EDUARD BUCHNER

Cell-free fermentation

Nobel Lecture, December 11, 1907

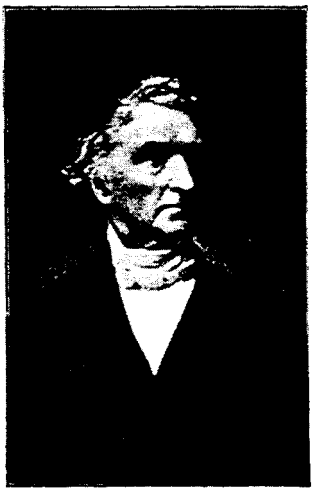
I would first ask the assembled company to allow me to express my sincere gratitude for having been so highly honoured with the distinction of speaking today before the Royal Swedish Academy of Sciences, to which at one time a Scheele and a Berzelius belonged and which at the present time counts Arrhenius among its members, all men whose achievements fill every chemist with admiration. The work on which I have to report lies on the boundary between animate and inanimate nature. I therefore have reason to hope that I can interest not only the chemists but also the wide circles of all those who follow the advance of biological science with close attention. It is difficult, however, for a person to be comprehensible and at the same time remain scientific, so I must ask you to bear with me.

If fruit juices or sugar solutions are left to stand in the open air, they show after a few days the processes which are covered by the name of fermentation phenomena. Gas is seen to develop, the clear solution becomes cloudy and a deposit appears which is called yeast. At the same time the sweet taste disappears and the liquid acquires an intoxicating effect. These observations are as old as the hills ; at any rate, these processes have been used since the most ancient times of the human race for the production offermented liquors. It is, however, only since the end of the eighteenth century - i.e. since Lavoisier - that we have known that during such a process sugar decomposes into carbon dioxide and ethyl alcohol. A short time later Gay-Lussac was able to show that the weight of the sugar reappears almost exactly in the sum of the weights of these two products of fermentation.

The part played by yeast remained for a long time obscure. It was believed that its appearance was of a secondary nature and it was regarded as an inferior kind of precipitation product. The old name for yeast, "Faex cerevisiae", and the expression which has taken root in our language, "die Hefe des Volkes" (the yeast of the people), meaning the outcasts of the nation, also point to this view. Certainly, as early as 1680, the Dutch naturalist Van Leeuwenhoek, who has been called the father of microscopic observation, had established the fairly regular spherical or elliptical shape of yeast, but he

did not succeed in convincing his colleagues of the vegetable nature of yeast.

It was only in the thirties of last century that three researchers, Cagnard Latour in Paris, Theodor Schwann in Berlin and Friedrich Kützing in Nordhausen, reported almost simultaneously that yeast consisted of live cells of a plant. Though priority in the recognition of the fact belongs to the Frenchman, who limited himself principally to microscopic observations, Schwann, who also based his work on experiment, was the first to provide the strict proof, and Kützing extended his investigations not only to yeast but also to



Theodor Schwann (1810-1882). Founder of cytology, who introduced a new epoch in the study of organisms by establishing that plants and animals are built of elementary components.

mother of vinegar, which converts ethyl alcohol into vinegar. The fermentation processes thus appeared as a result of the life activity of micro-organisms.

This vitalistic view, however, received a very mixed reception among the naturalists. In particular there was no lack of keen, even derisive criticism from the greatest chemists of the time, Berzelius, Liebig and Wöhler. Berzelius[1] called the new concept of yeast a scientific-poetic fiction. "If the coalescence of the globules of yeast can be ascribed to the presence of vegetable life, the same reason might well be assumed for the coalescence of globules of clay or calcium phosphate". Liebig and Wöhler[2], however, published a jeering satire in which it was stated that by means of an excellent microscope they had seen the yeast creatures swallow the sugar from the solution, that

this was instantaneously digested in the stomach and that this digestion could be seen immediately and most positively from the subsequent evacuation of excrement. "In a word, these infusoria gobble sugar, and discharge ethyl alcohol from the intestine and carbon dioxide from the urinary organs."

This attitude of total rejection on the part of the foremost chemists is understandable. It was indeed only a few years earlier (1828) that Wöhler had succeeded in artificially producing urea, a substance which had previously been conceived as a type of all substances produced in animal bodies

Justus Liebig (1803-1873). Unrivalled pioneer in the field of organic chemistry, who by his studies on plant nutrition first provided a rational basis for agriculture; the inspiring teacher, to whom Germany owes her first teaching laboratory.

only, under the influence of the life force. No sooner had it been realized, said Liebig, that all life processes in plants, just as in animals, must be conceived as physical and chemical processes, than along came unscientific people and tried to make acts of life out of simple chemical processes.

