The Synthetic Activities of the Cell.*

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UNLIKE the chemist, the animal cell has a very limited choice of raw materials from which synthesis must start. These are the components of the common foodstuffs. When they have undergone the preliminary processes of digestion they provide in all about thirty substances which may be regarded as available for the building up of new compounds by the cell. Given these raw materials, can we in every instance indicate which is the likely starting-point for the synthesis of substances the constitution of which is known or partly known? This question must still be answered in the negative for such well-known products as cholesterol and the unconjugated acids of bile.

With the purine bases, which are components of nuclear material and therefore present in all cells, this question of indicating the probable raw material for their synthesis must also be answered in the negative, but with less emphasis than in the case of cholesterol and the bile acids. The amino acid histidine, which contains a five-membered ring similar to that found in the purine bases, is their most likely precursor on structural grounds. But we have as yet no experimental evidence that indicates clearly their origin from this amino acid in the body. Until this has been proved, it is perhaps useless to speculate as to the chemical processes involved in the transformation.

An example of a synthetic product which bears a fairly close structural relationship to two of the amino acids which are found in proteins is adrenaline. These two amino acids, phenylalanine and tyrosine, have practically the same carbon skeleton as adrenaline. Either of them might give rise to adrenaline by successive oxidation, methylation of the amino group, and loss of carbon dioxide. That a part of the necessary oxidation process can be brought about by means of an oxidising enzyme, tyrosinase, has already been demonstrated.

If, however, we consider whether a process of this kind, operating in the cells of the adrenal gland under specific conditions, gives rise to adrenaline, difficulties appear. When we remember that the oxidation of one substance may only take place if another is reduced; that a reaction taking place in one compound may only be possible when some other reaction takes place alongside it—in other words, that in the living cell there is a continuous and complex interplay of chemical reactions—then it is not surprising that the discovery of the mechanisms by which adrenaline is formed, although a simple problem at first sight, is probably in reality very complicated.

The case of thyroxine is comparable with that of adrenaline. Thyroxine is a relatively simple chemical substance which could conceivably be produced by the oxidation of diiodotyrosine. The last-named compound has recently been shown to be present in the thyroid gland and this makes

the presumption that it is the mother substance of thyroxine all the stronger. Yet it has not been demonstrated either *in vitro* or *in vivo* that thyroxine may be produced from diiodotyrosine.

We may now pass on to consider a process in which the raw material is known with certainty but the chemical reactions by which the synthesis takes place are relatively obscure. I refer to the production of fat from carbohydrate. Lawes and Gilbert, in their classical experiments at Rothamsted on the fattening of farm stock, showed indubitably that animals can produce fat from starch. Since the starch is converted into glucose in the alimentary canal prior to absorption, we may consider glucose as the starting-point of the synthesis.

The most likely chemical explanation of the origin of the fatty acids is that they are built up, two carbon atoms at a time, from some simple reactive substance which is first produced by degradation of glucose. Acetaldehyde and pyruvic acid have both been suggested as probable participants in a reaction of this kind, the former condensing with itself as in the well-known aldol condensation, the latter either with acetaldehyde or with some higher aldehyde containing an even number of carbon atoms produced in the earlier stages of the reaction. By both of these methods it has been shown that *in vitro* aldehydes with an even number of carbon atoms in a straight chain can be built up step by step and these by oxidation can be readily converted into the corresponding fatty acids. Unsaturated linkages in the chain may be produced by either method, so that this requirement in the hypothetical synthetic method is also satisfied. Further, the condensation takes place in weakly alkaline solution or under the catalytic influence of certain organic bases, so that drastic treatment is not necessary to bring out the reaction. So far, this evidence for the mechanism of synthesis of the fatty acids is purely chemical and the grounds on which it can be put forward are largely chemical ones. It is known also that acetaldehyde and pyruvic acid, the intermediates postulated in this hypothesis, can be produced in the body. But there the question rests for the present so far as higher animals are concerned. When we know what the conditions are which set the process of fat synthesis going, and when we are able to reproduce them at will in animals, it may be possible to determine what are the intermediate substances concerned.

More success has been achieved by a study of the formation of fatty acids in micro-organisms. Bacteria which form butyric acid from glucose have been found to produce in addition both lactic acid and acetaldehyde. These same bacteria will also produce butyric acid from pyruvic aldol though not from aldehyde ammonia, aldol, or pyruvic acid itself. Neuberg and Arinstein, who investigated this type of fermentation, conclude that pyruvic aldol is the precursor of the butyric

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acid when it is formed from carbohydrate. But it has also been shown that in this so-called butyric fermentation fatty acids containing an even number of carbon atoms higher in the series than butyric are formed, namely, hexoic and octoic acids. This makes it appear probable that the processes by which these lower members of the series of fatty acids are formed in bacterial fermentation may be the same as those by which higher members of the series are formed in animals.

