M. H. Hoffmann on Fermentation.

placed towards the extreme angle of the mouth. Teeth wanting. Pectoral fins wanting. Body linear, with a filiform caudal extremity. Dorsal and anal fins of equal length; the former commences above the end of the guttural sac, and exhibits one more strongly developed and elongated ray; the anal runs up to the aperture of the guttural sac. The anus must also open into this aperture. Body naked, with no trace of scales.

I do not know where to place this form, to which I give the name of *Porobronchus linearis* (Pl. III. D). Its place is perhaps in the vicinity of *Saccopharynx*, Mitch., which certainly does not belong to the Apodes, and, like the above genus, forms the commencement of some new family, or of one which is not yet well established.

The specimens described are in the Collection of the British Museum.

XXXVII.—Mycological Investigation upon Fermentation. By M. HERMANN HOFFMANN*.

ALTHOUGH the phenomenon of fermentation long since attracted the attention of observers, its origin was still involved in considerable obscurity, and various hypotheses, amongst others that of spontaneous generation, had been invented to explain it. In order to solve this problem definitively, M. Hoffmann undertook a series of experiments, of which he gives an account in the 'Botanische Zeitung' of Berlin for 1860, Nos. 5 & 6.

1. If the juice extracted from some vegetables be examined by the microscope, it is found to contain here and there, not only cells similar to yeast, but also spores of *Mucedinæ* (such as *Cladosporium, Stemphylium,* &c.), some of which have even begun to germinate. These would be sought in vain in the interior of the fruits furnishing the juice under examination; so that it is extremely probable that they are derived from their surface.

Boiling water kills the germs of yeast-cells. Hence, if gooseberries, before being crushed, be immersed for four to ten seconds in boiling water, it is only after the lapse of four days that a fermentation with evolution of gas makes its appearance in the expressed juice, and then but feebly. If gooseberries be placed for three-quarters of an hour in cold water, and agitated from time to time, the water, when decanted, will be found to contain a small quantity of ferment, which may be employed as yeast, and which will evolve carbonic acid with a solution of sugar. When the surface of a gooseberry is scraped with a blunt knife,

* Translated from the 'Bibliothèque Universelle,' 1860, p. 337, by W.S. Dallas, F.L.S. and the matter removed is examined by the microscope, the same spores which occur in the expressed juice of the fruit are recognized, mixed with impurities of all sorts; the brown spores of *Stemphylium* and *Cladosporium*, and colourless fragments of *Oidium*, *Monilia*, *Torula*, &c. If the matter scraped off be placed in distilled water, and protected from all access of dust from without, there will be at the end of twenty-four hours dense groups of germinating filaments, and numerous cells of yeast in every stage of budding and fragmentation, and with all the varieties of form which characterize the yeast of the juices of fruits. Infusoria are also frequently met with. The expression *yeast* is therefore a collective denomination, and not the name of a species of plant.

2. The cells of yeast from beer or spirit in fermentation are generally more similar than those of vegetable juices which have undergone no boiling; nevertheless they are not only round or oval, but also cylindrical. To ascertain what they really are, two methods may be adopted. The first, which has already frequently been employed, consists in cultivating yeast, and examining what forms of plants it gives origin to. This is the method employed by Kützing, who obtained from it some Sporotricha and a Mucor. The second, in which the yeast is produced directly by means of the fungi which are presumed to be its cause, was employed by M. Bail; he made use of Ascophora elegans, a species of Mucor, and Penicillium glaucum; and M. Hoffmann states that he cannot but confirm the results obtained by these experiments.

The observations of M. Hoffmann were made on a large scale in breweries, and on a small scale in cultivations of small portions of yeasts. In the breweries, large tufts of *Penicillium* glaucum make their appearance on the yeast which has been thrown out; *Penicillium breve*, Corda, and Ascophora elegans, are also seen, but in smaller quantities. By cultivation on a small scale, taking all possible precautions to prevent the access of spores from without, M. Hoffmann has observed the development of the fungi above mentioned, together with *Periconia hyalina*. His experiments were made by pouring a few drops of water into a test-tube inclined obliquely, placing in it a few fragments of fresh yeast, and stopping the mouth of the tube with wadding to prevent the access of any impurities from without. He soon saw the yeast produce mycetoid filaments.

