

Physiological Theory Of Fermentation

By Pasteur, Louis

1879

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Introductory Note

Louis Pasteur was born at Dole, Jura, France, December 27, 1822, and died near Saint-Cloud, September 28, 1895. His interest in science, and especially in chemistry, developed early, and by the time he was twenty-six he was professor of the physical sciences at Dijon. The most important academic positions held by him later were those as professor of chemistry at Strasburg, 1849; dean of the Faculty of Sciences at Lille, 1854; science director of the Ecole Normale Superieure, Paris, 1857; professor of geology, physics, and chemistry at the Ecole des Beaux Arts; professor of chemistry at the Sorbonne, 1867. After 1875 he carried on his researches at the Pasteur Institute. He was a member of the Institute, and received many honors from learned societies at home and abroad.

In respect of the number and importance, practical as well as scientific, of his discoveries, Pasteur has hardly a rival in the history of science. He may be regarded as the founder of modern stereo-chemistry; and his discovery that living organisms are the cause of fermentation is the basis of the whole modern germ-theory of disease and of the antiseptic method of treatment. His investigations of the diseases of beer and wine; of pebrine, a disease affecting silk-worms; of anthrax, and of fowl cholera, were of immense commercial importance and led to conclusions which have revolutionized physiology, pathology, and therapeutics. By his studies in the culture of bacteria of attenuated virulence he extended widely the practise of inoculation with a milder form of various diseases, with a view to producing immunity.

The following papers present some of the most important of his contributions, and exemplify his extraordinary powers of lucid exposition and argument.
To The Memory Of My Father

Formerly A Soldier Under The First Empire Chevalier Of The Legion Of Honor

The longer I live, the better I understand the kindness of thy heart and the high quality of thy mind.

The efforts which I have devoted to these Studies, as well as those which preceded them, are the fruit of thy counsel and example.

Desiring to honor these filial remembrances, I dedicate this work to thy memory.

L. Pasteur.

Author's Preface

Our misfortunes inspired me with the idea of these researches. I undertook them immediately after the war of 1870, and have since continued them without interruption, with the determination of perfecting them, and thereby benefitting a branch of industry wherein we are undoubtedly surpassed by Germany.

I am convinced that I have found a precise, practical solution of the arduous problem which I proposed to myself - that of a process of manufacture, independent of season and locality, which should obviate the necessity of having recourse to the costly methods of cooling employed in existing processes, and at the same time secure the preservation of its products for any length of time.

These new studies are based on the same principles which guided me in my researches on wine, vinegar, and the silk-worm disease-principles, the applications of which are practically unlimited. The etiology of contagious diseases may, perhaps, receive from them an unexpected light.

I need not hazard any prediction concerning the advantages likely to accrue to the brewing industry from the adoption of such a process of brewing as my study of the subject has enabled me to devise, and from an application of the novel facts upon which this process is founded. Time is the best appraiser of scientific work, and I am not unaware that an industrial discovery rarely produces all its fruit in the hands of its first inventor.

I began my researches at Clermont-Ferrand, in the laboratory, and with the help, of my friend M. Duclaux, professor of chemistry at the Faculty of Sciences of that town. I continued them in Paris, and afterwards at the great brewery of Tourtel Brothers, of Tantonville, which is admitted to be the first in France. I heartily thank these gentlemen for their extreme kindness. I owe also a public tribute of gratitude to M. Kuhn, a skillful brewer of Chamalieres, near Clermont-Ferrand, as well as to M. Velten of Marseilles, and to MM. de Tassigny, of Reims, who have placed at my disposal their establishments and their products, with the most praiseworthy eagerness.

L. Pasteur.

Paris, June 1, 1879.

Section I.

Part I.

