JOHN PAUL

Cell Biology

A Current Summary

PREFACE

A number of more or less fortuitous circumstances have led to the production of this speculative essay on protein synthesis.

In the first place the monograph *Production of Antibodies* (Burnet and Fenner, 1949) was due for revision. The theoretical approach adopted in the monograph appears to have provoked considerable interest and to have helped to initiate some fruitful experimental work. It seemed desirable, therefore, to attempt to bring the account up to date.

The second stimulus was our current intense interest in the genetics of influenza virus and the findings by Ada and Perry of the unique importance of ribonucleic acid (RNA) in its structure. Influenza virus and the susceptible cell represents almost the only system in which chemical and genetic aspects of replication can be conveniently studied together.

In the 1949 discussion of antibody production much use was made of the analogy with the formation of adaptive enzymes. The great advances recently made in the understanding of enzyme synthesis in micro-organisms provided a third reason for broadening the scope of any new discussion of antibody production.

Finally, when a first draft had been nearly completed Green's paper appeared in which the self-marker concept of Burnet and Fenner was tentatively applied to the phenomena of carcinogenesis and tumour transplantation. This stimulated a further extension.

If the essay has any virtue, it may be in stimulating workers in one or other of four very different fields to appreciate how developments in all four are converging towards a common point of view.

I am indebted to Dr A. Gottschalk for help in the discussion of enzyme action and to my biological colleagues for reading other parts of the manuscript.

F. M. BURNET

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CHAPTER 1

INTRODUCTION: ENZYME ACTION AND PROTEIN SYNTHESIS

THE ESSENCE OF LIFE is the replication of specific pattern.

We are concerned with an attempt to understand the significance of this, to point out the difficulties of considering, even at a purely theoretical level, the application of the standard physico-chemical approach to biological matters at this level and to try to develop a series of concepts in terms of macromolecular pattern which may make such matters more amenable to an effective scientific approach.

No one could have the slightest hope of producing a lasting achievement from such an attempt. It seems to be of the nature of the relation between the human mind and the events which make up the universe that the approach to control and understanding is a process in which success leads always to the envisaging of more problems than it solves. At every stage in the past and at every stage in the future, the advancing edge of knowledge in every field has been and will be in a state of confusion. There are phases when the emergence of a new technique or, more rarely, of a fertile generalization allows a swift development of a new area in which ignorance and confusion can be replaced by understanding and the possibility of control and utilization for the satisfaction of human desires. But the edge where ignorance lies beyond the zone of *ad hoc* hypothesis and inadequate experimental technique is always there. Speculation and tentative generalization, as well as the search for and development of new technical approaches, are the legitimate weapons to take us further toward the always receding periphery.

We shall be concerned almost wholly with the properties of protein and nucleic acid simply because these are the types

of biological material which (i) seem to be of central importance to the problem, (ii) show evidence of specific function beyond other types of material, and (iii) have been susceptible to a wide variety of experimental approaches at both biological and chemical levels. Possibly the most important reason of all is that in the field of work that has interested me personally, immunology and virology, there are many striking examples of specificity in which the need for some concept of macromolecular pattern seems specially urgent. We cannot for a moment forget about the importance of other components of living organisms beyond functional protein and nucleic acid. Morphology depends on the laying-down, in appropriate fashion, of a host of structural materials —proteins like keratin and collagen, cellulose and chitin amongst the polysaccharides, with many types of inorganic reinforcements, silica or carbonates and phosphates of calcium.

There is, too, a relatively strict control of the inorganic ions, which in animals show their characteristically different distribution in intra- and extra-cellular environments. And even at the more conventionally functional level we have the enormous array of lipids in organisms. In higher animals we find fats that appear to be a relatively simple means for the storage of fuel but, in addition, a wide range of phospholipids clearly of more importance than has yet been ascribed to them, and sterols of many types, some of them hormones of high importance and great subtlety of action. Polysaccharides, mucoids and mucoproteins, such as the lipids, fulfil a wide range of functions in the animal body, as structural and lubricating components, as stores of fuel (glycogen, for instance) and as highly specific patterns conferring serological character on cells and hormonal character on agents such as gonadotrophins. No less than the pattern of an enzyme or an antigen, the distribution and functioning of all these agents

is implicit in the patterns carried by the fertilized ovum. Their biosynthesis under gene control is just as important a series of problems as those with which we shall be concerned.

