
erythro-4-hydroxy-D$_S$-proline



cis-3-hydroxy-L-proline
allo-3-hydroxy-L-proline
threo-3-hydroxy-L$_S$-proline



trans-3-hydroxy-L-proline
3-hydroxy-L-proline
erythro-3-hydroxy-L$_S$-proline

Cis and *trans* refer to the relative positions of the hydroxyl and carboxyl groups in each compound.

5.4. Use of allo

Where only the configuration of the α-chiral centre of a naturally occurring α-amino acid has been ascertained and that of a second chiral centre is unknown so that names constructed according to 5.1 or 5.2 cannot be assigned, the diastereoisomer is given the same trivial name with the prefix allo. Such diastereoisomers have the same configuration as the "parent" amino acid at α-C and the opposite configuration to that of the parent at the second chiral centre. The examples are names that were assigned before the configuration of the second chiral centre had been ascertained.

Examples: allo-L-isoleucine; allo-5-hydroxy-L-lysine; allo-β-hydroxy-L-glutamic acid; allo-L-threonine.

Note that the configuration at the α centre is ascertained (a) by direct chemical correlation with substances of known configuration, or (b) by determination of absolute configuration by the methods of Bijvoet *et al.* (14, 15), or (c) tentatively, from the results of studies of biological properties, in particular response to either

L-amino-acid oxidase or D-amino-acid oxidase, or (d) by examination of the change in optical rotation with change in the acidity of a solution of the substance.

Where a second chiral centre is present, it has been customary in the past to designate the first diastereoisomer described as the L- (or D-) amino acid. The second diastereoisomer, when found or synthesized, is then assigned the same name but with the prefix allo-. Where choice of the names on these grounds is impossible or inappropriate, such designations as "isomer A" and "isomer B" are frequently employed until the full configurational relationships are known. Alternatively, the symbol ξ (Greek xi) for "unknown configuration" may be used if more precise specification seems desirable, for example, as in the name (2*S*)-2-amino-5ξ-hydroxyhexanoic acid, $CH_3CHOH(CH_2)_2CH(NH_2)COOH$.

2AA-6. Optical Rotation

Where the configurational relationship of the α-carbon atom has not been established definitively, or where it is desired to emphasize the actual direction of rotation of an enantiomer of known configuration, the direction of rotation in a specified solvent is shown by a plus or a minus sign in parentheses or by the prefixes *dextro* or *levo* in lower case italic letters.

Examples: (+)-6-hydroxytryptophan, or *dextro*-6-hydroxytryptophan; (+)-glutamic acid, or *dextro*-glutamic acid (for dextrorotatory (in water) L-glutamic acid).

2AA-7. Peptides[7]

7.1. Construction of Names

Since peptides are, formally, aminoacylamino acids, the products of the condensation of two or more α-amino acids whereby the elements of water are eliminated between the α-carboxyl group of one amino acid and the α-amino group of the adjacent amino acid, they are named with use of the radical names ending in "yl." Thus, if amino acids "X-ine" and "Y-ine" (NH_2—X—COOH and NH_2—Y—COOH) condense in this order, the dipeptide (NH_2—X—CO-NH—Y—COOH) is named "X-yl-ine." If they condense in the reverse order, the dipeptide (NH_2—Y—CONH—X—COOH) is named "Y-yl-X-ine." Tripeptides and higher oligopeptides are named similarly, *i.e.*, X-yl-Y-yl-Z-ine (or -an or -ic acid).

$NH_2CH(COOH)(CH_2)_2CONHCH(CH_2SH)CONHCH_2COOH$

γ-L-glutamyl-L-cysteinylglycine

7.2. Use of Prefixes in Peptide Names

The configurational prefixes are placed immediately before the trivial name of each unit, whether radical, residue, or terminal. The prefixes and radical names, with the exception of the achiral glycine or glycyl, are set off from each other by hyphens (6). (But see note following 7.4.)

Examples: L-alanyl-L-leucine, L-alanyl-D-leucine, DL-alanyl-DL-leucine (mixture of four isomers), glycyl-L-alanine, L-alanylglycine, L-leucyl-L-phenylalanyl-L-isoleucylglycine, glycyl-L-leucyl-L-alanine, L-alanylglycyl-L-leucine.

7.3. Order of Names of Residues[7]

The name of a polypeptide begins with the name of the radical that carries the free α-amino group (NH_2-terminal amino acid) followed in order by the name or

names of the residue(s) of the internal α-amino acid(s). The final N-substituted amino acid carries the free carboxyl group (COOH-terminal amino acid). Formulas are usually written according to the same pattern, the NH_2-terminal at the left and the COOH-terminal at the right. The formula of reduced glutathione is written.

7.4. Names of Simple Polymers of α-Amino Acids

Simple polymers of amino acids may, if preferred, be named with prefixes indicating the number of amino-acid units involved. More complex polymers are referred to as polyglycine, poly(L-lysine), etc. (7).

Examples: glycylglycylglycine may be named triglycine, but not (diglycyl)-glycine; L-leucyl-L-leucyl-L-leucyl-L-leucine may be named tetra-L-leucine, or tri-L-leucyl-L-leucine.

Note: Although the units in a peptide chain—usually in abbreviated form—are usually separated by hyphens, it is necessary to use arrows when formulating a cyclic peptide, the arrow being pointed from the carboxyl of one unit to the amino group of the unit attached to that carboxyl group (6).

7.5. Conformation of Polypeptide Chains

Abbreviations and symbols recommended by CBN for the description of the conformation of polypeptide chains may be found in *J. Mol. Biol.*, **55**, 299 (1970), *Biochemistry*, **9**, 3471 (1970), and elsewhere.