On the Relations Existing Between Oxygen and Yeast

It is characteristic of science to reduce incessantly the number of unexplained phenomena. It is observed, for instance, that fleshy fruits are not liable to fermentation so long as their epidermis remains uninjured. On the other hand, they ferment very readily when they are piled up in heaps more or less open, and immersed in their saccharine juice. The mass becomes heated and swells; carbonic acid gas is disengaged, and the sugar disappears and is replaced by alcohol. Now, as to the question of the origin of these spontaneous phenomena, so remarkable in character as well as usefulness for man's service, modern knowledge has taught us that fermentation is the consequence of a development of vegetable cells the germs of which do not exist in the saccharine juices within fruits; that many varieties of these cellular plants exist, each giving rise to its own particular fermentation. The principal products of these various fermentations, although resembling each other in their nature, differ in their relative proportions and in the accessory substances that accompany them, a fact which alone is sufficient to account for wide differences in the quality and commercial value of alcoholic beverages.

Now that the discovery of ferments and their living nature, and our knowledge of their origin, may have solved the mystery of the spontaneous appearance of fermentations in natural saccharine juices, we may ask whether we must still regard the reactions that occur in these fermentations as phenomena inexplicable by the ordinary laws of chemistry. We can readily see that fermentations occupy a special place in the series of chemical and biological phenomena. What gives to fermentations certain exceptional characters of which we are only now beginning to suspect the causes, is the mode of life in the minute plants designated under the generic name of ferments, a mode of life which is essentially different from that in other vegetables, and from which result phenomena equally exceptional throughout the whole range of the chemistry of living beings.

The least reflection will suffice to convince us that the alcoholic ferments must possess the faculty of vegetating and performing their functions out of contact with air. Let us consider, for instance, the method of vintage practised in the Jura. The bunches are laid at the foot of the vine in a large, tub, and the grapes there stripped from them. When the grapes, some of which are uninjured, others bruised, and all moistened by the juice issuing from the latter, fill the tub - where they form what is called the vintage - they are conveyed in barrels to large vessels fixed in cellars of a considerable depth. These vessels are not filled to more than three-quarters of their capacity. Fermentation soon takes place in them, and the carbonic acid gas finds escape through the bung-hole, the diameter of which, in the case of the largest vessels, is not more than ten or twelve centimetres (about four inches). The wine is not drawn off before the end of two or three months. In this way it seems highly probable that the yeast which produces the wine under such conditions must have developed, to a great extent at least, out of contact with oxygen. No doubt oxygen is not entirely absent from the first; nay, its limited presence is even a necessity to the manifestation of the phenomena which follow. The grapes are stripped from the bunch in contact with air, and the must which drops from the wounded fruit takes a little of this gas into solution. This small quantity of air so introduced into the must, at the commencement of operations, plays a most indispensable part, it being from the presence of this that the spores of ferments which are spread over the surface of the grapes and the woody part of the bunches derive the power of starting their vital phenomena. ¹ This air, however, especially when the grapes have been stripped from the bunches, is in such small proportion, and that which is in contact with the liquid mass is so promptly expelled by the carbonic acid gas, which is evolved as soon as a little yeast has formed, that it will readily be admitted that most of the yeast is produced apart from the influence of oxygen, whether free or in solution. We shall revert to this fact, which is of great importance. At present we are only concerned in pointing out that, from the mere knowledge of the practices of certain localities, we are induced to believe that the cells of yeast, after they have developed from their spores, continue to live and multiply without the intervention of oxygen, and that the alcoholic ferments have a mode of life which is probably quite exceptional, since it is not generally met with in other species, vegetable or animal.

Another equally exceptional characteristic of yeast and fermentation in general consists in the small proportion which the yeast that forms bears to the sugar that decomposes. In all other known beings the weight of nutritive matter assimilated corresponds with the weight of food used up, any difference that may exist being comparatively small. The life of yeast is entirely different. For a certain weight of yeast formed, we may have ten times, twenty times, a hundred times as much sugar, or even more decomposed, as we shall experimentally prove by-and-by; that is to say, that whilst the proportion varies in a precise manner, according to conditions which we shall have occasion to specify, it is also greatly out of proportion to the weight of the yeast. We repeat, the life of no other being, under its normal physiological conditions, can show anything similar. The alcoholic ferments, therefore, present themselves to us as plants which possess at least two singular properties: they can live without air, that is without oxygen, and they can cause decomposition to an amount which, though variable, yet, as estimated by weight of product formed, is out of all proportion to the weight of their own substance. These are facts of so great importance, and so intimately connected with the theory of fermentation, that it is indispensable to endeavour to establish them experimentally, with all the exactness of which they will admit.

