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PROTEIN STRUCTURE AND CLINICAL PROBLEMS*

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The proteins have always been an object of interest to chemists and biologists. Their investigation, however, was for long hampered by the old vitalistic ideas which regarded protoplasm as fundamentally living and therefore beyond the scope of the chemist. Despite the fact that as early as 1746 Beccari succeeded in isolating gluten from wheat flour, and in 1859 Ritthausen had developed methods for preparing vegetable proteins in a relatively pure and stable form, the attack on animal proteins was still held back by devotion to vitalism. The decisive blow to this old doctrine was struck by Hoppe-Seyler's crystallization of the protein haemoglobin in 1866. This achievement was followed by the crystallization of a number of other proteins of animal origin, culminating in the production of crystalline egg albumin in 1892 by Hofmeister.

Experimental clinicians and biologists have been mainly interested in proteins on account of their nutritional value. It was clearly recognized that, though all proteins provide necessary nitrogen in assimilable form, they differ among themselves in nutritional value, as shown classically by the work of Hopkins and his school, and this led to the concept of "essential amino-acids." Proteins are also important in determining water balance and the osmotic pressure of the blood, and there is an increasing recognition of their significance in immunity. But our whole conception of the structure of tissues in the body, and their relationship to the internal environment, has undergone a complete change since the introduction of labelled or tagged elements into the study of metabolism. Before the introduction of this new technique, proteins, and other tissue constituents such as minerals and so forth, were considered as being in more or less static equilibrium, and a protein molecule once made and stabilized stayed in its intact state in the plasma or tissue.

We now know from a study of the metabolism of amino-acids containing labelled or tagged elements that proteins are steadily being broken down and built up, and that the whole of the protein system of the body is in dynamic equilibrium with the amino-acid content of the tissue fluids and the blood serum. Schoenheimer, in his classical experiments (Schoenheimer *et al.*, 1939; Schoenheimer, 1942), has used deuterium and ^{15}N labelled amino-acids for the study of protein turnover *in vivo*. He showed that the half-life of liver proteins in the adult rat is seven days. Sprinson and Rittenberg (1949) were able to extend these investigations and to develop an elaborate quantitative method for studying nitrogen balance and protein synthesis in the rat. By the use of suitably labelled amino-acids it was possible to study what they termed the metabolic pool in both the

rat and man and to work out the rate at which amino-acids were passed out of this metabolic pool into the body. Again, they showed that in the rat the half-life of the total proteins was about 17 days, of the liver, plasma, and internal organ proteins some 6-7 days, and of the total carcass proteins 21 days. In the case of man the total turnover of protein occurred in about 80 days, while the liver and serum proteins were turned over in 10 days and the lung, brain, bone, skin, and principally muscle in 158 days. Tarver and Reinhardt (1947) were able to make some similar observations by studying the metabolism of methionine containing ^{35}S in normal and hepatectomized dogs, and they showed that the incorporation of tagged methionine into body proteins could take place without the intervention of the liver. The administration of ^{35}S containing methionine resulted in a rapid increase in the concentration of radioactive sulphur in plasma proteins, the five-hour value being twice that at two hours. The intestinal mucosa surprisingly absorbed very large quantities of the labelled amino-acids, indicating that the intestinal mucous membrane must play an important part in protein synthesis. Extensive experiments in this field have shown that the injection of such amino-acids is followed by their very rapid uptake into the body proteins, particularly the proteins of the visceral organs. It would appear that from this storehouse the amino-acids are distributed for incorporation into other tissues. A full review of this fascinating subject is given in a recent paper by Borsook (1950).

From the constitutional point of view, clinician and biologist have been content up to the present with the knowledge that proteins are composed of amino-acids joined together through the classical CO-NH linkage. The proteins possess a very large number of amino-acids joined together in this manner, and therefore are bodies of high molecular weight. It stands to reason that, if the molecular weight can vary from 10,000 to 1,000,000 or over, the disposition of the peptide chains, or strings of amino-acids, must be of considerable importance. How, in fact, are these strings orientated in space? Are they long single chains, are they branched chains arranged in a definite manner, or are they just a jumble like a badly wound ball of string? This problem has fascinated physical and stereo-chemists from the very time that the peptide-linkage theory of protein structure was accepted.