The intensive study during the last few years of the processes of alcoholic fermentation and the chemical events which occur in muscular contraction has revealed such close similarities that we are becoming forced to accept the view that certain fundamental activities of the living cell, whether animal or vegetable, may be carried out by an almost identical mechanism. It may therefore be that we shall eventually discover the reactions responsible for the synthesis of fat in animals by investigating the processes by which it occurs in vegetable forms, such as bacteria or moulds.

Even if these reactions be the right ones, the problem still remains as to how they are accurately controlled within the cell. Some of the substances concerned in them are diffusible and very reactive, and we should have to explain how diffusion away from the site of reaction is prevented. To overcome difficulties of this kind it is becoming common to invoke the intervention of surface forces. There is, however, not much experimental evidence as yet which helps us to explain by such intervention the mechanism of synthetic processes even of such a simple kind as the reversal of an enzyme action.

Freundlich in his Liversidge Lecture to the Chemical Society last year described experiments on the influence of charcoal in modifying the velocity of certain reactions. These showed that the stability of a substance on a surface may be greater than in homogeneous solution under similar conditions. It must be borne in mind, however, that in these experiments very large quantities of charcoal were used compared with the amounts of the substances the equilibria of which were being studied. So much so that in the reaction in alkaline solution the bromoethylamine was practically completely adsorbed and the reaction was taking place entirely on the charcoal surface. Can one postulate such conditions during the continuous synthesis of substances in the cell, glycogen from glucose, for example? It seems to me that to explain the rapid accumulation of synthetic products such as fat or glycogen which we observe in cells, something more than a shift in the equilibrium of the reactions due to surface forces is necessary. Such a condition favouring synthesis could only operate for a time until the surface became saturated. We must therefore postulate some additional mechanism whereby the synthesised product is removed from the sphere of action, for if it diffused off the surface again it would be subject to the equilibrium conditions which are present in the solution. It may be that the arrangements in the cell are such that

only small amounts of the substrate are dealt with at a time, so that complete synthesis is achieved and the synthetic product removed.

We have also to consider how the synthetic product is protected in the cell from the disruptive agencies which exist there. Arrangements for this purpose must be present, since we know that substances may accumulate in cells which contain enzymes that hydrolyse them. Whatever these arrangements are, they appear in certain instances to be closely associated with the life of the cell, for after death they cease to operate and the synthetic product is again broken down. However difficult it is to form a conception of them, it may be necessary to do so, since they must form a part of any system which is put forward to explain synthesis as a result of the intervention of surface phenomena.

We may now consider two syntheses in which there is little or no doubt about the raw materials or some of the chemical reactions involved. These are the production of glycogen and of proteins.

It has been proved that when glycogen breaks down in the liver it gives rise to glucose. Lohmann and also Barbour have succeeded in obtaining glycerol extracts of liver and muscle which hydrolyse it, but the product appears to be a trisaccharide and not glucose. No enzyme which by itself hydrolyses glycogen to glucose has yet been obtained from animal tissues. It is of interest that pancreatic and salivary amylase produce isomaltose from glycogen. These results suggest that there may be some configurational difference between glycogen and starch which accounts for their difference in behaviour with diastatic enzymes. Be that as it may, it appears natural to assume that the synthesis of glycogen from glucose in the cell is brought about by the simple reversal of a hydrolysis which may be catalysed by enzymes under appropriate conditions. These conditions have, however, not yet been realised *in vitro*. Barbour was unable to demonstrate any synthesis of glycogen from the trisaccharide produced by the muscle enzyme even in highly concentrated solutions.

This failure to obtain evidence of the synthesis of glycogen from the products of its hydrolysis makes it legitimate to consider whether we are right in adopting the orthodox view that the synthesis of glycogen from glucose in the living cell is brought about by a reversal of action of the enzyme or enzymes which hydrolyse it. There are other facts which merit consideration in any discussion of its mode of synthesis in the body. When an animal is fed liberally with glucose or fructose, it converts a part of them into glycogen in the liver. The evidence for this is indubitable. It implies, therefore, either a conversion of fructose into glucose before the condensation to glycogen occurs, or a conversion of both into some common form of hexose which then undergoes the condensation. Further, there is a considerable amount of accredited evidence that many substances not belonging to the sugar group can be converted into glucose in the animal body. Such substances, therefore, must be regarded as potential glycogen formers. Whatever the processes

may be which eventually result in the production of a hexose from these diverse substances, the most remarkable thing about them, to my mind, is that the hexose is always *d*-glucose. We have no satisfactory explanation for this striking stereochemical performance, but the facts suggest that the condensation of the two—or three—carbon units to a hexose is brought about under such specific conditions of strain that only the *d*-glucose configuration can result, much as coins must be minted in a definite mould to become currency.

