NATURALLY OCCURRING PEPTIDES

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General

This review is intended to bring together information about a class of substances of which, until recently, very few examples were known in Nature. The substances discussed contain peptide bonds of the general type ·CHR·CO·NH·CHR'·, yield α-amino- or α-imino-carboxylic acids (amino-acids) on hydrolysis, and differ from the proteins in that they have smaller molecules or that they embody a much smaller variety of amino-acid species. The upper limit for molecular weight has been arbitrarily placed at 10,000 (about 100 amino-acid residues). There is, in fact, no sharp dividing line between peptides and proteins and it is thus accidental that such low-molecular proteins as cytochrome-*c*,[1] ribonuclease,[2] and the disaggregated form of insulin [3] are excluded. However, the 10,000-molecular-weight limit corresponds roughly with a limitation of the power to pass through Cellophane on dialysis, and is therefore convenient, since dialysability is often the only evidence as to molecular weight cited by authors. The most marked difference between peptides and proteins is the greater tendency of the latter to undergo denaturation. Typical denaturation phenomena are not shown by any of the substances discussed here, and some of the proteins of lower molecular weight are also extremely resistant to denaturing agents. Otherwise, the general physical and chemical properties of the peptides are only different from those of proteins in ways that can be directly correlated with smaller molecular size. The alternative criterion—lack of variety of amino-acid residues—is employed mainly on account of the polymer of D-glutamic acid found in *Bacillus anthracis* etc. (see below), which is a high-molecular substance, although it is not usually referred to as a protein. No other such substances have yet been described. The absence of one, or of a few, amino-acid species from a protein, as with gelatin or zein, has not led to inclusion. Whilst some of the substances described below contain a small variety of amino-acids, no evidence as to their molecular weight has been presented; it is therefore desirable to retain the criterion.

The study of peptides has scarcely advanced beyond the descriptive stage; the structures of few of the compounds mentioned have been thoroughly elucidated, and classification according to chemical structure or biological function would seem premature. The substances have been arranged in three main groups according to biological origin: (*a*) found in animals or higher plants; (*b*) found exclusively in fungi or related micro-

[1] J. Wyman, jun., *Advances in Protein Chemistry*, 1948, **4**, 410.
[2] I. Fankuchen, *ibid.*, 1945, **2**, 387.
[3] H. Gutfreund, *Biochem. J.*, 1948, **42**, 544.

organisms; and (*c*) found exclusively in bacteria. An outline is given of the salient chemical and biological features for each type, with key references to the literature. No attempt has been made to deal with the important and extensive subject of the experimental techniques used in studies of peptides.

However, studies of natural peptides are not confined in interest to the substances themselves, but are felt to have very important implications for the study of proteins and of the metabolism of nitrogenous compounds generally.

Proteins [4, 5, 6, 7] have been shown to be large molecules possessed of the most diverse biochemical activities, which are fundamental to the continuance of life. They are made up from a very limited number of species of amino-acid residue, joined for the most part in peptide linkage. Only about twenty different species of amino-acid have yet been discovered in proteins isolated from the whole range of living organisms,[8, 9] and most of these are present in every protein. This implies a degree of standardisation of the "working parts" of living organisms that has usually been explained in terms of highly specific geometrical arrangements of the chemical groupings in protein molecules, leading to highly specific "secondary valency" interactions with other molecules, large and small.[10] The study of simpler peptides has already helped in elucidating the nature of interactions of this kind,[10] and has further implications for the study of proteins, that are discussed below.

Peptides as Model Substances in the Study of Proteins.—Peptides of known structure and purely synthetic origin have been extensively used in studies of the specificity of proteolytic enzymes, in electro-chemical investigations bearing on proteins, and so forth. These lie outside the scope of this article. The structures of the majority of known natural peptides have not yet been elucidated, so they are less useful for these purposes. However, owing to their relatively simple molecular structure, they have already proved of value in testing methods of amino-acid analysis [9] and methods of determining the mode of linkage of amino-acid residues. Methods for the latter purpose have so far depended mostly on identification of free functional groups [11—16]

[4] C. L. A. Schmidt, "The Chemistry of the Amino Acids and Proteins", Thomas, Springfield, Ill., 1938.

[5] D. Jordan Lloyd and A. Shore, "Chemistry of the Proteins", Churchill, London, 1938. [6] M. V. Tracey, "Proteins and Life", Pilot Press, London, 1948.

[7] Thorpe's Dictionary of Applied Chemistry, Vol. X. Article on "Proteins and Peptides", Longmans, London, in the press.

[8] H. B. Vickery and C. L. A. Schmidt, *Chem. Reviews*, 1931, **9**, 169.

[9] A. J. P. Martin and R. L. M. Synge, *Advances in Protein Chemistry*, 1945, **2**, 1.

[10] K. Landsteiner, "The Specificity of Serological Reactions", Harvard University Press, Cambridge, Mass., 1945; L. Pauling, p. 275.

[11] R. M. Herriott, *Advances in Protein Chemistry*, 1947, **3**, 169.

[12] H. S. Olcott and H. Fraenkel-Conrat, *Chem. Reviews*, 1947, **41**, 151.

[13] S. W. Fox, *Advances in Protein Chemistry*, 1945, **2**, 155.

[14] F. Sanger, *Biochem. J.*, 1945, **39**, 507.

[15] R. R. Porter and F. Sanger, *ibid.*, 1948, **42**, 287.

[16] F. Sanger, *ibid.*, 1949, **44**, 126.

and of products of partial hydrolysis. Studies of partial hydrolysates of proteins have not so far yielded very much structural information. The subject has been reviewed in detail to 1941.[17] Since then, not many structural studies have been made of " true " proteins, although concrete evidence has been obtained of the extreme complexity of their partial hydrolysates. Natural peptides are proving to be very valuable substances with which to test the methods used in such studies. Glutathione [13] and gramicidin *S* [18] are perhaps at present the best examples. There are grounds for attaching greater significance to products of partial hydrolysis effected with acid or alkali than to those obtained enzymically. Peptides obtained in the former ways are excluded from consideration here. Of the few peptides arising by enzymic hydrolysis of proteins that have been characterised at all, only casein phosphopeptone, hypertensin, and strepogenin seem to merit detailed description here.

As the chemical structure of the simpler peptides becomes known, it will become possible to interpret their physical properties in considerable detail, and this will give much greater significance to future interpretations of the structure of true proteins based on their physical properties. This applies to such properties as solubility, osmotic pressure, sedimentation, diffusion, electrophoretic migration, and so forth.[19] However, perhaps the most promising field for such developments is that of *X*-ray crystallography. We can hope that *X*-ray studies of peptides of known chemical structure will lead to the elucidation of complete crystal structures, and that knowledge of such simpler crystal structures will help towards the elucidation of the more complex structures of true proteins. Many of these occur as crystals giving beautiful *X*-ray diffraction pictures that can as yet be interpreted only in the most general terms.[2, 20] The complete crystal-structure analysis of benzylpenicillin by Crowfoot and her colleagues,[21] with its implications for the chemical formulation of that group of substances, is an important step forward.

