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THE MECHANISM OF ENZYME ACTIONS[1]

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IN making up the program of the symposium on enzymes for this meeting, the speaker was asked to discuss the mechanism of enzyme actions. The ground to be covered was not further specified. In thinking over the possible topics to be considered, it was soon evident that everything related to an enzyme action might be included, but this would make the treatment a hopeless one. Perhaps it would be as well not to attempt an exhaustive review, but rather to present some personal conclusions and relations based upon the experimental and theoretical work and study over a considerable period of time, as time is reckoned by the individual.

Ten years ago, in a monograph on enzyme action, the speaker wrote a chapter on the mechanisms of such actions. The conclusions presented there in rather elementary fashion have been supplemented since, but unfortunately, the relations have not been simplified. Rather the complexities of the problems have become more generally recognized, and while the simpler relations may still be said to hold, they furnish only the beginnings of the real study of the problem of the mechanisms of such actions.

It would be rather easy to present a number of facts of enzyme actions and to draw conclusions limited to the cases in point from them. To such an audience as this the facts of enzyme actions are known. To repeat them is unnecessary and also boring. To present some more general relations and views may perhaps be useful, more for the purpose of raising questions than of answering them.

[1] Presented at the Symposium on the Chemistry of the Enzymes held by the Divisions of Agricultural and Food Chemistry and of Biological Chemistry of the American Chemical Society, Cleveland, Ohio, September 11, 1934.

In the first place, what is meant by mechanism of enzyme actions? Enzyme actions are chemical actions; enzymes are materials causing chemical changes in various substances. This raises the question of the mechanism of chemical actions and reactions in general. In a monograph published six years ago by the American Chemical Society, F. O. Rice[2] discussed the "Mechanism of Homogeneous Organic Reactions." He brought out clearly the difficulties involved in such studies and stated[3]: "It is not a matter for discouragement that the mechanism of organic reactions is, in great part, so uncertain. . . . It does not seem likely, however, that there will be any remarkable advance by proceeding along classical lines, and we may look for this only through the development of some new method. . . ." In view of the more complex nature of enzyme reactions involving substances in the colloidal state, etc., in comparison with the homogeneous organic reactions which were treated by Rice, the outlook for understanding the mechanism of enzyme actions is not hopeful, to say the least.

Enzyme actions have been and are included in the group of catalytic actions. This does not add anything to the understanding, either of enzyme actions or of catalytic actions, so nothing more will be said of this relationship, or comparison, or classification, or whatever it may be called.

Having put forward the worst imaginable view, it will now be possible to go ahead with the statements of some of the relationships which have been proposed.

To any one working with enzyme actions, the rates of chemical reactions or the amounts of changes in definite times, brought about or influenced by these enzymes, is the predominatingly important factor. The accurate experimental determination and interpretation of such rates obviously is an essential feature of enzyme studies. In an attempted evaluation of the results of such studies a most disconcerting fact appears. There is no standardized method of studying these actions. Apparently, most of those who have made extended studies of the velocities of enzyme actions have developed what may be called individualistic methods of carrying out the experiments or of presenting these results in mathematical form.

The experimental methods of measuring enzyme changes may first be considered. For a few enzyme actions the methods appear to be simple and have been generally adopted. For most, however, individualism runs wild. For example, for such a comparatively simple action as fat or ester hydrolysis, in the methods used, one chemist, apparently to fix conditions, adds a mixture of sodium oleate, calcium chloride and albumin to the enzyme material; another adds nothing at all but allows the acid formed progressively in the reaction to do what damage it may; one uses a stalagmometric method successfully, another is unable to obtain results with it; buffers of different compositions are added, although it is recognized that each such buffer may modify the action in its own way; and so on. This list could be elaborated endlessly and with other enzymes. All this may be said to have culminated in the protease work of Northrup, who uses twelve different methods of protease testing to determine similarities and differences in his crystalline protease preparations.

Then, on the theoretical side, the most obvious way of handling an experimental series of results mathematically is to apply the reaction velocity equations to them. The simple monomolecular reaction rate, involving the substrate, early was found to hold either not at all or only for limited ranges of change for many enzyme actions. Then came modifications of this equation, factors added to account for the products of reaction combining with the enzyme and so removing it from the sphere of action, of the reaction taking place in steps, etc., all accounting or reproducing mathematically the changes within more or less limited ranges. Terms to include adsorption were introduced. Empirical terms were proposed. The most extreme treatment was that of Nelson and Hitchcock[4] who developed an empirical equation, containing four constants, to reproduce the results on the hydrolysis of cane sugar by invertase. Many of the equations suggested, both theoretical and quasi-theoretical, are well known to those here to-day.

