An Address
ON
THE DYNAMIC SIDE OF BIOCHEMISTRY.

DELIVERED TO THE PHYSIOLOGICAL SECTION OF THE BRITISH ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE.

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[AFTER giving a history of the growth of organic chemistry in relation to physiology, which he traced back to Justus Liebig, Dr. Hopkins continued as follows:]

We know first of all that the raw material of metabolism is so prepared as to secure that it shall be in the form of substances of small molecular weight; that the chief significance of digestion, indeed, lies in the fact that it protects the body from complexes foreign to itself. Abderhalden has ably summarized the evidence for this and has shown us also that, so far as the known constituents of our dietaries are concerned, the body is able to maintain itself when these are supplied to it wholly broken down into simple *Bausteine*, any one of which could be artificially synthesized with the aid of our present knowledge. Dealing especially with the proteins, we have good reason to believe that the individual constituent amino-acids, and not elaborate complexes of these, leave the digestive tract, while Folin, Van Slyke, and Abel have recently supplied us with suggestive evidence for the fact that the individual amino-acids reach the tissues as such and there undergo change. But still more important, when things are viewed from my present standpoint, is the fact that recent work gives clear promise that we shall ultimately be able to follow, on definite chemical lines, the fate in metabolism of each amino-acid individually; to trace each phase in the series of reactions which are concerned in the gradual breakdown and oxidation of its molecule. Apart from the success to which it has already attained, the mere fact that the effort to do this has been made is significant. To those at least who are familiar with the average physiological thought of thirty years ago it will appear significant enough. So long as there were any remains of the instinctive belief that the carbonic acid and urea which leave the body originate from oxidations occurring wholly in the vague complex of protoplasm, or at least that any intermediate products between the complex and the final excreta could only be looked for in the few substances that accumulate in considerable amount in the tissues (for instance, the creatin of muscle), the idea of seriously trying to trace within the body a series of processes which *begin* with such simple substances as tryosin or leucin was as foreign to thought as was any conception that such processes could be of fundamental importance in metabolism. However vaguely held, such beliefs lasted long after there was justification for them; their belated survival was due, it seems to me, to a certain laziness exhibited by physiological thought when it trenched on matters chemical; they disappeared only when those accustomed to think in terms of molecular structure turned their attention to the subject. But it should be clearly understood that the progress made in these matters could only have come through the work and thought of those who combined with chemical knowledge trained instinct and feeling for biological possibilities. Our present knowledge of the fate of amino-acids, as of that of other substances in the body, has only been arrived at by the combination of many ingenious methods of study. It is easy in the animal, as in the laboratory, to determine the end-products of change; but, when the end result is reached in stages, it is by no means easy to determine what are the stages, since the intermediate products may elude us. And yet the whole significance of the processes concerned is to be sought in the succession of these stages. In animal experiments directed to the end under consideration, investigators have relied first of all upon the fact that the body, though the seat of a myriad reactions and capable perhaps of learning, to a limited extent and under stress of circumstances, new chemical accomplishments, is in general able to deal only with what is customary to it. This circumstance has yielded two methods of determining the nature of intermediate products in metabolism. Considerations of molecular structure will, for instance, suggest several possible lines along which a given physiological substance may be expected to undergo change. We may test these possibilities by administering various derivatives of the substance in question. Only those which prove on experiment to be fully metabolized, or to yield derivatives in the body identical with those yielded by the parent substance, can be the normal intermediate products of its metabolism. All others may be rejected as not physiological. In a second method dependent upon this eclecticism of the body substances are administered which so far differ from the normal that, instead of suffering a complete breakdown, they yield some residual derivative which can be identified in the excreta, and the nature of which will throw light upon the chemical mechanism which has produced it. For instance, a substance with a resistant (because abnormal) ring structure, but possessing a normal side chain, may be used to demonstrate how the side chain breaks down. Again, we may sometimes obtain useful information by administering a normal substance in excessive amounts, when certain intermediate products may appear in the excreta. Another most profitable method of experiment is that in which the substance to be studied is submitted to the influence of isolated organs, instead of to that of the whole animal. Under these conditions a series of normal reactions may go on, but with altered relative velocities, so that intermediate products accumulate; or again when, as may happen, the successive changes wrought upon a substance by metabolism occur in different organs of the body, this use of isolated organs enables us to dissect, as it were, the chain of events. Extraordinarily profitable have been the observations made upon individuals suffering from those errors of metabolism which Dr. Garrod calls "metabolic sports, the chemical analogues of structural malformations." In these individuals Nature has taken the first essential step in an experiment by omitting from their chemical structure a special catalyst which at one point in the procession of metabolic chemical events is essential to its continuance. At this point there is arrest, and intermediate products come to light.