Finally, it should be pointed out that many of the peptides occur in Nature as mixtures of related compounds, similar in general structure, but differing in detail. The ergot alkaloids, gramicidins, tyrocidines, and penicillins are well-established examples of such families, and many more will be found. Such mixtures often behave in many respects as though they were pure homogeneous substances, and the establishment of their heterogeneity has tried existing techniques to their limits. Workers in this field tend therefore to be highly sceptical of claims to have isolated " pure, homogeneous proteins ", since by analogy with the peptides proteins might also be expected to occur in families. The molecule being so large, detection of heterogeneity would require an altogether higher order of technique.

[17] R. L. M. Synge, *Chem. Reviews*, 1943, **32**, 135.

[18] R. Consden, A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1947, **41**, 596.

[19] E. J. Cohn and J. T. Edsall, " Proteins, Amino Acids and Peptides ", Reinhold Publishing Corp., New York, 1943.

[20] J. Boyes-Watson, E. Davidson, and M. F. Perutz, *Proc. Roy. Soc.*, 1947, *A*, **191**, 83.

[21] D. Crowfoot, *Ann. Review Biochem.*, 1948, **17**, 115.

Meanwhile, the critical application of existing techniques raises serious doubts as to the validity of most claims so far made for the homogeneity of protein preparations.

"Abnormal" Structural Features of Peptides and their Possible Occurrence in Proteins.—Peptides may also, because they have lower molecular weights and because their study is technically simpler, provide the first examples of structural features that subsequently prove to be present in proteins. (However, reasons are given in the following section why peptides so far studied may not be representative in this respect.) Strepogenin, hypertensin, and casein phosphopeptone are in fact derived from proteins by enzymic breakdown, and other peptides such as the posterior-pituitary hormones and various of the antibacterial substances from *Bacillus* spp. may be products of breakdown of proteins brought about by the somewhat violent conditions of extraction.

Nevertheless, many of the amino-acid species present in natural peptides have not been revealed in hydrolysates of proteins studied by appropriate methods. This is true for β-alanine, $\alpha\gamma$-diaminobutyric acid, ornithine, penicillamine, etc. Amino-acid residues possessing abnormal steric configurations occur in many of the fungal and bacterial products described below. Attempts to demonstrate the occurrence of D-amino-acid residues in proteins have not, however, been generally successful, and have been complicated by racemisation.[22, 23] Reference should, however, be made to claims for the occurrence of D-amino-acids in hydrolysates of *Lactobacillus arabinosus* [24] and *Bacillus brevis*.[25]

Many of the peptides described below seem to have a "normal" open unbranched peptide-chain structure. Others, however, present "abnormal" features, whose occurrence in proteins deserves serious consideration. Thus gramicidin *S* and the components of tyrothricin are closed-chain "cyclopeptides". Glutathione, and probably the pteroyl peptides and the capsular substance of *Bacillus anthracis*, contain glutamic acid residues linked through their γ-carboxyl group. Gramicidin embodies a residue or a precursor of ethanolamine in such a way that its basic properties are masked; lycomarasmin and the ergot alkaloids may contain α-hydroxy-α-amino-acid residues. Similar types of linkage of these and other hydroxy-amino- and thiolamino-compounds should be searched for in proteins. Penicillin, similarly, illustrates ways in which an aldehyde group (*e.g.*, of a sugar residue in a glycoprotein) could combine with thiol groups or with an enolised peptide bond. Numerous peptides embody acyl residues that are not amino-acid in character (*e.g.*, pteroylglutamic acid and related compounds, penicillins, polymyxins, and ergot alkaloids) and could serve as models for the linking of acyl groups in some of the conjugated proteins.

[22] A. Neuberger, *Advances in Protein Chemistry*, 1948, **4**, 297.

[23] *Biochem. Soc. Symposia*, No. 1, "The Relation of Optical Form to Biological Activity in the Amino-acid Series", University Press, Cambridge, 1948.

[24] M. S. Dunn, M. N. Camien, S. Shankman, and H. Block, *J. Biol. Chem.*, 1947, **168**, 43.

[25] A. S. Konikova and N. N. Dobbert, *Biokhimiya*, 1948, **13**, 115.

Casein phosphopeptone has already proved useful in elucidation of the linking of phosphoryl residues in phosphoproteins.

Distribution and Physiological Function of Peptides.—It is still very difficult to generalise with any confidence about the biological rôle of peptides. A very high proportion of those so far described possesses some notable physiological activity—as hormones, poisons, growth promoters, or inhibitors, and so on. This is the result of researches aimed specifically at concentrating substances possessing particular biological activities. Such active substances have been found in a wide variety of chemical classes, and none of these types of biological activity has so far been found to be invariably due to peptides. However, of those antibiotic substances about whose chemical structure sufficient has been published to permit classification, a high proportion appears to be peptides or peptide derivatives. Work and Work [26] suggest that some of these may act as specific inhibitors of stages in protein synthesis, and prove to be valuable aids for elucidating that process in micro-organisms.

The occurrence, in toxic substances from bacteria and fungi, of stereoisomers of substances commonly found in higher organisms calls for special comment in this connection. Possible interpretations of the rôle of D-amino-acids have been discussed in a special publication.[23] Attention should also be drawn to the occurrence of antibiotic substances which, though not peptides, are sufficiently closely related in structure to be possible antagonists for reactions in which peptides are involved. Certain of the antibiotics from *Fusarium* spp.[27] and aspergillic acid [28, 28a] closely simulate peptides, whilst streptomycin and gliotoxin are rather more remotely analogous.

What is manifestly needed is objective study of peptides in living organisms irrespective of any immediately obvious physiological activity that they possess. Very little work on such lines has been done, and much of that, such as the earlier studies of peptidæmia, is technically inadequate. However, techniques for studying peptides have much improved in recent years. A very large proportion of the reliable physiological information so far available is due to the critical and careful studies of H. N. Christensen and his colleagues, making use of the Van Slyke ninhydrin CO_2 determination. Many of their data are referred to below.

Peptides without doubt occur in great variety during enzymic breakdown of proteins, as in tissue autolysis, in the intestinal tract, in breakdown of proteins by bacteria, and so forth. Over-all properties of such mixtures have been extensively studied, but very few chemical individuals from them have been characterised. It is noteworthy that at most a small proportion of the peptides present in the mammalian intestine are absorbed as such ;

[26] T. S. Work and E. Work, " The Basis of Chemotherapy ", Oliver and Boyd, Edinburgh, 1948, p. 227.

[27] A. H. Cook, S. F. Cox, T. H. Farmer, and M. S. Lacey, *Nature*, 1947, **160**, 31 ; A. H. Cook, S. F. Cox, and T. H. Farmer, *ibid.*, 1948, **162**, 61 ; P. A. Plattner, U. Nager, and A. Boller, *Helv. Chim. Acta*, 1948, **31**, 594 ; P. A. Plattner and U. Nager, *ibid.*, p. 665.

[28] J. D. Dutcher, *J. Biol. Chem.*, 1947, **171**, 321, 341.