As Dr. Dorothy Crowfoot Hodgkin (1949) points out, it is difficult to know whether it is wise at the present juncture to comment on protein structure or whether it would be better to wait until all the accurate analyses now being made throughout the world on protein crystals have been received and collated. There is no doubt that the greater accuracy of these methods of measurement has revealed

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that none of the theories so far considered will stand up, and that we are driven to the conclusion that the protein molecule consists of relatively long chains of peptides strung together by various bridges and possibly coiled or wound round in order to give a compact mass. There

seems to be enough evidence to show that proteins such as haemoglobin are composed of peptide chains which are folded on each other in the manner shown in Fig. 1.

Astbury and Street (1931) have pointed out that proteins can be classified as of either the fibre or the globulin type. The fibre proteins consist of silk fibroin, myosin (from muscle), and keratin (skin and hair), while the globular proteins are those in which clinicians are primarily interested—namely, globulins, albumins, and so forth.

It is proposed to take the study of

insulin molecule as the typical example of this vast and complicated subject of the configuration of the protein molecule.

Protein Structure as Exemplified by a Study of Insulin

Physical Studies.—Gutfreund (1948) has shown that the molecular weight of insulin is 47,000–48,000 and that the variable results obtained in previous estimations were due to the existence of an equilibrium between the 48,000 molecule and four apparently identical submolecules of 12,000 weight each. Further support for the 12,000 subunit in insulin comes from the finding that it passes through "cellophane" impervious to molecules of weight 21,000 and higher values.

Analysis.—Chibnall's (1946) analysis shows that the 12,000 weight subunit of insulin probably contains 106 amino-acid residues, of which 101 have been accounted for. These are: aspartic acid 5, glutamic acid 15, glycine 7, alanine 6, valine 8, leucine and isoleucine 14, phenylalanine 6, tyrosine 9, serine 6, threonine 2, lysine 2, histidine 4, arginine 2, proline 3, half-cystine 12. The remaining five residues probably are accounted for as a cumulative error in the numerous analyses. Insulin lacks methionine and tryptophan, but still contains 16 different amino-acids, and in any case methionine and tryptophan are present only in very small amounts in all but exceptional proteins.

Insulin has a high proportion of sulphur, like the keratins and certain pituitary hormones, and the cystine apparently accounts for all the sulphur of insulin, though this has been the subject of much controversy in the past (for example, du Vigneaud, 1927). Scott (1934) found that the addition of zinc, nickel, cobalt, or cadmium alone among metallic cations is necessary for the crystallization of insulin.

Main Structural Arrangement.—The most important contributions to the structural theory of insulin have come

from the laboratory of Professor Chibnall, at Cambridge. Early work indicated that there were a number of peptide chains in the insulin molecule, but it was not until 1945 that Sanger, working in his laboratory, was able to demonstrate that there were four chains in the insulin submolecule,

two with glycine and two with phenylalanine residues providing the free end amino-groups of the chains. The view was held that these chains were probably joined together by six cystine disulphide bridges (Fig. 2), and this has been substantiated by Sanger (1948, 1949). If these bridges are broken

the separate chains can be fractionated with ammonium acetate into two distinct groups, A and B. The A fraction is obtained from the glycine chains of insulin, and each chain has a molecular weight of about 2,500 and four cysteic acid residues. The B chains contain all the lysine, histidine, arginine, phenylalanine, proline, and (in the case of bovine insulin) all the threonine of the insulin submolecule, so that the two types of peptide chain in insulin are very different in structure.

In bovine and ovine insulins the A fractions contain glycine, alanine, valine, leucine, isoleucine, tyrosine, serine, cystine, aspartic acid, and glutamic acid only, whereas porcine insulin A chains have been found to contain some threonine also (Sanger, 1949).

Amino-acid Sequences.—Sanger has obtained by analysis a series of peptides which leave no doubt that part of the amino-acid sequence is: in the A chains (amino-group of glycine terminal and free) glycyl-isoleucyl-valyl-glutamyl-glutamyl-, and in the B chains (amino-group of phenylalanine terminal and free) phenylalanyl-valyl-aspartyl-glutamyl. Some of the aspartic and glutamic residues are in the form of their amides (that is, asparaginyl and glutaminyl residues). An internal sequence in the B chains was also determined by the labelling of the ϵ -amino group of the lysine side-chains. This sequence was: threonyl-prolyl-lysyl-alanine. This is of especial interest since threonine, proline, and lysine are among the rarest amino-acids in the insulin submolecule.