As you know, most ingenious use of this ready-made experimental material has added greatly to our knowledge of intermediate metabolism. Admirable use, too, has been made of the somewhat similar conditions presented by diabetes, clinical and experimental. Every day our knowledge of the dynamics of the body grows upon these lines.

I know that the history of all these efforts is familiar to you, but I am concerned to advertise the fact that our problems call for ingenuity of a special sort, and to point out that an equipment in chemical technique alone would not have sufficed for the successful attack which has been made upon them. But I am even more concerned to point out that the direct method of attack has been too much neglected, or has been in the hands of too few—I mean the endeavour to separate from the tissues further examples of the simpler products of metabolic change, no matter how small the amount in which they may be present; an endeavour which ought not to stop at the separation and identification of such substances, but to continue till it has related each one of them to the dynamic series of reactions in which each one is surely playing a part. The earliest attempts at tracing the intermediate processes of metabolism looked for information to the products which accumulate in the tissues, but it seemed to be always tacitly assumed that only those few which are quantitatively prominent could be of importance to the main issues of metabolism. It is obvious, however, upon consideration that the degree to which a substance accummulates is by itself no measure of its metabolic importance; no proof as to whether it is on some main line of change, or a stage in a quantitatively unimportant chemical by-path. For, if one substance be changing into another through a series of intermediate products, then, as soon as dynamical equilibrium has been established in the series, and to such equilibrium tissue processes always tend, the rate of production of any one intermediate product must

be equal to the rate at which it changes into the next, and so throughout the series. Else individual intermediate products would accummulate or disappear, and the equilibrium be upset. Now the rate of chemical change in a substance is the product of its efficient concentration and the velocity constant of the particular reaction it is undergoing. Thus the relative concentration of each intermediate substance sharing in the dynamic equilibrium, or, in other words, the amount in which we shall find it at any moment in the tissue, will be inversely proportional to the velocity of the reaction which alters it. But the successive velocity constants in a series of reactions may vary greatly, and the relative accumulation of the different intermediate products must vary in the same degree. It is certain that in the tissues very few of such products accumulate in any save very small amount, but the amount of a product found is only really of significance if we are concerned with any function which it may possibly possess. It is of no significance as a measure of the quantitative importance of the dynamical events which give rise to it.

To take an instance. The substance creatin has always asserted itself in our conceptions concerning nitrogenous metabolism because of the large amount in which it is found in the muscle. It may be of importance *per se*, and abnormalities in its fate are certainly important as an indication of abnormalities in metabolism, but we must remember that the work of Gulewitsch, Krimberg, Kutscher, and others has shown us that a great number of nitrogenous basic bodies exist in muscle in minute amounts. Maybe we shall need to know about each of these all that we now know, or are laboriously trying to know, about creatin, before the dynamics of basic nitrogen in muscle become clear. Fortunately for the experimenter, most of the raw materials required for tissue analysis are easily obtainable; there is no reason save that of the labour involved why we should not work upon a ton of muscle or a ton of gland tissue.

I am certain that the search for tissue products of simple constitution has important rewards awaiting it in the future, so long as physiologists are alive to the dynamical significence of all of them. Such work is laborious and calls for special instincts in the choice of analytical method, but, as I mentioned in an earlier part of this address, I am sure that high qualifications as an analyst should be part of the equipment of a biological chemist.

Modern Work upon Intermediate Metabolism: the Amino-Acids.

