CHAPTER I.

THE NATURE OF FERMENTATION AND ITS RELATION TO ENZYMES.

LIKE so many of the phenomena which are associated with the presence of life, fermentation has been the subject of careful study extending over centuries of time. It has been the object of experiment at the hands of many investigators, and its nature has been the theme of vigorous controversy. Hypothesis after hypothesis has been advanced to explain the various phenomena which it presents, each in turn marking an advance in knowledge, but none of them sufficing to account satisfactorily for the whole of the facts observed. Though within the past two centuries very substantial advances have been made, much still remains undiscovered.

The term fermentation was first applied to the process which leads to the formation of alcohol, the knowledge of which goes back to very remote antiquity. The name probably arose from the copious evolution of gas which accompanies the production of the spirit, and which gives the liquid in which it is taking place the appearance of a gentle ebullition.

It is not surprising that in early times very mistaken ideas arose as to the nature of the process, based upon this appearance. Wherever a disengagement or evolution of a gas was noticed it was thought to be due to similar causes. Hence we find some of the older writers classing with fermentation the effervescence which ensues when an acid is brought into contact with chalk. The evolution of gas in the animal intestine was held more correctly to be a kindred phenomenon.

Though many of the reactions thus grouped together were possessed of very little in common, one process which was of very early date was associated correctly with fermentation. This was the action of leaven in the preparation of bread. The evolution of gas was observed in connection with the raising of the dough, though no further resemblance to the alcoholic fermentation was recognised. Indeed the speculations concerning leaven and its action were of the wildest order, some writers comparing it to the hypothetical philosopher's stone, and attributing to it the power of transforming the dough into something resembling itself.

One fact of importance came out amidst all the mass of confusion, though its interpretation left much to be desired. This was the discovery that a very small quantity of leaven was capable of fermenting or transforming an almost indefinite amount of dough. The latter was however considered to be converted into leaven.

Another point of resemblance between these two fermentative processes was known at a comparatively early date. Just as the aeration of dough was associated with the presence of leaven, alcoholic fermentation was found to be accompanied by the formation of a deposit in the fermenting liquid, which took the form sometimes of a sediment, sometimes of a scum floating on the surface. By many writers considerable importance was attached to this deposit, and, as in the case of the leaven, some special occult force was attributed to it, by virtue of which it set up the changes which could be observed in the liquid. It was consequently called a *ferment*.

The true nature of this deposit, like that of leaven, for a very long period was not investigated, nor the part it plays at all understood.

About the end of the sixteenth century the process of putrefaction was associated with the two fermentations so far mentioned, and the three shown to have much in common. The confusion however which led to the association of effervescence with them was maintained for some considerable time. It was not till 1659 that de la Boë pointed out that they differ considerably from effervescence, the chief reaction of the latter being one of combination, while in the cases of fermentation it is one of decomposition.

The first clear pronouncement upon alcoholic fermentation was made by Becher in 1682, and it marks an epoch in the development of our knowledge of the subject. This author ascertained that only saccharine liquids are capable of undergoing alcoholic fermentation, and he showed that the alcohol does not exist as such in the original "must" of wine, as had been supposed, but is formed during the operation of fermentation. Becher considered its formation to be due to a kind of combustion of the sugar, as he ascertained that air is needed to set up the phenomenon.

About the same time the deposit or scum which had been observed as an invariable accompaniment of fermentation was examined microscopically by Leuwenhoek, who showed it to be composed of little ovoid or spherical globules, but he was unable to determine their nature.

Though the subject was somewhat vehemently discussed and various theories were advanced to explain the observed phenomena, but little real advance was made during the next century. The acetic fermentation was discovered and the similarity of putrefaction and fermentation in general insisted upon. But the nature of Leuwenhoek's globules remained undetermined, many observers, especially Fabroni, holding them to be of animal origin. The work of Lavoisier towards the close of the eighteenth century threw a flood of light upon the process. The great chemist studied quantitatively the relations of the sugar to the derivatives formed from it during the fermentation, and came to the conclusion that the operation consisted of a splitting up of the sugar into two parts, one of which became oxygenated to form carbonic dioxide, while the other was converted into alcohol. He said that if it were possible to recombine these two bodies, sugar would again be formed.