Enzymic Studies

Butler *et al.* (1948, 1949, 1950) have employed crystalline trypsin, chymotrypsin, and pepsin on crystalline insulin. Trypsin was found to have only a very slight action, if any, on the protein. This is in accord with the very low lysine and arginine content of the hormone (two residues of each per submolecule); these amino-acids must be present in synthetic peptides before trypsin can exercise its hydrolytic action on them (Bergmann and Fruton, 1937; and later).

Chymotrypsin and pepsin both readily degrade insulin to peptides of unequal sizes. In both cases a large fragment of weight 4,000–5,000 is produced from each submolecule, and the rest appears as smaller trichloroacetic-acid-soluble peptides of average size tetra- or hexa-peptide, though there is evidence that one of these soluble peptides derived from the chymotryptic fission of insulin is as large as 2,000 in molecular weight.

FIG. 1.—Suggested structure of haemoglobin. (a) Basal section showing folded polypeptide chain. (b) Vertical section showing packing. Taken from Perutz, *Proc. roy. Soc., A*, 1949, 195, 474 (Fig. 23).

FIG. 2.—Showing the six cystine disulphide bridges joining the four chains of the insulin submolecule.

The large precipitable fragments have been called the "core" of the insulin submolecule, since they have three or four of the six cystine disulphide bridges and, compared with insulin, are relatively resistant to digestion. The free amino-groups of the core formed by chymotryptic action (containing parts of the four original peptide chains of insulin) are those of glycine, valine, and leucine (insulin has glycine and phenylalanine).

Physical measurements on the insulin core (electrophoretic diagram by Dr. J. M. Creeth and diffusion and sedimentation by Dr. K. Pedersen at Upsala) have shown that it is in all probability a single substance of molecular weight about 5,000, whereas the core formed by the action of pepsin is inhomogeneous, the smaller material having a molecular weight of about 4,000. An intensive study of the composition of these fractions produced by enzymic action is being undertaken at the Courtauld Institute.

Many of these structural details from the work described above can be shown together in diagrammatic form. In the diagram below, amino-acids are indicated by abbreviations, and one submolecule of insulin (weight 12,000) is illustrated (Fig. 3). It has been assumed that the two

FIG. 3.—Key to abbreviations: φAl = phenylalanine; V = valine; Asp = aspartic acid; Glut = glutamic acid; Gly = glycine; IL = Isoleucine; Ty = Tyrosine; A = aromatic amino-acid residue (phenylalanine or tyrosine); -SS- = cystine disulphide linkage.

terminal acids, two histidine residues, four tyrosine residues, and 0 or 1 phenylalanine residue per weight of 5,000, a weight corresponding to about 45 residues. The true positions of the cystine disulphide bridges (-SS-) are unknown. The tyrosine and phenylalanine residues other than those designated by Ty or φAl are given as A, and these have been distributed throughout the extra-core part to conform with the fission of the molecule at these points into small peptides of average size about penta-peptides. It is not certain from the work of Lens (1948) that alanine (Al) lies at the carboxyl end of every peptide chain, as shown here.

As all this information is far in excess of that available for any other protein it is too early to say how representative of proteins in general it is, but there is no reason to suppose that it is not so.

Biological Activity of Insulin

The source of the activity of insulin remains unknown. Chemical studies have provided the most useful information on this point. A paper by Fraenkel-Conrat and Fraenkel-Conrat (1950), re-examining much previous work, concludes that the cystine disulphide bridges, the carboxyl groups, and about half of the tyrosine phenolic hydroxyl groups are essential for the activity. The aliphatic hydroxyl,

the imidazole, amide, and guanidyl groups appear to be dispensable.

No fragment of insulin provided by enzymic degradation with crystalline pepsin or chymotrypsin or by crude pancreatin or papain has shown any activity, whereas crystalline trypsin does not attack the protein and the activity is retained.

It is not possible to say definitely, however, whether the activity resides in a part or in the integrity of the whole molecule, for the enzymic degradations are not random processes and they may all involve fission within an active grouping (if such exists). In the enzyme inactivation quoted above, the loss of activity is approximately proportional to the loss of intact protein.

Other Protein Hormones

It must be clearly recognized that the account given of the structure of insulin is only the very barest outline of the vast amount of work in progress on this one protein. A description has been given in the hope that the magnitude of the task of determining the constitution of a single protein may be appreciated. Not only has the number of chains, etc., to be determined, and the number of amino-acids in each chain, but also the order in which they are arranged.