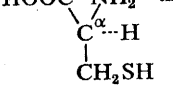
[1] Composition: N. Lozac'h (Chairman), S. P. Klesney (Secretary), K. Bláha, L. C. Cross, H. Grünewald, W. Klyne, K. L. Loening, J. Rigaudy. Associate Members: K. Hirayama, W. H. Powell, S. Veibel, F. Vögtle.

[2] In this document, a number of special procedures are used to form names based on the approved names of α-amino acids in an attempt to indicate important biochemical relationships. These procedures, yielding such names as alaninol (1.5), N^6-lysino (1.4.4), norleucin-6-yl (1.4.5), etc., should not be extended to other areas.

[3] Note that glutamate refers to glutamic acid whereas glutaminate refers to glutamine.

[4] This recommendation (6) arose from the fact that two different systems of numbering the atoms in the imidazole ring of histidine have been used for so long a time (biochemists generally numbering as 1 the nitrogen atom adjacent to the side chain and organic chemists designating it as 3).

[5] In L-cysteine, the α-carbon atom bears the four groups NH_2, CH_2SH, COOH, and H. They fall in this order of precedence as the atomic number of sulphur is greater than that of oxygen, and if L-cysteine is written in "steering-wheel" fashion,



the groups are in the *rectus* (*R*) or clockwise order, thus (2*R*)-cysteine.

[6] It has been customary in amino-acid nomenclature to apply the carbohydrate prefixes according to Anglo-American usage (Rule Carb-8), in which the prefixes refer to a sequence of consecutive but not necessarily contiguous asymmetric groups.

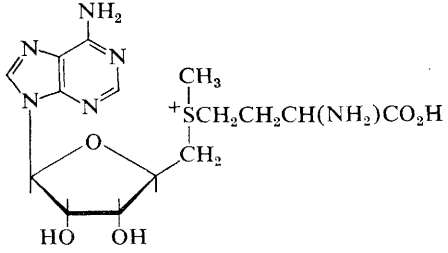
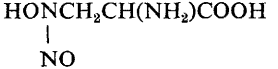
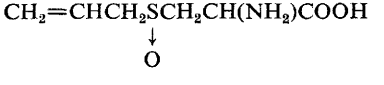
[7] More comprehensive recommendations for naming peptides, including the use of symbols and prefixes, are to be found in ref. 6 and 7.

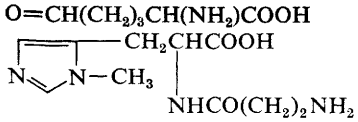
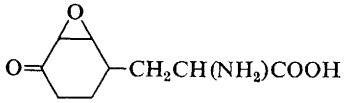
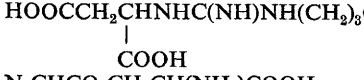
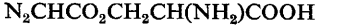
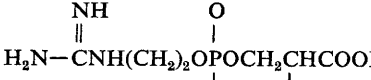
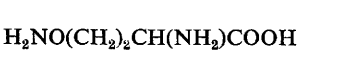
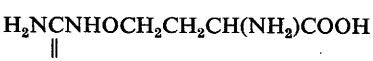
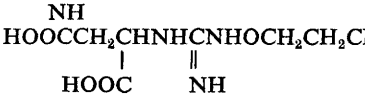
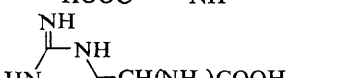
References

(1) Vickery, H. B., and Schmidt, C. L. A. (1931), *Chem. Rev.*, **9**, 169.
(2) Vickery, H. B. (1972), *Advan. Protein Chem.*, **26**, 82.
(3) Vickery, H. B. (1947), *J. Biol. Chem.*, **169**, 237.
(4) IUPAC, Definitive Rules for the Nomenclature of Amino Acids, (1960), *J. Amer. Chem. Soc.*, **82**, 5575.
(5) Addendum to ref. 4 (H. B. Vickery) (1963), *J. Org. Chem.*, **28**, 291.
(6) IUPAC–IUB, Symbols for Amino-Acid Derivatives and Peptides (1972), *J. Biol. Chem.*, **247**, 977; (1972), *Biochim. Biophys. Acta*, **263**, 205; also ref. 16.

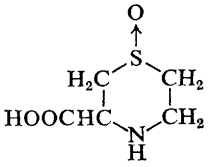
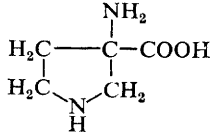
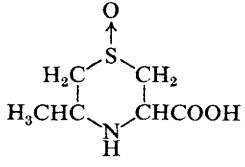
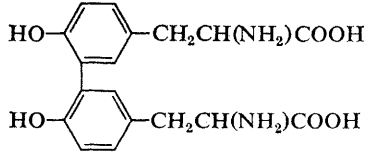
(7) IUPAC–IUB, Abbreviated Nomenclature of Synthetic Polypeptides (1972), *Biochem. J.*, **127**, 753; (1972) *Arch. Biochem. Biophys.*, **151**, 597; also ref. 17.
(8) IUPAC, Rules for the Nomenclature of Organic Chemistry, Sections A, B, and C (1971), Butterworths, London (Section C-421 in particular).
(9) IUPAC, Rules for the Nomenclature of Organic Chemistry, Section E, Fundamental Stereochemistry (Tentative Rules) (1970), *J. Org. Chem.*, **35**, 2849; (1970) *Biochim. Biophys. Acta*, **208**, 1, also ref. 18.
(10) Cahn, R. S., Ingold, C., and Prelog, V. (1966), *Angew. Chem., Int. Ed. Engl.*, **5**, 385, 511.
(11) Cahn, R. S., Ingold, C., and Prelog, V. (1966), *Angew. Chem.*, **78**, 413; (1956) *Experientia*, **12**, 81.
(12) Cahn, R. S. (1964), *J. Chem. Educ.*, **41**, 116.
(13) IUPAC–IUB, Tentative Rules of Carbohydrate Nomenclature (1972), *Biochemistry*, **10**, 3983; (1972) *Eur. J. Biochem.*, **21**, 455; also ref. 19.
(14) Bijvoet, J. M., Peerdeman, A. F., and van Bommel, A. J. (1951), *Nature (London)*, **168**, 271.
(15) Peerdeman, A. F., van Bommel, A. J., and Bijvoet, J. M. (1951), *Proc. Acad. Sci. Amsterdam*, **B54**, 16.
(16) Tentative Nomenclature Appendix No. 23 (June 1972) to the *IUPAC Information Bull.*
(17) *Pure Appl. Chem.* (1973), **33**, 437–444.
(18) *IUPAC Information Bull.* (1969), No. 35, 36–80.
(19) Tentative Nomenclature Appendix No. 7 (Sept. 1970) to the *IUPAC Information Bull.*