Attempts were made to produce an explanation of a purely chemical nature. Berzelius assumed that yeast caused the decomposition of sugar catalytically, simply by its presence as a contact substance or catalyst. There seemed to be analogies with many processes-for instance, with the action of very finely divided platinum on hydrogen peroxide which, in the presence of that contact substance, rapidly decomposes into water and oxygen, whilst the platinum apparently remains unchanged. Liebig gave as his opinion that yeast caused fermentation "in consequence of a progressive disintegration

which it suffers in the presence of air in contact with water"[3]. A disintegrating body would possess the ability to cause the same change in another substance touching it[4]. In their views, therefore, the only part played by the yeast was that of an organic compound in a state of continuous decomposition. The idea of their nature being that of living plants, however, was unilaterally ignored by the chemical authorities of the day.

The experimental investigations of the next decade, by E. Mitscherlich, H. Helmholtz, H. Schröder and others, even though they favoured the vital-

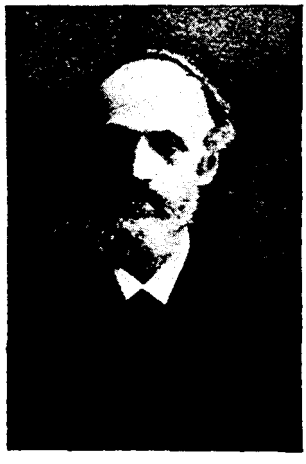
Louis Pasteur (1822-1895). Brilliant chemist and bacteriologist, who at the age of 22 investigated the isomerism of racemic and tartaric acid and hereby laid the foundation for a concept of the structure of molecules; who recognized micro-organisms not only as instigators of fermentation phenomena but also of infectious diseases; who by his discovery of vaccination against rabies has served all humanity.

istic concept, did not produce complete clarity. More extensive and more convincing indeed were the investigations of T. Bail in Danzig which, however, had unfortunately to be broken off for external reasons before any final conclusion had been reached.

It was only the systematic and striking experiments of Louis Pasteur, extending over a decade, which finally led to the *recognition* that in Nature without living organisms, without live yeast, no fermentation exists. This put an end to all disputes. Fermentation was seen to be a physiological act inseparably linked with the life processes of yeast.

Endeavours were now made to comprehend the phenomenon of fermen-

tation biologically and establish its origin in greater detail. Schwann had already conjectured that sugar fermentation coincided with the feeding processes of the yeast. A simpler assumption was made by Moritz Traube in Berlin[5] (1858), according to which there was in micro-organisms a certain chemical body which caused fermentation. Similar substances, chemically very active, which are known today as enzymes, had already been traced on many occasions in vegetable and animal bodies - for instance, diastase in germinating barley, which converts starch into sugar; pepsin or peptase, found



Moritz Traube (1826-1894). Received comprehensive academic training, but was forced by circumstances to become a wine-merchant for many years. He then became active as a private scientist in Berlin: science owes to him a large number of valuable investigations, in particular on oxidation phenomena and on biological problems.

in gastric juice by T. Schwann, which converts into solution and digests coagulated protein; and invertase, discovered by Berthelot in yeast cells, which decomposes cane sugar into glucose and fructose.

This enzyme theory as an explanation of fermentation phenomena found great favour in wide circles. Berthelot, Claude Bernard, Schönbein and Schaer, F. Hoppe-Seyler, G. Hüfner and particularly also Liebig supported it. The plant physiologists, however, principally Naegeli and Sachs, raised weighty objections. In particular, every attempt made to separate such an enzyme from the yeast cells had failed. In spite of extensive investigations, M. Berthelot, Adolf Mayer, Naegeli and Low, even Pasteur himself, the great experimenter had no success. Pasteur's words sound extremely resigned:[6] "In what does for me the chemical process of sugar decomposition con-

sist, and what is its intrinsic cause? I confess that I am completely in the dark about it. Can we say that the yeast nourishes itself on the sugar, only to give it off again as an excrement in the form of alcohol and carbon dioxide? Or must we say that the yeast in its development produces a substance of the nature of peptase which acts on the sugar and disappears as soon as it has exhausted itself, since we find no substance of this kind in the fermentation liquids ? I have no answer to the substance of these hypotheses. I neither accept them nor do I reject them, and I shall always try not to go beyond the facts." Twenty years later two abortive attempts to prove the existence of a soluble fermentation enzyme were published by Denys Cochin[7] of Pasteur's

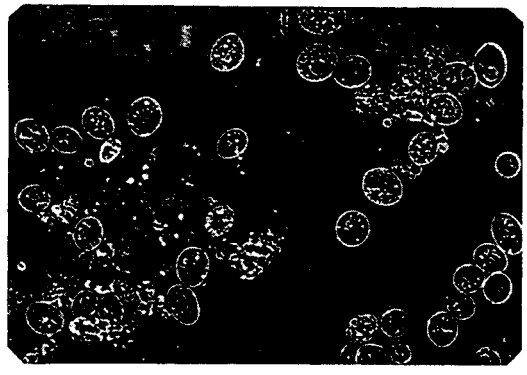


Fig. 1. Microscopic image of yeast, clearly revealing with a 600-fold magnification the substances contained in the cells and in particular the cell membrane (negative picture)[8].