To ascertain that beer-yeast is only the product of these little Fungi, which are so common everywhere and in all climates, M. Hoffmann placed in one of his tubes a solution of sugar, which does not ferment by itself, together with spores of *Penicillium glaucum*, shook the whole strongly, and then placed the tube in as oblique a position as possible. The spores, which contain air, and are therefore specifically very light, rose as usual; but instead of meeting the air, they came in contact with the wall of the tube, and often remained immersed in the fluid. The tube was agitated once a day; and as early as the second or third day a mycelium was developed around the spores, and at this point, and then only, an evolution of gas commenced. From this time the tube had to be shaken more frequently. This experiment, modified in many different ways, proves that the development of the gas is intimately connected with the vegetation of the Fungus. In course of time the liquid becomes acid, and the evolution of gas ceases. If it be examined by the microscope, besides a few fructiferous filaments (in their atypic state), it is found to consist of mycetoid filaments and spores in various stages of germination, besides a great many yeast-cells in all phases of development.

A fermentation of greater or less strength may be produced not only with the spores of *Penicillium*, but also with those of other Fungi. M. Hoffmann succeeded in setting up fermentation in fresh wort, in grape-sugar, cane-sugar, and boiled gooseberry juice, by adding to the fluids spores of *Ustilago carbo*, *Ascophora mucedo*, and *Stachylidium pulchrum*, and also by putting in rose-leaves infected by *Phragmidium incrassatum*, and *Uredo Rosæ*, and finally by means of *Torula fructigena*, Pers. The dust collected on books also produces fermentation. The liquid at first contains yeast and a greater or less number of *Bacteria*; finally, *Penicillia* or *Ascophoræ* are developed on its surface.

The yeast thus artificially produced has all the physical and chemical properties of the ordinary yeast of the juice of raw fruits. M. Hoffmann has even made leaven with yeast produced by means of the Fungi of the Rose. On the other hand, he never succeeded in producing fermentation or the formation of yeast by means of fresh spores of Agarics or *Boleti*. From this it follows that all Fungi cannot assist in the production of yeast. This property appears to depend upon their capacity of forming conidia by their filaments of germination, and also perhaps upon the fact of their having been produced upon parts of plants in good health, or dead or dying. M. Hoffmann is inclined to think that this property of decomposing and penetrating deeply into the surrounding fluids is due especially to the mycetoid filaments.

And now the question presents itself, what part do Fungi and Infusoria take in the decomposition and putrefaction of organized bodies?

If certain Fungi (and Infusoria) alone possess the property of

decomposing liquids containing sugar, and evolving gas, or, with the addition of oxygen, of causing the corruption and putrefaction of other organic liquids, then, by protecting these liquids from the Fungi, we should be able to preserve them in an incorruptible state. On this point Schröder has made a series of remarkable experiments, from which it appears that the dust of the atmosphere is in almost all cases the cause of the decomposition of organic liquids which have been boiled. He has, however, half abandoned the suspicion which he entertained that the spores of Fungi played an important part in this, on observing that when the liquids had been heated the spores no longer induced decomposition; and he has arrived at the result that the dust only produces this effect when the materials have been previously in contact with the free air. M. Hoffmann, on the contrary, believes that decomposition may be produced by means of the spores of Mucedineæ (supposing that they are not killed thereby), by placing them for an hour in the midst of liquids heated to 214° F.

Organic liquids, such as broth, saccharine solutions, gluewater, boiled apples, honey and water, &c. placed in test-tubes well closed with a cotton plug, and boiled for an hour, remained intact for three to eight months, notwithstanding the excessive heat of the summer of 1859. But the result of the following experiment was very different :- Before pushing in the plug of cotton, an iron wire of moderate strength was passed through it; to the lower extremity of this was attached a small glass tube, two inches long, closed at both ends, containing dry spores of the Fungus on which the experiment was to be made. A second iron wire, placed by the side of the former, was attached to the lower part of the small tube; when the liquid in the testtube had been boiled, and become cool, this served to break the two extremities of the small enclosed tube, and thus place the spores in contact with the liquid surrounding them. If these spores belonged to *Penicillium glaucum*, they rose to the surface, and in a few days covered it with a thick carpet of *Penicillium*. With the spores of Ustilago carbo and Stachylidium pulchrum, or dried beer-yeast, fermentation does not occur, or is produced very feebly, because the dried spores rise and float on the surface. If, in place of a small closed tube, an open tube be employed, the boiling vapour alone is sufficient to kill the spores, and in this case the liquid undergoes no alteration. Thus, although such experiments cannot be performed without some of the spores contained in the atmospheric dust arriving at the liquid, they would be killed by the boiling.

