CHAPTER VI

THE SOLUBLE FERMENT

Before we can form any idea of the magnitude of Béchamp's discoveries we must thoroughly realise the obscurity of the scientific views of the period. Not only were physical and chemical influences believed to be operative in the spontaneous generation of plant and animal life, but Dumas' physiological theory of fermentation had been set aside for the belief that this transformation anteceded the appearance of micro-organisms.

We have already seen that light was thrown upon this darkness by Béchamp's Beacon Experiment; we have now to study the

teaching he deduced from his observations.

At the date of the publication of his Memoir, scientists were so little prepared to admit that moulds could appear apart from the co-operation of some albuminoid matter that it was at first insisted that Béchamp must have employed impure sugar. On the contrary, he had made use of pure sugar candy, which did not produce ammonia when heated with soda lime. Yet his critics would not be satisfied, even by the fact that the quantity of ammonia set free by the moulds far surpassed any that could have been furnished by an impurity. Further evidence was given by the experiments that showed the development of microorganisms in mineral media, and these could not be accused of connection with anything albuminoid.

Béchamp was not, of course, the first to view and notice the moulds, the micro-organisms. That had been done before him. What he did was conclusively to demonstrate their atmospheric origin, and, above all, to explain their function. Anyone interested in this important subject cannot do better than study the second *Conference*, or chapter, of his great work *Les Microzymas*, where the matter is explained fully. Here we can only briefly

summarise some of its teaching.

The outstanding evidence that faced the Professor in his observations was the fact that the moulds, which appeared in sweetened water exposed to air, set free ammonia when heated with caustic potash. This was evidence that a nitrogenised organic substance, probably albuminoid, had been produced and

had served to constitute one of the materials necessary for the development of an organised being. Whence had it arisen? The Professor finds his answer by a study of nature. He describes how the seed of a flowering plant will germinate and the plant that appears will grow and develop, always weighing more than the seed sown originally. Whence were the chemical compounds derived that were wanting in the seed? The answer, he says, is elementary, and he goes on to explain how the organs of the young plant are the chemical apparatus in which the surrounding media (i.e. the water in the soil, in which it strikes its roots, supplying nitrogenous salts, and the atmosphere providing its leaves with carbonic acid and oxygen) are enabled to react and produce according to chemical laws the compounds whereby the plant is nourished and wherewith it builds up its cells and hence all its organs. In the same way behaves the spore of the mucorina, which the air carried to the sweetened solution. It develops, and in the body of the microscopic plant the air, with its nutrient contents, the water and the dissolved materials in the sweetened solution react and the necessary organic matter is elaborated and compounds are produced which were non-existent in the original medium. He goes on to explain that it is because the mucorina is a plant, with the faculty of producing organic matter, that it is able to develop in a medium that contains nothing organised. For this production of organic matter the help of certain minerals is indispensable. Béchamp here reverts to Lavoisier's explanation of the way in which water attacks glass and dissolves a portion of it, and himself shows how the moulds are thus supplied with the earthy and alkaline materials they need. The amount thus furnished is very small, so that the harvest of moulds is correspondingly limited. If, however, certain salts, such as aluminium sulphate, potassium nitrate or sodium phosphate, were added to the sweetened water large moulds resulted and the inversion of the sugar was proportionately rapid.

"The meaning of this," says Béchamp, "is that each of these salts introduced a specially favourable condition and perhaps helped in attacking the glass, which thus yielded a greater quan-

tity of its own substance." 1

But, even still, the mystery of fermentation was not quite clear without an explanation of the actual way in which the change in the sugar was brought about, that is to say, cane-sugar transformed into grape-sugar.

Here again, as we have already seen, Béchamp solved the difficulty by a comparison and likened the influence of moulds to the effects exercised upon starch by diastase, which, in solution, possesses the property of causing starch to break up at a high temperature, transforming it first into dextrin and then into

Béchamp proved his comparison to be correct by rigorous experiments. By crushing the moulds which appeared in his solutions he found that the cells that composed them secreted a soluble ferment and that the latter was the direct agent in transforming the sugar, and he made a very clear demonstration of this also in regard to beer-yeast. For instance, just in the same way the stomach does not work directly upon food, but only indirectly through a secretion called gastric juice, which contains pepsin, a substance more or less analogous to diastase and which is the direct agent of the chemical changes that take place in the digestive organ. Thus it is by a soluble product that beer-yeast and certain other moulds bring about the chemical change that alters the type of sugar. Just as the stomach could not transform food without the juice it secretes, so yeast could not change sugar

without a soluble ferment secreted by its cells.