Institute. Through his failure, however, Naegeli was led to set up a new fermentation theory according to which the catalytic action which decomposes the sugar was due directly and exclusively to the living plasma of the yeast cells. This assumption can at least claim the merit that it inspired further experiments. For now the question was: are any special actions attributable to the contents of the yeast cells at all?

Yeast cells must be regarded as small bubbles filled with a semi-liquid body, the protoplasm, surrounded on the outside by a comparatively firm cell membrane (Fig. 1).

This membrane, without openings so far as can be concluded from microscopic observation, must nevertheless be pierced with fine pores which enable the absorption of nourishment and discharge of excretion substances. To the inner side of the cell membrane, furthermore, there is attached a

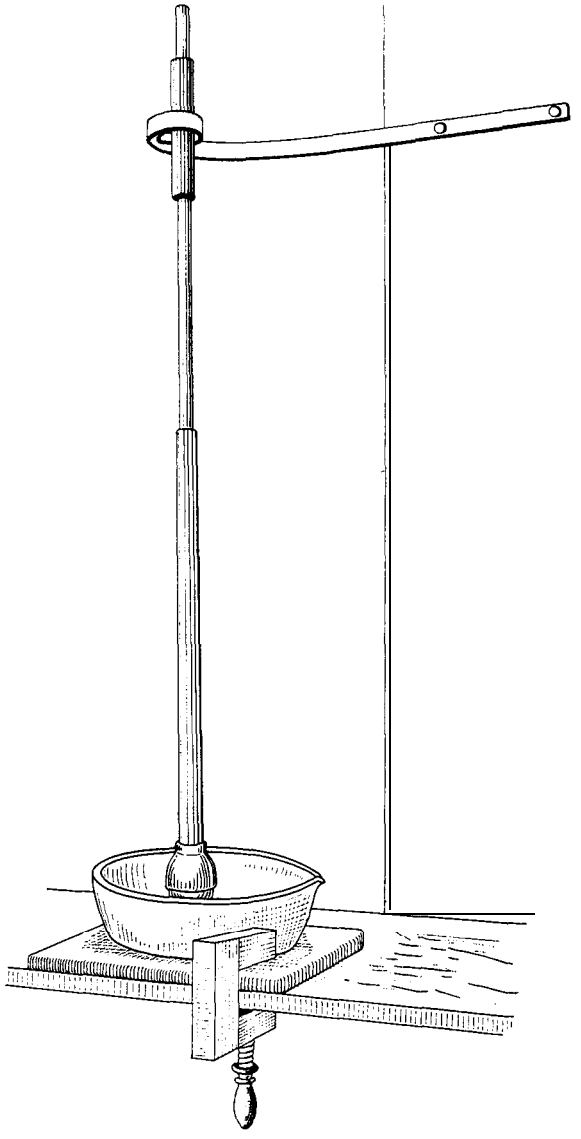


Fig.2. Device for crushing yeast[13].

special layer of plasma, called the plasma envelope. This, too, controls the exit and entry of substances. High-molecular substances will probably be unable to get out of the cells at all. Attempts have now been made to extract the substances contained in micro-organisms by digestion with water for a number of weeks, and further by boiling with glycerine solutions or also

with sodium hydroxides. It is certain, however, that by this means only parts of the contents can be isolated and even these, most probably, only in a changed state.

For the chemical investigation of the cell contents, it was necessary to remove the membrane and the plasma envelope by crushing them to pieces. Furthermore, all chemically active solvents and the use of higher temperatures had to be avoided. Finally, it was important that the process should reach completion in the shortest possible time, which would exclude any change whilst the operation was proceeding. These guiding principles represent the outcome of many discussions with my brother, the bacteriologist Hans Buchner of Munich, who died at an early age.

Still in 1878, Naegeli and Löw[9] had declared : "The difficulties of yeast analysis, when the compounds, not the elements, are involved, consist in the fact that the cells, owing to their smallness, cannot be pulverized, ruptured or burst, so as to separate mechanically the contents from the membrane", and even in 1895 G. Clautriau in Brussels[10] formed from yeast with quartz sand and water-glass a "stone of yeast" *(pierre de levure)* which was dried and then ground in a mill, so that in this way the glycogen of the yeast cells might be isolated!