A number of other protein hormones await investigation. All the hormones of the anterior lobe of the pituitary are of a protein character. Many of them have been isolated as chemically pure proteins, but up to the present little or no work has been done on their configuration. The investigator is severely handicapped by the scarcity and great expense of pituitary hormones, and it may well be a considerable time before our knowledge of such bodies as the growth hormone, lactation hormone, and the gonadotropic factors can be brought up to the same standard as that of insulin. Should the importance of these hormones require any stressing, it would only be necessary to refer to the overwhelming clinical interest in the adrenocorticotrophic hormone at the present moment. Until comparatively recently this hormone was known only to experimental biologists, endocrinologists, and biochemists, but to-day, in its abbreviated form of A.C.T.H., its name is on every clinician's tongue.

Recent work at the Mayo Clinic has shown that the injection of this hormone into patients with rheumatoid arthritis will produce an astonishing remission of symptoms. From the protein chemist's point of view one of the most interesting developments has arisen through the work of Li (1949), who has shown that it is possible to produce by peptic hydrolysis a low-molecular-weight fragment possessing all the activity of the intact protein. It would appear that this low-molecular-weight substance is a peptide containing 6, 7, or 8 amino-acids.

The work of Morris and his collaborators (Cortis-Jones, Crooke, Henly, Morris, and Morris, 1950) has indicated that this low-molecular-weight substance may be present in active preparations of the whole proteins even without prior hydrolysis. Morris and his collaborators have shown that it is possible to separate the clinically active low-molecular-weight fraction from inactive A.C.T.H. protein by ultrafiltration. It is obvious that if the constitution of the peptide can be determined its synthesis will go a long way towards solving the problems of the "A.C.T.H.-cortisone" situation.

During our work on insulin in the Courtauld Institute we have always hoped to find an active fraction, and the

search for such a substance still goes on despite the fact that we have so far had no success.

It will not be necessary to point out that the synthesis of proteins is at present quite out of the question. Great advances have been made in the synthesis of polypeptides, and there now exist simple and economical methods of making these substances in the laboratory. This has been reviewed recently (Dodds, 1950).

Proteins of Toxic Agents

That, in certain circumstances, proteins can act as highly toxic substances has been known for a very long time. The death produced by anaphylaxis is a well-known phenomenon and is associated with the injection of proteins. Vaughan and his co-workers (1913) showed that it was possible, by treating harmless proteins with dilute alkalis, to produce highly toxic substances capable of causing an immediate and sometimes fatal fall of blood pressure when injected into animals.

This was regarded as an entirely academic subject, and received very little attention until Sir Edward Mellanby (1946) produced his startling work on canine hysteria. Briefly, Mellanby was able to show that hysteria can be produced in dogs whose diet consists of some 60% of bread made from flour which has been subjected to a process known to the milling industry as "improvement." It has been known for over a century that flour, when stored for long periods, would give a loaf of a lighter character and better texture than one made from flour freshly milled. The storage of flour is an uneconomical procedure, and cereal chemists therefore studied the nature of this ageing problem and were able to develop methods of treating flour in order to age it artificially. Most improvers are oxidizing agents, but one of the most popular and convenient is gaseous nitrogen trichloride, used in the "agene process." Not only is the flour "improved" by this treatment, but it is simultaneously bleached. It is certainly safe to say that over 90% of the flour of Great Britain and the United States has been subjected to this treatment. Despite the fact that there has been official uneasiness about improvers ever since their introduction, it is again safe to say that any immediate danger from the treatment never entered anybody's head, official or otherwise.

To return to the experiments of Mellanby, he was able to prove conclusively that only those dogs fed on bread made from agene-treated flour suffered from hysteria. The control dogs fed on identical flour, but untreated, remained perfectly healthy and free from symptoms. This publication by Mellanby, and the showing of his film on hysteria, caused a profound sensation in nutritional circles, and also among millers and legislators.

Moran and his colleagues (Bentley *et al.*, 1950) at the Research Association of the British Flour Millers were able to prove that the agene process attacked the protein of the flour, and that powerful toxic hysteria-producing substances could be formed by the treatment of gluten with agene. Further, this toxicity could be induced not only in flour but also in such inert proteins as casein and zein; and in a brilliant series of papers from Moran's laboratory the whole nature of the process has been elucidated. Thus the toxic fraction has been concentrated and finally obtained in crystalline form, and later its constitution and synthesis have been worked out.

