THE ROLE OF ENZYMES IN CHEMICAL CHANGES.*

C. H. MARYOTT, B. S., PH. D.

Formerly it was thought that the chemical changes involved in the process of living matter sprung from a special vital force, unlike any of the material forces with which we are familiar. The assumption of this vital force was a natural sequel to the lack of knowledge concerning the nature of the changes involved in the growth and decay of plant and animal structures. Later as a number of products formerly obtained only from living organisms were made in the laboratory by familiar chemical methods, the need of any special vital agent disappeared, for it was seen that many of the chemical changes occurring in nature were not essentially different from those producible in the laboratory. The beauty and mystery of the transformations of plant and animal life, however, did not thereby suffer, for it was seen that each living cell constituted a laboratory most complete and wonderfully equipped, where reaction of the most complex nature was carried out with an ease that defied imitation. A study of the means by which these reactions are accomplished, has revealed the importance of a class of substances known as enzymes, elaborated by living cells. In their action they resemble the inorganic catalyzers and conform in general to the behavior of such.

A catalyzer is a substance which by its presence alters the rate of a chemical change, without taking, of itself, a permanent part in the reaction. There are many chemical changes going on around us which proceed so slowly under ordinary conditions that they are difficult or impossible of detection within a moderate length of time. Thus cane sugar dissolved in water, very slowly reacts with the water and yields dextrose and levulose. In the presence of hydrochloric acid, or of invertase, an enzyme procured from yeast, the change takes place rapidly, and under suitable conditions might be completed within a few hours. Neither the hydrochloric acid nor the invertase would be used up during the process, but would be present in the same amount and in the same condition after the reaction as they were before it. Likewise oxygen and hydrogen when mixed, under ordinary conditions, do not combine perceptibly, but if finely divided platinum is placed in the mixture, rapid union results, which may ignite the mixture.

Besides the fact that catalyzers accelerate reactions and that they undergo no permanent change themselves, there are several characteristics generally ascribed to them. One of the most conspicuous of these is the property which they possess of effecting an amount of chemical change out of all proportion to the amount of catalyzer used, the merest trace often causing a pronounced action; colloidal platinum will cause the decomposition of 1,000,000 times its weight of hydrogen peroxide, and invertase will act upon 200,000 times its weight of sugar.

Another characteristic of catalyzers of importance in connection with enzymes is the fact that catalytic agents do not affect the conditions of equilibrium in any

* Read at a monthly meeting of Faculty and Senior Classes, Baylor University Colleges of Medicine and Pharmacy.

chemical change. There are many reactions which do not proceed to completion, but the reaction apparently stops before all of the original reacting substances are combined. Thus fats will react with water to form glycerin and free fatty acids, but the reaction will come to an apparent halt before all of the fat is transformed. Under exact conditions there will be a definite ratio between the quantities of unchanged fat and the amounts of glycerin and free acid. The explanation of this condition is that we are dealing here not with a single reaction, but with two oppositely directed reactions, and each tends to undo the effect of the other. While it is true that fat and water react to give glycerin and acid, it is equally true that the glycerin and acid react to give the original fat and water.

The relative amount of fat that will be decomposed will be determined by the conditions of temperature and the concentration of the various substances present. Inasmuch as both of these reactions are influenced to the same extent by a catalyzer, the relative amounts of the substances present when equilibrium is reached will be the same whether a catalyzer is used or not. The fact that in such reversible reactions, the catalyzers affect with reactions alike, is of great importance physiologically, for it makes it possible that under certain conditions a substance such as a fat may be broken down by an enzyme into products which can be absorbed, and under different conditions, by this same enzyme, these products can be combined again. Thus in the body the same enzyme may effect decompositions, or it may bring about syntheses.