We know that the first change suffered by an α-amino-acid when it enters the metabolic laboratories is the loss of its amino group; and, thanks to the labours of Knoop, Neubauer, Embden, Dakin, and others, we have substantial information concerning the mechanism of this change. The process involved in the removal of the amino group is not a simple reduction, which would yield a fatty acid, or substituted fatty acid, nor a hydrolytic removal which would leave an α-hydroxy-acid; but the much less to be expected process of an oxidative removal, which results in the production of a keto-acid. If the direct evidence for this chemically most interesting primary change were to be held insufficient (though there is no insufficiency about it) its physiological reality is strongly supported by the proof given us by Knoop and Embden that the liver can resynthesize the original amino-acid from ammonia and the corresponding keto-acid. This profoundly significant observation is part of the evidence which is continually accumulating to show that all normal chemical processes of the body can suffer reversal. The next step in the breakdown involves the oxidation of the keto-acid, with the production of a fatty acid containing one carbon less than the original amino-acid. This in turn is oxidized to its final products along the lines of the β-oxidation of Knoop, two carbon atoms being removed at each stage of the breakdown. All this is true of the aliphatic α-amino-acids, and, with limitations, of the side chains of their aromatic congeners. In the case of certain amino-acids the course of breakdown passes through the stage of aceto-acetic acid. This happens to those of which the molecule contains the benzene ring, and Dakin has enabled us to picture clearly the path of change which involves the opening of the ring. This particular stage does not seem to occur in the breakdown of the aliphatic amino-acids, save in the case of leucin; the rule and the exception here being alike easy of explanation by considerations of molecular structure.

But direct breakdown on the lines mentioned is far from being the only fate of individual amino-acids in the body. The work of Lusk, completed by that of Dakin, has shown us that of seventeen amino-acids derived from protein no less than nine may individually yield glucose in the diabetic organism, and there are excellent grounds for believing (indeed, there is no doubt) that they do the same to a duly regulated extent in the normal organism. The remainder have been shown not to yield sugar, and there is therefore a most interesting contrast in the fate of two groups of the protein *Bausteine*. Those which yield sugar do not yield aceto-acetic acid, and those which yield the latter are not glycogenic. One set, after undergoing significant preliminary changes, seems to join the carbohydrate path of metabolism, the other set ultimately joins a penultimate stage in the path which is traversed by fats.

I will here venture to leave for one moment the firm ground of facts experimentally ascertained. Unexplored experimentally, but quite certain so far as their existence is concerned, are yet other metabolic paths of prime importance, along which individual amino-acids must travel and suffer change. We know now from the results of prolonged feeding experiments upon young growing animals, which I myself, as well as many others, have carried out, that all the nitrogenous tissue complexes, as well as the tissue proteins, can be duly constructed when the diet contains no other source of nitrogen beside the amino-acids of protein. The purin and pyrimidin bases, for instance, present in the nuclear material of cells certainly take origin from particular amino-acids, though we have no right to assume that groups derived from carbohydrates or fats play no part in the necessary syntheses. While recent years have given us a wonderfully clear picture as to how the nucleic acids and the purin bases contained in them break down during metabolism, we have as yet no knowledge of stages in their synthesis. But it is clear that to discover these is a task fully open to modern experimental methods, and, though a difficult problem, it is one ready to hand. Again, in specialized organs substances are made which are of great importance, not to the structure, but to the dynamics of the body. These have become familiar to us under the name of hormones. We know the constitution of one of these only—adrenalin. The molecule of this exemplar has a simple structure of a kind which makes it almost certain to be derived from one of the aromatic amino-acids. It is clearly open to us to discover on what lines it takes origin. Facts of this kind, we may be sure, will form a special chapter of biochemistry in the future. I would like to make a point here quite important to my main contention that metabolism deals with simple molecules. As a pure assumption it is often taught, explicitly or implicitly, that, although the bowel prepares free amino-acids for metabolism, only those which are individually in excess of the contemporary needs of the body for protein are directly diverted to specialized paths of metabolism, and these to the paths of destructive change. All others—all those which are to play a part in the intimacies of metabolism—are supposed to be first reconstructed into protein, and must therefore again be liberated from a complex before entering upon their special paths of change. But there is much more reason (and some experimental grounds) for the belief that the special paths (of which only one leads to the repair or formation of tissue protein) may be entered upon straightway. Mrs. Stanley Gardiner (then Miss Willcock) carried out some feeding experiments a few years ago, and in discussing these I pointed out that they offered evidence of the direct employment for special purposes of individual amino-acids derived as such from the bowel. It seemed at the time that the argument was misunderstood or felt to carry little weight, but later Professor Kossel[1] quoted my remarks with approval and expressed agreement with the view that the *Bausteine* of the food protein must, in certain cases, be used individually and directly.