As the result of this work an entirely new approach to the activity of the central nervous system has been provided, and from the academic point of view a detailed study of compounds in the series of those produced in the laboratories at St. Albans should prove of very great interest.

It can thus be seen that proteins used for food can be turned into highly toxic substances, at least for animals, by processes which have been regarded for decades as quite harmless. We are not concerned here with the clinical implications of this work, but it is perhaps reassuring to be able to say that up to the present, even with enormous intakes of highly agenzized flour, no toxic symptoms have been detected in the human subject.

Bacteriological Toxins

The exotoxin produced by Type A *Clostridium botulinum* forms an example of the group of bacterial toxins of very high lethal power. Like diphtheria and tetanus toxins, botulinus toxin has now been isolated and crystallized (Lamanna, McElroy, and Eklund, 1946; Abrams, Kegeles, and Hottle, 1946). Physico-chemical characterization by electrophoresis, sedimentation, diffusion, and viscosity measurements (Putnam, Lamanna, and Sharp, 1946, 1948; Kegeles, 1946; Abrams, Kegeles, and Hottle, 1946) has shown it to be a single homogeneous heat-labile protein with the solubility properties of a globulin. Serological data also show that the toxin acts as a single substance (Lamanna and Doak, 1948).

All the three toxins referred to have somewhat spectacular potencies, but botulinus toxin is the most potent by a considerable margin, and is indeed the most poisonous substance known. Some comparative data are as follows:

Toxicities of Purified Bacterial Toxins (in Terms of Minimum Lethal Dose for 20-g. Mouse; data from Pappenheimer, 1947)

Diphtheria	..	..	..	175 MLD per mg. toxin
Tetanus	..	..	..	10×10^6 MLD per mg. toxin
Botulinus	..	..	..	30×10^6 MLD per mg. toxin

These high potencies are of great biochemical interest when the possible modes of action of the toxins are considered. In this respect botulinus toxin is outstanding when its toxicity is examined on a molecular basis, as its molecular weight is about 1,000,000 (Putnam, Lamanna, and Sharp, 1946; Kegeles, 1946), compared with values of approximately 70,000 for the other toxins. Accordingly, it may be calculated that the lethal dose for a mouse contains only 20,000,000 molecules: toxæmia may thus be produced at a dosage level of one molecule per cell, and even at this concentration only a relatively small proportion of the tissue can actually be affected.

It is now established that the toxicity of the pure crystalline toxin is 220×10^6 LD₅₀ per mg. nitrogen for 20-g. mice, or approximately 30×10^6 LD₅₀ per mg. toxin. Calculated on a weight basis, it follows that the LD₅₀ for a 75-kg. man would be 1.2×10^{-7} g., or only 0.12 µg. Alternatively, it has been computed (Pappenheimer, 1947) that 1 mg. of the toxin would be sufficient to kill more than 1,000 tons of guinea-pig. Considering the figure for a man again, if one takes the proportion of water in the body as 85% one may calculate that a toxin concentration of approximately 2×10^{-12} g./ml. of total body water would be lethal in 50% of cases. This figure is even more impressive when the high molecular weight of the toxin (1×10^6) is taken into account; thus the lethal concentration of toxin within the body is only 2,000 molecules per ml. of total water.

First culture of the organism gives solutions of approximately 1×10^6 LD₅₀ (mouse) per ml. Of such a solution (which might be compared with spoiled food) only 0.04 ml. would be required for a fatal dose for a man. Botulinus toxin, moreover, is unique among the bacterial toxins in that it is active by mouth.

It is difficult to visualize any direct mechanism whereby such a small number of molecules can cause death, and

it has been suggested (Pappenheimer, 1947) that the toxin acts not by inhibiting an enzyme action, nor by acting as an enzyme itself, but rather by blocking the synthesis of an essential enzyme. Other workers (Putnam, Lamanna, and Sharp, 1948) have speculated on the enzymatic synthesis of a cellular poison from some normal metabolite. Characteristic of its action, as also of diphtheria and tetanus toxin, is the latent period before the onset of symptoms, the time being roughly inversely proportional to the logarithm of the dose. The suggestion (Guyton and MacDonald, 1947) that this period is associated with the slow diffusion to be expected of a large molecule may be discounted, as Burgen, Dickens, and Zatman (1948) have shown that the latent period and duration of paralysis are prolonged by lowering the temperature to an extent much greater than would be accounted for on a purely physical basis. Death generally occurs within two or three days of the onset of symptoms and is due to respiratory paralysis.