If a start has to be made, however, it must be with the proteins and nucleic acids. They are the constituents *universally* present in living material. The smallest and simplest viruses have no constituents other than protein and nucleic acid. All enzymes are protein with or without prosthetic groups or coenzymes of other nature. And wherever protein is synthesized in an organism we find nucleic acid present. There are striking functional differences between the two classical types of nucleic acids, those containing deoxyribose (DNA) and those with ribose as the sugar component (RNA). In DNA the purine bases are adenine and guanine and the pyrimidines, thymine and cytosine, in RNA thymine is replaced by uracil. There are hints that perhaps small amounts of other nucleic acids may exist, derived largely from the unusual composition of the DNA of bacteriophage T_2 where the cytosine is replaced by 5-hydroxymethylcytosine. It is, however, still orthodox to keep the two types separate and to ascribe to DNA the essential function of carrying the genetic features of all higher organisms, and to look to RNA for some function intimately related to the synthesis of protein.

The characteristic patterns with which we are concerned in experimental biology are those which confer specificity on functional proteins, enzymes, hormones, antibodies and antigens. The central feature of this or any other discussion of macromolecular pattern must inevitably be the nature of the specificity of such proteins and the ways by which the patterns concerned are synthesized or replicated.

It may be that, in the nucleus and in the course of replication of bacterial viruses, protein synthesis is directly controlled by DNA. Elsewhere it seems highly probable that RNA is in some way the controlling agent that confers

specificity on the protein being synthesized in the cell. Any discussion of the material basis of life—the means by which replication of pattern is possible—must today be centred on the behaviour of proteins and ribonucleic acids.

Many biologists would probably accept the optimistic point of view that the further understanding of biological processes, including those which we include as dependent on specific pattern, is merely a matter for the continued application of the currently successful methods of physical and of chemical study. It is obviously necessary at the present time to use cruder concepts such as those of immunology or genetics, but eventually these should be expressible in physico-chemical terms. Anyone who claims that standard methods have nearly reached the limit of their effectiveness must first attempt to indicate clearly those aspects of living chemistry which are not accessible now, or will not eventually be accessible to the standard methods of chemical study. Biochemists can point to a continuing series of successes in the isolation, analysis and often the synthesis of substances of biological significance. The synthesis of the polypeptide hormone oxytocin by du Vigneaud and colleagues (1953) is the latest major achievement, one which might well be regarded as a prelude to eventual success in defining the structure of functional proteins. Oxytocin, however, contains only eight amino acid residues and its synthesis presented an extremely difficult problem to the chemists. The smallest 'standard' proteins with a molecular weight around 17,000 contain about 150 amino acid residues. Some exceptional proteins such as insulin are smaller, with a molecular weight about 6000; the great majority are, however, larger and proportionally complex. Chemical methods are, from their nature, only applicable to pure compounds, i.e. to molecular species which can be collected into a large uniform population. In the case of any large biological molecule, the various

fragments into which it can be split must also be sorted out into pure substances and characterized. Then follows the effort to reassemble the parts, to synthesize material of the same structure and with the functional activity of the original. Without ever being able to state the precise point at which technique must break down, we can yet be quite certain that no conceivable development of organic chemistry will provide us with the detailed structure of trypsin or of the particular nucleic acid that can transfer a new antigenic quality from one pneumococcus to another.

There is another feature of the work on oxytocin that calls for comment. The synthetic material, like the natural hormone, provokes contraction of the smooth muscle of the uterus, but there is nothing as yet to indicate what part of its chemical structure is primarily responsible for that action. Even more remote is any knowledge of how the target substance, whatever it may be, of the smooth muscle is related to the oxytocin structure.

Perhaps it may underline our ignorance to recall that the most poisonous protein known, botulinus toxin, appears to be a simple protein with no other components than the amino acids common to all our protein foodstuffs. Someone once pointed out that botulinus toxin contained all the amino acids necessary for the growth of the young rat! Not the slightest clue has been published as to any correlation of its chemical structure with its toxicity. The suggestion by Payling Wright (1955) that botulinum toxin may act as an enzyme perhaps on choline-acetylase at cholinergic end organs is based only on pharmacological evidence.