On p. 70 of Les Microzymas Professor Béchamp commences an account of some of the experiments he undertook in this connection. Here may be found the description of an experiment with thoroughly washed and dried beer-yeast, which was mixed with a little more than its weight of cane-sugar and the mixture carefully creosoted, the whole becoming soft and by degrees completely fluid. Béchamp provides a full explanation of the action. He shows that the yeast cell is like a closed vesicle, or a container enclosing a content, and that it is limited in space by a membranous envelope. In the dried state, in which he made use of it for his experiment, it yet contained more than seventy per cent of water, no more perceptible to touch than the amounton an average eighty per cent of the body-weight-contained in the human body. He explains how the living yeast, in its natural state, on contact with water allows nothing of its content to escape except excretory products, but in contact with the sugar it is, as it were, irritated and the enveloping membrane permits the escape of water with certain other materials held in solution, and it is this fluid that liquefies the mixture of yeast and sugar. The escape of the fluid Béchamp shows to be due to the physical process osmosis, by which a solution passes through a permeable

¹ Les Microzymas, p. 84.

membrane. Thus having obtained his liquid product he diluted it with water and left it to filter.

Meanwhile Béchamp performed another experiment; namely, he dissolved a small piece of cane-sugar in water and found that no change was produced when this was heated with alkaline copper tartrate. He then took another small piece of sugar and heated it to boiling point with very dilute hydrochloric acid; he neutralised the acid with caustic potash and made the solution alkaline; he then added his copper reagent and heated it, whereupon reduction took place, a precipitate being produced which was at first yellow and then red. By means of the acid the sugar had been inverted, that is to say, transformed into a mixture of glucose and levulose (a constituent of fruit sugar), which reduced the cupric copper of the blue reagent to cuprous copper which was precipitated as the red oxide.

Béchamp then returned to the liquid that had been filtering, and found that when he barely heated it with the alkaline copper tartrate reagent the change in the sugar was effected. This proved to him that something besides water had escaped from the yeast, something that, even in the cold, had the power of rapidly inverting the sugar.

Professor Béchamp here points out1 two facts that must be clearly demonstrated. First, that without the escaping element yeast in itself is inoperative, for when steeped in water, with the alkaline copper tartrate reagent added, reduction is not affected. Secondly, that heat destroys the activity of the escaping element, for yeast brought to the boil with a little water to which sugar is added does not, even after time has been allowed for it to take effect, produce the inversion; the alkaline copper tartrate reagent is not reduced. In short, he discovered that heat destroys the activity of the ferment secreted by yeast and moulds of all sorts, just as heat destroys the activity of sprouted barley, of diastase and of other soluble ferments, that is, ferments capable of being dissolved in a fluid.

Béchamp further discovered sodium acetate to be another agent especially efficient in promoting the passage of the soluble contents through the cell walls. To dried yeast he added some crystals of that salt, experimenting on a sufficiently large quantity. The mixture became liquid and was thrown upon a filter. One part sodium acetate to ten or more of yeast he found sufficient to effect the liquefaction. He then took the filtered liquid

and added alcohol to it, and a white precipitate appeared. He collected this in a filter and washed it with alcohol to free it from the sodium acetate. The alcohol being drained off, the precipitate was dried between folds of filter papers and then it was taken up with water. There resulted a solution and an insoluble residue. This last was coagulated albumen, which came from the yeast in solution, but was rendered insoluble by the

coagulating action of the alcohol.

"As to that portion of the precipitate which has been dissolved, alcohol can precipitate it again," says Béchamp.1 "This new precipitate is to beer-yeast what diastase is to sprouted barley or synaptase to almonds; it is the principle that in the yeast effects the inversion of the cane-sugar. If some of it is dissolved in water, cane-sugar added and the solution kept for several minutes in the water bath at 40°, the alkaline copper tartrate proves that the sugar has been inverted. The action is also very rapid at the ordinary temperature, but slower in proportion to a lesser amount of the active product; which explains the slowness of the reactions obtained with certain moulds that I could only utilise in small quantity. All this proves that the cause of the inversion of the sugar is pre-formed in the moulds and in the yeast, and as the active matter, when isolated, acts in the absence of acid, this shows that I was right in allying it to diastase."