The pharmacology of the toxin has been investigated by many workers (for example, Edmunds and Long, 1923; Dickson and Shevsky, 1923; Bishop and Bronfenbrenner, 1936; Guyton and MacDonald, 1947), and it has been established that the site of action is at the neuromuscular junction. In an investigation of the various factors involved in the paralysis, Burgen, Dickens, and Zatman (1948) utilized the isolated rat phrenic nerve diaphragm preparation of Bülbirg (1946). It was shown that conduction in the nerve was unaffected by the paralysis, and that the block differs from that produced by curare in that motor nerve tetani are maintained. Diaphragms from rats actively immunized with toxoid were not affected by the toxin.

The oral toxicity of the substance has often been correlated with the reported resistance to proteolytic enzymes (Pappenheimer, 1947, 1948; Putnam, Lamanna, and Sharp, 1948), though the problem is then raised of how such a large molecule could be absorbed through the wall of the gut. Such resistance, however, is not well established, as so much of the work on which this conclusion was based was done on very impure toxin preparations.

The preparation of the pure crystalline toxin by the American workers has raised the possibility of new approaches to the problem of the mode of action, and investigations have been started both in this country and in the U.S.A. Already the toxin has been characterized by a complete elemental and amino-acid analysis (Buehler, Schantz, and Lamanna, 1947), but this gave no clue to its action, no abnormal amino-acid nor characteristic toxic grouping being revealed.

In this country, work has been designed to establish whether the toxin is, in fact, resistant to proteolytic enzymes and, if not, whether a toxic fragment may be split off the main molecule by such treatment. The experiments of Li and of Morris and his colleagues, already referred to, would lend a certain amount of support to the possibility that some active fraction may be split off the molecule.

The investigation is complicated by the fact that the initial culture of the organism results in a very low concentration of the toxin and the subsequent working-up tends to be rather long and involves too many precipitation steps, to which the toxin is peculiarly sensitive. Investigations on the possible utility of the modern methods of separation by electrophoresis and of concentration by ultracentrifugation are at present in hand.

Virus Proteins

The bacterial toxins mentioned in the previous section are, relatively speaking, straightforward protein poisons,

though we have really no knowledge of their mode of action. An entirely different type of toxic protein has been discovered in the plant virus diseases. Largely due to the work of W. M. Stanley (1940, 1943), the causative agents of certain plant diseases, such as tobacco plant disease, bushy stunt disease, and similar conditions, have been found to be of a protein character. Stanley and his colleagues have actually prepared crystalline specimens of a number of these substances. If a plant is injected with a small quantity of these crystals, the disease will be reproduced in the plant, and analysis of the infected plant will at a later date show a great increase in this crystalline material, and we have the paradoxical phenomenon of an infective agent in crystalline form apparently reproducing itself.

At present there is no adequate explanation for this, but it would certainly be a most reasonable assumption that the crystalline protein alters the synthetic process of the cells of the plant so that they continue to produce more of this crystalline protein, and that this in turn produces the outward and visible signs of the disease.

These crystalline protein viruses have been analysed for their amino-acid content, and work is at present being conducted on a number of them in the same manner as described for insulin. It has been shown that by passage through various plants the virulence of these crystalline substances can be varied and that the variation is accompanied by a change in the chemical constitution of the protein. We do not know as yet whether the viruses of human and animal diseases such as rabies and yellow fever and so forth belong to the same family as the plant viruses, but we do know that it is possible to alter their virulence and activity by passage. An immediate classical example is the work of Pasteur on rabies and the work on yellow-fever virus. This modification of the protein structure of plant viruses leads to the question of immunity.