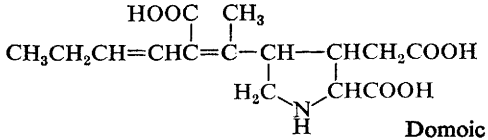
Appendix A: Naturally Occurring α-Amino Carboxylic Acids Bearing Unique Trivial Names[a] (excluding those listed in Table I)

Structure	*Trivial name*	*Biochemical name(s)* [c]	*Systematic name(s) or other names* [d]
	Abrine [b]	N^{α}-Methyltryptophan	
	*S*-Adenosylmethionine	*S*-(5′-Deoxy-5′-adenosyl)methionine	*S*-5′-[(3-Amino-3-carboxypropyl)-methylsulfonio]-5′-deoxy-adenosine
		N^{5}-[4-(Hydroxymethyl)anilino]-glutamine	Glutamic 5-{[2-(4-hydroxy-methyl)phenyl]hydrazide}
	Agaritine		
	β-Alanine [a, b]		3-Aminopropionic acid
	Alanosine	3-(Hydroxynitrosamino)alanine	2-Amino-3-(hydroxynitrosamino)-propionic acid
	Albizzine	3-Ureidoalanine	2-Amino-3-ureidopropionic acid
	Alliin	*S*-Allylcysteine *S*-oxide	3-(Allylsulfinyl)alanine
	Alloenduracididine [b]	(2*R*)-Enduracididine	
	Allohydroxylysine [b]	Allo-5-hydroxy-L$_8$-lysine	*threo*-5-Hydroxy-L$_8$-lysine; (5*S*)-5-hydroxy-L-lysine
	Allohydroxyproline [b]	Allo-4-hydroxy-L$_3$-proline	*threo*-4-Hydroxy-L$_8$-proline; *cis*-4-hydroxy-L-proline; (4*S*)-4-hydroxy-L-proline
	Alloisoleucine [b]	Allo-L$_8$-isoleucine	*threo*-L$_8$-Isoleucine
	Allokainic acid [b]	(See kainic acid)	
	Allothreonine [b]	Allo-L$_8$-threonine	(2*S*,3*S*)-Threonine

	Allysine Anserine	6-Oxonorleucine π-Methylcarnosine; N^{α}-(β-Alanyl)-π-methylhistidine	2-Aminoadipaldehydic acid N^{α}-(3-Aminopropionyl)-π-methylhistidine
	Anticapsin	3-(2,3-Epoxy-4-oxocyclohexyl)-alanine	α-Amino-5-oxo-7-oxabicyclo-[4.1.0]heptane-2-propionic acid
	Argininosuccinic acid	N^{ω}-(1,2-Dicarboxyethyl)-arginine	*N*-{[(4-Amino-4-carboxybutyl)-amino]iminomethyl}aspartic acid [a]
	Azaserine	O^{3}-(Diazoacetyl)serine	3-[(Diazoacetyl)oxy]alanine; serine diazoacetate ester
	Bonellidine	N^{2}-(γ-Aspartyl)lombricine	*N*-(1-Carboxy-2-hydroxyethyl)-asparagine 2-guanidinoethyl hydrogen phosphate (ester)
	Butyrine [a, b]		2-Aminobutyric acid
	Canaline		2-Amino-4-(aminooxy)butyric acid
	Canavanine	N^{ω}-Amidinocanaline	2-Amino-4-(guanidinooxy)-butyric acid
	Canavaninosuccinic acid	N^{ω}-(1,2-Dicarboxyethyl)canavanine	{*N*-[(3-Amino-3-carboxy-propoxy)amino]iminomethyl}-aspartic acid
	Capreomycidine	2-(Hexahydro-2-iminopyrimidin-4-yl)glycine; deoxytuberactidine	α-Aminohexahydro-2-imino-4-pyrimidineacetic acid
	Carnosine	N^{α}-(β-Alanyl)histidine	N^{α}-(3-Aminopropionyl)-histidine

Appendix A (*cont.*)