The chief thing to realize is that as a result of modern research the conception of metabolism in block is, as Garrod puts it, giving place to that of metabolism in

compartments. It is from the behaviour of simple molecules we are learning our most significant lessons.

Now interest in the chemical events such as those we have been dealing with may still be damped by the feeling that, after all, when we go to the centre of things, to the bioplasm, where these processes are initiated and controlled, we shall find a *milieu* so complex that the happenings there, although they comprise the most significant links in the chain of events, must be wholly obscure, when viewed from the standpoint of structural organic chemistry. I would like you to consider how far this is necessarily the case.

The highly complex substances which form the most obvious part of the material of the living cell are relatively stable. Their special characters, and in particular the colloidal condition in which they exist, determine, of course, many of the most fundamental characteristics of the cell; its definite yet mobile structure, its mechanical qualities, including the contractility of the protoplasm, and those other colloidal characters which the modern physical chemist is studying so closely. For the dynamic chemical events which happen within the cell, these colloid complexes yield a special *milieu*, providing, as it were, special apparatus, and an organized laboratory. But in the cell itself, I believe, simple molecules undergo reactions of the kind we have been considering. These reactions, being catalysed by colloidal enzymes, do not occur in a strictly homogeneous medium, but they occur, I would argue, in the aqueous fluids of the cell under just such conditions of solution as obtain when they progress under the influence of enzymes *in vitro*.

There is, I know, a view which, if old, is in one modification or another still current in many quarters. This conceives of the unit of living matter as a definite, if very large and very labile molecule, and conceives of a mass of living matter as consisting of a congregation of such molecules in that definite sense in which a mass of, say, sugar is a congregation of molecules, all like to one another. In my opinion, such a view is as inhibitory to productive thought as it is lacking in basis. It matters little whether in this connexion we speak of a "molecule" or, in order to avoid the fairly obvious misuse of a word, we use the term "biogen," or any similar expression with the same connotation. Especially, I believe, is such a view unfortunate when, as sometimes, it is made to carry the corollary that simple molecules, such as those provided by foodstuffs, only suffer change after they have become in a vague sense a part of such a giant molecule or biogen. Such assumptions became unnecessary as soon as we learnt that a stable substance may exhibit instability after it enters the living cell, not because it loses its chemical identity, and the chemical properties inherent in its own molecular structure, by being built into an unstable complex, but because in the cell it meets with agents (the intracellular enzymes) which catalyse certain reactions of which its molecule is normally capable.

Exactly what sort of material might, in the course of cosmic evolution, have first come to exhibit the elementary characters of living stuff, a question raised in the Presidential Address which so stirred us last year, we do not, of course, know. But it is clear that the living cell as we now know it is not a mass of matter composed of a congregation of like molecules, but a highly differentiated system; the cell, in the modern phraseology of physical chemistry, is a system of co-existing phases of different constitutions. Corresponding to the difference in their constitution, different chemical events may go on contemporaneously in the different phases, though every change in any phase affects the chemical and physico-chemical equilibrium of the whole system. Among these phases are to be reckoned not only the differentiated parts of the bioplasm strictly defined (if we can define it strictly) the macro-nuclei and micro-nuclei, nerve fibres, muscle fibres, etc., but the material which supports the cell structure, and what have been termed the "metaplasmic" constituents of the cell. These last comprise not only the fat droplets, glycogen, starch grains, aleurone grains, and the like, but other deposits not to be demonstrated histologically. They must be held, too—a point which has not been sufficiently insisted upon—to comprise the diverse substances of smaller molecular weight and greater solubility, which are present in the more fluid phases of the system—namely, in the cell juices. It is important to remember that changes in any one of these constituent phases, including the metaplasmic phases, must affect the equilibrium of the whole cell system, and because of this necessary equilibrium-relation it is difficult to say that any one of the constituent phases, such as we find *permanently* present in a living cell, even a metaplasmic phase, is less essential than any other to the "life" of the cell, at least when we view it from the standpoint of metabolism. It is extremely difficult and probably impossible by any treatment of the animal to completely deprive the liver of its glycogen deposits, so long as the liver cells remain alive. Even an extreme variation in the quantity is in the present connexion without significance because, as we know, the equilibrium of a polyphasic system is independent of the mass of any one of the phases; but I am inclined to the bold statement that the integrity of metabolic life of a liver cell is as much dependent on the coexistence of metaplasmic glycogen, however small in amount, as upon the coexistence of the nuclear material itself; so in other cells, if not upon glycogen, at least upon other metaplasmic constituents.