[28a] G. Dunn, G. T. Newbold, and F. S. Spring, *Nature*, 1948, **162**, 779.

the gut wall seems selectively to transmit free amino-acids.[29, 30] Mixed peptides, obtained by enzymic or acid hydrolysis of proteins, when injected into the blood-stream, are rather slowly utilised by the tissues, and a considerable proportion of them is excreted through the kidneys.[31] However, peptides derived from different proteins behave differently in this respect.[32] The blood-plasma contains only small amounts of peptides.[33, 34] Peptides are liberated when blood clots.[34] Red blood corpuscles, muscle, liver, and other tissues contain much larger concentrations of peptides. Of these, a high proportion is accounted for by the ubiquitous glutathione (together with anserine and carnosine in muscle). Other peptides, however, are certainly present.[29, 33, 35, 36, 37] Peptides are normally present in urine in addition to such conjugates as benzoylglycine (hippuric acid).[31, 32, 38, 39] In a recent study of pathological urines, some individual peptides were observed, one of which may be serylglycylglycine.[40] "Bound" amino-acids or peptides have likewise been reported in extracts of plant tissues [41, 42, 43a, 43b] and in culture media after the growth of bacteria.[43]

All the tissues and fluids mentioned above contain also free amino-acids, and it is naturally surmised that the peptides may be intermediate stages in the interconversion of amino-acids and proteins, which Schoenheimer and his colleagues showed to occur at a very considerable rate. Unfortunately, the more actively an intermediate takes part in a chain of reactions, the lower is its concentration. It is even possible to visualise protein synthesis or breakdown occurring as an enzyme reaction without detectable intermediates, as in the breakdown of amylose to maltose by β-amylase.[44] When techniques have been devised for the isolation and characterisation of peptides present in living tissues, isotopic tracers should prove of great

[29] H. N. Christensen, D. G. Decker, E. L. Lynch, T. M. Mackenzie, and J. H. Powers, *J. Clin. Invest.*, 1947, **26**, 853.

[30] C. E. Dent and J. A. Schilling, *Biochem. J.*, 1949, **44**, 318; H. N. Christensen, *ibid.*, p. 333.

[31] H. N. Christensen, E. L. Lynch, and J. H. Powers, *J. Biol. Chem.*, 1946, **166**, 649.

[32] H. N. Christensen, E. L. Lynch, D. G. Decker, and J. H. Powers, *J. Clin. Invest.*, 1947, **26**, 849.

[33] H. N. Christensen and E. L. Lynch, *J. Biol. Chem.*, 1946, **163**, 741.

[34] *Idem*, *ibid.*, 1946, **166**, 87.

[35] H. N. Christensen, J. A. Streicher, and R. L. Elbinger, *ibid.*, 1948, **172**, 515.

[36] H. N. Christensen, J. T. Rothwell, R. A. Sears, and J. A. Streicher, *ibid.*, 1948, **175**, 101.

[37] H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley, and P. H. Lowy, *ibid.*, 1948, **174**, 1041; *ibid.*, 1949, **179**, 705.

[38] H. E. Sauberlich and C. A. Baumann, *ibid.*, 1946, **166**, 417.

[39] R. D. Eckhardt, A. M. Cooper, W. W. Faloon, and C. S. Davidson, *Trans. N.Y. Acad. Sci.*, 1948, Ser. 2, **10**, 284.

[40] C. E. Dent, *Biochem. Soc. Symposia*, No. 3, "Partition Chromatography", in the press; *Biochem. J.*, 1947, **41**, 240.

[41] H. B. Vickery, *J. Biol. Chem.*, 1925, **65**, 657, and previous papers.

[42] P. Haas, T. G. Hill, and B. R. Wells, *Biochem. J.*, 1938, **32**, 2129.

[43] H. Proom and A. J. Woiwod, *J. Gen. Microbiol.*, 1949, **3**, 319.

[43a] P. Haas and T. G. Hill, *Biochem. J.*, 1931, **25**, 1472.

[43b] T. Ohira, *J. Agr. Chem. Soc. Japan*, 1939, **15**, 370; 1940, **16**, 1, 293.

[44] M. A. Swanson, *J. Biol. Chem.*, 1948, **172**, 805.

value in assessing their metabolic function. Some data of this kind exist for glutathione.[45] The syntheses of leucylglycine from glycine [46] and of peptide material from leucine [37] have also been demonstrated using tracer techniques. Nevertheless, the first requirement still is for non-discriminatory study of the peptides occurring in living organisms. Whether or not such studies will throw much light on protein synthesis and breakdown, they may have other important results that cannot at present be predicted.

Descriptive

Peptides from Animals and Higher Plants.—*Casein phosphopeptones* (*lactotyrines*) are obtained by treating casein with trypsin, with or without previous peptic digestion. They have been studied in considerable detail by a number of authors, and there seems to be substantial unanimity that each molecule contains eight or more amino-acid residues, belonging almost exclusively to the species glutamic acid, serine, and *iso*leucine.[47—53] The numerous phosphate residues are esterified to hydroxyl groups of serine. The larger peptides in this group may additionally contain aspartic acid residues.[51] Partial acid hydrolysis of casein phosphopeptones yields phosphoserylglutamic acid, which probably furnishes the free amino-group of the polypeptides.[51—53] The data on phosphopeptone thus suggest that there is a high degree of localisation of the *iso*leucine and phosphoserine residues within the structure of the casein molecule. There is evidence that the phosphoric ester groupings may prevent trypsin from attacking the phosphopeptone.[50, 54]

Hypertensin (sometimes also called *angiotonin*) is a blood-pressure-raising substance obtained by allowing a proteolytic enzyme (renin) from the kidney to act on serum-protein of the globulin fraction. A similar reaction occurring *in vivo* may be responsible for the clinical condition of renal hypertension, which may be realised experimentally by promotion of renal ischemia. P. Edman [55] has carried the purification of hypertensin further than other workers, and has given a full review of the literature. A somewhat similar product ("*pepsitensin*") results from the action of pepsin on serum-protein. Edman's hypertensin preparations were dialysable through Cellophane, and diffusion measurements suggested a molecular weight of the order of 3000. The material yielded much histidine on hydrolysis, and, of the usual amino-acids, appeared to lack arginine, cystine, methionine, threonine, and phenylalanine, and to contain little tyrosine.

[45] K. Bloch and H. S. Anker, *J. Biol. Chem.*, 1947, **169,** 765.
[46] F. Friedberg, T. Winnick, and D. M. Greenberg, *ibid.*, p. 763.
[47] M. Damodaran and B. V. Ramachadran, *Biochem. J.*, 1941, **35,** 122.
[48] J. Lowndes, T. J. R. Macara, and R. H. A. Plimmer, *ibid.*, p. 315.
[49] C. Rimington, *ibid.*, p. 321.
[50] T. Posternak and H. Pollaczek, *Helv. Chim. Acta,* 1941, **24,** 921.
[51] *Idem, ibid.*, p. 1190.
[52] P. A. Levene and D. W. Hill, *J. Biol. Chem.*, 1933, **101,** 711.
[53] B. H. Nicolet and L. A. Shinn. See *Ann. Review Biochem.*, 1947, **16,** 236.
[54] T. Posternak and S. Grafl, *Helv. Chim. Acta,* 1945, **28,** 1258.
[55] *Arkiv Kemi., Min., Geol.*, 1945, **22,** *A,* No. 3.

The methods of purification used both by A. A. Plentl and I. H. Page [56] and by Edman [55] are similar to those used in isolations of histidine. E. Cruz-Coke [57] has provided some data on the behaviour of hypertensin with ion-exchange adsorbents. Taken together, these results suggest a considerable localisation of the histidine residues within the parent molecule of serum-protein. Hypertensin appears to be stable to boiling water or for a few hours to boiling dilute hydrochloric acid, but is readily inactivated by alkali and various other agents, including proteolytic enzymes. Plentl and Page [58] have studied the attack by various enzymes as a possible approach to elucidating the chemical structure. It seems probable that, like the phosphopeptones, hypertensin and pepsitensin are not chemical individuals, but families of similar peptides.