A consideration of all the data which have been accumulated regarding the synthesis of glycogen makes it probable that more than the mere reversal of enzyme action is concerned. It is certain that the cells in which it occurs must be supplied with oxygen. Fletcher and Hopkins showed with muscle that its structure must be maintained, and with liver the synthesis certainly only takes place in the intact organ. Do not these facts point to the conclusion that it is only the living cell that can bring about this synthesis? And if this be so, cannot we go further and suggest that the substances from which glycogen is produced, or bodies derived from them, must first become bound up with, or at some stage form an integral part of, the living structure before they are converted into glycogen? The evidence at least suggests that some such conception of the process may not be far from the truth.

We may now pass to the consideration of the synthesis of proteins. In the early part of this century, due largely to the elegant methods introduced by Emil Fischer, rapid advances were made in our knowledge of the structure of proteins. These advances led to a picture of protein structure which has become generally accepted, namely, that the protein molecule is formed by the union of amino acids through an amide linkage.

The investigation of the structure of proteins, which are closely allied in origin, composition, and general chemical behaviour by immunological and in part by chemical methods, has taught us how intricate the mechanism must be by which they are built up. The facts brought out by the classical work of Dakin and Dale on the albumin of the duck's and hen's egg serve to exemplify this. The only chemical difference that could be shown between these two proteins was concerned with the disposition in the molecule of some of the leucine, aspartic acid, and histidine. But when used as antigens in the anaphylactic reaction they were markedly specific.

These results indicate that the chemical structure of the molecule is different in proteins which are very similar both in general chemical properties and in biological origin. They suggest that the protein molecule produced by a particular type of cell is always built up in a distinctive and, so far as we can determine, an unvarying pattern. We may deduce from this that although the general method of protein synthesis—that is to say, the mechanism by which amino acids are joined up—may be the same in all cells, yet there must be arrangements in the cell which enable only

one particular, final pattern to result from the synthesis.

What are the methods by which the amino acids are caused to combine? The use of proteolytic enzymes in an attempt to bring about synthesis under conditions which have been partially successful with other substances has often been tried and nearly as often has failed. Two examples of protein synthesis by enzyme action have been described. The most acceptable of these is Taylor's production of a protamine by a glycerol extract of clam liver from the products of its complete hydrolysis. The other is the preparation of so-called plasteins from the products of the partial hydrolysis of certain proteins by pepsin. A comprehensive review of the subject of plastein formation by Wasteneys and Borsook, who have themselves made notable contributions to this problem, has led them to the belief that in this phenomenon we have a true resynthesis of protein from some of the more complex of its hydrolytic products.

Even if this be accepted as a possible explanation, it still leaves many questions regarding protein synthesis unanswered, and not the least difficult of these is the problem of how the separate amino acids are brought together to form the specific proteose substrates which one must postulate as combining—and in a definite order—to produce the particular protein which is characteristic of the cell which synthesises it.

The process by which the substance of the cell is increased, the building up of protoplasm, is one which must be closely allied to protein synthesis, since the material we call protoplasm is constituted for the greater part of amino acids, united, so far as can be ascertained, by the same sort of linkage that we find in proteins. The protoplasm of the dead cell responds to all the tests by which we identify protein, it is subject to the action of hydrolytic agents in the same way, and yields identical products when hydrolysed.

Ought we not therefore to look for some of the mechanisms of protein synthesis in the processes which operate when the living cell grows, and can we by any stretch of imagination account for this by a reversal of the action of one or more hydrolytic enzymes? It appears inconceivably difficult to do so. The extreme specificity of the reaction, which necessitates that at a given phase of the synthesis one particular amino acid and that one alone can be added as the next link in the molecule, requires such a multiplicity of enzymes and such a remarkable degree of control of their action as to be almost outside the range of probability. When we remember, however, that one of the prime attributes of life is that it is a dynamic condition, it does become possible to form a conception of protein synthesis in relation to that fact. The experiments of Willstätter and others have shown that to some extent the specificity of enzymes is accounted for by the 'carrier' with which they are associated. It is not inconceivable that a catalyst capable of bringing about the union of amino acids in the living cell and ultimately fashioning its protoplasm may be attached to or associated with a

'carrier' which, instead of having a fixed configuration, as with the enzymes that we can extract from the dead cell, has one which is continually varying, this dynamic state being characteristic of the living material of the cell.