Now we should refuse to speak of the membrane of a cell, or of its glycogen store, as living material. We should not apply the term to the substances dissolved in the cell juice, and, indeed, would hardly apply it to the highly differentiated parts of the bioplasm if we thought of each detail separately. We are probably no more justified in applying it, when we consider it by itself, to what, as the result of microscopic studies, we recognize as "undifferentiated" bioplasm. On ultimate analysis we can hardly speak at all of living matter in the cell; at any rate, we cannot, without gross misuse of terms, speak of the cell life as being associated with any one particular type of molecule. Its life is the expression of a particular dynamic equilibrium which obtains in a polyphasic system. Certain of the phases may be separated, mechanically or otherwise, as when we squeeze out the cell juices, and find that chemical processes still go on in them; but "life," as we instinctively define it, is a property of the cell as a whole, because it depends upon the organization of processes, upon the equilibrium displayed by the totality of the coexisting phases.

I return to my main point. The view I wish to impress upon you is that some of the most important phenomena in the cell, those involving simple reactions of the type which we have been discussing, occur in ordinary crystalloid solution. We are entitled to distinguish fluid (or more fluid) phases in the cell. I always think it helpful in this connexion to think of the least differentiated of animals' cells—to consider, for instance, the amoeba. In this creature a fluid phase comes definitely into view with the appearance of the food vacuole. In this vacuole digestion goes on, and there can be no doubt, from the suggestive experimental evidence available, that a digestive enzyme, and possibly two successive enzymes (a pepsin followed by a trypsin), appear in it. It is now generally admitted that digestion in the amoeba, though intracellular, is metaplasmic. The digestion products appear first of all in simple aqueous solution. Is it not unjustifiable to assume that the next step is a total "assimilation" of the products, a direct building up of all that is produced in the vacuole into the complexes of the cell? If there be any basis for our views concerning the specificity of, say, the tissue proteins, they must apply to the amoeba no less than to the higher animal, and we must picture the building up of its specific complexes as a selective process. The mixture of amino-acids derived from the proteins of the bacteria or other food eaten by it may be inharmonious with their balance in the amoeba. Some have to be more directly dealt with, by oxidation or otherwise. If the digestive hydrolysis occur outside the complexes, we may most justifiably assume that other preparative processes also occur outside them. We need not think of a visible vacuole as the only seat of such changes. Similar fluid phases in the cell may elude the microscope, and the phenomena would be just as significant if reactions occur in the water imbibed by the colloids of the cell or present in the intramicellar spaces of the bioplasm. It is always important to remember that 75 per cent. of the cell substance consists of water.

All of these considerations we may apply to the tissue cells of the higher animal. To my mind, at least, the