Strepogenin is the name given to a growth-factor for certain bacteria first shown to be present particularly in tryptic digests of various proteins.[59] Subsequent work indicated that concentrates of this principle could stimulate the growth of animals. It appeared to be peptide in character, and to promote growth additional to that obtained with the best available mixtures of free amino-acids.[60] Woolley has put forward evidence that strepogenin is a derivative of glutamic acid, and has found that various peptides of glutamic acid possess strepogenin activity, although not to a degree comparable with concentrates of strepogenin.[61] Phenylalanine and lysine were absent from Woolley's concentrates.[62] From diffusion measurements the molecular weight of strepogenin was estimated at 300–500.[63] Evidence was further obtained that strepogenin may embody a free amino-group of a glycine residue and be derived by tryptic or acid hydrolysis from the neighbourhood of terminal glycyl groups [14] in intact insulin or trypsinogen, though not in casein.[62] Recently some possible amino-acid sequences at such points in the insulin molecule have been mentioned.[64]

It is of particular interest that the activity of strepogenin can be inhibited competitively by the plant-wilting substance lycomarasmin (see below), and that, conversely, strepogenin competitively inhibits the activity of lycomarasmin. The same relations extend on the one hand to the synthetic glutamic acid derivatives possessing strepogenin activity, and on the other to a group of homologous aspartic acid derivatives, which appear to be structurally related to lycomarasmin.[63, 65] This seems to be the clearest example of biochemical antagonism of structurally analogous substances yet revealed in the peptide series.

Protamines. This group of basic substances, found associated with nucleic acid in the sperm of certain fish, has been subjected to intensive

[56] *J. Biol. Chem.*, 1945, **158,** 49. [57] *Ciencia,* 1945, **6,** 101.
[58] For refs. see A. A. Plentl, J. H. Page, and F. R. Van Abeele, *J. Biol. Chem.*, 1946, **163,** 49.
[59] H. Sprince and D. W. Woolley, *J. Amer. Chem. Soc.*, 1945, **67,** 1734.
[60] D. W. Woolley, *J. Biol. Chem.*, 1946, **162,** 383.
[61] *Idem, ibid.*, 1948, **172,** 71. [62] *Idem, ibid.*, 1947, **171,** 443.
[63] *Idem, ibid.*, 1946, **166,** 783.
[64] *Idem, Fed. Proc.*, 1948, **7,** 200; *J. Biol. Chem.*, 1949, **179,** 593.
[65] *Idem, J. Biol. Chem.*, 1948, **176,** 1299.

study for much longer than any of the other substances described here. Apart from the interest of their biogenesis, Kossel considered that they had interest as especially simple members of the group of proteins, suitable for model studies. Subsequent work has fully confirmed that they possess a much simplified qualitative amino-acid composition, and there is further physical evidence that some of them have molecular weights less than 10,000.[66, 67] Kossel [68] reviewed the whole subject in great detail to 1927. Since that date, interest has largely been concentrated on *clupein* (from herring) and *salmine* (from salmon), the latter of which has found application for combining with insulin for injection. These protamines both contain arginine as their only basic amino-acid, and there are two residues of arginine per residue of neutral amino-acid present. The neutral amino-acids alanine, valine, proline, and serine are common to both,[68—71] and proline appears in each case to be the terminal residue possessing a free basic group.[68, 69, 71, 72] *iso*Leucine [70, 71] and glycine [71] are present in salmine, but do seem not to have been demonstrated in clupein, and the converse is true for hydroxyproline.[69—71] Differences have also been noted in behaviour on methylation [73] and treatment with alkali.[74] However, the two protamines appear to be so similar as to merit more detailed comparative study. The Reviewer [17] has summarised structural studies by partial hydrolysis, especially of clupein, and suggests that the structural conclusions reached by Felix and his colleagues by actual isolation of peptides from partial hydrolysates made with acid are preferable to those of Waldschmidt-Leitz and his colleagues which were reached by non-isolative studies of enzymic digests. The last-mentioned authors have since described some interesting work involving isolations from tryptic digests, which does not, however, call for reversal of this judgment.[75—77] Particularly in view of the demonstration by Felix's school that clupein is heterogeneous,[78] it seems best to regard the protamines, like so many other peptides, not as pure substances, but as families of compounds closely related in chemical structure.

The histones [68] embody a much wider variety of amino-acid residues, and there is little evidence that their molecular weight is low ; they are therefore not considered in this article.

[66] E. Waldschmidt-Leitz, F. Ziegler, A. Schäffner, and L. Weil, *Z. physiol. Chem.*, 1945, 1931, **197**, 219.

[67] V. Plaskéev, N. Yarovenko, and A. Passynski, *Compt. rend., Acad. Sci. U.R.S.S.*, **49**, 580.

[68] A. Kossel, " The Protamines and Histones ", Longmans, Green & Co., London, 1928.

[69] K. Felix and A. Mager, *Z. physiol. Chem.*, 1937, **249**, 111.

[70] R. J. Block and D. Bolling, *Arch. Biochem.*, 1945, **6**, 419.

[71] G. R. Tristram, *Nature*, 1947, **160**, 637.

[72] R. R. Porter and F. Sanger, *Biochem. J.*, 1948, **42**, 287.

[73] S. Edlbacher, *Z. physiol. Chem.*, 1919, **107**, 52.

[74] J. Roche and M. Mourgue, *Compt. rend.*, 1946, **222**, 204.

[75] E. Waldschmidt-Leitz and F. Turba, *J. pr. Chem.*, 1940, **156**, 55.

[76] E. Waldschmidt-Leitz, J. Ratzer and F. Turba, *ibid.*, 1941, **158**, 72.

[77] E. Waldschmidt-Leitz, *Beiheft Z. Ver. dtsch. Chem.*, No. 45 ; *Chemie*, 1942, **55**, 62

[78] K. Felix and K. Dirr, *Z. physiol. Chem.*, 1929, **184**, 111.

Pituitary hormones. An interesting position exists with regard to the hormones of the *posterior lobe*, which has been reviewed at some length by G. W. Irving and V. du Vigneaud [79] and by B. F. Chow.[80] The two generally recognised principles, the so-called *pressor* and *oxytocic hormones*, have been isolated and separated from one another, by chromatographic separation on ion-exchange adsorbents.[81] The substances responsible appear to be peptides of molecular weight 600—1300,[82, 83] and both contain much cysteine and tyrosine ; the pressor principle contains also arginine.[82] The resulting difference in basicity probably accounts for the separation by adsorption and the differences in electrophoretic behaviour. For a long time it was held, particularly by Abel, that both activities were due to a single large molecule, and recently extracts of the gland have yielded an apparently homogeneous protein, of molecular weight approx. 30,000, possessing both the activities to about the same extent (molecule for molecule) as the individual active peptide preparations. Since the latter are isolated from the gland by somewhat drastic procedures, it is reasonable to assume that they are liberated from the " parent protein " during the isolation. This does not yet seem to have been established by direct experiment, but it is suggestive that the two active peptides are resistant to digestion by pepsin, whereas the " parent protein " is digested by pepsin, though without loss of either activity. An enzyme inactivating the pressor, but not the oxytocic substance, has recently been described.[84]