The question before us is whether yeast is in reality an anaerobian ² plant, and what quantities of sugar it may cause to ferment, under the various conditions under which we cause it to act.

The following experiments were undertaken to solve this double problem: - We took a double-necked flask, of three litres (five pints) capacity, one of the tubes being curved and forming an escape for the gas; the other one, on the right hand side (Fig. 1), being furnished with a glass tap. We filled this flask with pure yeast water, sweetened with 5 per cent. of sugar candy, the flask being so full that there was not the least trace of air remaining above the tap or in the escape tube; this artificial wort had, however, been itself aerated. The curved tube was plunged in a porcelain vessel full of mercury, resting on a firm support. In the small cylindrical funnel above the tap, the capacity of which was from 10 cc. to 15 cc. (about half a fluid ounce) we caused to ferment, at a temperature of 20 degrees or 25 C. (about 75 F.), five or six cubic centimetres of the saccharine liquid, by means of a trace of yeast, which multiplied rapidly, causing fermentation, and forming a slight deposit of yeast at the bottom of the funnel above the tap. We then opened the tap, and some of the liquid in the funnel entered the flask, carrying with it the small deposit of yeast, which was sufficient to impregnate the saccharine liquid contained in the flask. In this manner it is possible to introduce as small a quantity of yeast as we wish, a quantity the weight of which, we may say, is hardly appreciable. The yeast sown multiplies rapidly and produces fermentation, the carbonic gas from which is expelled into the mercury. In less than twelve days all the sugar had disappeared, and the fermentation had finished. There was a sensible deposit of yeast adhering to the sides of the flask; collected and dried it weighed 2.25 grammes (34 grains). It is evident that in this experiment the total amount of yeast formed, if it required oxygen to enable it to live, could not have absorbed, at most, more than the volume which was originally held in solution in the saccharine liquid, when that was exposed to the air before being introduced into the flask.

Some exact experiments conducted by M. Raulin in our laboratory have established the fact that saccharine worts, like water, soon become saturated when shaken briskly with an excess of air, and also that they always take into solution a little less air than saturated pure water contains under the same conditions of temperature and pressure. At a temperature of 25 C. (77 F.), therefore, if we adopt the coefficient of the solubility of oxygen in water given in Bunsen's tables, we find that 1 litre (1 3/4 pints) of water saturated with air contains 5.5 cc. (0.3 cubic inch) of oxygen. The three litres of yeast-water in the flask, supposing it to have been saturated, contains less than 16.5 cc. (1 cubic inch) of oxygen, or, in weight, less than 23 milligrammes (0.35 grains). This was the maximum amount of oxygen, supposing the greatest possible quantity to have been absorbed, that was required by the yeast formed in the fermentation of 150 grammes (4.8 Troy ounces) of sugar. We shall better understand the significance of this result later on. Let us repeat the foregoing experiment, but under altered conditions. Let us fill, as before, our flask with sweetened yeast-water, but let this first be boiled, so as to expel all the air it contains. To effect this we arrange our apparatus as represented in the accompanying sketch. (Fig. 2.) We place our flask, A, on a tripod above a gas flame, and in place of the vessel of mercury substitute a porcelain dish, under which we can put a gas flame, and which contains some fermentable, saccharine liquid, similar to that with which the flask is filled. We boil the liquid in the flask and that in the basin simultaneously, and then let them cool down together, so that as the liquid in the flask cools some of the liquid is sucked from the basin into the flask. From a trial experiment which we conducted, determining the quantity of oxygen that remained in solution in the liquid after cooling, according to M. Schutzenberger's valuable method, by means of hydrosulphite of soda,³ we found that the three litres in the flask, treated as we have described, contained less than one milligramme (0.015 grain) of oxygen. At the same time we conducted another experiment, by way of comparison (Fig. 3). We took a flask, B, of larger capacity than the former one, which we filled about half with the same volume as before of a saccharine liquid of identically the same composition. This liquid had been previously freed from alterative germs by boiling. In the funnel surmounting A, we put a few cubic centimetres of saccharine liquid in a state of fermentation, and when this small quantity of liquid was in full fermentation, and the yeast in it was young and vigorous, we opened the tap, closing it again immediately, so that a little of the liquid and yeast still remained in the funnel. By this means we caused the liquid in A to ferment. We also impregnated the liquid in B with some yeast taken from the funnel of A. We then replaced the porcelain dish in which the curved escape tube of A had been plunged, by a vessel filled with mercury. The following is a description of two of these comparative fermentations and the results they gave.