It has long been known that the dust of inhabited houses contains spores. If an organic liquid which has been boiled be placed in a narrow-necked bottle and left unstopped, its surface is covered in a few days with tufts of *Mucedineæ*, arising from the spores which have fallen from the air. Protecting these liquids from these spores is the object of the methods of preservation of Appert and others. The above experiments furnish a fresh proof that spontaneous generation must be placed amongst dreams.

The following experiment proves that, notwithstanding the free action of the air, there is no formation of Infusoria, Fungi, or Bacteria when measures have been taken to prevent the liquid from containing any germs of these organisms. A small alembic is half filled with an organic liquid, and closed with a pierced cork, through which is passed a small glass tube of one or two lines in diameter, of which the free extremity is bent down, for one or two inches, in the form of a hook; the liquid is boiled for an hour, the extremity of the tube being closed with wadding, which is removed after the liquid has become quite cool; the organic matter will then be in free and direct communication with the oxygen of the external air, and, notwithstanding, it will remain intact, without any formation of Infusoria or Moulds for six months and more, even during the hottest summer-evidently because the spores diffused in the atmosphere cannot penetrate to it.

Fermentation is therefore only a simple division of the groups of organic atoms, which is essentially connected with the presence of yeast. M. Hoffmann leaves the question, whether the carbonic acid is a secretion of the interior of the yeastcell, or produced by its outer surface, undecided. Vesicles of gas are never seen in the interior of one of the active and normal cells; nevertheless, carbonic acid might be contained in the interior in a dissolved state (as in the blood). M. Hoffmann says that he does not see how this question can be solved directly by experiment. It is certain that the development of carbonic acid in a saccharine solution is immediately connected with the yeast-cells; this is proved by the following experiment. amongst others :- If a saccharine solution, or freshly prepared wort, contained in a test-tube, be divided by a thick plug of wadding, and yeast be poured into the upper portion, it is only in this that a fermentation will take place, which may be carried on until the complete disappearance of the sugar. After the lapse of some days, some bubbles of gas pass through the plug of wadding, and increase by degrees until they form a stratum of gas of 2 to 6 lines in thickness, which completely isolates the inferior liquid, but the sugar remains in this without alteration.

"When we see what an energetic and penetrating influence

the vegetation of certain Fungi exerts upon organic liquids, we are no longer surprised," says M. Hoffmann, "at the devastations which it causes among plants. We can no longer dispute upon the question whether the Fungi are the cause, or a concomitant of these maladies, and we must admit that, under certain combinations of temperature, atmospheric condition, &c. these effects are due naturally to these Fungi and Infusoria, and see in them redoubtable enemies of plants and animals, and perhaps also of man."

With regard to the potato-disease, M. Hoffmann refers to the valuable discoveries of M. Speerschneider, but gives a succinct account of the results of his own experiments, from which he draws the following conclusion :—After continued heavy rain, accompanied by a low temperature and want of sun, *Peronospora Solani* is developed in prodigious quantity on the leaves of the potato plant, and kills them, as if they had been burnt. The mature spores fail to the ground, and, when the moisture and heat are sufficient, germinate and send down their filaments into the tubers, of which the bark is still tender; the filaments reach the interior of the tubers, introduce themselves into the cells, of which they decompose the walls and amylaceous grains, and thus cause the destruction of the tuber.

This furnishes the indication of a rational treatment for the cure or prevention of the potato-disease. As soon as, under the atmospheric combinations above described, the foliage of the potato-plants appears to be attacked by a whitish blight (*Peronospora*), and appears as if burnt, there is danger of rotting of the tubers—at least unless dry weather supervene. The best method is to cut off the stalks, and it would be well to water the ground above the tubers with milk of lime or a solution of chloride of lime, or to sulphur it, as has been done with the grapes, with the object of destroying the spores of the fungus.

If the disease has begun to affect the potatoes, it would be necessary, before heaping them up, to wash and dry them. The removal of the herbage appears to be of no consequence when the tubers are developed. According to M. Hoffmann's observations, the period of this development, in the part of Germany where he dwells, is seven weeks after the first planting of the tubers, fourteen weeks after planting for late potatoes, and twelve weeks after planting for spring potatoes. Under any circumstances, the removal of the leafy parts towards the end of August cannot be prejudicial.

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