Most of the hormones of the *anterior lobe* [80, 85] appear to be protein in character, although material possessing *adrenocorticotrophin* activity may be ultra-filtered through Cellophane,[85, 86] and there is evidence that the *thyrotrophin* may also have a rather low molecular weight.[85]

Secretin. This hormone, that stimulates pancreatic secretion, has been isolated from intestine in a crystalline state (for bibliography see refs. 87, 88). It appears to have molecular weight of approx. 5000. Recently some new data on its amino-acid composition have become available.[89]

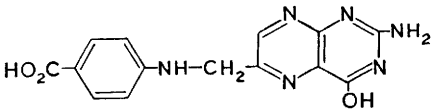
Glutathione. This tripeptide, γ-L-glutamyl-L-cysteinylglycine, with the corresponding —S·S— compound, is a very widely distributed tissue component, often occurring in high concentrations. Its structure has been established by synthesis.[90, 91] Its metabolic function is by no means

[79] G. W. Irving, jun., and V. du Vigneaud, *Ann. N.Y. Acad. Sci.*, 1943, **43**, 273.
[80] B. F. Chow, *Advances in Protein Chemistry*, 1944, **1**, 153.
[81] A. M. Potts and T. F. Gallagher, *J. Biol. Chem.*, 1944, **154**, 349.
[82] *Idem, ibid.*, 1942, **143**, 561.
[83] M. Rosenfeld, *Bull. Johns Hopkins Hosp.*, 1940, **66**, 398.
[84] H. Croxatto, W. Badia, and R. Croxatto, *Proc. Soc. Exp. Biol. Med.*, 1948, **69**, 422.
[85] A. White, *Physiol. Reviews*, 1946, **26**, 574.
[86] Dr. C. J. O. R. Morris, private communication.
[87] G. Ågren and E. Hammarsten, *J. Physiol.*, 1937, **90**, 330.
[88] G. Agren, *ibid.*, 1939, **94**, 553.
[89] P. Edman and G. Ågren, *Arch. Biochem.*, 1947, **13**, 283.
[90] C. R. Harington and T. H. Mead, *Biochem. J.*, 1935, **29**, 1602.
[91] V. du Vigneaud and G. L. Miller, *J. Biol. Chem.*, 1936, **116**, 469.

clear, despite a vast amount of experimentation since its discovery by F. G. Hopkins more than twenty years ago.

Carnosine and anserine. These well-known compounds, respectively β-alanyl-L-histidine and β-alanyl-1-methyl-L-histidine, are major components of muscle tissue, for which, likewise, no metabolic function has been clearly established.

Derivatives of pteroic and p-*aminobenzoic acid.* Recent work has helped to elucidate the chemical nature of certain growth-factors for animals and micro-organisms belonging to the "B complex", and their relation to *p*-aminobenzoic acid, a known growth-factor and naturally occurring antagonist of sulphonamide drugs. Although several of these products have only been isolated from micro-organisms, it is convenient to consider all together. A very full review has been published by T. H. Jukes and E. L. R. Stokstad.[91a] The structure of pteroic acid



has been established by degradative [92–94] and synthetic [95–98] studies. Pteroyl-L-glutamic acid has been isolated from liver [99, 100] and synthesised.[95–98] The pteroyl derivative of what appears to be a peptide composed of three residues of glutamic acid has been isolated from a bacterial fermentation product (*Corynebacterium* sp.).[92, 93, 101] It can be degraded to pteroylglutamic acid by partial alkaline hydrolysis,[92] and synthetic studies [102, 103] suggest that it may have the structure pteroyl-γ-glutamyl-γ-glutamylglutamic acid.[103a] The pteroyl derivative of what may be a heptapeptide embodying seven glutamic acid residues has been isolated from yeast.[104] It is inactive towards micro-organisms, but acts as a growth-factor for chicks. It appears to be closely related with a polypeptide, also isolated from yeast [105] and embodying ten or eleven residues of L-glutamic acid, one of unidentified α-amino-acid per residue of *p*-aminobenzoic acid, and one amidic NH_2. The molecular size of all these compounds other than pteroylglutamic acid must be regarded as uncertain until more physical data have been published.

Other acyl derivatives. Although they are not, strictly, peptides, mention should be made of certain acylamino-acids. *Pantothenic acid* (αγ-dihydroxy-

[91a] *Physiol. Reviews*, 1948, **28**, 51.
[92] E. L. R. Stokstad *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 5.
[93] B. L. Hutchings *et al.*, *ibid.*, p. 10.
[94] J. H. Mowat *et al.*, *ibid.*, p. 14.
[95] C. W. Waller *et al.*, *ibid.*, p. 19.
[96] M. E. Hultquist *et al.*, *ibid.*, p. 23.
[97] R. B. Angier *et al.*, *ibid.*, p. 25.
[98] J. H. Boothe *et al.*, *ibid.*, p. 27.
[99] J. J. Pfiffner *et al.*, *ibid.*, 1947, **69**, 1476.
[100] E. L. R. Stokstad, B. L. Hutchings, and Y. SubbaRow, *ibid.*, 1948, **70**, 3.
[101] B. L. Hutchings *et al.*, *ibid.*, p. 1.
[102] J. H. Mowat *et al.*, *ibid.*, p. 1096.
[103] J. H. Boothe *et al.*, *ibid.*, p. 1099.
[103a] This seems to have been conclusively established by J. Semb *et al.* (*ibid.*, 1949, **71**, 2310).
[104] J. J. Pfiffner *et al.*, *ibid.*, 1946, **68**, 1392.
[105] S. Ratner, M. Blanchard, and D. E. Green, *J. Biol. Chem.*, 1946, **164**, 691.

N-$\beta'\beta'$-dimethylbutyryl-β-alanine, otherwise pantoyl-β-alanine) is a vitamin and a growth-factor for micro-organisms that is very widely distributed among living organisms. Evidence has recently been presented that it may occur in a conjugated form in tissues.[106] Other well-known acyl derivatives are found in urine and have been called " detoxication products " ; *e.g.*, *benzoylglycine* (*hippuric acid*), *a*δ-*dibenzoylornithine* (*ornithuric acid*), *phenylacetylglutamine, etc.* δ-*Acetylornithine* has been isolated from *Corydalis*.[107]

Miscellaneous. Cobalt- and phosphorus-containing substances have recently been isolated from liver, possessing very high activity for the treatment of pernicious anæmia, and also growth-factor activity for certain bacteria.[108—112] The molecular weight appears, from *X*-ray and diffusion measurements, to be 1500—3000. It is not yet clear whether these substances are peptides.[112a] E. L. Smith's earlier preparations were associated with peptide material and varied considerably in amino-acid composition. Enzymic attack permitted purification by fractionation procedures already employed, suggesting that the active material was unchanged, whereas associated contaminants had been digested.

