any longer retaining the view of spontaneous generation. It would just be as sensible to suppose that in the impregnation of the ovum of higher animals it is not the spermatozoa, but some organic molecule accidentally introduced at the same time which causes the development of the ovum, as to suppose that it is not bacteria or their spores but some organic molecule manufactured in or specially altered by passing through the air which produces bacteria in organic fluids and tissues.

CHAPTER XI.

RELATION OF MICRO-ORGANISMS TO FERMENTATION.

Summary of what has preceded with reference to fermentation. Relation between 'vital' and 'chemical' fermentations: theories of fermentation. Liebig's views. Alcoholic fermentation: *Pasteur's experiments and conclusions. Résumé.* Butyric fermentation. Formation of pigment by bacteria *Schroeter: Cohn.* Viscous fermentation. Lactic fermentation: *Pasteur: Lister.* Other fermentations, especially the putrefactive: *Lemaire; Cazeneuve* and *Livon: Paul Bert.* Conclusions.

We must now pass on to the relation of these bodies to fermentations, and I will here merely indicate the chief points without entering into a discussion on the subject And first, I may say that it is now admitted by Dr. Bastian, s well as by other observers, that organisms are present in all fermenting fluids. This statement was formerly denied, on account of the imperfection of the methods of examination.

We have already seen in the first part of this work that the cause of fermentation in organic substances was the entrance into them of solid particles held in suspension in air. We have also seen that the cause of the development of organisms in fluids and tissues was the entrance into them of particles suspended in the air. We also know that in all fermentations organisms are present, and that in the absence of organisms no fermentation occurs. What more likely, then, than that the particles which cause fermentation, and the particles which originate organisms, are one and the same? that in fact the fermentation of a fluid is the result of the growth of organisms in it?

The process in these 'vital' fermentations may be brought into the same category as that in fermentation by the 'unformed' ferments, if we suppose that the immediate cause of the chemical change in the former instance is some chemical substance resembling ptyalin, pepsin, &c. No doubt there is this difference between ordinary fermentations and those due to a chemical substance, that in the former case the ferment itself increases in quantity. This difference would, however, be easily reconciled if we were to suppose that each organism was a former of the ferment, even though to an extremely limited degree. The process in these 'vital' fermentations, of which we may take the alcoholic as an example, would then be the same as in the so-called chemical fermentations. Thus, to speak of the case of the saliva, ptyalin is not a chemical compound formed spontaneously, nor is it the result of any sort of double decomposition; it is a substance formed as the result of the vital action of certain living cells. According to the view under consideration, the alcoholic ferment would be likewise the product of the vital action of certain cells, the yeast cells. The ptyalin itself has not the power of self-multiplication, but the cells which form it produce it continuously; the ferment of alcohol would not have the power of self-multiplication, but the cells which form it produce it continuously. This explanation agrees completely with the contrast between the effects of ptyalin on starch, and those of yeast on sugar. The ptyalin is more or less immediate in its effect. It is a very active formed ferment. The yeast acts slowly because the ferment is only produced as the plant grows. Hence the explanation of the rapid action of the one and of the progressive action of the other. The apparent self-multiplication of the ferment in the alcoholic case would be due to the fact that the producers of it are free—float freely in the fluid—and hence ultimately no drop of it can be taken which will contain sufficient ferment to act without the ferment-producers being also present. In the case of the saliva, the ferment-producer is fixed, and the ferment is obtained alone and apart from its originator, hence it does not multiply. The same causes which arrest the production of ptyalin arrest also the alcoholic fermentation, for they destroy the living cells which form the ferment.

The case of emulsin would be exactly the same as that of yeast. The emulsin itself does not multiply, but the seed, the producer of it, does. Sow a seed of the bitter almond, and there springs up a plant bearing numerous seeds, numerous producers

of emulsin. Sow a yeast cell, and there follows the growth of numerous yeast cells, each producing a quantity of the ferment.