Though the fermentation of sugar containing solutions must have been the subject of a great deal of attention in very early times, it was not until nearly the middle of the nineteenth century that the production of alcohol and carbon dioxide was associated with the activities of the yeast cells, not until after Pasteur had completed his researches on the subject was full credence given to the belief that the yeast cell was the active instrument in alcoholic fermentation. Other ferments were known previous to that time for Reaumur as far back as 1752 had discovered the gastric enzyme pepsin, and Kirchoff in 1814 discovered the diastasic properties of sprouting barley. Emulsin and many other ferments were known. The catalytic nature of these enzymes was realized, for Berzelius in 1837 had compared them to the inorganic catalyzer, and had anticipated the tremendous importance which these enzymes have on vital processes. These enzymes just mentioned were held to act in an essentially different manner from those in yeast, lactic and butyric fermentations, which Pasteur had proved took place within the living cells and which were then believed to be possible only in conjunction with the living processes of these cells. Ferments were accordingly classified into organized and unorganized ferments. This classification prevailed until 1896, when Buchner showed that the activity of the yeast cells was due to a substance, which he called zymase, which could be separated from the cellular structure and which caused the breaking down of glucose into alcohol and carbon dioxide, independently of any living matter. The term "organized ferment" lost its original significance and since then all processes of fermentation have been looked upon as due to unorganized, non-living enzymes. At present enzymes are considered as intra-cellular or extra-cellular, depending upon whether their activity takes place normally within the living cell or outside of it.

The extra-cellular enzymes, those that are excreted into a fluid external to the cell, such as the common digestive enzymes, pepsin and trypsin, are the best known. Considerable difficulty surrounds the study of the intra-cellular enzymes, for in many cases it is impossible to separate them from the cells, and their activity must be studied while within the cell structure.

In the classification of the enzymes, they are usually grouped either according to the type of chemical reaction they accelerate, or according to the nature of the substrate that they act upon. Of the various chemical changes which take place in the living world that of hydrolysis is one of the most frequent and most important. Many substances, particularly plant and animal substances, when in the presence of water, react with the water, the molecules of the substance taking up water molecules and breaking down into simpler substances. Thus a cane-sugar molecule will react with a water molecule and break down into a molecule of dextrose and one of levulose. This process is very slow unless a catalyzer is present, but in the presence of even a very small amount of invertase, it takes place rapidly. The chemical process involved in the digestion of carbohydrates, proteins, and facts, is one of hydrolysis. Enzymes which bring about rapid hydrolysis are known as hydrolytic enzymes, and are spoken of as amylolytic, proteolytic or lipolytic, according to the substance they act upon. They are also sometimes classed as amylases, proteinases and lipases, the ending *ase* being a general suffix added to the name of the substrate. There are many other important hydrolytic enzymes besides those mentioned.

The oxidizing enzymes, or oxidases, form another widely occurring and important class, as they doubtless take an essential part in life processes. Closely related to the oxidases are the catalyses and peroxidases, which are nearly everywhere present in living matter. The catalases cause liberation of oxygen from hydrogen peroxide, and the peroxidases bring about oxidations in the presence of peroxides. These last are of particular interest, inasmuch as oxidation effected by hydrogen peroxide and the peroxides as a class, seem to resemble more closely the oxidations taking place in the tissues, than does any other type of oxidation, and it is thought probable that the peroxidases play a very important role in body metabolism. Little, however, of an exact nature is known about the metabolism enzymes. Other important groups are the sugar-splitting, or glycolytic, and protein-coagulating enzymes.

So far it has been impossible to separate any enzyme in a perfectly pure form, so that little of a definite nature is known regarding their chemical characteristics. They are all soluble in water, but are insoluble in strong alcohol, chloroform, acetone and the like. Glycerin dissolves most of them but not so well as does water. As they are colloidal substances and occur associated with protein matter, any attempt to precipitate them, throws them down always contaminated with large amounts of impurities from which it is impossible to separate them, as the methods that would be required for their purification destroys their activity. It is doubtless due to their colloidal nature and the tendency they therefore have to form adsorption compounds with other colloids, that many of the intracellular enzymes cannot be separated from the cell proteins.

The activity of enzymes is very markedly affected by slight changes in temperature. If raised above a certain temperature, usually about 50° C., their activity rapidly falls off owing to a gradual destruction of the enzyme. All enzymes if in a moist condition are destroyed by boiling, though if in a dry state they may be heated, frequently to higher temperatures.[1] Each enzyme acts best at the temperature in which it acts in nature. For plant enzymes this is about 25° C., and for animal enzymes about 40° C.

Enzymes are likewise usually very sensitive to the reaction of the medium in which they function. Some, like pepsin, act best in a faintly acid solution, others, like trypsin, in one faintly alkaline, while some act best in neutral solutions.