About the year 1815 analyses by Gay-Lussac, Thénard and de Saussure fixed definitely the composition of sugar and alcohol. During the early years of the present century indeed the views of Lavoisier were the basis of much research from the chemical side, and the work resulted in determining the changes which take place in the sugar.

During the same period inquiries were made by many observers into the nature of the globules and their relation to the fermentation. Astier in 1813 and Desmaziéres in 1826 adhered to Fabroni's view that they were of animal origin, Astier holding that they could only live at the expense of the sugar which they decomposed.

Shortly afterwards Cagniard de Latour, repeating Leuwenhoek's experiments, saw that the globules consisted of a definite organism, capable of reproduction by budding, and belonging apparently to the vegetable kingdom. He concluded that probably they disengaged the carbonic dioxide and fermented the liquid by some effect of their vegetation.

This discovery, which is really the basis of the present views of the subject, was also made almost simultaneously by Schwann at Jena, and by Kützing at Berlin, who were confirmed by Quevenne, Turpin and Mitscherlich. The organism was referred by some to the Fungi and by others to the Algae, but its true systematic position was first ascertained by Meyen, who pronounced it a fungus and placed it in a new genus, to which he gave the name *Saccharomyces*.

These researches laid the foundation for the more complete and satisfactory views of Pasteur, whose investigations have thrown so much light upon the whole process of fermentation. The association of a definite organism with the decomposition of the sugar and the idea that the latter was in some manner connected with the manifestation of its vital processes, removed the question from the realm of chemistry in the narrower sense and gave it a place among the problems of physiology. Pasteur in studying the subject from the latter point of view made the very important discovery that the production of alcohol is accompanied by the coincident formation of glycerine and succinic acid, and he determined by quantitative methods that about 4 per cent. of the sugar which disappears during the progress of a fermentation gives rise to these two new derivatives.

Pasteur came to the conclusion that the exercise of the fermentative power by the Saccharomycete, or yeast, was connected with nutrition in the absence of free oxygen, and that it was really the expression of the effort of the organism to obtain oxygen in the absence of a free supply. In this connection some investigations of Lechartier and Bellamy may be recalled. These observers made a number of experiments with ripe succulent fruits, which they kept for several months in an atmosphere devoid of oxygen. Under these conditions the fruits gave off continuously a certain quantity of carbon dioxide, and at the end of the time of observation the pulp contained a measurable amount of alcohol. Microscopic observation showed that this fermentation took place in the absence of yeast cells, and was in fact carried out by the living substance of the cells of the pulp. Pasteur confirmed these observations and regarded them as strengthening his hypothesis.

Schützenberger has argued with some force against the view that the decomposition of the sugar is related to the respiratory process of the yeast, preferring to regard it as ministering to its nutrition. A discussion of this point must however be left to a subsequent chapter.

Though the views of the Pasteur school did not at once meet with universal acceptance, being opposed strongly by Liebig and by Naegeli, both of whom supported a theory of molecular vibration as explaining the decomposition of the sugar, no doubt is now entertained that the living protoplasm of the vegetable cell is the ultimate cause of the fermentative process, and that the latter is the expression of some activity connected with the maintenance of its life. That it is not a specific peculiarity of the protoplasm of the yeast cell, but is shared by that of much higher plants, is equally clearly shown by the work of Lechartier and Bellamy already alluded to.

The influence of living cells of yeast in the fermentation or leavening of dough, and that of other vegetable organisms in the processes of putrefaction established during the present century show how essentially similar these processes are to the alcoholic fermentation. Micro-organisms have been found capable of setting up also many other decompositions comparable with all these.