As was later revealed, Lüdersdorff in Berlin[11] had, as far back as 1846, crushed yeast cells on a sheet of ground glass with the aid of a glass roller, a task which, however, took an hour for 1 g of yeast. When dextrose was added to the pulp thus obtained, "not one single bubble of gas" was given off, and the vitalistic theory thus seemed confirmed. The difficulties of grinding yeast disappeared, however, as was shown for the first time in 1872 by Marie von Manasséin in Wiesner's botanical institute in Vienna[12], when pulverized rock crystal or sand was added at the same time. The pestle then gets the necessary grip. In this way, micro-organisms had already been ground by Adolf Mayer, by A. Fernbach and by Amthor, before the start of my experiments.

If one part of quartz sand and one-fifth part of diatomite, by weight, are added to yeast, the initially dust-dry substance can be ground in a large mortar with a heavy long-shafted pestle (Fig. 2) within a few minutes (Fig. 3). The mass becomes dark-grey and doughy. (Experiment.)

The damp condition shows that liquid has come out of the interior of the cell. If the thick dough is now wrapped in strong canvas and placed in the hydraulic press (Fig. 4), a liquid juice is squeezed out when a pressure increased gradually up to go kg per square centimetre is applied. (Experiment.) Within a few hours 500 cc of liquid can be obtained from 1000 g of yeast, so

that considerably over half of the total cell content is expressed. For developing this method, Professor Martin Hahn particularly deserves great credit. Participating in the first experiments in the Munich Institute of Hygiene as assistant to my brother, he proposed the use of diatomite and a hydraulic press.

"Pressed yeast juice", a yellow-brown liquid, of which I am here handing round samples, smells pleasantly of yeast and in transmitted light appears transparently clear, though in incident light it appears opalescent. On heating, it soon separates flakes of coagulated protein and on further heating this

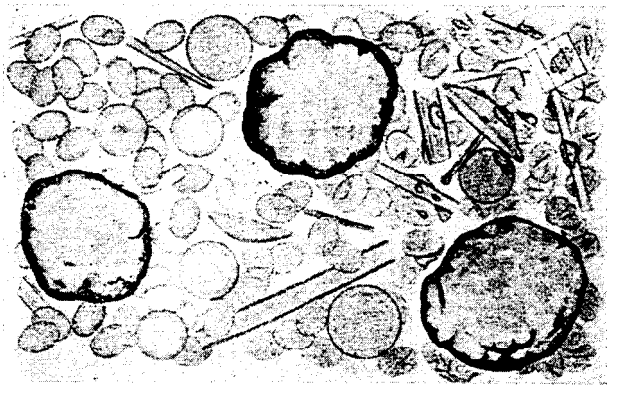


Fig. 3. Diagrammatic representation of the crushing process: yeast ground with quartz sand and diatomite until the cells burst, the contents thereof emerging in the form of mucous lumps from the cell membrane[14].

formation may be so extensive that when the container is inverted scarcely any liquid flows out. (Experiment.) The presence of coagulable protein in the interior of micro-organisms has thus been established for the first time. If the expressed juice *(Pressaft)* is diluted with a quantity of water and hydrogen peroxide is added, a violent foaming begins as a result of oxygen formation. By this means we prove the presence of catalase, an enzyme, discovered by O. Löw, which is known to be present in almost all liquids of vegetable or animal origin, e.g. in blood. If sugar solution is added to freshly expressed yeast juice, a strong formation of gas sets in after a little while. In these containers there is expressed juice which has been mixed with concentrated sugar syrup for some hours. The active frothing of carbon dioxide bubbles and the formation of a thick layer of foam show that a fermentation process has started. When sugar is dissolved in expressed juice at blood heat,