If, further, we could assume that the variations in the configuration of the carrier were cyclic, always going through a definite series of phases, it might be possible to account for the fact that at any particular phase of the cycle the configuration would be such as to favour the synthetic union of one particular amino acid rather than any other because of its spatial arrangement. The assumption of cyclic changes in simple or complex living organisms is not new in physiology, and it is not unlikely that they occur in parts even of the cell itself.

The possibility that protein synthesis is associated with some part of the cell which is undergoing cyclic changes, and is thus alive, raises the interesting question of the site of this and possibly other syntheses, such as those of fat and glycogen. Are all parts of the cell, that is to say, both nucleus and protoplasm, to be regarded as alive in the sense I have indicated, and therefore to be considered as regions in which syntheses depending on life can be brought about?

It is necessary not to confuse the terms irritability and life. It is true that what we term irritability is usually taken to imply that the tissue which shows it is living; but taking the nerve fibre as an example, it would appear that the maintenance, for a time at least, of the irritability of protoplasm and its restoration when it has disappeared—but not irreversibly—does not require the presence of the nucleus. It may require oxygen or the presence of certain ions, but this may merely mean that the labile state of the protoplasm has been upset by the products of the cell's activity, and removal of these will restore it to its irritable condition.

We have no evidence that irritability as a manifestation of what we call life is more than the possession of extremely labile structures, sensitive to minute environmental changes. The nucleus, on the other hand, is essential to the continuous life of the cell and its growth. It appears also to determine very largely the magnitude of the respiratory processes which occur in it at rest, though not necessarily the excessive respiration observed in the recovery from functional activity. Can we therefore go so far as to say that the nucleus is the seat of those synthetic activities of the cell which appear to depend on its living character rather than on its irritability, or have we to regard the protoplasm as equally living, so that it is able to reproduce itself and in addition bring about such syntheses as those of fat and glycogen?

There is something to be said in favour of the idea that the protoplasm is not living in the sense in which the nucleus is, and therefore is less likely to be the seat of certain synthetic processes. It is, I think, quite a tenable view that protoplasm is made up, largely but not entirely, of combinations of amino acids such as we find in the proteins, and

that it is synthesised by the nucleus to serve special as well as certain general requirements. These special requirements must and do vary greatly with each type of cell.

Consider the mammalian erythroblast. The principal substance in its protoplasm is the protein hæmoglobin. It is doubtful whether this cell exists except to produce hæmoglobin. When it has matured, the nucleus degenerates and disappears, leaving the red blood corpuscle. Along with this the respiratory activity of the cell practically disappears too, and there is not much discoverable in it except hæmoglobin. It is certainly now not living in the sense in which its progenitor, the erythroblast, was. Is it not reasonable to suggest, therefore, that the production of hæmoglobin as the erythroblast grows and matures is a function of the nucleus and not of the protoplasm of this cell? All semblance of further synthesis of hæmoglobin certainly disappears when the nucleus goes. The adoption of such a view does not imply that having produced the protoplasm of a cell the nucleus has nothing more to do with it. We know that in some indefinable way the nucleus in most cells controls the structure of the protoplasm and maintains its lability, but we have no knowledge as yet of the mechanism by which this is brought about.

One property of proteins, which may account in a general way for the presence of protein-like structure in protoplasm, is their buffering power. The nucleus is probably the most labile part of the cell. The chemical reactions proceeding in it may demand an environment that has to be finely controlled as regards changes in reaction or the concentration of certain ions. The protoplasm may thus serve as a protective layer between the nucleus and the external world, guarding it from changes which would otherwise terminate its existence. The known properties of the proteins, both chemical and physical, may be useful to this end, to which they are almost ideally suited.

Even if we can accept as possible the unorthodox view that the nucleus is the only living part of the cell, and is therefore the only part that can bring about syntheses which depend upon life, it does not solve our difficulties in explaining how they are achieved. It merely narrows down the possible sites in the cell in which they occur. The nucleus itself is a complex structure, and we have as yet few experimental methods for elucidating it. Most biologists would, I think, agree that the cell has arisen by a process of evolution from something simpler and eventually from non-living materials. It cannot have come as a 'bolt from the blue'. If we regard the nucleus as the only living part of the cell, then we may justly regard the protoplasm as something that has been acquired or developed in the process of evolution and is now necessary to its existence. We do not know definitely, however, of nuclear material which is living and devoid of its protoplasmic envelope unless such an arrangement is present in the bacteria. But the investigation of filterable viruses has given an indication that material possessing the

prime attribute of life, namely, the power of reproducing itself, exists possibly in simpler forms than we find in the smallest visible organisms.