The following statement was made some years ago:[5] "The three concepts—chemical reaction, chemical equation and mathematical equation—are supposed to describe the same phenomenon in any given case. Actually they do so only as an ideal condition, and the possibility of deviation becomes greater with increasing complexity of the reactions and with decreasing care in the use of terms and expressions." Evidently, enzyme actions involving unknown enzyme materials and mixtures, on the one hand, and changes in complex materials, such as proteins, etc., on the other hand, are not readily amenable to simple theoretical treatment.

What does all this mean? Is there any way out of this apparent muddle, if it is a muddle? First, it will be necessary to philosophize a little. The measurements and studies were made by chemists of various kinds and degrees. Now, it is possible to divide chemists into three groups. There are, first, the self-starters; second, those who must be cranked occasionally; and third, those who must be towed their whole

[2] American Chemical Society, Monograph Series, Monograph No. 39, Chemical Catalog Co., Inc., New York.
[3] Page 19.
[4] *Journal American Chemical Society*, 43: 26–32, 1921.
[5] "The Chemistry of Enzyme Actions," 1924, p. 36.

lives. For the purposes in view, only the first two classes need be considered, the self-starters and the cranked. The third group, the so-called towees, add much at times to the vociferousness of the proceedings and help to fill the journals; but for a true study of the value of their concepts such mass action for once must be given small consideration. Caution must be used in determining what methods are of real value.

To what does all this lead? Different methods are used by different workers. Each worker interprets his results from a different view-point. In the main, this means that this branch of science is new and in the making. The methods are not standardized, and it may be said that each contributor adds something to help build up the structure of enzyme actions. For example, Levene[6] finds that hydrolysis of certain dipeptides by hydrogen ion and by erepsin are based upon the same type of actions, Bergmann is following this up and extending these relations, while Waldschmidt-Leitz has been separating a number of peptidases and is developing a branch of the subject whose implications are not by any means clear. The hydrolysis of cane sugar by invertase, perhaps the most carefully and most accurately studied of all enzyme actions, leaves much to be asked for, if a real knowledge of the mechanism of its action is desired.

In general, the study of the kinetics of enzyme actions has not thrown any conclusive light on the mechanism of such changes. Perhaps the only conclusion which seems justified at present and which is widely accepted is that addition compounds of enzyme and substrate are formed which then break down to form the products of enzyme actions. Such addition compounds have not been isolated as chemical individuals. The evidence for their presence is indirect and to that extent perhaps doubtful, but to assume their presence is useful and possibly true.

Can anything further be said of the mechanism of enzyme actions? As is well known, because of the nature of the materials, the experimental study is extremely difficult. However, it is desired to present some views, which are perhaps personal, but which represent, at least to one chemist, some of the directions which enzyme studies are taking.

In the first place, a real and great advance has been made in the obtaining of enzyme materials as crystalline proteins of constant properties, first by Sumner for urease, and then by Northrop for pepsin and trypsin and by Sherman for amylase. This part of the subject of enzymes does not properly come under the topic of the mechanism of such actions. Indirectly it will play a most important part, as it will be possible to work with more definite materials. It also has a bearing upon another phase of the enzyme problem to be discussed presently. The fact that these enzyme preparations are protein in character, and in fact have been considered to be pure proteins, is a matter which can not be overlooked. At various times the question was raised, whether protein material was a necessary constituent of the active enzyme. Careful experimental studies are required to throw light on these questions. For example, for the enzyme pepsin, the view was advanced recently that the active enzyme was not necessarily protein but could be transferred from one protein substance to another. This view was shown to be erroneous, and as far as evidence is at present available, these crystalline proteins act as the enzymes. What the chemical composition of other enzymes may prove to be can not be foretold. It would seem that lipases and esterases are also protein in composition, and that at the other extreme is invertase, which has been found always to contain nitrogen, although possibly to only a small percentage, even in its most highly purified state. It is of interest that the two enzymes, lipase and invertase, at opposite extremes as far as chemical composition is concerned, have just been brought together with regard to certain relations involving the mechanisms of their actions. This relationship will be considered presently.

The second point of advance to which it is desired to draw attention has to do with the influence of added substances on enzyme actions. Added substances can modify many chemical reactions and especially the velocities of the reactions. It is therefore not surprising that added substances should modify enzyme actions most profoundly in many instances. If this were all that were involved—a listing of enzyme actions whose velocities were changed—there would be little or no justification for presenting these relations here. It is desired, however, to present a point of view which is based upon some of these relations; a point of view which some believe may aid in throwing light on the meaning and hence the mechanism of enzyme actions.