following considerations appeal. It is noteworthy that all the known complexes of the cell—the proteins, the phosphorous complexes, the nucleic acids, etc.—are susceptible to hydrolysis by catalytic agents, which are always present, or potentially present. If the available experimental evidence be honestly appraised, it points to the conclusion that only to hydrolytic processes are the complexes unstable. Under the conditions of the body they are, while intact, resistant to other types of change, their hydrolytic products being much more susceptible. Since hydroclastic agents are present in the cell we must suppose that there is, at any moment, equilibrium between the complexes and their water-soluble hydrolytic products, though the amount of the latter present at any moment may be very small. Now, I think we are entitled to look upon assimilation and dissimilation, when very strictly defined, as being dependent upon changes in this equilibrium alone. They are processes of condensation and hydrolysis respectively. Substances which are foreign to the normal constitution of the complexes—and these comprise not only strictly extraneous substances, but material for assimilation not yet ready for direct condensation, or metabolites which are no longer simple hydrolytic products—do not enter or re-enter the complexes. They suffer change within the cell, but not as part of the complexes. When, for instance, a supply of amino-acids transferred from the gut reaches the tissue cell, they may be in excess of the contemporary limits of assimilation; or, once more, individual acids may not be present in the harmonious proportion required to form the specific proteins in the cell. Are we to suppose that all nevertheless become an integral part of the complexes before the harmony is by some mysterious means adjusted? I think rather that the normality of the cell proteins is maintained by processes which precede actual condensation or assimilation. Conversely, when the cell balance sets towards dissimilation, the amino-acids liberated by hydrolysis suffer further change outside the complexes. So when a foreign substance, say benzoic acid, enters the cell, we have no evidence, experimental or other, to suggest that such a body ever becomes an integral part of the complexes. Rather does it suffer its conjugation with glycine in the fluids of the cell. So also with cases of specific chemical manufacture in organs. When, for instance, adrenalin—a simple, definite crystalline body—appears in the cells of the gland which prepares it, are we to suppose that its molecule emerges in some way ready-made from the protein complexes of the gland, rather than that a precursor derived from a normal hydrolytic product of these proteins or from the food supply is converted into adrenalin by reactions of a comprehensible kind, occurring in aqueous solution, and involving simple molecules throughout? While referring to adrenalin, I may comment upon the fact that the extraordinarily wide influence now attributed to that substance is a striking illustration of the importance of simple molecules in the dynamics of the body.

It should be, of course, understood—though the consideration does not affect the essential significance of the views I am advancing—that the isolation of reactions in particular phases of the cell is only relative. I have before emphasized the point that the equilibrium of the whole system must, to a greater or less degree, be affected by a change in any one phase. A happening of any kind in the fluid phases must affect the chemical equilibrium and, no less, the physico-chemical equilibrium, between them and the complexes or less fluid phases. A drug may have an "action" on a cell, even though it remain in solution, and it may have a specific action because its molecular constitution leads it to intrude into, and modify the course of, some one, rather than any other, of the numerous simple chemical reactions proceeding in the cells of different tissues.

Specific Catalysts.

But I must now turn from consideration of the reactions themselves to that of their direction and control. It is clear that a special feature of the living cell is the organization of chemical events within it. So long as we are content to conceive of all happenings as occurring within a biogen or living molecule all directive power can be attributed in some vague sense to its quite special properties.

But the last fifteen years have seen grow up a doctrine of a quite different sort, which, while it has difficulties of its own, has the supreme merit of possessing an experimental basis and of encouraging by its very nature further experimental work. I mean the conception that each chemical reaction within the cell is directed and controlled by a specific catalyst.

Considering the preparation made for it by the early teaching of individual biologists, prominent among whom was Moritz Traube, it is remarkable that belief in the endo-enzyme as a universal agent of the cell was so slow to establish itself, though in the absence of abundant experimental proof scepticism was doubtless justified. So long as the ferments demonstrated as being normally attached to the cell were only those with hydroclastic properties, such as were already familiar in the case of secreted digestive ferments, the imagination was not stirred. Only with Buchner's discovery of zymase and cell-free alcoholic fermentation did the faith begin to grow.

Remembering, however, the great multiplicity of the reactions which occur in the animal body, and remembering the narrow specificity in the range of action of an individual enzyme, we may be tempted to pause on contemplating the myriad nature of the army of enzymes that seems called for. But before judging upon the matter the mind should be prepared by a full perusal of the experimental evidence. We must call to mind the phenomena of autolysis and all the details into which they have been followed; the specificity of the proteolytic ferments concerned, and especially the evidence obtained by Abderhalden and others, that tissues contain numerous enzymes, of which some act upon only one type of polypeptide, and some specifically on other polypeptides. We must remember the intracellular enzymes that split the phosphorous complexes of the cell; the lipases, the amylases, and the highly specific invert ferments, each adjusted to the hydrolysis of a particular sugar. We have also to think of a large group of enzymes acting specifically upon other substances of simple constitution, such as the arginase of Kossel and Dakin, the enzyme recently described by Dakin, which acts with great potency in converting pyruvic aldehyde into lactic acid, and many others. Nothing could produce a firmer belief in the reality and importance of the specialized enzymes of the tissues than a personal repetition of the experiments of Walter Jones, Schittenhelm, Wiechowski, and others, upon the agents involved in the breakdown of nucleic acids; each step in the elaborate process involves a separate catalyst. In this region of metabolism alone a small army of independent enzymes is known to play a part, each individual being of proven specificity. The final stages of the process involve oxidations which stop short at the stage of uric acid in man, but proceed to that of allantoin in most animals. It is very instructive to observe the clean, complete oxidation of uric acid to allantoin, which can be induced *in vitro* under the influence of Wiechowski's preparations of the uric acid oxidase, especially if one recalls at the same time, in proof of its physiological significance, that this oxidase, though always present in the tissues of animals, which excrete allantoin, is absent from those of man, who does not.