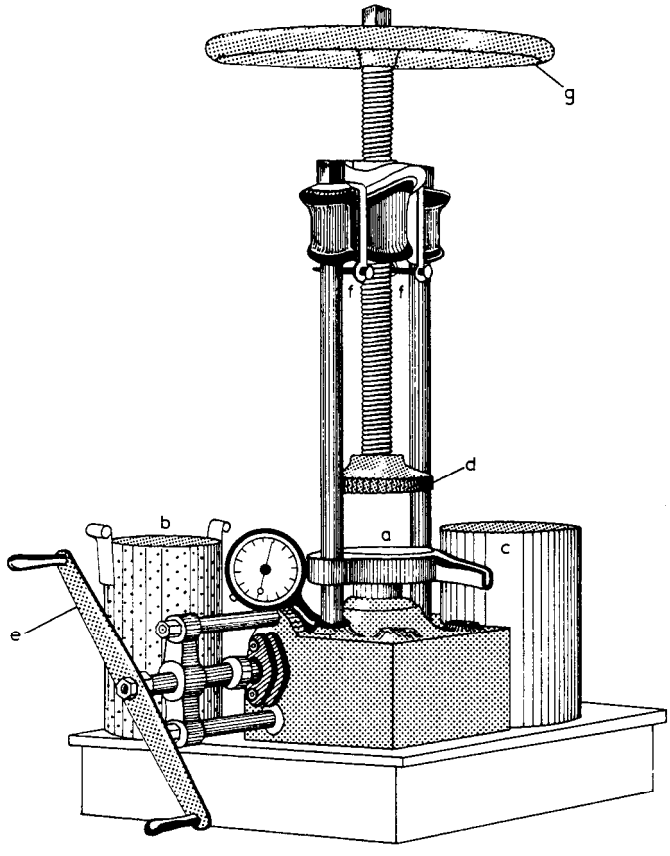


Fig. 4. Hydraulic press, supplied by Brinck and Hübner, machine manufacturers, Mannheim[15].

the phenomena are visible even after about a quarter of an hour. (Experiment.) Careful investigations have shown that the formation of carbon dioxide is accompanied by that of alcohol, and indeed in just the same proportions as in fermentation with live yeast.

The first question now was whether the few yeast cells still present in the expressed juice could in any way be considered to be the cause of the decomposition of the sugar. The answer to this is certainly in the negative, since their number is much too small. The juice can be filtered through a diatomite filter and even through biscuit-porcelain candles, without its action being completely destroyed. M. Delbrück and Lange have also shown in particular that even ten times the quantity of yeast cells normally present in

expressed juice cannot produce any fermentation phenomena in a concentrated sugar solution.

Furthermore, there was reason to assume that the fermentation action of expressed juice was attributable to pieces of live plasma present. This assumption contradicts in particular the behaviour of the expressed juice when antiseptic media are added. Especially, it is seen that toluene actually does prevent the action of live yeast on sugar, though not the action of expressed juice. The naked pieces of plasma, robbed of the protection of the cell membrane, would, however, necessarily be damaged far more in their life processes by toluene than would the uninjured yeast cells. A whole series of further experiments conclusively decides against that hypothesis. First of all, the expressed juice can be concentrated by evaporation at low temperature in a vacuum chamber and finally fully dried. The yellowish residue, reminding one of dried egg yolk (demonstration), is mainly soluble in water and still shows unchanged the fermentation action on sugar. Further, if the expressed juice is added to a quantity of alcohol and ether, a white precipitate is obtained, which can be transformed in the vacuum chamber into a dust-dry powder. (Experiment.) This also is largely soluble in water and causes strong fermentation when sugar is added. Finally, the yeast cells can be killed, without their fermentation action being destroyed. This can be done on the one hand by slow drying followed by heating for many hours - e.g. in a current of hydrogen at 110°C. The preparation so obtained has lost the capacity for growing in beer wort or wort gelatine, but still produces strong fermentation phenomena when sugar solution is poured over it. Altematively, in accordance with processes discovered by Professor Dr. R. Albert in my laboratory and improved upon by head-pharmacist Dr. Rapp, my colleague for many years, one can add the live yeast cells to large quantities of alcohol or acetone and finally wash them with ether. The air-dried "permanent yeast" thus obtained, also called "zymin", is incapable of growth but, when sugar solution is added, can produce an extraordinarily powerful fermentation.

If we now summarize the results of all these experiments, we establish that a separation of the fermentation effect from the live yeast cells can be carried out. To start off a fermentation process, no such complicated apparatus is needed, as the yeast cell is; rather is there a "cell-free fermentation". But even the assumption that the action of the expressed juice is attributable to the presence of still-living splinters of protoplasm must be regarded as refuted, since these hypothetical structures would need to be so small that they would

pass through a Chamberland biscuit-candle and, although deprived of the protection of a cell membrane, survive attacks which kill all micro-organisms. The active agent in the expressed yeast juice appears rather to be a chemical substance, an enzyme, which I have called "zymase". From now on one can experiment with this just as with other chemicals.