The fermentable liquid was composed of yeast-water sweetened with 5 per cent. of sugar-candy; the ferment employed was *sacchormyces pastorianus*.

The impregnation took place on January 20th. The flasks were placed in an oven at 25 degrees (77 F.).

Flask A, without air.

January 21st. - Fermentation commenced; a little frothy liquid issued from the escape tube and covered the mercury.

The following days, fermentation was active. Examining the yeast mixed with the froth that was expelled into the mercury by the evolution of carbonic acid gas, we find that it was very fine, young, and actively budding.

February 3rd. - Fermentation still continued, showing itself by a number of little bubbles rising from the bottom of the liquid, which had settled bright. The yeast was at the bottom in the form of a deposit.

February 7th. - Fermentation still continued, but very languidly.

February 9th. - A very languid fermentation still went on, discernible in little bubbles rising from the bottom of the flask.

Flask B, with air.

January 21st. - A sensible development of yeast.

The following days, fermentation was active, and there was an abundant froth on the surface of the liquid.

February 1st. - All symptoms of fermentation had ceased.

As the fermentation in A would have continued a long time, being so very languid, and as that in B had been finished for several days, we brought to a close our two experiments on February 9th. To do this we poured off the liquids in A and B, collecting the yeasts on tared filters. Filtration was an easy matter, more especially in the case of A. Examining the yeasts under the microscope, immediately after decantation, we found that both of them remained very pure. The yeast in A was in little clusters, the globules of which were collected together, and appeared by their well-defined borders to be ready for an easy revival in contact with air.

As might have been expected, the liquid in flask B did not contain the least trace of sugar; that in the flask A still contained some, as was evident from the non-completion of fermentation, but not more than 4.6 grammes (71 grains). Now, as each flask originally contained three litres of liquid holding in solution 5 per cent. of sugar, it follows that 150 grammes (2,310 grains) of sugar had fermented in the flask B, and 145.4 grammes (2,239.2 grains) in the flask A. The weights of yeast after drying at 100 C. (212 F.) were -

For the flask B, with air....1,970 grammes (30.4 grains).

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The proportions were 1 of yeast to 76 of fermented sugar in the first case, and 1 of yeast to 89 of fermented sugar in the second.

From these facts the following consequences may be deduced:

1. The fermentable liquid (flask B), which since it had been in contact with air, necessarily held air in solution, although not to the point of saturation, inasmuch as it had been once boiled to free it from all foreign germs, furnished a weight of yeast sensibly greater than that yielded by the liquid which contained no air at all (flask A) or, at least, which could only have contained an exceedingly minute quantity.

2. This same slightly aerated fermentable liquid fermented much more rapidly than the other. In eight or ten days it contained no more sugar; while the other, after twenty days, still contained an appreciable quantity.

Is this last fact to be explained by the greater quantity of yeast formed in B? By no means. At first, when the air has access to the liquid, much yeast is formed and little sugar disappears, as we shall prove immediately; nevertheless the yeast formed in contact with the air is more active than the other. Fermentation is correlative first to the development of the globules, and then to the continued life of those globules once formed. The more oxygen these last globules have at their disposal during their formation, the more vigorous, transparent, and turgid, and, as a consequence of this last quality, the more active they are in decomposing sugar. We shall hereafter revert to these facts.

3. In the airless flask the proportion of yeast to sugar was $1/89$; it was only $1/76$ in the flask which had air at first.