There is at present some disagreement regarding the extent to which the activity of enzymes is specific. Some take the view that enzymes having similar actions are the same. Accordingly there is no ground for belief that any difference exists between the ptyalin of the saliva, and the starch-splitting enzymes of the pancreas, muscle tissue, or plants. Others incline to the view that each enzyme from a different source is different and that each is limited in its action to some particular substance. Without taking such an extreme view we can with safety conclude that there is a very high degree of specificity in enzyme action. Lipases do not act upon starch, amylases do not act upon protein, neither do proteinases act upon fats. While all lipases effect the same action on fats, namely their hydrolysis into glycerin and fatty acid, they do not all act equally well under like conditions. The lipase of the stomach acts best in a slightly acid medium, that of the pancreas occurs as a zymogen and is activated by the bile.

The work of E. Fisher shows that in the case of sugar, and the polypeptides that there is an intimate relationship between the optical properties of the molecules of the substrate and the enyzme that acts upon it. He has likened the action of enzyme on a substrate to that of a key on a lock. The indications are that enzymes act only on substances having a chemical resemblance to themselves. It appears that the action of an enzyme is limited to a certain arrangement or grouping within the molecule, and a given enzyme will act only upon substances possessing this grouping.

There is much evidence that enzymes in acting upon substances first form temporary compounds with them, these subsequently break down as the substance changes, the enzyme is set free and immediately forms a new union with other molecules of the substance.

Many of the most important enzymes do not exist in the cells in an active form, but are excreted, or zymogens which require activation by the enzymes. These activating agents are called co-enzymes. Thus enterokinase activates trypsinogen and forms trypsin. There is an interesting class of substances which inhibit the action of enzymes, and are called anti-enzymes. Thus in blood serum there exists a substance which destroys the action of trypsin and is known as anti-trypsin; substances can be extracted from intestinal worms which prevent the digestive enzymes from acting. Antiferments are also developed in the blood of animals which have been injected with solutions of enzymes. Whether these anti-enzymes are true enzymes, or whether they act by forming more stable compounds with the enzymes has not been definitely settled.

The methods by which enzymes are formed and their action controlled by

[1] For behavior of enzymes at low temperatures see article by Joseph Samuel Hepburn in this issue.

the cells are not known, but they without doubt form a most important class of agents, through which the chemical operations of life are accomplished. Many of the tissues are abundantly supplied with them. Twenty or more have been shown to be present in the liver. The kidney and muscle tissue are also widely supplied with them.

Among the more familiar processes in which enzymes take a prominent part might be mentioned those of digestion. Here a certain amount of adaptation of secretion to diet seems possible. Lactase, an enzyme present in the intestine of infants, is absent or present in negligible amounts in adults, but constant feeding of lactase results in a revival of its secretion. This adaptability of secretion to diet, may have an important bearing on the ill effects frequently produced by a sudden change in the character of the diet. The oxidizing enzymes have already been mentioned in connection with metabolism. There is one anomaly which finds a probable explanation in a defective enzyme action, namely that of alkaptonuria. Tyrosin and phenyl-alanin, products derivable from protein, are normally completely oxidized in the system, but in alkapton are oxidized only partially to homogentisinic acid. Diabetes may find an explanation in the failure of the glycolytic enzymes of the tissue to oxidize glucose. The clotting of blood, the softening of tissue by pus, the destruction of germs by the leucocytes, rigor mortis, and the softening of tissue by autolysis are familiar illustrations. The formation of urea, of dextrose from glycogen and of glycogen from dextrose, the breaking down of neucleic acid in purins, and the oxidation of the purins to uric acid are other cases, which could be greatly multiplied.

THE BEHAVIOR OF ENZYMES AT LOW TEMPERATURES.*

JOSEPH SAMUEL HEPBURN, A. M., M. S., PH. D.

The influence of low temperatures on enzymes is a subject of growing importance to the chemist, the biologist, and the bromatologist. Problems in this field may be studied from either the potential or the kinetic side; for either the resistance of an enzyme to, or its activity at, low temperatures may be investigated. Various researches, conducted during the last half century, have demonstrated that enzymes survive exposure to low temperatures and also act as catalysts at such temperatures. The reports of these researches are widely scattered in the literature; and frequently the original papers may be obtained for consultation only with difficulty. It is the purpose of this paper, which is based on primary sources, to give a *resume* of our present knowledge of this subject. One section is devoted to the resistance of enzymes to low temperatures, and one to their activity at such temperatures.

* Communicated by the Author to the *Journal of the Franklin Institute,* and reprinted by permission. This contribution is based on a paper to be published by the writer in the *Biochemical Bulletin,* 1915, iv.
In this paper full details will be given of the researches of various investigators on the influence of low temperatures on enzymes, and a complete bibliography appended.