CHEMICAL SECTION.

Stated Meeting, held Thursday, November 5, 1903.

Fermentation without Yeast Cells: A Résumé of Buchner's Work.*

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I. HISTORICAL.

When fruit-juices or sugar-solutions are exposed to the air, the phenomena of fermentation gradually appear. A gas is evolved, the clear solution becomes turbid and a precipitate called yeast makes its appearance. At the same time, the liquid loses its sweet taste and acquires an alcoholic flavor and intoxicating properties. These observations are very ancient; fermented drinks have been prepared since the earliest times; but it is only since Lavoisier that it has been known that the chemical change is chiefly the separation of sugar into carbon dioxide and alcohol.

The rôle of the yeast long remained obscure. It was known that fruit-juices could be preserved if boiled and sealed up still hot; but this was ascribed to the absence of oxygen in the vessel. Leuwenhoek had ascertained in 1680 with the microscope the spherical or ellipsoidal shape of the individual constituents of yeast; but, instead of considering this a proof of organized structure, had regarded the substance as merely an albuminous precipitate, and compared the cells to starch granules. But in 1835-37 Cagniard de Latour and Theodor Schwann almost simultaneously made an important advance. The growth of the yeast was studied and its status as a living plant placed beyond doubt. It was shown that fruit-juices could be pre-

* Buchner has recently published a connected account of his researches (*Die Zymasegärung*, 1903). Those who wish to familiarize themselves with the revolutionary change which has been brought about in our views of fermentation, will find this book authoritative, fascinating and complete. It has been very freely used in the preparation of the present paper.

served by the boiling process even when there was a large supply of oxygen in the sealed vessel. Fermentation does not occur even when air is allowed access to the boiled juice, provided that the air has previously passed through a red-hot tube, or bubbled through strong sulphuric acid. On the other hand, the admission of ordinary air immediately causes fermentation and organisms simultaneously appear in the liquid.

Upon this basis was erected the "*vitalistic theory of fermentation*," according to which the cause of the phenomena is the life-processes of the yeast plant. Schwann assigned to the sugar the rôle of nutriment and to the alcohol and carbon dioxide that of excretion-products. This view was bitterly opposed by the chemists of the period, Liebig at their head. It is not remarkable that just after Wöhler's synthesis had dethroned the "vital principle" in organic chemistry chemists should object to converting what had been regarded as a chemical process into a vital act. Berzelius had already (1828) suggested that the action of the yeast was catalytic, and Liebig developed this idea into his "decomposition theory." He regarded the yeast as a body engaged in active putrefaction in the presence of which sugar became infected with the same change, just as a rotten apple may contaminate a good one, or a little soured wine produce a rapid transformation of good wine to vinegar under favorable conditions. This ill-defined conception had no experimental evidence in its favor. Dead and decaying yeast might have been regarded from this point of view—hardly the living, growing plant. But Liebig's influence was so immense that the idea held its ground until routed by the decisive experiments of Louis Pasteur, who proved in the most complete way that no putrefaction of the yeast occurs during fermentation; that the process depends upon the life of the yeast-cells, not upon their death, and that fermentation can occur in the entire absence of decomposing albuminous materials. The function of air in exciting fermentation in sterilized liquids he cleared up by filtering air through collodion wool, dissolving in alcoholic ether and examining the residue with the microscope. It was found

to consist of the same organisms which are present in fermenting liquids. The destruction of Liebig's theory was completed by the proof that substances engaged in active decomposition, like hydrogen peroxide or a warm solution of ammonium nitrite, did not arouse fermentation.

Thus the vitalistic theory remained in complete possession of the field. But the mere fact that fermentation had never been observed in the absence of organisms does not prove that it is due to the life-processes of the yeast plant. There is an alternative possibility—that the cell contains a substance which acts catalytically on the sugar and brings about the transformation. This is the *enzyme theory*, stated by Moritz Traube in 1858, and that Pasteur himself believed in its possibility is shown by the fact that he made repeated unsuccessful attempts to extract the active substance from the cells by grinding them in a mortar, by freezing and then thawing—the idea being that the cell-wall might thus be burst and allow the contents to escape, and by immersing them in strong saline solutions so as to force the contents through the cell-walls by osmosis.