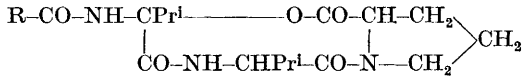
Among less well characterised substances, possibly peptides, mention should be made of a trypsin inhibitor from pancreas,[113] a pepsin inhibitor and other peptides from pepsinogen,[114] peptide material from milk,[114a] a basic compound from wheat (possessed of some antibacterial activity, and containing much arginine, cystine, and tyrosine [115, 116]), " allergens " from plant pollens,[117, 118] and components of bee venom [119, 120] and cobra venom.[121—122]

Peptides from Fungi.—*Penicillins.* No attempt is made to review the extensive data on these antibacterial substances (see refs. 123, 124). The complicated condensed ring-system of the penicillin molecule can be regarded as arising from reactions of the side-chains in the corresponding dipeptide, acyl-L-α-formylglycyl-D-β-mercaptovaline (penicillamine). The D-configura-

[106] G. D. Novelli, N. O. Kaplan, and F. Lipmann, *J. Biol. Chem.*, 1949, **177,** 97.
[107] R. H. F. Manske, *Canad. J. Res.*, 1937, **15,** *B*, 84.
[108] E. L. Smith, *Nature*, 1948, **161,** 638. [109] *Idem, ibid.*, 1948, **162,** 144.
[110] E. L. Rickes *et al.*, *Science*, 1948, **107,** 396.
[111] *Idem, ibid.*, 1948, **108,** 134.
[112] E. L. Smith and L. F. J. Parker, *Biochem. J.*, 1948, **43,** viii.
[112a] But see N. G. Brink *et al.*, *J. Amer. Chem. Soc.*, 1949, **71,** 1854.
[113] M. Kunitz and J. H. Northrop, *J. Gen. Physiol.*, 1936, **19,** 991.
[114] R. M. Herriott, *ibid.*, 1941, **24,** 325.
[114a] R. Aschaffenburg, *J. Dairy Res.*, 1946, **14,** 316.
[115] A. K. Balls, W. S. Hale, and T. H. Harris, *Cereal Chem.*, 1942, **19,** 279.
[116] A. K. Balls and T. H. Harris, *ibid.*, 1944, **21,** 74.
[117] H. A. Abramson, D. H. Moore, and H. H. Gettner, *J. Physical Chem.*, 1942, **46,** 192. [118] G. E. Rockwell, *J. Immunol.*, 1942, **43,** 259.
[119] R. Havemann and K. Wolff, *Biochem. Z.*, 1937, **290,** 354.
[120] W. Fassbender, *ibid.*, 1944, **317,** 246.
[121] F. Micheel and H. Emde, *Z. physiol. Chem.*, 1940, **265,** 266, and previous papers.
[122] B. N. Ghosh and D. K. Chowdhuri, *J. Indian Chem. Soc.*, 1943, **20,** 22.
[123] A. H. Cook, *Quart. Reviews*, 1948, **2,** 203.
[124] E. Chain, *Ann. Review Biochem.*, 1948, **17,** 657.

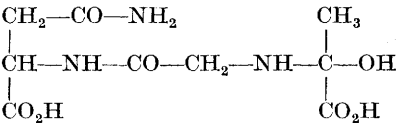
tion of the penicillamine moiety appears to be essential for the biological activity. Neither of the amino-acids has so far been found elsewhere in Nature, but L-α-formylglycine (aminomalonaldehydic acid) can be regarded as a simple oxidation product of L-serine, whilst the finding of β-mercaptovaline suggests that past claims to have isolated hydroxyvaline should be taken more seriously.

Ergot alkaloids. Members of this family, extracted from ergot of rye (*Claviceps purpurea*), have proved difficult to separate, owing to mixed-crystal and molecular-compound formation. A. Stoll [125] has given a useful review of the whole subject, together with a classification of the alkaloids. For details, reference should be made to papers from the schools of Stoll [126] and W. A. Jacobs.[127] All of the family embody one residue per mol. of the complicated condensed-ring indole derivative lysergic acid ($R{\cdot}CO_2H$). In *ergobasine* (*ergometrine*) this occurs as 2-*N*-lysergamidopropan-1-ol. The natural compound has proved of great value in obstetrics, and this substance and its optical stereoisomers have been partly synthesised. The other ergot alkaloids have not found so much application. On hydrolysis, each molecule yields one molecule each of lysergic acid, ammonia, D-proline, an α-keto-acid (pyruvic acid or ββ-dimethylpyruvic acid), and an α-amino-acid (L-leucine, L-valine, or L-phenylalanine). Stoll [125] on alkaline hydrolysis of *ergocornine* obtained lysergamide and dimethylpyruvoylvalylproline. Full experimental details do not yet seem to have been published; meanwhile, taking account of the suggestion of W. A. Jacobs and L. C. Craig [128] that the keto-acids and ammonia arise by breakdown of α-hydroxy-α-amino-acids (see also data on lycomarasmin, below), the following formula seems reasonable for ergocornine:



This interesting structure is only partly peptide in character, and has affinity with other products of *Fusarium* spp.[27] besides lycomarasmin.

Lycomarasmin. This substance was isolated from *Fusarium lycopersici* and has powerful leaf-wilting effects on tomato plants.[129] P. A. Plattner and his colleagues hydrolysed lycomarasmin and isolated glycine, aspartic acid, pyruvic acid, and ammonia. They suggested a provisional formula, which they recognised to have defects.[130] Recently D. W. Woolley has produced strong evidence in support of the following formulation: [131]



[125] *Experientia*, 1945, **1**, 250.
[126] F. Troxler, *Helv. Chim. Acta*, 1947, **30**, 163, and previous papers.
[127] F. C. Uhle and W. A. Jacobs, *J. Org. Chem.*, 1945, **10**, 76, and previous papers.
[128] *J. Biol. Chem.*, 1938, **122**, 419.
[129] P. A. Plattner and N. Clauson-Kaas, *Experientia*, 1945, **1**, 195.
[130] P. A. Plattner, N. Clauson-Kaas, A. Boller, and U. Nager, *Helv. Chim. Acta*, 1948, **31**, 860.
[131] *J. Biol. Chem.*, 1948, **176**, 1291.

The configuration of the α-hydroxyalanine residue is unknown. However, Woolley synthesised a mixture of the diastereoisomers, which possessed the appropriate biological activity and closely resembled lycomarasmin in a number of other respects. He also found that a number of derivatives of aspartic acid possess leaf-wilting properties.[65] The antagonism between some of these substances and strepogenin and its analogues has been referred to above.

Amanitine and phalloidine. These two highly toxic heat-stable substances have been isolated in a crystalline state from the well-known fungus *Amanita phalloides* (Death Cap). Phalloidine has been investigated in the greater detail.[132] It yielded on hydrolysis two compounds not previously found in Nature—a substance which seemed to be a hydroxytryptophan and L-*allo*hydroxyproline. The latter was identified with the original synthetic material of Leuchs. The hydroxytryptophan has special interest as a possible metabolic intermediate in the degradation of tryptophan to kynurenine.[133] Cystine and alanine were also found in the hydrolysate. No free amino- or carboxyl group was found, and no physical evidence as to molecular weight was presented. Amanitine [134] likewise appeared to be peptide in character, and probably to contain hydroxytryptophan.

Fumaryl-DL-*alanine* has been isolated from *Penicillium recticulosum.*[134a]

Peptides from Bacteria.—*Tyrothricin.* R. J. Dubos and R. D. Hotchkiss isolated peptides having antibacterial activity from strains of *Bacillus brevis.* These appeared to be in some way associated with bacterial protein, being set free by autolysis, extraction with acid alcohol, or by proteolytic enzymes, against which the peptides are resistant. The material so obtained is called tyrothricin. Simple fractionation of tyrothricin with solvents serves to separate the neutral *gramicidins* from the basic *tyrocidines.* These groups of substances are totally different in the nature of their antibacterial action. R. D. Hotchkiss [135] has given a very full and clear review of the earlier data on the isolation and chemistry of these peptides, and has also discussed their mode of action on living cells.