1. *Enzyme specificity*

If proteins were 'chosen' as the material basis of living matter for one reason more than another it may well have been for their potential versatility as specific catalysts. Next

to the formulation of the process of replication, an adequate generalization of the basis of enzyme action is the greatest prize for the academic biologist of the future.

There are various grades of specificity in enzymes even when we confine ourselves to enzymes catalysing well-defined reactions involving relatively small molecules as substrates. Some, notably lipases, appear to be specific only in regard to the type of linkage that is split. Others, of which β-glucosidases may be taken as examples, act only if the bond to be broken and the chemical pattern on one side of the bond are of a certain nature but are indifferent to the nature of the rest of the molecule. Still others appear to be specific for one substrate alone.

An enzyme is a functional concept and it may be that different enzymes have very little that is common at the level of chemical structure. Although it is easy enough to handle the extracellular enzymes of the digestive tract under biologically normal conditions, it is virtually impossible to provide an environment in which to test the function of intracellular enzymes that has any resemblance to the natural intracellular milieu. It is always found that there is a certain optimal range of pH for the activity of a given enzyme and very often there are other ionic requirements as well, a certain level of Ca^{++} ions, for instance.

At a more complex level it may be found that substances other than proteins are needed to allow the activity (or full activity) of enzyme on substrate. Sometimes diffusible substances of relatively small molecular size must be associated with protein to allow the system to function as an enzyme. The otherwise inert protein is then referred to as an apoenzyme, the diffusible component of the system as a coenzyme. There are other enzymically active complexes in which a non-protein prosthetic group is rather loosely combined with protein so that, by appropriate manipulations, it

can be removed or manipulated chemically without destruction of the protein position of the complex (apo-enzyme). Finally, there are many enzymes, probably a majority, which are simple proteins built up solely of amino acid units.

An enzyme is conventionally named according to the nature of the substrate and of the chemical change wrought on the substrate. But the 'biological' character of enzyme action becomes apparent when an attempt is made to say whether two enzymes from different organisms are the same or not. By testing them on the same substrate and following the course of purification by their activity on that substrate as a specific criterion, one may reach eventually two electrophoretically homogeneous protein solutions. These act on the same substrate and may, therefore, be called isodynamic. Detailed study, however, will almost always show (i) that the activity per microgram of one is greater than the other, (ii) that, if a range of different substrates is available for comparative study of the two enzymes, quantitative or perhaps absolute differences in the susceptibility of one or more of these substrates will be found.

It will, therefore, usually be impossible to define an enzyme in terms of its complete range of catalytic activity—in most instances it will be impractical to test more than a small fraction of the possible substrates. In practice, enzymes are recognized to be present as a result of tests on selected substrates that can be conveniently studied. The fact that enzymes of different provenance differ in the details of their action is simply something to be accepted as of the nature of things. Equally, we may soon have to recognize that many preparations conventionally regarded as of a single enzyme can be shown by refined methods to contain a mixture of related but not identical enzymes. These anomalies are based presumably on the fact that functionally similar proteins need not be built up of the same sequences of amino acids.

Insulins from different mammals are physically and physiologically similar, but their amino acid sequences differ. So every enzyme is, in whole or in part, a protein molecule and its access to substrate and its activity are probably both influenced by the nature of the groups adjacent to the enzymically effective groupings.