This is an appropriate place to point out that something similar to what has just been discussed regarding the alcoholic fermentation of sugar was established as far back as 1890 in respect of what is called urea fermentation, i.e. the conversion of urea into ammonium carbonate. At that time P. Miquel in Paris showed that this process, which gradually sets in in discharged urine, is actuated not directly by the vital activity of the bacteria which appear, but by the agency of a separable enzyme, urease. On the one hand, however, the chemical process in urea decomposition is extraordinarily simple and can be effected merely by heating with water to 120°, i.e. not comparable chemically with the process of sugar decomposition; on the other hand urease was seen to be highly sensitive, so that many confirmatory tests turned out negative. No conclusion regarding the existence of an enzyme of alcoholic fermentation could thus be drawn from urease, nor did anybody carry out any experiments in this direction. Finally, it should be mentioned that in 1894 Emil Fischer found in low organisms various enzymes which had hitherto remained unknown, such as maltase in beer yeast, lactase in lactose yeast and finally, in conjunction with P. Lindner, monilia invertase *in Monilia candida,* using also a process for rupturing the cells. Nothing is reported regarding attempts to find a fermentation enzyme. However, the treatises show at any rate that at that time similar problems were also occupying other professional colleagues. However, my work was by no means instigated by Emil Fischer's investigations, since it began as early as 1893 with the development of the crushing process, while Fischer's first publications appeared in 1894.

Attention should next be drawn to a further property of expressed yeast juice. When kept at ordinary temperature, this very quickly loses its activity. I was able to trace this remarkable behaviour to the presence of a proteolytic enzyme in the juice, endotryptase, which was first found by M. Hahn through the liquefaction which sets in when it is piled up on gelatine. Actually it can be seen that the fermenting power of the juice becomes lost still more rapidly when it is digested with digestive enzymes, e.g. fresh pancreatin or tryptase. The agent causing fermentation is thus destroyed by the presence of a digestive enzyme in the expressed juice, similarly to the case of high-molecular coagulable protein bodies of the expressed juice, which gradually disappear

when stored, so that old juice, no longer very active in inducing fermentation, can be heated to boiling point without flocculation.

The presence of a proteolytic enzyme in the interior of the yeast cells can, as was shown by R. and W. Albert, also be traced without difficulty in "permanent yeast", when the latter is allowed to stand under water and microscopic preparations stained by Gram's technique are manufactured at certain intervals of time with safranin counter-staining[16]. The unchanged permanent yeast then shows dark-blue, completely opaque cells, the same picture as is also given with this staining process by fresh live yeast.

After the damped permanent yeast has stood for some time, the substance whose presence governed the very dark coloration disappears. The cells after staining always appear lighter in colour, many of them directly coloured light-red, though many also still containing very dark-blue grains. The permanent yeast is here in a kind of transitional state.

If, however, the permanent yeast is digested for a longer period with water, the substances which assume a blue coloration when treated by Gram's technique disappear more and more; they presumably become chemically changed. The dark grains also disappear gradually. Finally the cells become almost all uniformly light red in colour. In the way described, therefore, post-mortem chemical digestive processes can easily be detected in the killed permanent yeast, such as must occur regularly after the natural death of all organisms.

There are now two directions in which my colleagues and I have occupied ourselves particularly in recent years. On the one hand it was obvious that we should strive for a preparation of zymase, and a separation from the proteins and the rest of the enzymes of the expressed juice. Experiments in this direction did not meet with any success worth mentioning, however, since all the separation processes used up to now had principally led merely to the destruction of a large part of the fermentation power. Even an enrichment of the live yeast, and hence the expressed juice, with zymase succeeded only within narrow limits.

On the other hand it appeared urgently necessary to follow up most carefully the chemical processes in cell-free fermentation. It was possible to hope that in this way many conclusions could be reached ; for any disturbing influence on the part of the yeast feeding processes was here excluded. It was first of all found that in cell-free fermentation alcohol and carbon dioxide were produced in equal weights, i.e. exactly as in the case of fermentation with live yeast. By contrast, we were able to confirm the assertions of the

English researchers A. Harden and W. I. Young that here the decomposition is by no means complete, but that a considerable quantity of the sugar (in certain cases up to and over 20 per cent) is built up into polysaccharides which cannot be directly reduced but which can be hydrolysed, and are not identical with glycogen. One must accordingly assume the presence in the expressed juice of a synthesizing so-called reverting enzyme. As regards the presence of the usual by-products of the fermentation, it was found that in cell-free fermentation very considerable quantities of glycerol and some acetic acid are formed, though no succinic acid and, at the most, only traces of fuse1 oils. This latter discovery agrees well with the fine investigations of Felix Ehrlich which show that the formation of fuse1 oils and succinic acid has nothing to do with the decomposition of sugar, but is attributable to the splitting up of amino acids by particular enzymes.