The proportion that the weight of yeast bears to the weight of the sugar is, therefore, variable, and this variation depends, to a certain extent, upon the presence of air and the possibility of oxygen being absorbed by the yeast. We shall presently show that yeast possesses the power of absorbing that gas and emitting carbonic acid, like ordinary fungi, that even oxygen may be reckoned amongst the number of food-stuffs that may be assimilated by this plant, and that this fixation of oxygen in yeast, as well as the oxidations, resulting from it, have the most marked effect on the life of yeast, on the multiplication of its cells, and on their activity as ferments acting upon sugar, whether immediately or afterwards, apart from supplies of oxygen or air.

In the preceding experiment, conducted without the presence of air, there is one circumstance particularly worthy of notice. This experiment succeeds, that is to say, the yeast sown in the medium deprived of oxygen develops, only when this yeast is in a state of great vigour. We have already explained the meaning of this last expression. But we wish now to call attention to a very evident fact in connection with this point. We impregnate a fermentable liquid; yeast develops and fermentation appears. This lasts for several days and then ceases. Let us suppose that, from the day when fermentation first appears in the production of a minute froth, which gradually increases until it whitens the surface of the liquid, we take, every twenty-four hours, or at longer intervals, a trace of the yeast deposited on the bottom of the vessel and use it for starting fresh fermentations. Conducting these fermentations all under precisely the same conditions of temperature, character and volume of liquid, let us continue this for a prolonged time, even after the original fermentation is finished. We shall have no difficulty in seeing that the first signs of action in each of our series of second fermentations appear always later and later in proportion to the length of time that has elapsed from the commencement of the original fermentation. In other words, the time necessary for the development of the germs and the production of that amount of yeast sufficient to cause the first appearance of fermentation varies with the state of the impregnating cells, and is longer in proportion as the cells are further removed from the period of their formation. It is essential, in experiments of this kind, that the quantities of yeast successively taken should be as nearly as possible equal in weight or volume, since, *ceteris paribus*, fermentations manifest themselves more quickly the larger the quantity of yeast employed in impregnation.

If we compare under the microscope the appearance and character of the successive quantities of yeast taken, we shall see plainly that the structure of the cells undergoes a progressive change. The first sample which we take, quite at the beginning of the original fermentation, generally gives us cells rather larger than those later on, and possessing a remarkable tenderness. Their walls are exceedingly thin, the consistency and softness of their protoplasm is akin to fluidity, and their granular contents appear in the form of scarcely visible spots. The borders of the cells soon become more marked, a proof that their walls undergo a thickening; their protoplasm also becomes denser, and the granulations more distinct. Cells of the same organ, in the states of infancy and old age, should not differ more than the cells of which we are speaking, taken in their extreme states. The progressive changes in the cells, after they have acquired their normal form and volume, clearly demonstrate the existence of a chemical work of a remarkable intensity, during which their weight increases, although in volume they undergo no sensible change, a fact that we have often characterized as "the continued life of cells already formed." We may call this work a process of maturation on the part of the cells, almost the same that we see going on in the case of adult beings in general, which continue to live for a long time, even after they have become incapable of reproduction, and long after their volume has become permanently fixed.

This being so, it is evident, we repeat, that, to multiply in a fermentable medium, quite out of contact with oxygen, the cells of yeast must be extremely young, full of life and health, and still under the influence of the vital activity which they owe to the free oxygen which has served to form them, and which they have perhaps stored up for a time. When older, they reproduce themselves with much difficulty when deprived of air, and gradually become more languid; and if they do multiply, it is in strange and monstrous forms. A little older still, they remain absolutely inert in a medium deprived of free oxygen. This is not because they are dead; for in general they may be revived in a marvellous manner in the same liquid if it has been first aerated before they are sown. It would not surprise us to learn that at this point certain preconceived ideas suggest themselves to the mind of an attentive reader on the subject of the causes that may serve to account for such strange phenomena in the life of these beings which our ignorance hides under the expressions of youth and age; this, however, is a subject which we cannot pause to consider here.