I will not trouble you with further examples. We have arrived, indeed, at a stage when, with a huge array of examples before us, it is logical to conclude that all metabolic tissue reactions are catalysed by enzymes, and, knowing the general properties of these, we have every right to conclude that all reactions may be so catalysed in the synthetic as well as in the opposite sense. If we are astonished at the vast array of specific catalysts which must be present in the tissues, there are other facts which increase the complexity of things. Evidence continues to accumulate from the biological side to show that, as a matter of fact, the living cell can acquire *de novo*, as the result of special stimulation, new catalytic agents previously foreign to its organization.

It is certain, from very numerous studies made upon the lower organisms, and especially upon bacteria, that the cell may acquire new chemical powers when made to depend upon an unaccustomed nutritive medium. I must be content to quote a single instance out of many. Twort has shown that certain bacteria of the *coli-typhosus* group can be trained to split sugars and alcohols which originally they could not split at all. A strain of

B. typhosus which after being grown upon a medium containing dulcite had acquired the power of splitting this substance, retained it permanently, even after passage through the body of the guinea-pig, and cultivation upon a dulcite-free medium. Similar observations have been made upon the Continent by Massini and Burri; the latter showed by ingenious experiments that all the individuals of a race which acquires such a new property have the same potency for acquiring it. No one, at the present time, will deny that the appearance of a new enzyme is involved in this adjustment of the cell to a new nutritive medium.

We have not, it is true, so much evidence for similar phenomena in the case of the higher animals. The milk-sugar splitting ferment may be absent from the gut epithelium before birth, and in some animals may disappear again after the period of suckling, but here we probably have to do with some simple alternation of latency and activization. But among the "protective" ferments studied by Abderhalden we have, perhaps, cases in which specific individuals appear *de novo* as the result of injecting foreign proteins, etc., into the circulation. Consider, moreover, the case of the reactions called out by simpler substances. We have seen that an enzyme separable from the kidney tissue can catalyse the synthesis no less than the breakdown of hippuric acid. Now the cells of the mammalian kidney have always had to deal with benzoic acid or chemical precursors of benzoic acid, and the presence of a specific enzyme related to it is not surprising. But living cells are not likely to have ever been in contact with, say, bromo-benzol, until the substance was administered to animals experimentally. Yet a definite reaction at once proceeds when that substance is introduced into the body. It is linked up, as we have seen, with cystein. Now, this reaction is not one which would proceed in the body uncatalysed; if it be catalysed by an enzyme, all that we know about the specificity of such agents would suggest that a new one must appear for the purpose. I have allowed myself to go beyond ascertained facts in dealing with this last point. But once we have granted that specific enzymes are real agents in the cell, controlling a great number of reactions, I can see no logical reason for supposing that a different class of mechanism can be concerned with any particular reaction.

If we are entitled to conceive of so large a part of the chemical dynamics of the cell as comprising simple metaplasmic reactions catalysed by independent specific enzymes, it is certain that our pure chemical studies of the happenings in tissue extracts, expressed cell juices, and the like, gain enormously in meaning and significance. We make a real step forward when we escape from the vagueness which attaches to the "bioplasmic molecule" considered as the seat of all change. But I am not so foolish as to urge that the step is one towards obvious simplicity in our views concerning the cell. For what, indeed, are we to think of a chemical system in which so great an array of distinct catalysing agents is present or potentially present; a system, I would add, which when disturbed by the entry of a foreign substance regains its equilibrium through the agency of new-born catalysts adjusted to entirely new reactions? Here seems justification enough for the vitalistic view that events in the living cell are determined by final as well as by proximate causes, that its constitution has reference to the future as well as the past. But how can we conceive that any event called forth in any system by the entry of a simple molecule, an event related qualitatively to the structure of that molecule, can be of other than a chemical nature? The very complexity, therefore, which is apparent in the catalytic phenomena of the cell to my mind indicates that we must have here a case of what Henri Poincaré has called *la simplicité cachée*.