We allowed ourselves to become particularly interested in research into the appearance of intermediate products of sugar decomposition during cell-free fermentation. Here it was seen that occasionally considerable quantities of lactic acid develop while in other cases, when the nature of the expressed juice is different, added lactic acid may even disappear in the fermentation. These results, confirmed in numerous experiments have convinced us that lactic acid itself or a close preliminary stage thereof should be regarded as an intermediate product of sugar decomposition. Thus in the first phase the sugar produces lactic acid, which then in the second stage decomposes into alcohol and carbon dioxide. This assumption corresponds perfectly to the views expressed theoretically by Adolf von Baeyer in 1870 regarding the mechanics of sugar decomposition.

As is known, the sugar molecule as it passes through lactic acid can easily be split by purely chemical means. The formation of this product from glucose takes place relatively very smoothly as soon as heated with potassium hydroxide. Then as regards the further decomposition of the lactic acid into alcohol and carbon dioxide, there is something to be said in favour of the. assumption expressed by H. Schade that thereby acetaldehyde and formic acid set in temporarily at the same time. Just how gentle the chemical attack needs to be in order to bring about a decomposition of the sugar in the directions referred to is shown by the work of E. Duclaux, who was able to convert sugar in an alkaline solution, merely by the effect of sunlight, both into lactic acid and also, under other conditions, into carbon dioxide and alcohol.

To support the hypothesis of an intermediate formation of lactic acid, we tried recently to ferment lactic acid with live yeast. In several instances we

appeared to succeed, in the absence of nutriments but in the presence of sugar, in causing added lactic acid to disappear by means of live yeast. The next task is to examine whether indeed alcohol and carbon dioxide are also formed in this process. Only then will lactic acid or a preliminary stage thereof be reliably proved to be an intermediate product.

When this objective is reached, however, we shall also be obliged to accept the notion that alcoholic fermentation is the action not of only one enzyme, but of two different ones, of which the first, which should perhaps specially be given the name of yeast zymase, transforms the sugar into lactic acid, whilst the second, the lactacidase, splits the lactic acid into alcohol and carbon dioxide. To explain the variable behaviour of expressed yeast juice in respect of lactic acid, as mentioned above, it can be assumed that both enzymes are present in the expressed juice in different quantities according to the physiological state of the original yeast. When there is a relative shortage of lactacidase, lactic acid will be accumulated, whereas when this enzyme is present to excess, even the added lactic acid will disappear in consequence of splitting. As Pasteur showed, no lactic acid can be found among the fermentation products of live yeast; the live cells manifestly produce yeast zymase and lactacidase, both to excess. Presumably the yeast zymase will be closely related to the enzyme of lactic acid bacilli, which also splits sugar into lactic acid.

In these truly difficult investigations I have been able to enjoy the distinguished assistance of Professor Dr. J. Meisenheimer, without whose energetic devotion we would never have made such progress.

If it should thus become necessary in the foreseeable future to talk not merely of *one* but of two different fermentation enzymes of the yeast cells, the situation will become even much more complicated by an observation made by Harden and Young. They showed that the fermenting power of an expressed juice can be increased extraordinarily by the addition of boiled expressed juice, so-called boiled juice, itself no longer capable of causing fermentation. The tests set up in London with expressed juice from English top yeast were confirmed in my laboratory with juice from bottom beer yeast. The English researchers further showed that by filtering the expressed juice through a Martin gelatine-filter it can be split into two parts ; neither of these by itself has any effect on sugar, but the re-united parts do have. They therefore assume in the expressed juice the existence of a dialysable chemical substance, unaffected by boiling, which is essential to the activity of the zymase. This so-called "co-enzyme" can also be partially replaced, according to the

discoverers, by the addition of phosphates, and I was able to show that also organic derivatives of phosphoric acid, e.g. lecithin and glycerophosphoric acid, perform similar services. Extremely remarkable, finally, were the results of experiments with which I have occupied myself very recently in conjunction with Dr. F. Klatte. The co-enzyme of the boiled juice appears to be destroyed when treated with certain enzymes of the expressed juice, so that the rapid decline in the fermenting power when expressed juice is stored should often be attributable less to a decomposition of the zymase by the endotryptase than to a destruction of the co-enzyme by other enzymes. For, in a number of cases we were actually able to establish that expressed juice which had been acting on sugar for four days at 22° and had thereby lost its fermenting power, regained all - and in some cases even doubled - its fermenting power on the addition of boiled juice, i.e. of co-enzyme. These experiments raise hopes of an insight into the extremely complicated nature of zymase.

However, the reason why the English researchers were more successful than I in discovering the co-enzyme, although I had already carried out tests with boiled juice many years previously[17], appears to lie simply in the particularly high co-enzyme content in the Munich yeast and in the lesser suitability of its juice for such experiments.