Considerable support is given to this view by the experiments of Musculus, on the mechanism of the ammoniacal fermentation of urine. This has been shown to be due to the growth of an organism in the urine (Micrococcus ureæ, Cohn). Musculus[1] demonstrated that, by adding absolute alcohol to ammoniacal urine, a precipitate was obtained which could be filtered and dried. This precipitate transformed urea into carbonate of ammonia. Its power was destroyed by exposure to a temperature of 80° C. This soluble ferment is a secretion of the micrococcus ureæ.

According to another view it may be that the organisms, while living in various substances, feed on them, and the products of the fermentation may be either the portion of the food which has been rejected by them, or products formed in and excreted by the organism.

Or it may be that, as Pasteur holds, the cause of the fermentation is not the production of a ferment but the breaking up of the chemical compounds by the growing plants in the search for nutriment, more especially for oxygen. It is quite clear that there must be such a breaking up. Or, again, it may be that in this breaking up of the organic compounds some of the molecules may rearrange themselves and form a ferment, and the presence of a ferment of this kind I consider the best explanation of some of the special fermentations, though I incline to hold that the ferment is excreted by the cell itself.

It is, however, probable that in different fermentations the process occurs in different ways. Thus in the pigment fermentations, as will be seen, the second is probably the correct explanation. The point of importance is that in any case there is nothing unreasonable in associating these changes with the growth of living cells; in fact all analogy points to such a relation.

The only other theory which is tenable in the presence of the facts stated, would be that the particles which cause fermentations are not the same as those which give rise to the growth of organisms, but that they are bodies which have only the

[1] Magnin, *Bacteria*.

power of causing fermentation, and are possibly either substances in a state of decomposition or special ferments. But then it is inconceivable that fermentation and the development of organisms should always be associated, or that organisms of the same form, *e.g.* the yeast cell, should always be present in the same fermentation. Supposing this view possible, we must assume that there is a special organic molecule for each fermentation, for otherwise we could not explain the occurrence of lactic fermentation in one flask, putrid fermentation in another, pigment fermentation in another, &c., all the flasks containing the same specimen of milk having been filled at the same time and kept under the same conditions.

Liebig originally propounded the view that fermentation was a change in organic fluids and tissues, set in motion by the access of oxygen or of bodies in a state of decomposition. He at that time regarded organisms as quite accidental. He supposed that when organic matter was exposed to the air, it underwent a slow process of oxidation which he termed *eremacausis*; and that this change, communicating itself to other molecules, caused them to break up or putrefy. In the presence of the facts stated as to the relation of dust to the fermentation of boiled and unboiled fluids, Liebig modified his views, and in his last publication he admitted that the yeast plant was in some way or other connected with the alcoholic fermentation, but he thought that the relation between them most frequently consisted in this:—that when the yeast cells died, they decomposed, and that the chemical change thus set agoing was propagated to the sugar, and caused it to break up into alcohol and carbonic acid.

That Liebig's theory of decaying matter is incorrect will be seen by a consideration of the facts mentioned on pages 200 and 210. Liebig was not latterly, however, absolutely opposed to the acceptance of the doctrine that living organisms are initiators of fermentative changes. On the contrary, to quote from Bastian, 'he slightly widened his views after the correlation of organisms with fermentations had become established, and endeavoured to show that the admitted actions of living units in initiating fermentations were but other exemplifications of his general doctrine, that fermentations are induced by certain communicated molecular movements, sometimes emanating from organic matter in a state of decay, and sometimes resulting from the vital processes of living units.'

Had Liebig left out the part of the view which holds that fermentations may be caused by movements, 'sometimes emanating from organic matter in a state of decay,' there would not have been much to find fault with. His theory would then have been merely another way of viewing the mode of action of these living bodies.

Certain definite facts are known which show that organisms do take part in certain fermentations, while I have already disproved Liebig's view that decaying matter has any power of causing these changes. The facts to which I am about to allude, when taken together with the constant presence of organisms in other fermentations of the same class not yet investigated, render it, to my mind, certain that living organisms are, probably in one or other of the ways indicated, the causes of these chemical changes.

Alcoholic Fermentation.

The first case of fermentation which was studied, and about which most is known, is the alcoholic fermentation. The facts and experiments on this subject are now so universally known that it would be superfluous for me to do more than summarise them here.