Gramicidin A, B, *etc.* The gramicidin fraction from tyrothricin yields, on repeated crystallisation from acetone, a product with constant amino-acid composition.[136, 137] The mother-liquors contain material differing somewhat in amino-acid composition and optical rotation.[136, 138] For a long time it appeared as if an individual substance had been obtained by crystallisation (*e.g.*, ref. 139). However, J. D. Gregory and L. C. Craig [140]

[132] H. Wieland and B. Witkop, *Annalen*, 1940, **543**, 171.
[133] A. Butenandt, W. Weidel, and E. Becker, *Naturwiss.*, 1940, **28**, 447; E. Becker, *ibid.*, 1941, **29**, 237.
[134] H. Wieland, R. Hallermeyer, and W. Zilg, *Annalen*, 1941, **548**, 1.
[134a] J. H. Birkinshaw, H. Raistrick, and G. Smith, *Biochem. J.*, 1942, **36**, 829.
[135] *Advances in Enzymology*, 1944, **4**, 153.
[136] A. H. Gordon, A. J. P. Martin, and R. L. M. Synge, *Biochem. J.*, 1943, **37**, 86.
[137] R. L. M. Synge, *ibid.*, 1949, **44**, 542.
[138] R. D. Hotchkiss, *J. Biol. Chem.*, 1941, **141**, 171.
[139] R. L. M. Synge and A. Tiselius, *Acta Chem. Scand.*, 1947, **1**, 749.
[140] *J. Biol. Chem.*, 1948, **172**, 839.

showed that such preparations are heterogeneous. They recognised a major component (*A*), a minor component (*B*) containing phenylalanine[140a] in addition to the residues present in (*A*), and other minor components (*C*, *D*, etc.) containing tyrosine. The following facts, though established with mixed material, are probably mostly true at least for gramicidin *A*. The molecular weight appears to be in the range 3000—5000 from measurements of diffusion [141, 142] and vapour-pressure lowering; [142] cryoscopic study gave results difficult to interpret.[142] Acid hydrolysis yields L-tryptophan and D-leucine, together with D-valine, L-valine, L-alanine, glycine,[135] and ethanolamine,[143] in amounts accounting adequately for all of the carbon and nitrogen; [137] free amino- and carboxyl groups are absent.[138] This implies some unconventional mode of linkage for the ethanolamine. Whatever this linkage is, it seems necessary to postulate a cyclopeptide structure. The following peptides have been isolated from mixed gramicidins by partial hydrolysis with acid: L-valylglycine,[144, 145] D-leucylglycine, L-alanyl-D-valine, L-alanyl-D-leucine,[137] L-valyl-L-valine, and D-valyl-D-valine.[146, 147] It seems unlikely, from the yields, that the first two can be derived from the same gramicidin. The data seem to suggest that residues both of D- and of L-valine exist in the molecule preformed, but there are possibilities of epimerisation that cannot be ignored. Gramicidin possesses free hydroxyl groups, which have been acetylated.[138] The action of sulphating [148, 149] and phosphorylating [150] agents has also been studied. A less toxic and more water-soluble product has been obtained by treating gramicidin with formaldehyde; [151] the chief reaction appears to be with the indole groups.[152] Solubility has also been increased by preparing the half-succinic ester, with and without additional formaldehyde treatment.[153] The biological activities of these and other derivatives have been studied.[154, 155]

Tyrocidines. These are a family of basic peptides, of which some at least differ in their content of tryptophan,[139] a difference which permits their separation by adsorption on charcoal.[139, 156] The fractions so obtained do not differ much in other properties. J. D Gregory (private communication) has fractionated tyrocidine by counter-current distribution.

[140a] Private communication from Dr. J. D. Gregory.
[141] K. O. Pedersen and R. L. M. Synge, *Acta Chem. Scand.*, 1948, **2**, 408.
[142] M. Tishler, J. L. Stokes, N. R. Trenner, and J. B. Conn, *J. Biol. Chem.*, 1941, **141**, 197.
[143] R. L. M. Synge, *Biochem. J.*, 1945, **39**, 355.
[144] *Idem*, *ibid.*, 1944, **38**, 285.
[145] *Idem*, *ibid.*, 1945, **39**, 351.
[146] H. N. Christensen, *J. Biol. Chem.*, 1943, **151**, 319.
[147] *Idem*, *ibid.*, 1944, **154**, 427.
[148] H. C. Reitz *et al.*, *J. Amer. Chem. Soc.*, 1946, **68**, 1024.
[149] *Idem*, *ibid.*, p. 1031.
[150] R. E. Ferrel, H. S. Olcott, and H. Fraenkel-Conrat, *ibid.*, 1948, **70**, 2101.
[151] J. C. Lewis *et al.*, *Science*, 1945, **102**, 274.
[152] H. Fraenkel-Conrat, B. A. Brandon, and H. S. Olcott, *J. Biol. Chem.*, 1947, **168**, 99.
[153] H. S. Olcott *et al.*, *Arch. Biochem.*, 1946, **10**, 553.
[154] H. Fraenkel-Conrat *et al.*, *Proc. Soc. Exp. Biol. Med.*, 1946, **63**, 302.
[155] O. Schales and G. E. Mann, *Arch. Biochem.*, 1947, **13**, 357.
[156] R. L. M. Synge and A. Tiselius, *Acta Chem. Scand.*, 1949, **3**, 231.

The amino-acid species found in tyrocidine are L-ornithine, L-valine, L-leucine, D-phenylalanine, L-proline, L-tyrosine, L-tryptophan, L-glutamic acid, and L-aspartic acid. The two last are probably present as glutamine and asparagine, an equivalent amount of ammonia being liberated in hydrolysis.[135, 157] These account for substantially all of the carbon and nitrogen of tyrocidine. The molecular weight appears, from diffusion experiments,[141] to lie in the range 1900—5100. The δ-amino-groups of ornithine and the phenolic groups of tyrosine are free,[158] but it is doubtful if carboxyl groups occur free.[138] A cyclopeptide structure without other abnormalities can therefore be postulated. The occurrence together of the first five amino-acid species listed above suggests a close relationship with gramicidin *S* (see below).

Gramicidin S. This antibacterial substance is produced by different strains of *Bacillus brevis* and was discovered by G. F. Gause and M. G. Brazhnikova.[159] The material is readily crystallisable, and recrystallised Soviet material appears substantially homogeneous by chemical analysis,[160] diffusion,[141] adsorption,[139] and counter-current-distribution studies.[161] The last method revealed the presence of several related compounds in an American preparation of gramicidin *S*. Gramicidin *S* yields, on hydrolysis, equimolar amounts of L-ornithine, L-valine, L-leucine, L-proline, and D-phenylalanine.[160] One free amino-group is present per stoicheiometric minimum unit, and this is the δ-amino-group of the ornithine residue.[162] A cyclopeptide structure is therefore postulated, embodying only α-peptide linkages. *X*-Ray studies of the hydrochloride and other salts and of the *N*-acetyl derivative, etc., impose a maximum molecular weight corresponding to two stoicheiometric minimum units. In this case a two-fold axis of symmetry must be present in the molecule. This could reasonably occur only by repetition twice of the same sequence of five amino-acids. The alternative possibility is a cyclopentapeptide molecule.[163] An extensive study of the products of partial hydrolysis by acid produced strong evidence for the amino-acid sequence being

-α-(L-valyl)-L-ornithyl-L-leucyl-D-phenylalanyl-L-prolyl-

though no differentiation was made between the cyclopentapeptide and cyclodecapeptide formulæ.[18] Evidence from cryoscopic [164] and diffusion [141] measurements is in favour of the latter. J. I. Harris and T. S. Work [165] have synthesised some analogous open-chain peptides. At present gramicidin *S* appears to be the most complicated natural peptide of which the structure can be formulated with reasonable certainty.