The mechanism of enzyme action may well differ in different cases, but there seems to be a sufficient concordance of opinion to allow some general statements. The most important is that enzyme action is initiated by union between enzyme and substrate. In a few instances it has been possible to provide a direct demonstration of such intermediate compounds. Since the enzyme molecule is in general very much larger than the substrate molecule, the combining groups of the enzyme represent only a small active patch on the surface of the molecule. Following Gottschalk (1953), we may ascribe to the co-enzyme, prosthetic group or active patch the function of providing the active grouping responsible for the attack on the susceptible group of the substrate molecule. For the rest of the molecule, the apo-enzyme, we can deduce several functions concerned with attachment to substrate and activation of the substrate. In general, we must assume a three-dimensional orientation of the substrate-attracting groups on the enzyme surface to allow a complementary 'fit' with substrate that is close enough to effect chemical union or allow electron or proton transfer to take place. In this fitting of complementary patterns, van der Waals force, hydrogen-bond force, dipolar and ionic forces may all play their part. Pauling (1948) has suggested that substrate is 'chemisorbed' to enzyme in such a way as to assume a strained configuration resembling the activated complex for the catalysed reaction. This conception of a complementary relationship which, however, is not a perfect fit and produces a potentially unstable complex, is one which seems to play

a particularly important role in the understanding of the interactions of biologically significant macromolecules.

In a slightly paraphrased form we may quote Gottschalk's (1958) conclusions in regard to enzyme action as follows. The ability of proteins to form surface profiles with specific patterns allows them to select as specific substrates compounds complementary to the patterns concerned. Charged, polar and hydrogen-bonding groups in the substrate-combining area of the enzyme attract and bind the substrate in a multi-point contact. By this contact between complementary but not completely juxtaposed groups, the enzyme protein with its rigid structure distorts the substrate to a configuration approaching that of the activated state. The whole process is directed towards lowering the energy of activation for the catalysed reaction as compared with the spontaneous one.

It will be the theme of this discussion that enzymes must be taken as the prototypes of functionally specific protein. This has been explicitly or tacitly recognized by many biochemists, as is shown by the great current activity both in classical enzymology and in the study of adaptive enzymes. It is specially significant that much of the work concerned in determining the conditions for protein synthesis use the activity of an adaptive enzyme as the index of synthesis of specific protein.

The production of a wide variety of specifically patterned proteins is a normal function of every living cell. It is characteristic, however, of any biological function that it can only be analysed and understood when ways become available by which it can be experimentally modified in response to a defined stimulus. When we are concerned with specific pattern of functional protein, the approach to understanding will require that by some manipulation we should induce a cell, a tissue or an organism to produce a recognizably new type of protein. In one sense this can be done by supplying glycine

or some other amino acid labelled with a radio isotype which will subsequently identify the protein in which it has been incorporated. This approach has many potentialities for the future, but the most relevant current approach is the study of the conditions under which functionally abnormal protein is produced. Three originally quite distinct disciplines have in recent years converged to contribute each in its way to this approach to the problem of protein biosynthesis. These are concerned with (i) adaptive enzyme production in micro-organisms under the stimulus of the appropriate substrate or inducer; (ii) the production of antibody in warm-blooded vertebrates in response to the appropriate administration of antigen; (iii) the production of new virus protein from the cell infected (stimulated) by pre-existent virus.

The constitution and character of proteins, the universality of enzymes and the basic biochemical resemblance of all living organisms demand that a general mechanism of protein synthesis must exist and that an adequate specification must be able to cover the phenomena of all three fields.

2. *Adaptive enzymes in micro-organisms*

One of the most elementary applications of enzymology is the provisional identification of coliform bacilli by their ability to ferment lactose. This is due to the action of an enzyme β-galactosidase which has become the classical instance of an adaptive enzyme. If a culture of *Escherichia coli* is grown in the absence of lactose or any other β-galactoside and then transferred to a lactose-containing medium, fermentation will commence only after a lag period during which β-galactosidase is accumulating in the cells. If, however, it has been grown in the presence of lactose, the cells contain a high complement of the enzyme and lactose fermentation commences immediately after the cells are brought into contact with the sugar.