The yeast plant (*Torula cerevisiæ*) is always present in a state of vitality during the alcoholic fermentation of sugar.

If yeast be raised to the temperature of 60° C., at which temperature the cells die, the fermentation of sugar no longer occurs. Here the cells are dead, and if Liebig's view be correct, that dead cells, not living ones, are the cause of the fermentation, the process ought still to go on.

The juice of the grape has no spontaneous tendency to undergo fermentation, as shown by the experiments of Van der Broeck, &c., formerly mentioned, and by the following experiment narrated by Pasteur. In a flask of the form shown in Fig. 68, A) the neck (*a*) of which was drawn out to a fine point, Pasteur boiled water which had been used to wash the outside of a grape, and which therefore, according to other experiments, contained abundant causes of the alcoholic fermentation. This point (a), which had been sealed, was heated, and plunged through the heated skin of a grape (Fig. 68, B). It was then broken in the interior of the grape, and, by causing a diminution of pressure inside of the flask, a drop or two of the juice was sucked into the boiled water. (The diminution in pressure was thus obtained. After heating the walls of the flask with the hand or a lamp, the orifice of the bent neck (Fig. 68, A, b) was sealed. When the point of a was broken off in the interior of the grape a little juice passed into the flask.) The orifice a was then sealed, and the orifice b opened. Here, then, he had in the flask unboiled grape juice, oxygen, water, dead organisms, and organic mole-

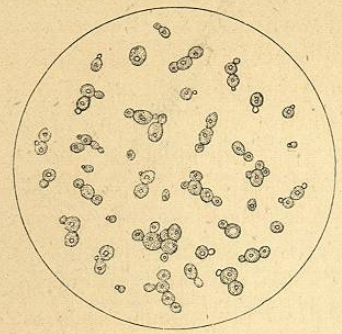

FIG. 67.—TORULA CEREVISIÆ. (AFTER PASTEUR.)

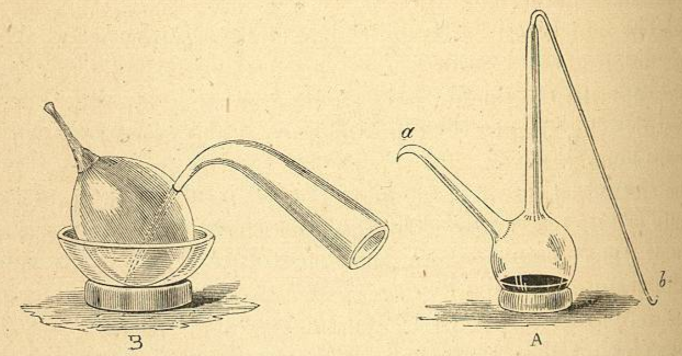

FIG. 68.—PASTEUR'S EXPERIMENT ON UNBOILED GRAPE JUICE.

A, the flask employed. a, The neck which is represented in B as plunged into the grape. b, The orifice of the bent neck at first sealed till the grape juice has been introduced and then opened and left open. (From Pasteur.)

cules, and yet no alcoholic fermentation occurred. This and other experiments[1] led Pasteur to the following conclusions:—

[1] See *Etudes sur la Bière.*

'The boiled juice of the grape never ferments when kept in contact with air which has been deprived of the germs suspended in it.

'Boiled grape juice ferments when a very small quantity of water is introduced, in which the surface of the grapes and of the branches of the vine have been washed.

'Grape juice does not ferment after the introduction of this water if the latter has been previously boiled and cooled.

'Grape juice does not ferment when a small quantity of the juice taken from the interior of a grape is introduced.'

Pasteur further shows that the apparently spontaneous commencement of fermentation in these fluids is due to the existence of spores of the torulæ in the air, though they are not, as a rule, present in great abundance. He also brings forward experiments to show how other fungi, such as *Mycoderma aceti*, can, when there is too little oxygen present, cause the splitting up of sugar into alcohol.