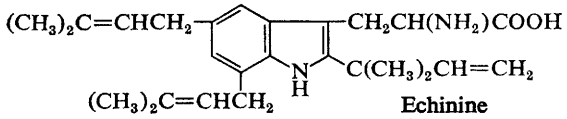
Structure	*Trivial name*	*Biochemical name(s)* [c]	*Systematic name(s) or other names* [d]
	Chondrine		3-Thiomorpholinecarboxylic acid 1-oxide; tetrahydro-2*H*-1,4-thiazine-3-carboxylic acid 1-oxide
$H_2NCH_2CH_2PO_3H_2$	Ciliatine [a]		(2-Aminoethyl)phosphonic acid
$H_2NCONH(CH_2)_3CH(NH_2)COOH$	Citrulline	N^5-Carbamoylornithine	2-Amino-5-ureidovaleric acid
	Cucurbitine		3-Amino-3-pyrrolidinecarboxylic acid
	Cycloalliin	5-Methylchondrine	5-Methyl-3-thiomorpholine-carboxylic acid 1-oxide
	Cycloserine [b] (oxamycin)		4-Amino-3-isoxazolidinone
$HO_3SCH_2CH(NH_2)COOH$	Cysteic acid	3-Sulfoalanine	
$HOOCCH(OH)CH_2SO_2CH_2SCH_2$-$CH(NH_2)COOH$	Dichrostachinic acid	*S*-[(2-Carboxy-2-hydroxyethyl-sulfonyl)-methyl]cysteine	
	Dityrosine	3,3′-Bityrosine	



Domoic acid

Dopa [b]

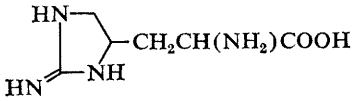
3-(Carboxymethyl)-4-(2-carboxy-1-methyl-1,3-hexadienyl)-proline



(3,4-Dihydroxyphenyl)alanine

5,7-Bis(3-methyl-2-butenyl)-2-(1,1-dimethylallyl)tryptophan

Echinine



Enduracididine

2-Aminodihydrohistidine (tautomer)

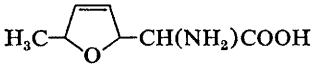
α-Amino-2-imino-4-imidazolidinepropionic acid

$CH_3CH_2SCH_2CH_2CH(NH_2)COOH$ Ethionine

S-Ethylhomocysteine

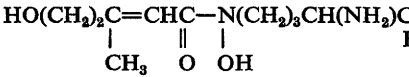
$HOCH_2CH_2C(CH_3)_2SCH_2CH(NH_2)COOH$ Felinine

S-(3-Hydroxy-1,1-dimethylpropyl)-cysteine



Furanomycin

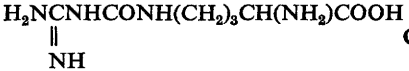
2-(2,5-Dihydro-5-methyl-2-furanyl)-glycine



Fusarinine

N^5-Hydroxy-N^5-(5-hydroxy-3-methyl-2-pentenoyl)-ornithine

2-Amino-5-[N-hydroxy-N-(5-hydroxy-3-methyl-2-pentenoyl)amino]-valeric acid



Gigartinine

N^ω-Amidinocitrulline; N^5-(amidinocarbamoyl)ornithine

5-(3-Amidinoureido)-2-aminovaleric acid

$HOOCCH(NH_2)(CH_2)_2CONHCH(CH_2SH)CONHCH_2COOH$ Glutathione

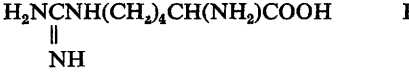
N-(N-γ-Glutamylcysteinyl)-glycine

$OCHN(OH)CH_2COOH$ Hadacidin [a]

N-Formyl-N-hydroxyglycine

Hippuric acid [b]

N-Benzoylglycine

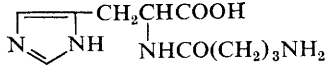
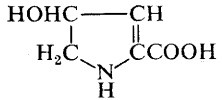
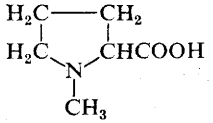
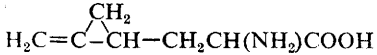
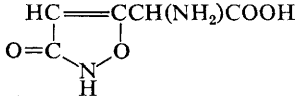
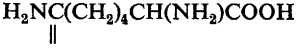
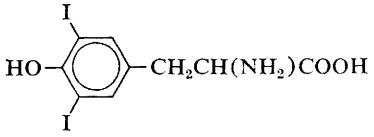


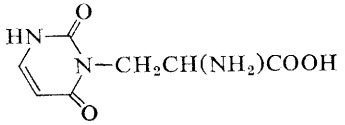
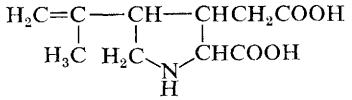
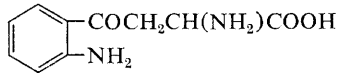
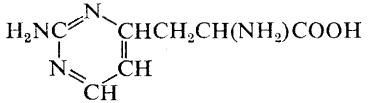
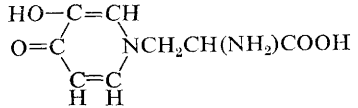
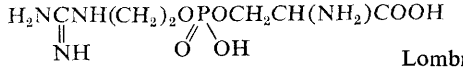
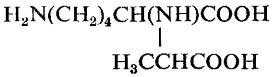
Homoarginine

N^6-Amidinolysine

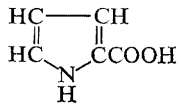
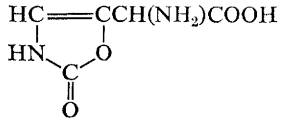
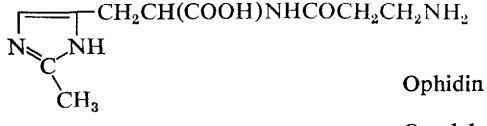
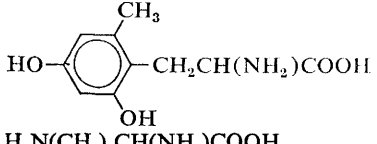
2-Amino-6-guanidinohexanoic acid