Distinguished audience. As you see, we are still far away from an understanding of the processes involved in the alcoholic fermentation of sugar, as well as from a more detailed description of the nature of the zymase. Quite the contrary, every step we take at present leads to fresh complications. We must be thankful, however, if the increasingly narrow and steep paths do not end up in an unclimbable cliff. Still, we have no reason to be particularly surprised at this unsatisfying situation, since we are still nowhere near any real understanding of the nature even of the simplest enzyme – e.g. even invertase, the chemical action of which has been perfectly known for a long time - in spite of all the praiseworthy experimentation carried out in recent times, in particular also by physico-chemists such as Bredig, Henri, Bodenstein, Euler, among others. For the present, all we know of all the enzymes is the way they act, and no way has been found up to now of preparing any of them.

Nevertheless, there is no cause whatsoever for discouragement. The progress made in the field of fermentation processes is clearly revealed when we compare our present knowledge with that of a few decades ago. The prob-

lems which faced the contemporaries of Berzelius, Liebig and Pasteur have been solved. The differences between the vitalistic view and the enzyme theory have been reconciled. Neither the physiologists nor the chemists can be considered the victors; nobody is ultimately the loser; for the views expressed in both directions of research have fully justified elements. The difference between enzymes and micro-organisms is clearly revealed when the latter are represented as the producers of the former, which we must conceive as complicated but inanimate chemical substances.

In one case, which seemed typical of processes strongly linked with the whole life of the cells, it was possible to trace the whole phenomenon to the relatively simple action of a chemical body in the interior of the cell. Just as it was earlier learnt how to produce urea without a living animal, in the test tube, without any life force, so it is seen here that an apparent action of live cells can take place without cells. The fermentation process becomes more comprehensible to us now that it is possible to separate it from the rest of the processes of life, just as the first step towards the explanation of the phenomena of combustion rested on the fact that it was possible to separate the generation of light and heat from the processes of oxidation. The way which first led to the proving of cell-free fermentation, the preparation of expressed juices, must be thought of as astonishingly simple, somewhat reminiscent of the story of Columbus' egg. For it is certainly obvious, in order to study the contents of a container, to open it first. The methods, however, which served here to extort the secrets from the yeast cells will be of great use in other similar cases. In co-operation with J. Meisenheimer and R. Gaunt I have already been able to apply them to acetic-acid and lactic-acid bacteria, which led to the demonstration of the fermentation enzymes concerned.

We are seeing the cells of plants and animals more and more clearly as chemical factories, where the various products are manufactured in separate workshops. The enzymes act as the overseers. Our acquaintance with these most important agents of living things is constantly increasing. Even though we may still be a long way from our goal, we are approaching it step by step. Everything is justifying our hopes. We must never, therefore, let ourselves fall into the way of thinking *"ignorabimus"* ("We shall never know"), but must have every confidence that the day will dawn when even those processes of life which are still a puzzle today will cease to be inaccessible to us natural scientists.

1. J. J. Berzelius, *Lehrbuch der Chemie,* translated by F. Wöhler, 3rd ed., 1839, Vol. 8, p. 84.
2. Written by Wöhler, though Liebig himself had made fun of it. Ann. *Pharm., 29 (1839) 100.* Cf. *Aus Liebig's und Wöhler's Briefwechsel,* Brunswick, 1888, Vol.1, p, 123.
3. *Die organische Chemie* in *ihrer Anwendung auf Agrikultur und Physiologie,* (1840), p. 232.
4. *Ann. Pharrn.,* 30 (1839) 262.
5. Cf. M. Traube, *Gesammelte AbhandIungen,* Berlin, 1899, p. 117.
6. *Ann. Chim. Phys.,* 58 [3] (1860) 360.
7. *Ann. Chim. Phys.,* 21 [5] (1880) 430.
8. This illustration was reproduced from P. Lindner, *Atlas der mikroskopischen Grundlagen der Gärungskunde,* Paul Parey, Berlin, 1903, Plate 56, with the kind permission of the author and publisher.
9. *Sitzungsber. Bayer. Akad. Wiss., Math. Phys. Kl.,* (1878) 161.
10. *Mem. Couronnés Acad. Roy. Belg.,* 53 (1895) 29.
11. *Poggendorff's Ann.,* 67 (1846) 408.
12. I. Wiesner, *Mikroskopische Untersuchungen,* Maier, Stuttgart, 1872, p. 126.
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15. This illustration was also reproduced from the monograph *Die Zymasegärung,* R. Oldenbourg, Munich, 1903, with the permission of the publishers.
16. For example, in accordance with the instructions in K. B. Lehmann and R.O. Neumann, *Bakteriologische Diagnostik,* 2nd ed., Munich, 1899, p. 455.
17. *Ber. Deut. Chem. Ges.,* 32 (1899) 2093, *Nos.* 258 and 265.