Seeing, then, that boiled and unboiled grape juice may be preserved unaltered in presence of air which has been heated, or in some way or other deprived of its dust, it is quite evident that alcoholic fermentation is not a spontaneous decomposition nor one which can be brought about by the action of the gases of the air alone. Seeing, also, that the yeast plant is always present when fermentation is going on, that anything which destroys the vitality or interferes with the growth of that plant arrests or interferes with the alcoholic fermentation, and that the introduction of dust which does not contain the torula cells is not accompanied by the alcoholic fermentation but by some other change, we must conclude that the particles which fall into fluids and give rise to alcoholic fermentations are intimately associated with the particles which give rise to yeast cells, and further that they are living particles subject to the same laws as the yeast cell itself. And seeing, further, that other plants when living under certain conditions are also capable of giving rise to the alcoholic fermentation, we must conclude that this change is a process due to changes in living tissues, that, in fact, the fermentation is the result of the life of these cells and plants, and that, therefore, the particles which, falling into fluids, give rise to the Torula cerevisiæ, and those which give rise to alcoholic fermentations, are one and the same; in fact that the growth of the yeast cell is in some way or other the *cause* of the alcoholic fermentation.

Butyric Fermentation.

Pasteur has also brought forward evidence of a similar nature as to the butyric fermentation. The organisms which produce butyric fermentation are bacilli (*Bacillus subtilis*, Cohn), which apparently live without free oxygen, and indeed are killed by it, and which when cultivated in various fluids, even in Pasteur's solution, cause butyric fermentation in all (see Fig. 69 and also Fig. 64).

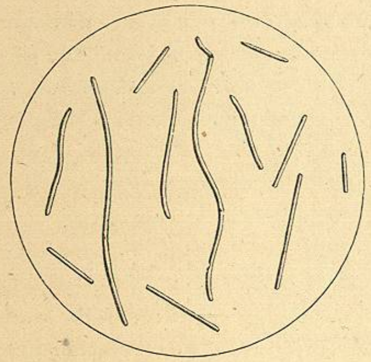

FIG. 69.—BACILLUS SUBTILIS, × 650. (AFTER COHN.)

Formation of Pigment by Bacteria.

Striking facts as to the association of a definite chemical change with the presence of organisms of a definite form were brought forward by Cohn and Schroeter with regard to 'pigment bacteria.'[1]

They showed that while many forms of organisms could grow on such soil as boiled potatoes, yet a definite pigment was produced only when one particular form was present. These organisms generally belonged to the group of 'micrococci,' though sometimes pigmentation was caused by bacteria, as in blue pus (see Fig. 70).

These pigments were sometimes scarlet, sometimes blue, sometimes soluble, sometimes insoluble; and when a variety of soils were inoculated from an individual specimen, the same colour, with the same chemical and other characteristics and the same organism, always resulted. Not only might these pigment bacteria grow on boiled potatoes, they could flourish

[1] *Beiträge zur Biologie der Pflanzen.*

on cheese, meat, white of egg, bread, starch, &c., the same pigment being invariably produced.

The conditions under which this pigment appeared were exactly those which were most favourable to the life of the organisms, while those in which it was absent were those in which the organisms could not develop.

Schroeter concludes from his investigations that these examples show what a manifold series of pigments may be produced 'by bacteria and bacteridia.' He adds that the organisms which form them can often be recognised as distinct owing to the difference in the pigment produced; the organisms which form the various pigments being often also distinctly separable by different morphological characters, and the different pigments behaving differently with reference to chemical reagents. He considers that it is not unjustifiable to hold that each separate pigment is formed by a distinct specific organism.

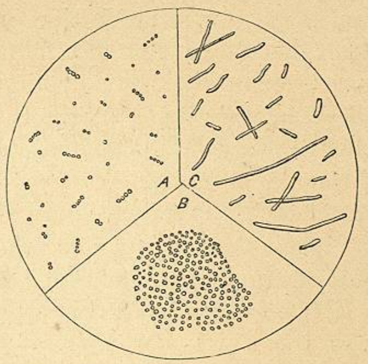

FIG. 70.—PIGMENT PRODUCING ORGANISMS.

A, Micrococcus prodigiosus. B, Micrococcus fulvis. C, Bacillus ruber.