It must not be supposed that I am blind to the fact that the phenomena of the cell present a side to which the considerations I have put before you do not apply. Paul Ehrlich, in his recent illuminating address to the International Congress of Medicine, remarked that if, in chemistry, it be true that *Corpora non agunt nisi liquida*, then, in chemiotherapy, it is no less true that *Corpora non agunt nisi fixata*. Whatever precisely may be involved in the important principle of "fixation" as applied to drug actions, it remains, I think, true that the older adage applies to the dynamic *reactions* which occur in the living cell. But there are doubtless dynamic phenomena in which the cell complexes play a prominent part. The whole of our doctrine concerning the reaction of the body to the toxins of disease is based upon the fact that when the cell is invaded by complexes other than those normal to it, its own complexes become involved. I must not attempt to deal with these phenomena, but rather proceed to my closing remarks. I would like, however, just to express the hope that the chemist will recognize their theoretical importance. He will not, indeed, be surprised at the oligo-dynamic aspects of the phenomena, startling as they are. When physico-chemical factors enter into a phenomenon the influence of an infinitely small amount of material may always be expected. It is a fact, for instance, as Dr. W. H. Mills reminds me, that when a substance crystallizes in more than one form it may be quite impossible to obtain the less stable forms of its crystals in any laboratory which has been "infected" with the more stable form, even though this infection has been produced by quite ordinary manipulations dealing with the latter. Here, certainly, is a case in which the influence of the infinitesimal is before us. But what I feel should arrest the interest of the chemist is the remarkable mingling of the general with the particular which phenomena like those of immunity display. In the relations which obtain between toxin and antitoxin, for example, we find that physico-chemical factors predominate, and yet they are associated to a high degree with the character of specificity. The colloid state of matter, as such, and the properties of surface determine many of the characteristics of such reactions, yet the chemical aspect is always to the front. Combinations are observed which do not seem to be chemical compounds, but rather associations by adsorption; yet the mutual relations between the interacting complexes are in the highest degree discriminative and specific. The chemical factor in adsorption phenomena has, of course, been recognized elsewhere; but in biology it is particularly striking. Theoretical chemistry must hasten to take account of it. The modern developments in the study of valency probably constitute a step in this direction.

It is clear to every one that the physical chemist is playing, and will continue to play, a most important part in the investigation of biological phenomena. We need, I think, have no doubt that in this country he will turn to our problems, for the kind of work he has to do seems to suit our national tastes and talents, and the biologist just now is much alive to the value of his results. But I rather feel that the organic chemist needs more wooing and gets less, though I am sure that his aid is equally necessary. In connexion with most biological problems physical and organic chemists have clearly defined tasks. To take one instance: In muscle phenomena it is becoming every day clearer that the mechanico-motor properties of the tissue, its changes of tension, its contraction and relaxation, depend upon physico-chemical phenomena associated with its colloidal complexes and its intimate structure. Changes in hydrogen-ion concentration and in the concentration of electrolytes generally, by acting upon surfaces or by upsetting osmotic equilibria, seem to be the determining causes of muscular movement. Yet the energy of the muscle is continuously supplied by the progress of organic reactions, and for a full understanding of events we need to know every detail of their course. Here, then, as everywhere else, is the need for the organic chemist.

But I would urge upon any young chemist who thinks of occupying himself with biological problems the necessity for submitting for a year or two to a second discipline. If he merely migrate to a biological institute, prepared to determine the constitution of new products from the animal and study their reactions *in vitro*, he will be a very useful and acceptable person, but he will not become a biochemist. We want to learn how reactions run in the organism, and there is abundant evidence to show how little a mere knowledge of the constitution of substances, and a consideration of laboratory possibilities, can help on such knowledge. The animal body usually does the unexpected.

REFERENCE.

[1] *Johns Hopkins Hospital Bulletin*, March, 1912.