II. ENZYMES IN GENERAL.

So far enzymes have been obtained only from plants and animals; it has not been possible to prepare them artificially. Most of them are to be regarded as albuminoids, but not all, for some seem to be very low in nitrogen or even to be free from it. They are produced directly from the living protoplasm, and many of them are similar to it in chemical character but not identical with it. They stand on the boundary between living and dead matter, but are not to be considered as living, for the characteristic signs of life, assimilation, reproduction and the organized structure, are lacking. Their extraordinary instability and chemical activity cannot be viewed as evidence of life, for there are substances like colloidal platinum and the diazo-compounds which share these peculiarities. The enzymes are soluble in water and the solution passes through a filter of biscuit-porcelain, by which all bacteria are stopped. A dissolved living organism is unthinkable. The chemical constitution of the enzymes

is extremely complex and they are probably all asymmetric in configuration. This is a natural assumption, since they are complex products of the organism and the different action of some enzymes on enantiomorphous sugars is only explicable on the ground of asymmetry in the enzyme itself.

The enzymes appear to take no part in the chemical changes which they bring about. They are found unaltered after the reaction and are able to produce a transformation which is quantitatively entirely disproportionate to the amount of the enzyme present. In this respect, as Berzelius recognized, their behavior is entirely analogous to that of other substances acting catalytically. Among inorganic catalyzers, especially the colloidal solutions of metals like platinum, show striking analogies with the enzymes. This is interesting because it shows that the chemical activity is very possibly connected with the huge surface-development of a substance held in colloidal solution. The solutions of the enzymes are also colloidal.

III. THE EXTRACTION OF THE CONTENTS OF THE YEAST-CELL.

The cell-wall of yeast is far too elastic to be ruptured by mere grinding in a mortar. But when yeast dried by compression is mixed with an equal weight of fine quartz-sand and one-third of its weight of infusorial earth and the mixture vigorously ground, the cell-walls are burst by the attrition of the quartz particles and their contents escape. At the same time, the mixture, which is at first a dry white powder, becomes moist, gray-brown and plastic. With 400 grams of the mixture this change takes place in less than five minutes. The mixture is then subjected to strong hydraulic pressure, and the cell-liquid escapes and is received in a vessel immersed in ice-water. The sand plays the chief rôle in bursting the cell; the object of the infusorial earth is to make the mixture less slimy, so that it supports pressure better and yields a clearer filtrate. Only a portion of the cells is opened in this operation. If anything like a complete extraction of the cell-contents is desired, the grinding must be repeated a number of times. In one experiment there were five repetitions of the grinding and compression,

and in all 700 cubic centimeters of cell-liquid were obtained. The last fraction showed strong fermenting action, and it was clear that the yeast was by no means exhausted.

The elasticity of the cell-wall explains the failure of Pasteur's attempt to extract the contents by freezing. When the liquid is frozen, the membrane is distended a little, but not burst, and upon thawing it remains intact. But if the yeast is *pulverized while frozen*, the cell is broken, and upon melting the contents escape. This is the explanation of the following experiment: When 500 grams of dry yeast are mixed with 500 grams of solid carbon dioxide and the mixture ground in a mortar, the mass, which is at first frozen to stony hardness, gradually becomes soft and semi liquid. Upon filtering with a pump, only 100 cubic centimeters of cell-liquid were obtained, but its fermenting power was energetic. This method has not been used much, owing to the expense, the small yield and the long grinding required.

IV. THE PROPERTIES OF THE CELL-LIQUID.

The liquid obtained by either of the preceding methods is yellow or brownish-yellow and almost clear. It smells and tastes strongly of yeast. The reaction is acid, and this is not due to carbonic acid, for the acid reaction is not removed by evaporation to one-half volume in a vacuum. Addition of strong potassium hydroxide, of mineral acids, or of acetic acid, causes such a copious coagulation of albumin that the whole mass often becomes solid. The same thing happens when the liquid is boiled. If it is only warmed gently (50°), the coagulation is slight, but the fermenting power disappears. Upon preservation, the fermenting power of the liquid is soon lost, and then, when it is boiled, the coagulation is much diminished, or does not take place at all. This loss of activity is rapid at ordinary temperatures, but even in a refrigerator it is very noticeable in one day, and complete in two or three.