At this point we must observe - for it is a matter of great importance - that in the operations of the brewer there is always a time when the yeasts are in this state of vigorous youth of which we have been speaking, acquired under the influence of free oxygen, since all the worts and the yeasts of commerce are necessarily manipulated in contact with air, and so impregnated more or less with oxygen. The yeast immediately seizes upon this gas and acquires a state of freshness and activity, which permits it to live afterwards out of contact with air, and to act as a ferment. Thus, in ordinary brewery practice, we find the yeast already formed in abundance even before the earliest external signs of fermentation have made their appearance. In this first phase of its existence, yeast lives chiefly like an ordinary fungus.

From the same circumstances it is clear that the brewer's fermentations may, speaking quite strictly, last for an indefinite time, in consequence of the unceasing supply of fresh wort, and from the fact, moreover, that the exterior air is constantly being introduced during the work, and that the air contained in the fresh worts keeps up the vital activity of the yeast, as the act of breathing keeps up the vigour and life of cells in all living beings. If the air could not renew itself in any way, the vital activity which the cells originally received, under its influence, would become more and more exhausted, and the fermentation eventually come to an end.

Part II.

We may recount one of the results obtained in other experiments similar to the last, in which, however, we employed yeast which was still older than that used for our experiment with flask A (Fig. 2), and moreover took still greater precautions to prevent the presence of air. Instead of leaving the flask, as well as the dish, to cool slowly, after having expelled all air by boiling, we permitted the liquid in the dish to continue boiling whilst the flask was being cooled by artificial means; the end of the escape tube was then taken out of the still boiling dish and plunged into the mercury trough. In impregnating the liquid, instead of employing the contents of the small cylindrical funnel whilst still in a state of fermentation, we waited until this was finished. Under these conditions, fermentation was still going on in our flask, after a lapse of three months. We stopped it and found that 0.255 gramme (3.9 grains) of yeast had been formed, and that 45 grammes (693 grains) of sugar had fermented, the ratio between the weights of yeast and sugar being thus $0.255/45 = 1/176$. In this experiment the yeast developed with much difficulty, by reason of the conditions to which it had been subjected. In appearance the cells varied much, some were to be found large, elongated, and of tubular aspect, some seemed very old and were extremely granular, whilst others were more transparent. All of them might be considered abnormal cells.

In such experiments we encounter another difficulty. If the yeast sown in the non-aerated fermentable liquid is in the least degree impure, especially if we use sweetened yeast-water, we may be sure that alcoholic fermentation will soon cease, if, indeed, it ever commences, and that accessory fermentations will go on. The vibrios of butyric fermentation, for instance, will propagate with remarkable facility under these circumstances. Clearly then, the purity of the yeast at the moment of impregnation, and the purity of the liquid in the funnel, are conditions indispensable to success.

To secure the latter of these conditions, we close the funnel, as shown in Fig. 2, by means of a cork pierced with two holes, through one of which a short tube passes, to which a short length of indiarubber tubing provided with a glass stopper is attached; through the other hole a thin curved tube is passed. Thus fitted, the funnel can answer the same purposes as our double-necked flasks. A few cubic centimetres of sweetened yeast-water are put in it and boiled, so that the steam may destroy any germs adhering to the sides; and when cold the liquid is impregnated by means of a trace of pure yeast, introduced through the glass-stoppered tube. If these precautions are neglected, it is scarcely possible to secure a successful fermentation in our flasks, because the yeast sown is immediately held in check by a development of anaerobian vibrios. For greater security, we may add to the fermentable liquid, at the moment when it is prepared, a very small quantity of tartaric acid, which will prevent the development of butyric vibrios.