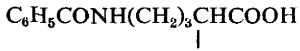
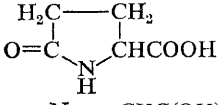
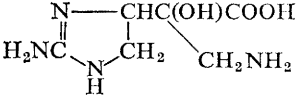
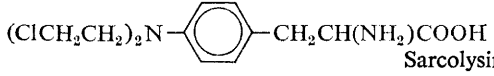
Appendix A (*cont.*)

Structure	*Trivial name*	*Biochemical name(s)* [c]	*Systematic name(s) or other names* [d]
	Homocarnosine	N^{α}-(4-Aminobutyryl)histidine	
$H_2NCONH(CH_2)_4CH(NH_2)COOH$	Homocitrulline	N^{6}-Carbamoyllysine	2-Amino-6-ureidohexanoic acid
$HSCH_2CH_2CH(NH_2)COOH$	Homocysteine		2-Amino-4-mercaptobutyric acid
	Homoisoleucine [b]		2-Amino-4-methylhexanoic acid
$CH_3S(CH_2)_3CH(NH_2)COOH$	Homomethionine	5-(Methylthio)norvaline	2-Amino-5-(methylthio)valeric acid
$HOCH_2CH_2CH(NH_2)COOH$	Homoserine		2-Amino-4-hydroxybutyric acid
	Hydroxyminaline	2,3-Didehydro-4-hydroxyproline	4-Hydroxy-2-pyrroline-2-carboxylic acid
	Hygric acid	*N*-Methylproline	
	Hypoglycin A	3-(2-Methylenecyclopropyl)alanine	α-Amino-2-methylenecyclopropanepropionic acid
$HO_2SCH_2CH_2NH_2$	Hypotaurine [a]		2-Aminoethanesulfinic acid
	Ibotenic acid	2-(3-Oxo-4-isoxazolin-5-yl)-glycine	α-Amino-3-oxo-4-isoxazoline-5-acetic acid
	Indospicine		6-Amidino-2-aminohexanoic acid
	Iodogorgoic acid	3,5-Diiodotyrosine	

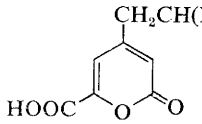
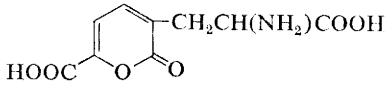
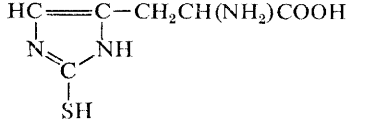
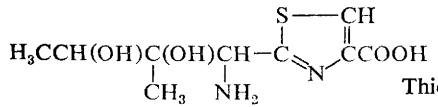
$CH_3CH(COOH)CH_2SCH_2CH(NH_2)COOH$	Isobuteine	*S*-(2-Carboxypropyl)cysteine	3-[(2-Carboxypropyl)thio]alanine
$H_2N(CH_2)_3CH(NH_2)CH_2COOH$	Isolysine [a] (β-lysine)		3,6-Diaminohexanoic acid
$(CH_3)_2CHCH(COOH)SCH_2CH(NH_2)COOH$	Isovalthine	*S*-(1-Carboxy-2-methylpropyl)-cysteine	
	Isowillardine	3-(Uracil-3-yl)alanine	α-Amino-3,6-dihydro-2,6-dioxo-1(2*H*)-pyrimidinepropionic acid
	Kainic acid	3-(Carboxymethyl)-4-isopropenyl-proline	2-Carboxy-4-isopropenyl-3-pyrrolidineacetic acid
	Kynurenine	3-Anthraniloylalanine	
$(CH_3)_3N^+(CH_2)_4CH(NH_2)COO^-$	Laminine	N^6-Trimethyllysine; N^6-trimethyllysine betaine	2-Amino-6-(trimethylammonio)-hexanoate
$CN(CH_2)_2NHCO(CH_2)_2CH(NH_2)COOH$	Lathyrus factor	N^5-(2-Cyanoethyl)glutamine	
	Lathytine (tingitanine)	3-(2-Amino-4-pyrimidinyl)alanine	α,2-Diamino-4-pyrimidinepropionic acid
	Leucenol (mimosine)	3-[3-Hydroxy-4-oxo-1(4*H*)-pyridinyl]alanine	
	Lombricine	*O*[3]-(2-Guanidinoethoxy)-phosphinicoserine	
	β-Lysine [b] (see isolysine)		
	Lysopine	N^2-(1-Carboxyethyl)lysine	

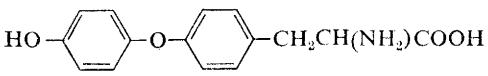
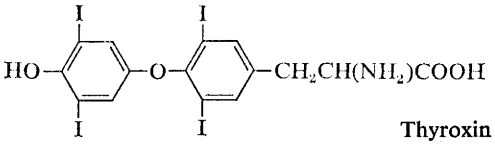
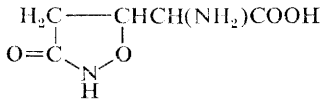
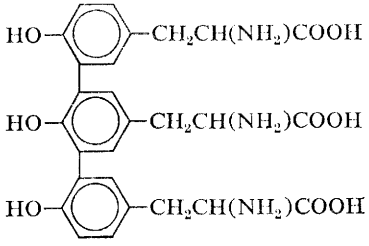
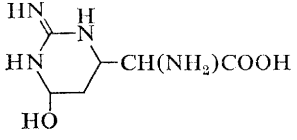
Appendix A (*cont.*)