It is now well known that the immune bodies circulating in the blood and body fluids consist of modified globulins. The constitution of these globulins is at present unknown, and only intensive work like that on insulin will solve the differences between the immune body globulins and the normal serum globulins. When one thinks of the complexity of the insulin molecule, and when one bears in mind the fact that proteins are continuously being destroyed and built up again in the body, it becomes a matter of wonderment and astonishment that the injection of an antigen on two or three occasions will produce immunity in the animal body, lasting in some cases for a period of years and in others apparently for a lifetime, as in the case of yellow fever. What is the mechanism that teaches the cells to alter the constitution of the globulins synthesized by them so that immune bodies will be produced and, moreover, continue to be produced for years? Again, it is possible only to speculate on this, and we can only adopt the hypothesis supported by Burnet and Fenner (1949), and known as the self-marker theory, that the antigen remains for a very long period in the body cells capable of producing antibodies, and that the antigen acts on the cells so that they synthesize the immune globulins.

Conclusion

I trust that I have succeeded in indicating that this vast problem must be of immediate importance to anyone concerned with the investigation or treatment of disease. When one contrasts the great complexity of the protein molecule with the fact that millions of these substances are continuously being built up and disintegrated in the human

body, and moreover rebuilt to precisely the same structure, one cannot help but speculate about the controlling mechanism. It is rather a terrifying thought that the whole of the protein in the human body is replaced in roughly 160 days, and at the present time we can only speculate on the mechanism controlling this elaborate resynthesis, where even a single amino-acid must not be out of place if the hormone is to have its activity or the antibody its potency.

It might well be that, when the mechanism of perhaps the most complex problem in human biochemistry is understood, we shall find the answer to many problems, possibly even the mechanisms of senescence and of cancer.

I should like to express my thanks to the President and Censors of the Royal College of Physicians for the honour of giving the Bertram Louis Abrahams Lecture, and my deep appreciation of the help given to this work and the preparation of the lecture by Professor A. C. Chibnall, F.R.S. It is a pleasure also to record the great assistance given me by Dr. D. N. Baron and by my colleagues in the Courtauld Institute who work on the proteins—Dr. J. M. Creeth, Dr. D. M. P. Phillips, Mr. P. A. Charlwood, and Dr. Joan Stephen.

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REGULATION OF SECRETION OF CORTICAL HORMONES*

BY

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There are several reasons why information about the control of the rate of secretion from an endocrine gland may be of interest. One is theoretical: an understanding of the factors regulating glandular activity gives us an insight into the part the gland plays in keeping up the *milieu intérieur*. The others are practical. First, in substitution therapy, such knowledge enables us to adapt the quantity of administered hormone to the requirements of the patient, as these vary with the circumstances. Secondly, in trying, for therapeutic reasons, to enhance or inhibit the action of a normal endocrine gland we have to know the conditions which will produce the desired effects on the gland.

Before discussing the information at present available about the control of adrenocortical activity, let me remind you of two fundamental facts concerning the physiology of the adrenal cortex. First, the gland, though secreting continuously, contains practically no stores of the biologically active hormones (Vogt, 1943), and, if synthesis were stopped while secretion were allowed to proceed at a normal speed, would lose its total hormone content in about 6–12 seconds. This means that synthesis is proceeding without interruption in the living condition, and explains the gland's large blood flow and high oxygen consumption. Secondly, the adrenal cortex has no secretory nerves, so that any nervous control over its activity would have to be exerted indirectly.

Experimental Methods

The knowledge we have of the control of adrenocortical secretion is almost entirely derived from animal experiments. A few introductory words on methods will be necessary. All available methods have their uses and their limitations, and the best picture of the gland's performance will be obtained by combining the information each of those methods can provide.

The oldest method is that which determines the experimental conditions which lead to hypertrophy of the cortex. It is suited to the investigation of all stimuli which can be applied over prolonged periods.

For stimuli of short duration suitable methods are the histological or chemical (Sayers *et al.*, 1945) determination of adrenal lipids, cholesterol, or ascorbic acid. Any acceleration of cortical secretion is reflected by an initial depletion in adrenal lipid or ascorbic acid usually followed by a restoration of the stores. This restoration may occur even if the accelerated secretion is maintained, either by prolonged action of the same stimulus or by a new one. Thus depletion is, as a rule, a sign of increased cortical secretion, while full stores of lipid or of ascorbic acid may be found in the later phases of increased activity as well as in the resting condition. Errors in interpretation may therefore arise if these methods are used in the investigation of prolonged or multiple stimuli.

Finally, cortical secretion may be measured directly by assaying adrenal venous blood for its hormone content (Vogt, 1943). The main use of this method is in acute experiments on anaesthetized animals or in perfused glands.

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