V. ACELLULAR FERMENTATION.

When sugar is dissolved in the liquid, or a strong sugar-solution added to it, the phenomena of fermentation mani-

fest themselves almost at once—so rapidly that is possible to show the process as a lecture-experiment. Thus in one experiment in which 100 cubic centimeters of cell-liquid were mixed with sugar: 6 cubic centimeters of gas were evolved in the first fifteen minutes; in another similar experiment, 6.5 cubic centimeters of gas in the same time. This gas is pure carbon dioxide, for it renders lime-water turbid, and when collected over mercury is completely absorbed, if a little solution of caustic potash is admitted to the tube. The process has been followed quantitatively, and it has been shown that carbon dioxide and alcohol are produced in approximately equal quantities. It will be remembered that this is also the case in ordinary yeast-fermentation as shown by the familiar equation, due to Gay Lussac:

In this respect, and in the considerable development of heat which occurs, acellular fermentation is similar to ordinary fermentation. But there are some important differences. Thus, the cell-liquid obtained by the methods just discussed, slowly ferments glycogen, dextrin and even starch, which is not the case with living yeast-cells. Again, yeast-cells act upon glucose more rapidly than upon fructose, so that a fermenting solution of invert-sugar gradually becomes more and more lævo-rotatory. But the cell-liquid ferments glucose and fructose with exactly the same speed, so that the volume of CO_2 , liberated in the two cases, is exactly the same after the same lapse of time. Of course, the same volume of cell-liquid was taken in the two experiments, and the same weight of glucose in one as of fructose in the other. The most probable explanation of this interesting fact is that glucose and fructose differ in the speed with which they diffuse through the cell-wall, and when the membrane is removed and the liquid acts directly upon the sugar the difference disappears.

It is well known that small quantities of glycerine and succinic acid are so constantly produced in ordinary fermentation that Pasteur regarded them as products of the process in the same sense as alcohol and carbon dioxide. Now

it is so difficult to estimate glycerine and succinic acid in such a mixture as the cell-liquid that Buchner does not regard his experiments as quite conclusive, but they indicate strongly that in the acellular fermentation these two substances are not produced at all. We shall have to regard them, therefore, as products of the nutrition of the cells, having no direct connection with the fermentation process.

VI. THE CAUSE OF FERMENTATION IN THE ABSENCE OF YEAST-CELLS.

The most important fact that emerges from the experimental results just stated is that the complex mechanism of the yeast-cell is not necessary for the fermentation process. The phenomena occur in its absence. But is this conclusion certain? Is it not possible that a sufficient number of cells have passed through the cloth of the hydraulic press and escaped the following filtration to account for the results? This objection seems serious, but it is easily met. The acellular fermentation is too rapid and energetic to be caused by the few hundreds of cells which could be present in the liquid. Millions would be necessary. More conclusive is the fact that the cell-liquid can be passed through a Berkefeldt filter and then through a Chamberland filter of biscuit porcelain without losing its fermenting power. This operation completely frees it from cells, as has been shown by bacteriological methods.

Driven from the conception that the yeast-cell is the active agent, the advocates of vitalistic views retired a little and took up a new position. True, fermentation occurs in the absence of the yeast-cell, but the protoplasm of the cell is still living. This escapes from the cell when the wall is broken and gets into the liquid. It is to the action of this living protoplasm upon the sugar that fermentation is due.

It is difficult to speak with proper moderation of an explanation which requires us to think of a "living liquid," soluble in water—of a "living" something which passes unchanged through filters impervious to all organisms—of a "living" substance which has no structure, no assimilation, and no reproduction. Anyone who advocates this view

should be required to explain exactly what he means by life, since it is clear that his conception of the term differs profoundly from that of normal men in general. We might as well regard the carbon dioxide of the lungs or the oxygen from plant-leaves as living.

This view may be dismissed as not possessing the definiteness which we have a right to demand of a scientific hypothesis. When, as in this case, a great step has been made in the separation of a complex phenomenon into its elements, the attempt to retain the old explanation by altering a definition is always futile and retrogressive. It may be worth while to mention a few facts which make the explanation—so far as it is capable of definite formulation—entirely inadmissible. Toluene in 1 per cent. solution entirely arrests the growth and fermenting action of yeast-cells, but does not interfere with acellular fermentation. We should have to assume, therefore, that after the removal of the cell-wall the naked living protoplasm becomes much less sensitive to the action of the toluene than it was before. The active agent in the cell liquid is precipitated as a white powder by adding a mixture of alcohol and ether. It can even be dissolved in water and the precipitation repeated without destroying the fermenting power. Yeast-cells are immediately killed by this treatment and it is impossible that living protoplasm should survive it.

