615. *Some Considerations on Autosynthesis in Bacteria.*

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An autosynthetic mechanism which takes into account the mutual influence of protein and nucleic acid is discussed in relation to various experimental findings.

In this paper we shall discuss certain general characteristics of the autosynthetic processes occurring in cells and shall endeavour to relate the conclusions to the experimental observations contained in the immediately preceding and other papers. Each component of the bacterial cell obeys an autocatalytic law when growth takes place. Here we shall concern ourselves only with the three most important types of substance encountered in living systems : proteins, ribose nucleic acid, and deoxyribose nucleic acid. A large amount of experimental evidence, which includes the results given in the preceding paper, suggests that the nucleic acids play an important part in the formation of proteins (see, for instance, Caspersson, *Soc. Exp. Biol. Symposium* No. 1, " Nucleic Acid," p. 127), and the theoretical implications of this interrelation will be examined in detail.

Picture of Autosynthesis.—In the consideration of autosynthesis certain analogies from the non-living world provide a useful basis for the formulation of ideas. The first of these is the analogy with polycondensation reactions, in which an indefinite extension of existing structures occurs by the addition of new monomer units, sometimes in a repeating pattern if these units are of more than one kind.

This kind of idea is an essential of the simplest theories of autosynthesis which seek merely to provide a mechanism for the formation of polymerised materials. These theories, which consider autosynthesis only from the rather restricted point of view of the formation of proteins, owe their origin to the fact that the action of proteolytic enzymes can, under suitable conditions, be reversed. Behrens and Bergmann (see Bergmann, *Adv. Enzymol.*, 1941, **1**, 92) showed, for instance, that benzoylphenylalanylglycylglycylanilide was formed from benzoyl-l-phenylalanylglycine and glycylanilide in the presence of cysteine-papain. The product being insoluble is precipitated and is thereby protected from the degradative action of the enzyme. Peptide synthesis in cells might occur by this sort of mechanism in cases where the product is not precipitated if there were other suitable means of protection. Incorporation into a nucleoprotein complex might provide the required type of stabilisation, and Chantrenne (quoted by Brachet, *Soc. Exp. Biol. Symposium* No. 1, " Nucleic Acid," p. 215) has suggested that in protein synthesis the function of nucleic acid is to provide this.

This simple mechanism suffers from one serious drawback in that it fails to give a picture of the formation of proteins possessing specific properties. The specificity of a protein molecule must involve the arrangement of amino-acids in the peptide chain. [It has been ascribed by Pauling (*Endeavour*, 1948, **7**, 43) to the folding of the chain, and since this is probably governed by the arrangement of amino-acids in the molecule—in particular by the relative positions of polyfunctional amino-acids capable of forming cross-linkages—these two views are equivalent.]

The production of specific molecules by the processes outlined above would involve the controlled action of a large number of proteolytic enzymes, each of which was specific for the peptide bond between two particular amino-acids.

Although these enzymes show a certain degree of specificity this, in general, seems to be limited, which means that in polypeptides synthesised by them the arrangement of amino-acids would be comparatively indiscriminate. The probability of an arrangement which would confer enzymatic properties on the molecule is small, and the number of abortive syntheses giving rise to metabolically useless products should far outweigh fruitful syntheses. It could be argued that the nucleic acids, in virtue of some feature of molecular arrangement, might combine preferentially with the active molecules and stabilise them, leaving the inactive molecules to undergo degradation to simple peptides and amino-acids which could be re-polymerised. It is extremely difficult, however, to reconcile an undisciplined mechanism of this type with the efficiency which is usually characteristic of biological processes.

Although random synthesis may be operative in the chance evolution of new protein patterns, it clearly cannot be responsible for the bulk of protein synthesis in the cell and some other factor must be operative.

It is at this stage that another idea derived from the non-living world must be brought into the discussion. This is the analogy between autosynthesis and crystal growth. In the

formation of crystals, a chaotic assembly of atoms or molecules is converted into an ordered structure. Once nuclei have been formed, the factor which encourages further growth and compels the disordered molecules into an ordered array is the existing pattern of forces on the crystal surface.