In an extended investigation[7] of lipase or esterase actions of extracts of a number of tissues and tumors of different animals, the ester-hydrolyzing actions on a number of different simple esters were determined under standardized conditions. Any one tissue gave definite, reproducible amounts of relative hydrolyses on these esters under the definite conditions. That is to say, if the tissue hydrolyzed twice as much of one ester as of another in one case, it did so in every experiment. Different tissues gave different amounts of the relative hydrolyses, so that it was possible in a number of cases to identify the tissue by means of the relative ester-hydrolyzing actions of its extract. Added proteins did not modify these relative actions, nor did

[6] P. A. Levene and H. S. Simms, *Jour. Biol. Chem.*, 62: 711, 1925.

[7] K. G. Falk and associates in various publications.

mixing two tissue extracts modify the actions which were found to be additive in these cases. If, however, a highly active preparation, such as pancreas extract, was tested alone, and then after the addition of different proteins, it was found that one protein would increase the hydrolytic action on one ester and another protein the hydrolytic action on another ester. There was a selective or directive influence on the action of the enzyme due to these added proteins. Such relations were clearly pointed out in 1925 by Platt and Dawson[8] and extended recently.[9] It seems that with impure enzyme preparations the proteins and perhaps other substances present interfere with the influence of added protein. With purer enzyme, added protein exerts a specific directive effect. Similar results are being reported by Nelson and Saul with invertase, in a paper which is appearing this week in the September issue of the *Journal of the American Chemical Society*. Here, only amounts of actions are involved, but the experimental methods permit of a high order of accuracy. The hydrolytic action on cane sugar of highly purified invertase at pH 3.0 is increased by added protein. The action of crude invertase is not influenced by such added protein.

It is possible to speculate endlessly about the meaning and significance of these results. Undoubtedly, they are of the highest importance for the proper understanding of the mechanism of enzyme actions, especially since it is probable that other enzyme actions will be found to show similar relations. Only a few points will be mentioned and these only sketchily. Certain phenomena of enzyme behavior have been explained by assuming the active enzyme to be a definite chemical grouping or part of a molecule, stabilized by the remainder of the generally colloidal molecule. This view has been widely publicized in recent years. Some remarks may perhaps be permitted in this connection. In the first place, Sumner[10] pointed out the indefiniteness of this point of view, which is flexible enough to meet any number of experimental results. Secondly, this point of view is not of recent origin. It seems to be the most obvious way of looking at the facts of enzyme actions chemically. It was put forward by Perrin[11] in 1905, by Matthews and Glenn[12] in 1911, by Röhmann and Shmanine[13] in 1912, by the speaker[14] in 1918, by Willstätter[15] in 1922, and possibly by a number of others whose publications have been overlooked.

It is a convenient way of thinking about the phenomena, but in itself is quite incomplete. This raises the next question: What might be the true nature of the combination between enzyme material and proteins which may be added or which are already present? Apparently the classical valence theory is inadequate. To call the compounds "adsorption compounds" covers a volume of ignorance. It is to be hoped that a view of chemical combination, possibly an outgrowth of the older valence theories, possibly a development of energy relationships and including the quantum theory in some form, will develop which will permit a rational formulation and description of these combinations.

The mechanism of enzyme actions, as a rule, is taken to apply to simplified enzyme actions in the laboratory. There is, however, a more profound view which may be taken. That is, the mechanism of enzyme actions in the living organism may be considered. Following the discussion of the influence of proteins on such different enzymes as lipase and invertase, it is evident that enzymes in every living organism must be influenced by the apparently inactive materials present. Sometimes these influences or substances may exert directive actions, as with proteins on lipase; sometimes they may increase the actions as shown with proteins on invertase under the special conditions or with proteins on the hydrolyzing action of papain on glyceryl triacetate,[16] sometimes the actions may be decreased, and finally, the possible action of one enzyme on another in the living organism must be considered.

The enzyme in the living organism never acts alone or in a pure state; the external factors play a possibly predominating rôle. Perhaps the enzyme may be called the hereditary factor, and what actually occurs in any given case depends upon the other substances present or the environment. This is an interesting thought to play with but must not as yet be taken too seriously. The mechanism of enzyme actions in the living organism offers a vast field for study. A beginning has been made. Time permits only a reference. In the breakdown of glucose by yeast, the various steps in the process whereby different products are obtained under different conditions are gradually being elucidated. The scheme of Neuberg[17] and quite recently that of Meyerhof and Kiessling[18] are undoubtedly familiar. Here, a number of enzyme actions are involved and the possible complications are many, as