Schroeter points out that the pigments are definite chemical substances formed by the bacteria from organic, albuminous materials, and that the process is therefore quite analogous to the formation of alcohol by the yeast plant or of lactic acid by other bacteria.

Cohn further found that these same organisms developed readily and produced the same pigment in *artificial cultivating fluids containing ammonia and a carbonate, but no trace of albumen.* Once obtained in this fluid they could be propagated indefinitely, the same pigment being constantly produced. In this instance we have an example of a definite change brought about by the growth of a definite form of organism. No *spontaneous* change ever occurs in Cohn's artificial fluids resulting in the formation of these pigments, and yet as soon as these organisms, which are associated with definite pigments when growing on albuminous soil, are introduced into these mineral solutions, the same pigments appear. That in the latter case the pigment is formed by the bacteria is evident, for it is a complex organic substance, closely related to the aniline colours, and yet in this instance it is derived from a few inorganic salts. It is therefore a substance formed by the living plants, and, as before remarked, there is nothing remarkable in this production of pigment by living cells. It is only what is constantly occurring in many animal and vegetable cells, and in the latter it is formed from inorganic compounds. Indeed, it would be against all chemical experience that by any purely chemical process a complex organic compound would be so readily built up from a few inorganic compounds of a very simple kind.

These facts absolutely prove that the pigment fermentation was the result of the *life* of the organism, for the pigment must have been built up by it and could not have originated from chemical changes propagated to the fluid. If then Dr. Bastian is correct in his opinion that 'Liebig's view, if it be true at all, must be true for *the whole* of the processes which are essentially included under the term fermentation,' these facts alone overturn Liebig's theories.

Viscous Fermentation.

Similar facts are known with regard to the viscous fermentation of sugar. This is a transformation of sugar into gum mannite, and carbonic acid, and results in the formation of a viscid, ropy fluid. Pasteur found that organisms of a special form were present in such instances, and these organisms when sown in Pasteur's solution always caused this viscous fermentation. The kind of proof is exactly the same as in the other instances of fermentation, and need not be repeated here.

Lactic Fermentation.

An instance in which the causal relation of organisms to a fermentative process is absolutely demonstrated is the lactic fermentation of milk.

This fermentation was investigated several years ago by

Pasteur,[1] who showed that it was exactly analogous to the alcoholic fermentation, and that it was caused by a special organism. On examining fluids which were undergoing lactic fermentation he observed that minute round or oval cells were constantly present. These organisms when transferred to fluids capable of nourishing them always produced lactic acid. To prove that these organisms were the cause of the fermentation he made a decoction of yeast, to which he added sugar and chalk. When they were sown in this fluid lactic fermentation occurred, resulting in the formation of large quantities of calcium lactate.

This fermentation has of late been more fully studied by Mr. Lister, and as his experiments bear on the whole subject of fermentation, I shall refer to them somewhat at length.

As I have previously mentioned, Mr. Lister[2] found that milk had no inherent tendency to undergo the lactic fermentation; in fact, unless it was brought into a dairy or into contact with dairy vessels or workers, all sorts of fermentations would occur rather than the lactic. On examining milk undergoing lactic fermentation he found that an organism of a definite and easily recognisable form was constantly present, while in milk which was not brought into the dairy, and which did not undergo lactic fermentation, this form of organism was absent.

'This organism is a motionless bacterium occurring most commonly in pairs, but frequently in chains of 3, 4, or more individuals, each segment being of somewhat rounded form, more or less oval, with the long diameter in the direction of the length of the chain, and often showing, on careful focussing, a line across their central part, indicating transverse segmentation. They vary in diameter. . . full-sized specimens measuring about $\frac{1}{20000}$th inch.'

The question now arose, Was this organism, so constantly present in milk which was undergoing the lactic fermentation, the cause of that change or not? Was some other bacterium the active agent, or was it some hypothetical organic molecule?

Mr. Lister solved these points in the following beautiful manner. He prepared a series of flasks containing milk which

[1] *Annales de Chim. et de Phys.*, vol. lii. p. 407.
[2] *Transactions of the Pathological Society of London*, 1878.