Polymyxins (*aërosporin*). What appear to be members of a family of

[157] H. N. Christensen, L. Uzman, and D. M. Hegsted, *J. Biol. Chem.*, 1945, **158**, 279.
[158] H. N. Christensen, *ibid.*, 1945, **160**, 75.
[159] P. G. Sergiev (ed.). "Sovyetskii Gramitsidin i Lecheniye Ran", Medgiz, Moscow, 1943.
[160] R. L. M. Synge, *Biochem. J.*, 1945, **39**, 363.
[161] Dr. L. C. Craig, private communication.
[162] F. Sanger, *Biochem. J.*, 1946, **40**, 261.
[163] D. Crowfoot and G. M. J. Schmidt, unpublished.
[164] A. N. Belozersky and T. S. Paskhina, *Biokhimia*, 1945, **10**, 344; *Lancet*, 1944, II, 716.
[165] *Nature*, 1948, **161**, 804.

peptide-like antibacterial substances have been isolated from strains of *Bacillus polymyxa*.[166, 167, cf. 168] Polymyxins *A*, *B*, *C*, *D*, and *E* have so far been described. The data and publications are concisely summarised in three recent abstracts [169—171] and more fully in a forthcoming publication.[172] The polymyxins are recognised as individuals on the basis of distinctive chromatographic behaviour or of yielding distinctive hydrolysis products, or of both. So far, most of the strains studied have each yielded polymyxin of a single type. On acid hydrolysis, L-$\alpha\gamma$-diaminobutyric acid, D-leucine, phenylalanine, L-threonine, serine, and an optically active saturated fatty acid have been identified. For data on molecular weights see P. H. Bell *et al.* (ref. 172, p. 187).

Licheniformin. A component of culture filtrates of *Bacillus licheniformis* active against *Mycobacterium tuberculosis* was first described by R. K. Callow and P. d'A. Hart,[173] who reported a positive Sakaguchi reaction and diffusion through Cellophane (see also refs. 174, 175). R. K. Callow and T. S. Work (private communication) state that licheniformin has been resolved into three biologically active peptides of similar amino-acid composition.

Bacitracin. This material, isolated from a strain of the *Bacillus subtilis* group and possessing antibacterial activity, was discovered by B. A. Johnson, H. S. Anker, and F. L. Meleney.[176, cf. 177] G. T. Barry, J. D. Gregory, and L. C. Craig [178] have produced evidence of the homogeneity of the active material in a commercial preparation. After hydrolysis, they found phenylalanine, leucine, *iso*leucine, glutamic acid, aspartic acid, lysine, histidine, cystine, and an unidentified basic amino-acid. Methionine, valine, threonine, serine, proline, and arginine were absent. A dipeptide of phenylalanine and *iso*leucine, and a peptide containing phenylalanine and ornithine, were also isolated from the hydrolysate. Some D-amino-acids were present. The permeability of membranes to bacitracin [177] suggests a molecular weight less than 2000.

Subtilins. A number of other antibacterial preparations from *Bacillus subtilis* have been described under this name.[*e.g.*, 179—185] The main

[166] G. C. Ainsworth, A. M. Brown, and G. Brownlee, *Nature*, 1947, **160**, 263.
[167] P. G. Stansly, R. G. Shepherd, and H. S. White, *Bull. Johns Hopkins Hosp.*, 1947, **81**, 43. [168] R. G. Shepherd *et al.*, *J. Amer. Chem. Soc.*, 1948, **70**, 3771.
[169] G. Brownlee and T. S. G. Jones, *Biochem. J.*, 1948, **43**, xxv.
[170] T. S. G. Jones, *ibid.*, p. xxvi.
[171] J. R. Catch, T. S. G. Jones, and S. Wilkinson, *ibid.*, p. xxvii.
[172] P. H. Long *et al.*, *Ann. N.Y. Acad. Sci.*, 1949, **51**, 853—1000.
[173] *Nature*, 1946, **157**, 334.
[174] R. K. Callow, R. E. Glover, and P. d'A. Hart, *Biochem. J.*, 1947, **41**, xxvii.
[175] R. K. Callow *et al.*, *Brit. J. Exp. Path.*, 1947, **28**, 418.
[176] *Science*, 1945, **102**, 376. [177] H. S. Anker *et al.*, *J. Bact.*, 1948, **55**, 249.
[178] *J. Biol. Chem.*, 1948, **175**, 485.
[179] E. F. Jansen and D. J. Hirschmann, *Arch. Biochem.*, 1944, **4**, 297.
[180] J. C. Lewis *et al.*, *ibid.*, 1947, **14**, 415.
[181] J. J. Stubbs *et al.*, *ibid.*, p. 427. [182] J. C. Lewis *et al.*, *ibid.*, p. 437.
[183] K. P. Dimick *et al.*, *ibid.*, 1947, **15**, 1.
[184] R. E. Feeney, H. D. Lightbody, and J. A. Garibaldi, *ibid.*, p. 13.
[185] C. H. Hassall, *Nature*, 1948, **161**, 317.

evidence as to their peptide nature is their dialysability, and loss of biological activity with proteolytic enzymes. In this last, they differ from polymyxins.[186]

Other antibiotics. A number of other such bacterial products has been described,[187–190] some of which may be peptides, *e.g.*, *colistatin*, *diplococcin*, *nisin*, *eumycin*, *bacillin* (*and an inhibitor thereof*), *actinorubin*, *lavendulin*, *streptolin*, etc.

Capsular substance of Bacillus anthracis *and related species.* Capsulated strains of *Bacillus anthracis* and related *Bacillus* spp. produce this substance in their capsules and culture media.[191, 192] H. N. Rydon [193] has made a useful review of work on this topic. On acid hydrolysis, the substance appears to yield only D-glutamic acid.[194] The material in the capsules has a molecular weight of 50,000 or more when undegraded, but breaks down rather readily, and the material in the media has a molecular weight of a few thousand. There is evidence for the occurrence of both α- and γ-linkages. The γ-linkages appear to be fewer in number and more labile. The peptide has been esterified with methanol,[195, 196] and, through the ester, converted into the amide, polyglutamine.[196] Studies have been made of the reactivity of the peptide and these derivatives with sulphating agents,[148, 149] *iso*cyanates,[197] and phosphorylating agents.[150] W. E. Hanby, S. G. Waley, and J. Watson [198] have synthesised a polymeric α-peptide of L-glutamic acid possessing properties similar to those of the capsular substance.

Tuberculin. Peptide material has been repeatedly observed in tuberculin preparations, and some of the larger peptide molecules present have biological activity similar to that of the tuberculin proteins.[199, 200]

Other bacterial products. So far, despite fairly extensive studies of biologically active materials from pathogenic bacteria, few, if any, products falling within the scope of this review have been reported.[201] The claim that scarlet-fever toxin has a low molecular weight [202] has been made doubtful by subsequent work.[201]

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