Structure	*Trivial name*	*Biochemical name(s)* [c]	*Systematic name(s) or other names* [d]
	Mimosine [b] (see leucenol)		
	Minaline	Tetradehydroproline	2-Pyrrolecarboxylic acid
	Muscazone	3-(2-Oxo-4-oxazolin-5-yl)glycine	α-Amino-2-oxo-4-oxazoline-5-acetic acid
$H_2NC(NH)NH(CH_2)_3$-CHCOOH | $NHCH(COOH)CH_2CH_2COOH$	Nopaline	N^2-(1,3-Dicarboxypropyl)arginine; *N*-(1-carboxy-4-guanidinobutyl)-glutamic acid	
	Norleucine [b]		2-Aminohexanoic acid
$HOOCCH(NH_2)(CH_2)_2CONHCH(CH_3)CONHCH_2COOH$	Norophthalmic acid	*N*-[*N*-γ-Glutamylalanyl)glycine	N^5-[1-((Carboxymethyl)-carbamoyl)ethyl]glutamine
	Norvaline [b]		2-Aminovaleric acid
$H_2NCNH(CH_2)_3CHCOOH$ | | $NH \quad NHCH(CH_3)COOH$	Octopine	N^2-(1-Carboxyethyl)arginine	
$H_2N-(CH_2)_3CHCOOH$ | $NHCH(CH_3)COOH$	Octopinic acid	N^2-(1-Carboxyethyl)ornithine	
	Ophidin	2-Methylcarnosine; N^{α}-(β-alanyl)-2-methylhistidine	
	Orcylalanine	(2,4-Dihydroxy-6-methylphenyl)-alanine	
$H_2N(CH_2)_3CH(NH_2)COOH$	Ornithine	5-Aminonorvaline	2,5-Diaminovaleric acid

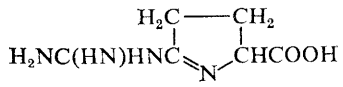
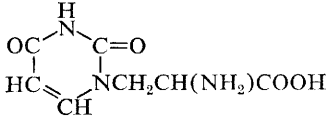
	Ornithuric acid	N^{α},N^{δ}-Dibenzoylornithine	
	Oxamycin [b] (see cycloserine)		
	Oxypinnatanine	N^{5}-[2,5-Dihydro-3-(hydroxymethyl)-2-furanyl]-4-hydroxyglutamine	
$HOCH_2C(CH_3)_2CH(NH_2)COOH$	Pantonine	3,3-Dimethylhomoserine	2-Amino-4-hydroxy-3,3-dimethylbutyric acid
$HOCH_2C(CH_3)_2CHOHCONH(CH_2)_2COOH$	Pantothenic acid [a]	*N*-Pantoyl-(β-alanine); 3-pantoamidopropionic acid	*N*-(2,4-Dihydroxy-3,3-dimethylbutyryl) (β-alanine)
$(CH_3)_2C(SH)CH(NH_2)COOH$	Penicillamine	3-Mercaptovalline; 3,3-dimethylcysteine	2-Amino-3-mercapto-3-methylbutyric acid
$C_6H_5CH_2CONHCH_2COOH$	Phenaceturic acid	*N*-(Phenylacetyl)glycine	
	Picolinuric acid	*N*-Picolinoylglycine; *N*-(2-pyridylcarbonyl)glycine	
	Pinnatanine	4-Hydroxy-N^{5}-[2-(hydroxymethyl)-1,3-butadienyl]-glutamine	
	Piperidinic acid [a, b]		4-Aminobutyric acid
	Pyroglutamic acid	5-Oxoproline; 5-pyrrolidone-2-carboxylic acid	5-Oxopyrrolidine-2-carboxylic acid
	Roseanine [a]		3-Amino-2-(2-amino-2-imidazolin-4-yl)-2-hydroxypropionic acid; 2-amino-α-(aminomethyl)-α-hydroxy-2-imidazoline-4-acetic acid
$HOOC(CH_2)_2CH(COOH)NH(CH_2)_4CH(NH_2)COOH$	Saccharopine	*N*-(5-Amino-5-carboxypentyl)-glutamic acid; N^{6}-(glutar-2-yl)-lysine	2-Amino-6-[(1,3-dicarboxypropyl)-amino]hexanoic acid
	Sarcolysine	4-[Bis(2-chloroethyl)amino]-phenylalanine	

Appendix A (*cont.*)

Structure	*Trivial name*	*Biochemical name(s)* [c]	*Systematic name(s) or other names* [d]
	Sarcosine [b]	*N*-Methylglycine	
	Stizolobic acid	3-(6-Carboxy-2-pyron-4-yl)-alanine	α-Amino-6-carboxy-2-oxo-2*H*-pyran-4-propionic acid
	Stizolobinic acid	3-(6-Carboxy-2-pyron-3-yl)-alanine	α-Amino-6-carboxy-2-oxo-2*H*-pyran-3-propionic acid
	Surinamine [b]	*N*-Methyltyrosine	
$HOOCCH(NH_2)(CH_2)_2CH(OH)$-$CH(NH_2)COOH$	Tabtoxinine	4-(Alanin-3-yl)threonine; 4-(2-amino-2-carboxyethyl)-threonine	2,6-Diamino-3-hydroxy-heptanedioic acid
$H_2NCH_2CH_2SO_3H$	Taurine [a]		2-Aminoethanesulfonic acid
$H_2NC(=NH)NH(CH_2)_2OP(O_2H)OCH_2CH(COOH)N(CH_3)_2$	Thallasemine	N^2,N^2-Dimethyllombrocine	*N*,*N*-Dimethylserine 2-guanidinoethyl hydrogen phosphate (ester); *N*,*N*-dimethyl-O^3-[(2-guanidinoethoxy)phosphinico]serine
$C_2H_5NHCOCH_2CH_2CH(NH_2)COOH$	Theanine	N^5-Ethylglutamine	
	Thiolhistidine	2-Mercaptohistidine	
	Thiostreptine		2-(1-Amino-2,3-dihydroxy-2-methylbutyl)-4-thiazolecarboxylic acid