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Whilst Pasteur's views were gradually making themselves felt, work in another direction was progressing which was destined to materially enlarge our views of the whole subject. Quite in the early part of the present century (1814) Kirchhoff observed that germinating barley contained something which was capable of liquefying starch-paste and that in the process it formed a kind of sugar. Kirchhoff associated this power with the albuminoid material or gluten of the grain. In 1823 the observation was repeated by Dubrunfaut, and in 1833 Payen and Persoz extracted from germinating barley the substance which effected the decomposition. They steeped the grain in water for some time and filtered off the extract. On adding a large excess of alcohol to the filtrate they obtained a white flocculent precipitate, which when dried and dissolved in water was found capable of converting starch-paste into sugar. The change induced by this substance, which they called *diastase*, was held to be a kind of fermentation. It appeared to act in the same way as the globules of Leuwenhoek, the nature of which was at that time undetermined, as already explained. At the same time it was clearly an unorganised substance, though very little was definitely known about it. On this account it was distinguished as an *unorganised ferment*.

Two years earlier Leuchs had noticed that saliva possessed the same property as the germinating barley in that it was capable of converting starch-paste into sugar. In 1845 Mialhe showed that a preparation of diastase could be prepared from this animal secretion by the same process as Payen and Persoz had adopted for their barley extract.

This discovery was followed by others. In 1836 Schwann demonstrated the existence of *pepsin* in gastric juice, and showed that it decomposed indiffusible albuminous bodies into others that were capable of passing through membranes. Berthelot found that a watery extract of yeast, quite free from the cells of the plant, was capable of converting cane-sugar into two other sugars of simpler composition. Liebig and Woehler discovered a similar body in the seeds of the almond, which decomposed amygdalin with the formation of sugar and other products, and they were again struck by the similarity of the action to that of yeast in alcoholic fermentation. About 1860 Brücke prepared from the mucous membrane of the stomach the ferment first observed by Schwann which split up insoluble proteids, causing the formation of soluble peptone.

All these various ferments were recognized as associated in some way with the living substance of either animal or vegetable cells, and they were soon held to play an important part in the life of the organism from which they were extracted.

Two classes of ferments appeared to be thus indicated, the one a living organism, working only during its own processes of growth and multiplication; the other consisting of substances which could be extracted by solvents from the cells in which they were formed, and capable of setting up decompositions apart from the life of such cells. The two categories were consequently called *organised* and *unorganised* or *soluble* ferments. The term *enzyme* is now generally used to indicate the latter class.

In studying these various bodies and the ultimate relations of the one class to the other it is evident that attention must be given to the vital phenomena of protoplasm, and to the changes which take place in its substance during the manifestation of its life. The work of recent years has thrown much light upon the various operations that take place in cells, and we now know that these are for the most part, if not entirely, regulated by the behaviour of the living substance.

Various views have been put forward as to the arrangement of the living protoplasm. Though this is a matter which can by no means be regarded as finally established, there is some probability that it is disposed in the form of a network, the meshes of which are occupied by a material which is similar in composition but which is not living. The great characteristic of protoplasm is its instability; it is continually undergoing decomposition and reconstruction. Some of the residues of its breaking down are capable of being built up again into its substance; others are thrown off from it. Of these some are eliminated entirely from the organism, others are retained within it to carry on some of the more subordinate processes of its life or its nutrition.

An animal or vegetable cell is hence the centre of very vigorous activity; work is going on within it in the direction of incorporating material for the growth of the living substance, or of preparing material brought to it, so that it may be capable of such incorporation. Again some of its substance may be undergoing decomposition with a view to supplying the energy which it needs for the maintenance of its vital processes.

The chemical changes involved may be of three kinds. The decompositions may involve the incorporation of material into the actual substance of the protoplasm and the subsequent splitting off of various residues from the latter. Such appear to attend the formation of the various enzymes such as diastase; also the formation of fat, starch, and other compounds which can be seen in various cells. Other changes may take place without the establishment of such an intimate relationship with the protoplasm. They may be carried out by the protoplasm outside its own substance, the materials affected not being incorporated in it while the change is taking place. Such decompositions have been alluded to by various writers as caused by the fermentative action of protoplasm itself.