E. coli strains which possess β-galactosidase as a constitutive enzyme are not found in nature, but have been produced by rapid alternate cultivation in media containing glucose and lactose respectively as sole carbon source (Cohen-Bazire and Jolit, 1953). Under these conditions a constitutive enzyme will clearly provide a survival advantage to a mutant in which it appears. Monod and Cohn (1952) find that the constitutive enzyme is biochemically and immunologically identical with that induced in the standard strain. It is of interest that this holds also for the β-galactosidases produced by *Shigella sonnei* and *Aerobacter aerogenes*. The similar enzymes produced by species of *Lactobacillus* and *Saccharomyces* are, however, quite distinct immunologically from the β-galactosidase of *E. coli* and from each other. Manson, Pollock and Tridgell (1954) have recently reported similar findings for penicillinase. Pollock's standard strain of *Bacillus cereus* produces a small but measurable amount of penicillinase in the absence of the specific inducer. This is functionally and immunologically identical with the induced enzyme. The same identity of constitutive and adaptive penicillinase was shown for a *B. subtilis* strain. Enzymes from *cereus* and *subtilis* were, however, quite distinct immunologically and showed significant functional differences. It will be necessary, therefore, to assume that, basically, constitutive and adaptive enzymes are produced by the same processes.

The production of the adaptive enzyme β-galactosidase has been closely followed by Monod, Cohn and collaborators. Cohn and Torriani (1953) showed that, from bacteria in which adaptive enzyme production had been induced, an immunologically identifiable protein Gz could be obtained which was absent in cells grown in the absence of an inducer. The normal cells, however, have a physically similar protein Pz which reacts with anti-Gz serum but differs from Gz in

three significant respects. Pz is not antigenic in the full sense of being able to provoke the formation of antibody when injected in a rabbit, it has no enzymic action, and it is destroyed by trypsin, while Gz is not. Since only those bacteria which have Pz are capable of responding to produce β-galactosidase, there is evidently some significant relationship between Pz and the synthesis of the enzyme Gz. There does not, however, appear to be any evidence for the conversion of Pz to Gz. Gz is produced exclusively under conditions allowing the synthesis of new protein. The usual stimulus to the production of the enzyme is a substrate such as lactose; but Monod, Cohen-Bazire and Cohn (1951) showed that induction need not necessarily be by a substrate. Melibiose, for instance, is a potent inducer, but is not a substrate for the enzyme. Inducers must have an intact galactosidic radical, but their activity as inducers is quite independent of their affinity for the enzyme.

Monod and Cohn's (1952) view is that the specific inducer combines transitorily or otherwise with some cell component and that it is this complex which provokes the synthesis of β-galactosidase. The complex is a short-lived one as there is a rapid disappearance of the lactose-fermenting capacity on transfer of the culture to medium not containing inducer. The evidence indicates that all the conditions needed for protein synthesis must be provided if the production of adaptive enzyme is to occur. Monod *et al.* (1952) find that all necessary amino acids must be present and that there is a linear relation between growth, as measured by total protein synthesis, and induced synthesis of enzyme.

This galactosidase system of *E. coli* is the most extensively studied example of adaptive enzyme formation, but there are many other examples which have been recognized since Karstrom first pointed out the difference between adaptive and constitutive enzymes. Some of these will need to be

mentioned in the discussion. Pollock's (1953) work on the production of penicillinase by *B. cereus* is probably the most interesting. It has still to be discovered, however, whether the almost startlingly effective response of this organism to penicillin has any direct biological significance. It may be that an ability to deal with antibiotics produced by other micro-organisms is needed for the survival of saprophytic bacteria like *B. cereus* in nature. On the other hand, there is also the possibility that penicillin has an essentially accidental action on a mechanism evolved to deal with an unrelated metabolite having some structural features in common with penicillin. The existence of small but definite amounts of similar enzyme in culture fluids from *B. cereus* grown in the complete absence of penicillin points strongly towards the latter conclusion. No indication of the nature of the normal metabolite seems to have been obtained.

In this example only a transient contact with penicillin is needed to produce a persisting capacity to synthesize penicillinase in a penicillin-free medium. Growth of the induced culture in penicillin-free medium gives rise to a linear production of penicillinase despite the logarithmic increase in the total protein with growth of the culture. The inducer, therefore, is apparently not a self-replicating agent nor is any mechanism which it may call into existence capable of replicating itself.

As we have already mentioned, the current concept of protein synthesis suggests a process in which nucleic acids are intimately concerned and the first approach to an analysis of adaptive enzyme formation will be in relation to DNA and then to RNA function.

There is good evidence that DNA has no *immediately* necessary part to play in the formation of adaptive enzymes. The capacity to produce an adaptive enzyme of some specific character in the presence of the appropriate inducer is a