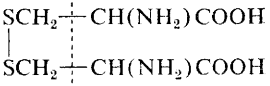
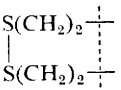
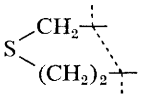
	Thyronine	4-(4-Hydroxyphenoxy)phenyl-alanine	O^4-(4-Hydroxyphenyl)tyrosine
	Thyroxin	3,5,3′,5′-Tetraiodothyronine	O^4-(4-Hydroxy-3,5-diiodophenyl)-3,5-diiodotyrosine
	Tingitanine [b] (see lathytine)		
	Tricholomic acid	2-(3-Oxo-5-isoxazolidinyl)-glycine	α-Amino-3-oxo-5-isoxazolidine-acetic acid
	Trityrosine [e]	3,3′; 5′,3″-Tertyrosine	
	Tuberactidine	2-(Hexahydro-6-hydroxy-2-imino-pyrimidin-4-yl)glycine; 6-hydroxycapreomycidine	α-Aminohexahydro-6-hydroxy-2-imino-4-pyrimidineacetic acid
	m-Tyrosine [b]	(3-Hydroxyphenyl)alanine	

Appendix A (*cont.*)

Structure	*Trivial name*	*Biochemical name(s)* [c]	*Systematic name(s) or other names* [d]
	Viomycidine	1,5-Didehydro-5-guanidinoproline	2-Guanidino-1-pyrroline-5-carboxylic
	Willardine	3-(Uracil-1-yl)alanine	α-Amino-3,4-dihydro-2,4-dioxo-1(2*H*)-pyrimidinepropionic acid

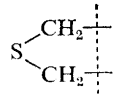
[a] Some amino acids that are not α-aminoalkanoic acids are included; their trivial names are so marked. No D-amino acids are included, hence L has been omitted from the names. No α-betaines are included. [b] Indicates no structure is given. [c] 'Biochemical names' are based on the names of amino acids defined in this document, and attempt thereby to indicate biochemical relationships as far as possible. [d] Systematic or other names avoid the use of names not included among the most common α-amino acids (Table I) and avoid the radical-prefix names described in Recommendation 2AA-1.4.4. [e] As this name implies tyrosyltyrosyltyrosine, tertyrosine (column 3) should be used.

Appendix B: Trivially Named Di(α-amino acids) Other than Peptides

Name [Symbol]	*Systematic names*	*Formula*
	B1. Two α-Amino Acids Connected by S or S—S or S—CH_2—S	
Cystine [(Cys)$_2$; Cys]	2,2′-Diamino-3,3′-dithiobis(propionic acid) 3,3′-Dithiobis(2-aminopropionic acid) 3,3′-Dithiodialanine	
Homocystine [(Hcy)$_2$; Hcy]	2,2′-Diamino-4,4′-dithiobis(butyric acid) 4,4′-Dithiobis(2-aminobutyric acid)	
Cystathionine [Ala; Hcy(Ala); Hcy]	*S*-(Alanin-3-yl)homocysteine *S*-(2-Amino-2-carboxyethyl)homocysteine 2-Amino-4-[(2-amino-2-carboxyethyl)thio]-butyric acid	

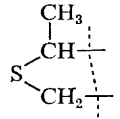
Lanthionine [Cys(Ala); Ala; Cys]

S-(Alanin-3-yl)cysteine
S-(2-Amino-2-carboxyethyl)cysteine
2,2′-Diamino-3,3′-thiobis(propionic acid)



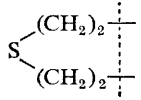
Methyllanthionine [Cys(3MeAla); 3MeAla; Cys]

S-(3-Methylanalin-3-yl)cysteine
2-Amino-3-[(2-amino-2-carboxyethyl)thio]-butyric acid



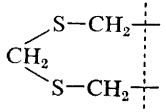
Homolanthionine [Hcy(Abu)]

2,2′-Diamino-4,4′-thiobis(butyric acid)
4,4′-Thiobis(2-aminobutyric acid)



Djenkolic acid [$Cys_2(CH_2)$]

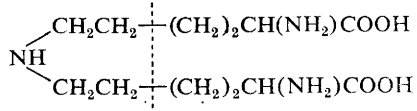
3,3′-Methylenedithiobis(2-aminopropionic acid)
3,3′-(Methylenedithio)dialanine
Methylenebiscysteine



B2. Lysinonorleucines:[a] Two α-Amino Acids Connected by N

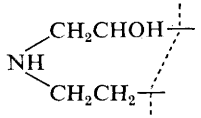
Lysinonorleucine [Lys(ωNle)]
[$HN(\omega Nle)_2$]

6-(N^6-Lysino)norleucine
N^6-(Norleucin-6-yl)lysine
N^6-(5-Amino-5-carboxypentyl)lysine
Bis(6-norleucin-6-yl)amine