Here an ordered structure catalyses its own formation. In autosynthesis, similar factors could govern the duplication of pre-existing structures, the arrangement of the units in a molecule of the polymerised material dictating the crystallisation and formation of replica molecules in its vicinity.

The simplest autosynthetic systems, typified by the viruses, are nucleoproteins, and an entity of this type must provide the model for the further development of this idea.

The hypothesis will now be advanced that autosynthesis depends essentially upon a co-ordination of the following kind : in the synthesis of protein, the nucleic acid, by a process analogous to crystallisation, guides the order in which the various amino-acids are laid down; in the formation of nucleic acid the converse holds, the protein molecule governing the order in which the different nucleotide units are arranged. (This would mean that if the proteolytic enzymes did form part of the autosynthetic mechanism, they would necessarily operate in close association with nucleic acid, and that the processes of synthesis and stabilisation would coincide.)

The hypothesis that the configuration of a nucleic acid can control the arrangement of amino-acids in a protein raises the question of the molecular interpretation of this process. Astbury (*Soc. Exp. Biol. Symposium* No. 1, " Nucleic Acid," p. 66) points out that the spacing between the residues in a polypeptide is approximately equal to that in a nucleic acid. This suggests some sort of correspondence between the units in the two kinds of polymer. In a protein, about 23 different amino-acids may occur, whereas in a nucleic acid only 5 basic units are found—two pyrimidine nucleotides, two purine nucleotides, and ribose phosphate. Clearly there cannot be a one-to-one correspondence between the position of an individual amino-acid in the protein part of a nucleoprotein and the position of an individual nucleotide in the nucleic acid part. If, however, it is assumed that, in the synthesis of a protein at the surface of a nucleic acid polymer, the amino-acid side-chain which is guided into a particular place depends on the nature and relative position of two adjacent nucleotide units, the difficulty can be overcome. Twenty-five different internucleotide arrangements are possible, and this is of the right order to give correspondence with the number of different possibilities in a protein chain.

If the nucleoprotein is accepted as a model of autosynthesis, and the reciprocity between the two components gives rise to their mutual synthesis, the cell must be considered to contain a large number of different autosynthetic nucleoprotein units, the protein parts of which possess specific enzymic properties. In the normal course of events the protein part is formed in excess and, in addition to contributing to the actual autosynthetic process, participates as an enzyme in the metabolism of the cell.

Mathematical Formulation and Experimental Relations.—We may now attempt to formulate mathematically the ideas which have been developed in the previous section, and to examine the extent to which they agree with the experimental findings.

The mutual dependence of protein and nucleic acid in autosynthesis may be expressed in the following equations :

For the synthesis of protein we may write

$$dP/dt = \alpha X \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

(where P signifies protein and X nucleic acid)

and for the synthesis of nucleic acid we can write

$$dX/dt = \beta P \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Interrelated equations of this sort, which typify the most elementary degree of organisation imaginable, have been considered previously by Hinshelwood and Lewis (*Proc. Roy. Soc.*, 1947, *B*, **135**, 321) who showed that they were consistent with a steady state in which both X and P increase autocatalytically. Hence we can also write for the formation of X and P,

$$dP/dt = kP \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

and

$$dX/dt = kX \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (4)$$

(k being the growth constant)

From equations (1), (2), (3), and (4) we obtain

$$X/P = \beta/k = k/\alpha \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

and it can easily be shown that

$$k = (\alpha\beta)^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

These notions can be applied to the behaviour of nucleic acids and proteins in cells. Equation (5) would indicate a complex relation between X/P and k if both α and β showed wide simultaneous variations. In the foregoing paper, however, it has been shown that the ribose nucleic acid content per unit of cell nitrogen is approximately proportional to k for cells grown under a wide variety of conditions. (The total cell nitrogen contains from 5 to 25% of purine-nitrogen according to the rate at which the cells have grown, the remainder being protein-nitrogen. This disturbance is not, however, sufficiently large to mask the general form of the relation between the ribose nucleic acid and the protein of the cells.) The experimental result would receive a simple interpretation, therefore, if X were identified essentially with ribose nucleic acid (R), and if α were taken to be approximately constant over a wide range of conditions, the effect of which on the growth rate would be expressed mainly by changes in β.