When yeast-cells are killed by being soaked in acetone or in alcohol the vital functions of growth and assimilation cease, but the fermenting power remains, although the protoplasm is undoubtedly dead. In one experiment some of these *dead* cells were ground with quartz and infusorial earth and subjected to pressure just as previously described. The resulting liquid—which possessed a strong fermenting power—was precipitated by a mixture of alcohol and ether. The solid was dissolved in water to a clear light-brown liquid and again precipitated with alcohol and ether. The liquid produced by dissolving this final precipitate in water fermented sugar vigorously. A “living” liquid extracted from dead yeast-cells and passing still living through the treatment just described is simply unthinkable.

The experimental results, therefore, without any admixture of speculation amount to this: that the cell-liquid contains a substance which gives rise to that separation of the sugar-molecule which we call fermentation. This substance cannot be regarded as living, for it can be dissolved in water filtered through biscuit porcelain and precipitated again, dried and heated to over 100° without losing its activity. It is contained abundantly in dead yeast and can be extracted by exactly the same methods which apply to the living cells. In short, it behaves in exactly the same way as other unstable chemical compounds.

Now, the preceding paragraph—which contains nothing but pure fact from beginning to end—is simply a brief statement of the enzyme theory of fermentation. Moritz Traube's speculation has become a fact. Yeast contains an enzyme which is the active cause of alcoholic fermentation and the process must be classified with the other enzyme actions: it has been transferred from the domain of the biologist to that of the chemist.

VII. EXPERIMENTS DIRECTED TOWARD THE ISOLATION OF ZYMASE.

Buchner has given the name *zymase* to the enzyme of yeast, and has made many interesting experiments on its separation from the cell-liquid. That these attempts have not led to its preparation in pure condition is not surprising, for zymase, like all enzymes, is unstable, and the cell-liquid is a very complex mixture.

When the cell-liquid is simply frozen in a tall cylinder and then allowed to melt, it separates into two layers. The upper one is colorless, and consists mostly of water; the lower is red-brown, dense and ferments sugar much more intensely than the original liquid.

When the cell-liquid is allowed to fall into a mixture of alcohol and ether, a white precipitate is produced, which contains all the zymase. This is filtered at a pump and dried in a desiccator over sulphuric acid. It is a dry, white powder, which represents about 15 per cent. by weight of the original liquid. When 2 grams of it were mixed with

sugar and water in a long tube, the fermentation was so energetic that in fifteen minutes the mass frothed over at the top. The powder can be dissolved in water and the precipitation by alcohol-ether, repeated without injury to the enzyme, if the operation is rapidly performed. Long contact with alcohol reduces or destroys the fermenting power.

However, the powder prepared by the preceding method is not pure zymase, or anything like it. In fact, it can be shown that, in all probability, the zymase is only a very small fraction of its mass. When cell-liquid is dropped into a small quantity of alcoholic ether, the albumins are precipitated, but not the zymase, so that the precipitate has no fermenting power. On the other hand, when a large quantity of alcoholic ether is employed, the zymase also precipitates. But when equal volumes of the cell-liquis are employed in the two experiments, the weight of the two precipitates is almost exactly the same, though one contains all the zymase and the other apparently contains none of it.*

Instead of alcoholic ether, acetone can be employed in this method, but its use offers no special advantage. Wood alcohol entirely destroys the enzyme, for both precipitate and filtrate are without action on sugar.

When the cell-liquid is carefully evaporated to dryness at 35° under reduced pressure, a yellowish powder is obtained, which has a pleasant odor of yeast and is completely soluble in water. This possesses all the fermenting power of the original liquid, and retains it unchanged for a year or more. Eight hours heating at 85° did not affect the activity.