[8] *Biochem. Jour.*, 19: 869, 1925.
[9] K. G. Falk, *Jour. Biol. Chem.*, 96: 53, 1932.
[10] SCIENCE, 78: 335, 1933.
[11] J. Perrin, *Jour. Chim. Physique*, 3: 50, 1905.
[12] A. P. Mathews and T. H. Glenn, *Jour. Biol. Chem.*, 9: 29, 1911.
[13] F. Röhmann and T. Shmanine, *Biochem. Zeit.*, 42: 235, 1912.
[14] K. G. Falk, SCIENCE, 47: 423, 1918.
[15] R. Willstätter, *Ber. Chem. Ges.*, 55B, 3601, 1922.
[16] K. G. Falk, *Jour. Biol. Chem.*, 103: 363, 1933.
[17] *Cf.* the review by W. Fuchs, *Sammlung chemischer und chemisch-technischer Vorträge*, 27: 1, 1922.
[18] O. Meyerhof and W. Kiessling, *Biochem. Z.*, 267, 313–48, 1934.

you all know. Such studies, the results of which can be carried over to glucose breakdown in bacterial metabolism with necessary modification for any given case, and then perhaps brought into relation to muscle metabolism, would be a real triumph for a more useful understanding of the mechanism of such actions.

It would be possible to go on indefinitely in this strain. However, just one more thought will be presented. One of the points which it is desired to emphasize here is the action of added substances on enzyme actions. In other words, in these phenomena of living matter and of life processes it is the system as a whole which must be considered. This thought is not new, but it is frequently overlooked or ignored, possibly because of the aim to make the study of chemical phenomena objective as far as possible. In another field of chemistry, the simple ionic theory which treated of ions as independent entities has come to be modified to include the properties and actions of the solvent, of the ions on each other, of the influence of non-ionized substances on the properties of the solvent and of the ions, etc. It would be possible to give many other chemical illustrations, especially from the field of organic reactions. In every case, for a proper understanding of the reaction, all the factors and their interrelationships must be included. It is therefore obvious that in the complex mixtures of living matter the reciprocal influences of the constituents must be considered. For life processes, therefore, the understanding of the functioning of any one of the parts, and consequently also of the functioning of the whole, must necessarily treat of the system as a whole. This point of view is in contradistinction to the present trend of physics, to work down toward the ultimate particles of energy or matter. These two aims are not in contradiction, both are needed for a complete understanding of the phenomena involved. But further, in considering the system as a whole and the influences of various substances on enzyme actions and the surprising results obtained at times, it might appear as if these studies are being developed from the view of "Emergent Evolution." Although the latter might perhaps be considered as a philosophy of ignorance, yet it sets definite problems and raises questions which may or may not be answerable.

If the mechanism of enzyme actions were better understood, it would be an approach from the chemical side to the science of life itself. The biologist is working toward simpler units of cell constituents. Apparently, the genes are the simplest such units so far achieved. Their molecular weights are perhaps in the neighborhood of 50,000. Davenport[19] considers that they probably are enzymes and presents views relative to their development and actions, analogous to some of the views presented here. This is a tempting subject, and much of interest may be expected in this field in the near future.

Finally, in considering the mechanism of enzyme actions as outlined here, several specific questions may be asked. What is meant by "protein molecule" and by "pure protein"? What new concepts of forces or means of combination must be developed to account for the reactions observed? How do proteins, and perhaps other substances, act in modifying certain enzyme actions? And finally what sort of a mechanism in the living organism permits of the continuity of the enzyme formations and actions which are needed for the continuance of the given life process?

OBITUARY

MICHAEL IDVORSKY PUPIN

IN the small village of Idvor, not far from Belgrade, in the Austrian province of Banat, now a part of Yugoslavia, Michael Idvorsky Pupin was born on the fourth day of October, 1858.

His parents, Constantine and Olympiada, were Serbian peasants who could neither read nor write; they were prosperous and highly esteemed members of the community. From them he inherited a remarkably strong physique, an exceptional mental endowment and an oriental imagination.

His formal education was begun in the village school of Idvor, where he learned reading, writing and arithmetic, and was continued in the schools of Panchevo and Prague.

Eventually, while a student in Prague, he became so incensed at the Teutonic oppression of the Slavs in Bohemia that he decided to emigrate to America, where, he had come to believe from what he had learned in the schools at Panchevo and Prague, real freedom was to be found, and where, he thought, a young immigrant might make his way to fortune.

Late in March of the year 1874 he landed, practically penniless, as an immigrant in New York City. Shortly after landing, in an encounter with a crowd of newsboys, whose gibes at his headgear, a red fez, had aroused his resentment to fighting pitch, he demonstrated his ability to take care of himself. An onlooker, a Delaware farmer, impressed by his performance, offered him a job on his farm, which he declined, since his duties would have included the milking of cows, which in accordance with Serbian tradition was a job for women. Another offer of a job, on a Delaware farm, bearing a satisfactory stamp

[19] C. B. Davenport, *Scientific Monthly*, August, 1934, pp. 104–108.