Such a state of affairs is by no means unlikely. It would mean that the formation of protein under the directing influence of nucleic acid is an approximately constant function. On the other hand, it would suggest that the influence of the total protein of the cell on the formation of nucleic acid varies rather widely. The enzymic composition of cells is known to be susceptible of great variations, and this probably entails considerable changes in the relation of dX/dt to P, *i.e.*, in β.

In a very general way this result could be expressed by saying that the nucleic acid is the seat of the more stable properties of the cell, while the protein is the seat of those properties which are more liable to modification by the environment.

The Two Kinds of Nucleic Acid.—According to equation (5) the plot of nucleic acid content per unit of cell-nitrogen against growth rate should pass through the origin. There is, however, no reason to suppose that the curve should be more than approximately linear. The diagrams of the preceding paper show in fact a linear relation for ribose nucleic acid (R) and growth rate, and despite the scatter of the points it seems clear that the line is directed towards the origin. It might have been expected that such a relation would hold rather for the total nucleic acid than for the single component, but if the plot is actually made of total nucleic acid there is a finite intercept (of about 0·2 unit) as the growth rate tends to zero. This would appear to suggest that the equations for autosynthesis outlined in the last section apply essentially to the ribose nucleic acid itself, and that the contribution of the deoxyribose nucleic acid (D) to the synthesis of the cell-protein is much smaller in magnitude.

The question of the function of the deoxy-acid now arises. It is present in approximately constant amount per cell, and is therefore clearly an important agent in determining cell division. It is not even approximately a constant fraction of the ribose nucleic acid. The ribose nucleic acid per cell, virtually R/D, is related to k much as R/P is related to it (cf. Fig. 3 of the preceding paper), which suggests another interdependence of the approximate form $dD/dt = \gamma R$.

The possible implication that deoxyribose nucleic acid is directly formed from the ribose nucleic acid is not at first sight a very acceptable one, though perhaps it cannot be entirely rejected. If by some means this transformation occurred, and if the structure of the ribose–deoxyribose system became unstable when a critical amount of the second were built up, some sort of explanation of cell division would be provided.

What seems much more likely, however, is that the deoxyribose acid is formed indirectly from the ribose component by a process of depolymerisation (accompanied by an unknown degree of nucleotide breakdown) and then resynthesis after conversion of ribose into deoxyribose (or replacement of the former by the latter). In this case we might have dD/dt proportional to the concentration, $\varkappa$, of a substance derived from ribose nucleic acid, $\varkappa$ being proportional, or roughly proportional, to the total amount of the ribose nucleic acid in the cell.

Towards the end of the growth cycle when the medium becomes depleted of phosphorus there is in fact an extensive conversion of the ribose nucleic acid into deoxyribose nucleic acid (experiments to be published), as shown by the fact that division (with maintenance of the constant amount of deoxy-acid per cell) continues for a considerable time after phosphorus starvation sets in.

Ribose nucleic acid in certain respects (*e.g.*, in alkaline hydrolysis) is a less stable structure

than deoxyribose nucleic acid. This property may possibly make it a suitable source of certain nucleotides, and possibly even those which after further transformations are the raw material for the synthesis of deoxyribose nucleic acid itself. If this idea were correct a relation similar to that found for the proportionality of dD/dt and R might well be explained. Clearly it is premature to attempt to reach a definite decision on this matter.

The relative stability of the deoxy-component, and its constancy per cell, perhaps reflect its biological function, in so far as it is believed to be the principal seat of the unchanging hereditary characters, in contrast with the more changeable ribose nucleic acid component. This appears in part to be responsible for the bulk of the protein synthesis, and will presumably be the seat of the variable and adaptable characters.

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