VIII. DEAD YEAST.

Yeast is dried as far as possible by pressure, and slowly introduced through a sieve into a large quantity of acetone in a flat dish. The yeast is allowed to remain in the liquid ten minutes. It is then filtered and subjected to a second acetone treatment. Finally, the acetone is removed by

* It may be pointed out *en passant* that this experiment constitutes another disagreeable difficulty for the advocates of living protoplasm. They are forced to assume that the protoplasm is killed by a small quantity of alcoholic ether, but resists perfectly the action of a large quantity.

washing with a little ether, and the product heated to 45° for a time to drive off the ether. The result is a dry, white powder. It contains no living cells, for it is incapable of growth or reproduction, as has been shown by convincing experiments. Its fermenting action is intense. A tube half filled with a liquid containing 2 grams of dead yeast and 1 gram of sugar foamed over forty minutes after the beginning of the experiment. Dead yeast retains its activity almost unchanged for six months or more. All these facts are inexplicable by the hypothesis of living protoplasm, but they are natural consequences of the enzyme explanation.

IX. CONSEQUENCES.

The great result of Buchner's work is that the yeast-cell is non-essential to the fermentation process. Its only function is to produce and store up the zymase. Fermentation itself is a purely chemical process. We are now in a position to answer the old question whether fermentation takes place inside or outside the yeast-cell. Since the zymase never passes through the unbroken cell-wall the inside of the cell is the theatre of the process. The sugar goes to meet the enzyme by diffusion through the membrane and when we consider the speed with which fermentation progresses it is clear that the cell-membrane must be the seat of osmotic phenomena of exceptional intensity.

Zymase is the first example of a new class of enzymes—those which never leave the cell during its life. These may be called endo-enzymes. Most enzymes, when secreted, leave the cell and exert their chemical activity in an outside liquid. It will be observed that the cell loses some of its importance in biology as a result of this work. It seems that for every specific chemical act of the cell there is a specific enzyme which can do the same thing in the cell's absence. When the process of dissection and explanation of the cellular activities is finished what will remain as actual vital performance?

The scientific importance of Buchner's discovery is immense, but there is no prospect of any direct practical application. The synthesis of zymase, while a possibility,

is a very remote one, and in the meantime it is far simpler to employ yeast than to extract the cell-liquid and use it for fermentation. For in yeast new zymase is constantly produced, while in the cell-liquid or the precipitates made from it, this is impossible. Indirectly the practical results of the discovery will be of vast value. It points the way, for instance, to researches on the production of zymase in the yeast-cell, and the ascertainment of the most favorable conditions for the production and storing up of the enzyme would produce a revolution in fermentation technique. The attainment of this information is now perfectly possible and work directed toward it has already begun.

CENTRAL MANUAL TRAINING SCHOOL,
PHILADELPHIA, November, 1903.

THE PRODUCTION OF QUICKSILVER IN 1902.

The production of quicksilver in the United States during 1902, says Dr. Joseph Struthers in his report to the United States Geological Survey, amounted to 34,451 flasks of 76½ pounds each, valued at \$1,500,412, as compared with 29,727 flasks, valued at \$1,382,305, in 1901, an increase in quantity of 4,724 flasks and in value of \$118,107. California contributed the greater part of the output, amounting to 29,199 flasks, as compared with 26,720 flasks in 1901. Texas reported 5,252 flasks, as compared with 2,932 flasks in 1901, both States thus showing an increase in the production above that of the preceding year. Oregon, which furnished 75 flasks in 1901, reported no production during 1902, the quicksilver mining operations in that State being limited to development work.

The report contains a detailed review, by Mr. William Forstner, of the progress in quicksilver mining in California during 1902. As is well known, California has produced nearly the entire output of quicksilver in the United States, the quantity to be credited to that State since 1850 amounting to 1,913,258 flasks, of which the New Almaden mine, in Santa Clara County, has furnished more than 50 per cent.

The production of quicksilver in Texas during 1902, valued at \$228,620, as compared with a value of \$132,438 in 1901, shows a very active development of the industry. The entire output for both years was produced by the Marfa and Mariposa Mining Company, operating at Terlingua, Brewster County.

The exports of quicksilver for 1902 amounted to 13,247 flasks, valued at \$575,099, as compared with 11,219 flasks, valued at \$475,609, in 1901. Of the exports, 8,913 flasks, valued at \$383,578, went out of the port of San Francisco, nearly one-half going to Hongkong.