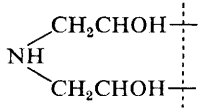
,5-Hydroxy[o^5Lys(ωNle)]

6-[N^6-(5-Hydroxylysino)]norleucine
N^6-(5-Hydroxynorleucin-6-yl)lysine
5-Hydroxy-N^6-(norleucin-6-yl)lysine
N^6-(5-Amino-5-carboxypentyl)-5-hydroxylysine

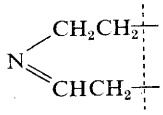
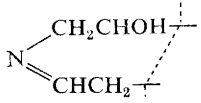
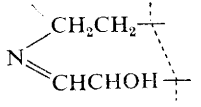
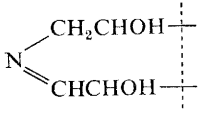


,5,5′-Dihydroxy[o^5Lys($o^5\omega$Nle)]
[$NH(o^5\omega Nle)_2$]

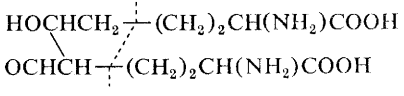
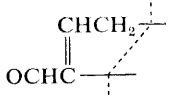
5-Hydroxy-6-[N^6-(5-hydroxylysino)]norleucine
5-Hydroxy-N^6-(5-hydroxynorleucin-6-yl)lysine
N^6-(5-Amino-5-carboxy-2-hydroxypentyl)-5-hydroxylysine



Appendix B (*cont.*)

Name [*Symbol*]	*Systematic names*	*Formula*
,N^6: 6′-Dehydro[Δ^6Lys(ωNle)]	6-(Δ^6-Lysino)norleucine N^6-(Norleucine-6,6-diyl)lysine N^6-(5-Amino-5-carboxypentylidene)lysine	
,N^6: 6′-Dehydro-5-hydroxy[Δ^6Lys($o^5\omega$Nle)]	5-Hydroxy-6-(Δ^6-lysino)norleucine N^6-(5-Hydroxynorleucin-6-yl)-Δ^6-lysine N^6-(5-Amino-5-carboxypentylidene)-5-hydroxylysine	
[Δ^6o^5Lys(ωNle)]	6-[Δ^6-(5-Hydroxylysino)]norleucine 5-Hydroxy-N^6-(norleucin-6-yl)-Δ^6-lysine N^6-(5-Amino-5-carboxy-2-hydroxypentylidene)-lysine	
,N^6: 6′-Dehydro-5,5′-dihydroxy-[Δ^6o^5Lys($o^5\omega$Nle)]	5-Hydroxy-6-[Δ^6-(5-hydroxylysino)]norleucine 5-Hydroxy-N^6-(5-hydroxynorleucin-6-yl)-Δ^6-lysine N^6-(5-Amino-5-carboxy-2-hydroxypentylidene)-5-hydroxylysine	

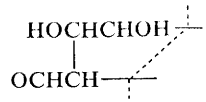
B3. Allysines:[b] Two (or More) Six-Carbon α-Amino Acids Connected by a Carbon–Carbon Bond

Allysine aldol [$(All)_2$] Di(allysine)	5-(6-Hydroxynorleucin-6-yl)-6-oxonorleucine [a]	
	6-Hydroxy-6-(6-oxonorleucin-5-yl)norleucine 2,10-Diamino-5-formyl-6-hydroxyundecanedioic acid	
Dehydro(allysine aldol)[$\Delta(All)_2$] Δ^5-(Allysine aldol)	2,10-Diamino-5-formyl-5-undecenedioic acid	

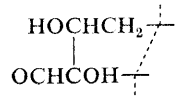
Syndesine [o(All)$_2$]
Hydroxy(allysine aldol)
Hydroxy(diallysine)

Hydroxyallysine-allysine

2,10-Diamino-5-formyl-6,7-dihydroxyundecanedioic acid

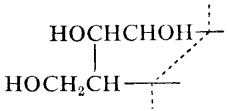


2,10-Diamino-5-formyl-5,6-dihydroxyundecanedioic acid

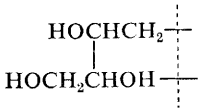


Syndesinol [o(All)$_2$H$_2$]
Reduced syndesine
Syndesine alcohol
Dihydroxydi(allysine)

2,10-Diamino-5,6-dihydroxy-7-(hydroxymethyl)undecanedioic acid

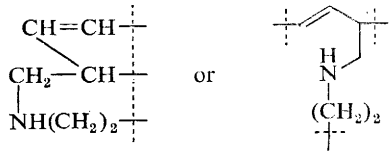


2,10-Diamino-5,6-dihydroxy-5-(hydroxymethyl)undecanedioic acid



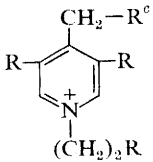
Merodesmosine

2,10-Diamino-5-{[(5-amino-5-carboxypentyl)-amino]methyl}-6-undecenedioic acid

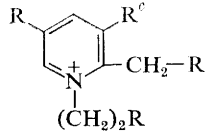


Desmosine

4-(4-Amino-4-carboxybutyl)-1-(5-amino-5-carboxypentyl)-3,5-bis(3-amino-3-carboxypropyl)pyridinium



Appendix B (*cont.*)

Isodesmosine	2-(4-Amino-4-carboxybutyl)-1-(5-amino-5-carboxypentyl)-3,5-bis(3-amino-3-carboxypropyl)pyridinium	

[a] For definition of lysino and norleucin-6-yl, see Section 1.4. [b] Allysine is defined in Appendix A as 6-oxonorleucine. [c] R = $(CH_2)_2CH(NH_2)COOH$.

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