If we agree that the cell has evolved from something simpler, then we might expect to find such elementary forms of life coexisting with it, did we but know how to look for them. The filterable viruses may represent such forms, and their

chemical characters may resemble those that we find in the nucleus of the cell. The ability to synthesise protein may be a property which living material only acquired at a late stage of its evolution, and that property may be one which in the process of time has come to be essential for the maintenance of the complex structure of the nucleus as we see it to-day.

An Anthropometric Investigation on Twins.

THE latest contribution to the now considerable mass of literature bearing on the resemblances and differences between twins is a valuable memoir by Dr. Percy Stocks ("A Biometric Investigation of Twins and their Brothers and Sisters", *Annals of Eugenics*, vol. 4, April 1930). The tests and measurements were made during 1925-27 on twin children and their brothers and sisters in elementary and central schools of the London County Council, the ages of the children ranging from three to fifteen years. The characters considered in the present memoir—others will be dealt with later—include height; weight; length, breadth, and horizontal circumference of head; interpupillary distance; blood pressure; pulse rate; respiration rate; eye colour; hair colour; facial resemblance on an arbitrary scale of four classes; and finger-prints. The total number of children examined was 832 (392 boys and 440 girls), of whom 563 were members of twin pairs, 7 were surviving members of triplets, a few were odd twins who had a brother or sister at school, and the remainder were either siblings of twins, or pairs of siblings unconnected with twins, the latter being necessary to form a comparative group.

An investigation on such data is obviously not a simple matter. It is desired to compare (1) monozygotic twins, (2) dizygotic twins, (3) other siblings, with regard both to individual measurements (means, standard deviations, etc.) and correlations or differences between pairs. As was first pointed out recently by Dr. R. A. Fisher, the proportions of monozygotic and dizygotic twins amongst pairs of the same sex may, *if a character is normally distributed*, be estimated from the available data and the frequency distribution of differences analysed, but this does not give wholly what is wanted. Separation of monozygotic from dizygotic pairs on the basis of facial resemblance only is by no means so satisfactory as the unpractised observer might suppose: Dr. Stocks concludes (p. 81) that only about two-thirds of like-sexed twins can be at once separated by facial resemblance into two groups labelled 'identical' and 'fraternal', and even for these groups other evidence suggests that the diagnosis is really incorrect in a small proportion. The final conclusion reached is that finger-prints form the best fundamental test, with anthropometric measurements (height and head measurements) to decide doubtful cases, and definite rules are laid down for the purpose.

Assuming this analysis made, there is a further trouble due to the great range of age amongst the

children observed, and differences of sex. Were the data subdivided into age and sex groups, the numbers in each group would be too few for valid comparison. This difficulty is surmounted by converting all measurements to standard age and sex, and only these converted or 'corrected' measurements are used. Further to increase the virtual numbers on which a comparison is based, by using relative deviations different measurements may be pooled together; for example, all the 'static characters', as Dr. Stocks terms them—height, the three head measurements, and interpupillary distance.

Finally, in an investigation of this kind, errors of measurement, and the errors involved in the fact that only single measurements were taken of such fluctuating characteristics as pulse and respiration rates, are of much more importance than they would be in most anthropometric investigations. To obtain information on these points and enable correction to be made, repeated observations were carried out on five individuals for fifty days. How important is this correction for errors and day-to-day variation may be seen from the fact that correlations between siblings are raised by about 90 per cent for pulse rate, 40 per cent for respiration rate, 20 to 30 per cent for blood pressure, though only by much smaller amounts of 0.25 to 2 per cent for the dimensional measures.

Making these corrections, mean correlations for the three groups work out as follows (Table XI., p. 103):

	Non-twin siblings.	Dizygotic twins.	Monozygotic twins.
Height	0.49	0.56	0.95
Head dimensions	0.45	0.45	0.83
Weight	0.38	0.46	0.94
Blood pressure	0.46	0.39	0.81
Pulse and respiration	0.27	0.39	0.86
Aggregate of 10 characters	0.42	0.47	0.85

The results suggest that correlations for non-twin siblings do not differ appreciably from those for dizygotic twins. Hereditary constitution appears to be more important in comparison with environment in regard to height and weight, rather less so in regard to head measurements, still less in regard to blood pressure, and least of all in pulse and respiration rates. Dizygotic twins are, in these data, on the whole inferior to their brothers and sisters in the physical measurements, but this is certainly not the case with monozygotic twins, except perhaps as regards weight.