A third class of reaction may take place in the cell without the actual intervention of the protoplasm at all. It is probable that processes of oxidation and reduction are taking place among the substances which occupy the meshes of the protoplasmic network, and that quiescent as the cell appears it is the seat of many chemical reactions of this kind. Thus the formation of sugar in the cells of leaves under the influence of chlorophyll, which probably involves the polymerisation of some form of aldehyde, need not necessarily involve the action of the living substance, as such polymerisation is very frequent among aldehyde bodies.

Some of the decompositions of the latter class may be distinguished from the others by the fact that though protoplasm is not immediately concerned in bringing them about, it prepares from its own substance an enzyme by which the transformation is effected. The secretion or formation of this new factor belongs to the first class of reactions mentioned, but the material once secreted is endowed with the power of setting up and maintaining the decompositions in question. The ultimate purpose of the secretion is usually the digestion of some form of food material to prepare it for incorporation into the living substance itself. But recently reason has been found to believe that the processes of oxidation and reduction are carried out by similar agencies. We can consequently recognise in many organisms both digestive and respiratory enzymes. So many of these bodies are now known that it does not seem very unreasonable to put forward the view that all decompositions of this kind will ultimately be found to be carried out by such a mechanism. The number of cases in which the direct rather than the indirect intervention of protoplasm appears to be involved is continually growing smaller as further work upon metabolism proceeds. A very important addition to our knowledge in this direction has been made during the past two years by Buchner, who has ascertained that even the earliest known fermentative process, the formation of alcohol from sugar, is carried out by a soluble ferment which can be extracted from the yeast cell.

The constructive processes which take place in the cell have not been so clearly shown to be carried out by such secretions. In most cases in which a building up of complex from relatively simple substances takes place in the cell, it is carried out by the direct intervention of the protoplasm. Hence the study of fermentation is mainly directed to processes of degradation whereby complex substances are broken down into more simple ones. To this point we shall return later.

We may consequently for the present define fermentation to be *the decomposition of complex organic material into substances of simpler composition by the agency either of protoplasm itself or of a secretion prepared by it.*

We find instances of both these methods in unicellular and in multicellular organisms, as well in the animal as in the vegetable kingdom. By far the greater number of instances of the fermentative activity of the protoplasm as apart from secreted enzymes may be found in the so-called *organised* ferments, the yeasts and the great group of micro-organisms or *Schizophyta*. So prominent indeed are these plants in their power of exciting various decompositions that they were originally regarded as the only "ferments," the fact that the "fermentation" was merely incident to their own biological process passing almost unnoticed. The theory that this power or property puts them into a class distinct biologically from other and higher plants is however quite disproved by the discovery that many of them provoke the decomposition associated with them by means of enzymes, which can with a little care be prepared and separated from them with almost as much ease as from the higher plants themselves. Moreover the enzymes they secrete are identical with those which are prepared by the latter.

It follows from this that those processes of fermentation in which the protoplasm is directly involved are intracellular. It is only comparatively lately that this has been realised in the case of many vegetable organisms. The presence of a cell-wall clothing the living substance would render impossible the contact necessary to produce externally the decompositions observed. This point still needs emphasising in the case of many of the bacterial fermentations. Not only in unicellular but in multicellular organisms also this can be observed. Instances are afforded by the alcoholic fermentation of ripe fruits noticed by Lechartier and Bellamy, and more recently studied by Gerber; by the transformation of glycogen into sugar in the muscles and possibly the liver of the higher animals, though it is not certain that the latter is not the seat of a fermentation by a variety of diastase. The power of forming acids possessed by various bacteria is shown also by the cells of the succulent parenchyma of the higher plants. Though acetic acid is formed by *Mycoderma aceti* from alcohol, and such parenchyma appears to form the acids it contains from sugar, the protoplasm in both cases is the active agent. The acids are more complex in the latter case, but this is probably due to the character of the metabolism of the two classes of cells respectively.

Turning to the processes of fermentation which are carried out by means of enzymes we find more complexity. In the simplest cases of unicellular plants and animals intracellular fermentation is most general. The complex substance, which is usually a food-material, is absorbed into the cell; an enzyme is secreted there, and the work of transformation follows. From various unicellular plants enzymes have been extracted