It was after Professor Béchamp had established these facts that he gave a name to this active matter. He called it zymase, from the Greek $\xi \psi \mu \eta$, ferment. The word, applied by him at first to the active matter of yeast and of moulds, has become a generic term. Later on he specially designated the zymases of yeast and of moulds by the name of zythozymase.

Béchamp's first public employment of the name "zymase" for. soluble ferments was in a Memoir on Fermentation by Organised Ferments, which he read before the Academy of Science on

4th April, 1864.2

The following year he resumed the subject³ and showed that there were zymases in microzoaires and microphytes, which he isolated, as Payen and Persoz isolated the diastase from sprouted barley. These zymases, he found, possessed generally the property of rapidly transforming cane-sugar into glucose, or grape-sugar. He discovered the anthrozyma in flowers, the morozyma in the

³ C. R. 59, p. 496.

Les Microzymas, pp. 71, 72.

¹ Les Microzymas, p. 72. ² Comptes Rendus 58, p. 601.

white mulberry and the *nephrozyma* in the kidney of animals. Finally, the following year, 1866, he gave the name *microzyma*¹ to his crowning discovery, which to him was the basic explanation of the whole question and which had not yet been made apparent to him when he immortalised his early experiments in his Memoir of 1857; but this we must leave for future consideration. We have here given these dates to show how long ago Professor Béchamp made a complete discovery of the nitrogenous substance formed in the yeast cell to which he gave the name of *zymase*.

Apart from the justice of giving credit where credit is due, for the mere sake of historical accuracy it is desirable that his own discovery should be publicly accredited to him. Instead, in the Encyclopædia Britannica² we find, in the article on "Fermentation" by Julian Levett Baker, F.I.C., that it is stated that "in 1897 Büchner submitted yeast to great pressure and isolated a nitrogenous substance, enzymic in character, which he termed zymase." Again, we take up A Manual of Bacteriology, by R. Tanner Hewlett, M.D., F.R.C.P., D.P.H.(Lond.), F.R.M.S., and we read: "Until 1897 no enzyme had been obtained which would carry out this change [alcoholic fermentation]; it only occurred when the living yeast-cells were present, but in that year Büchner, by grinding up the living yeast-cells, obtained a juice which decomposed dextrose with the formation of alcohol and carbonic acid. This 'zymase' Büchner claimed to be the alcoholic enzyme of yeast." Yet, once more, Professor and Mrs. Frankland, in their book Pasteur,4 while apologising for certain of the latter's erroneous views, write as follows: "In the present year [1897] the discovery has been made by E. Büchner that a soluble principle giving rise to the alcoholic fermentation of sugar may be extracted from yeast cells, and for which the name zymase is proposed. This important discovery should throw a new light on the theory of fermentation."

But "this important discovery," as we have here seen, was made nearly half a century before by a Frenchman!

It is true that Pasteur accused Béchamp of having taken his ideas from Mitscherlich. Not only was Béchamp able to disprove this, but he also showed that it was Pasteur who had followed the

German's views, and that, moreover, on a point on which the latter appeared to have been mistaken.¹

Thus it is clear that Béchamp was the first to give tangible proof not only of the air-borne origin of yeasts and moulds, but also of the means by which they are physiologically and chemically active. When he started work there was no teaching available for him to plagiarise, had plagiarism been possible to such a deeply versed and honest student of scientific history who, step by step, traced any observations that had preceded his own. Unfortunately it was he who was preyed upon by plagiarists, and, sad to relate, foremost among these seems to have been the very one who tried to detract from his work and who bears the world-famous name of Pasteur! Let us pause here to watch the latter's progress and the way in which he gained credit for Béchamp's great discovery of the invading hordes from the atmosphere, micro-organisms with their fermentative powers.

¹ Comptes Rendus de l'Académie des Sciences, 63, p. 451.

² Eleventh Edition. ³ Sixth Edition, p. 36.

See Chapter IX.

¹ Les Microzymas, pp. 76, 77.