The variation of the ratio between the weight of the yeast and that of the sugar decomposed by it now claims special attention. Side by side with the experiments which we have just described, we conducted a third lot by means of the flask C (Fig. 4), holding 4.7 litres (8 1/2 pints), and fitted up like the usual two-necked flasks, with the object of freeing the fermentable liquid from foreign germs, by boiling it to begin with, so that we might carry on our work under conditions of purity. The volume of yeast-water (containing 5 per cent. of sugar) was only 200 cc. (7 fl. oz.), and consequently, taking into account the capacity of the flask, it formed but a very thin layer at the bottom. On the day after impregnation the deposit of yeast was already considerable, and forty-eight hours afterwards the fermentation was completed. On the third day we collected the yeast after having analyzed the gas contained in the flask. This analysis was easily accomplished by placing the flask in a hot-water bath, whilst the end of the curved tube was plunged under a cylinder of mercury. The gas contained 41.4 per cent. of carbonic acid, and, after the absorption, the remaining air contained: -

Oxygen 19.7 Nitrogen 80.3
 100.0

Taking into consideration the volume of this flask, this shows a minimum of 50 cc. (3.05 cub. in.) of oxygen to have been absorbed by the yeast. The liquid contained no more sugar, and the weight of the yeast, dried at a temperature of 100 C. (212 F.), was 0.44 grammes. The ratio between the weights of yeast and sugar is $0.44/10 = 1/22.7^5$. On this occasion, where we had increased the quantity of oxygen held in solution, so as to yield itself for assimilation at the beginning and during the earlier developments of the yeast, we found instead of the previous ratio of $1/76$ that of $1/23$.

The next experiment was to increase the proportion of oxygen to a still greater extent, by rendering the diffusion of gas a more easy matter than in a flask, the air in which is in a state of perfect quiescence. Such a state of matters hinders the supply of oxygen, inasmuch as the carbonic acid, as soon as it is liberated, at once forms an immovable layer on the surface of the liquid, and so separates off the oxygen. To effect the purpose of our present experiment, we used flat basins having glass bottoms and low sides, also of glass, in which the depth of the liquid is not more than a few millimetres (less than $1/4$ inch [Fig. 5]). The following is one of our experiments so conducted: - On April 16th, 1860, we sowed a trace of beer yeast ("high" yeast) in 200 cc. (7 fl. oz.) of a saccharine liquid containing 1.720 grammes (26.2 grains) of sugarcandy. From April 18th our yeast was in good condition and well developed. We collected it, after having added to the liquid a few drops of concentrated sulphuric acid, with the object of checking the fermentation to a great extent, and facilitating filtration. The sugar remaining in the filtered liquid, determined by Fehling's solution, showed that 1.04 grammes (16 grains) of sugar had disappeared. The weight of the yeast, dried at 100 C. (212 F.), was 0.127 gramme (2 grains), which gives us the ratio between the weight of the yeast and that of the fermented sugar $0.127/1.04 = 1/8.1$, which is considerably higher than the preceding ones.

We may still further increase this ratio by making our estimation as soon as possible after the impregnation, or the addition of the ferment. It will be readily understood why yeast, which is composed of cells that bud and subsequently detach themselves from one another, soon forms a deposit at the bottom of the vessels. In consequence of this habit of growth, the cells constantly covering each other prevents the lower layers from having access to the oxygen held in solution in the liquid, which is absorbed by the upper ones. Hence, these which are covered and deprived of this gas act on the sugar without deriving any vital benefit from the oxygen - a circumstance which must tend to diminish the ratio of which we are speaking. Once more repeating the preceding experiment, but stopping it as soon as we think that the weight of yeast formed may be determined by the balance (we find that this may be done twenty-four hours after impregnation with an inappreciable quantity of yeast), in this case the ratio between the weights of yeast and sugar is 0 gr. 024 yeast/0 gr. 098 sugar = 1/4. This is the highest ratio we have been able to obtain.

Under these conditions the fermentation of sugar is extremely languid: the ratio obtained is very nearly the same that ordinary fungoid growths would give. The carbonic acid evolved is principally formed by the decompositions which result from the assimilation of atmospheric oxygen. The yeast, therefore, lives and performs its functions after the manner of ordinary fungi: so far it is no longer a ferment, so to say; moreover, we might expect to find it to cease to be a ferment at all if we could only surround each cell separately with all the air that it required. This is what the preceding phenomena teach us; we shall have occasion to compare them later on with others which relate to the vital action exercised on yeast by the sugar of milk.

We may here be permitted to make a digression.

In his work on fermentations, which M. Schutzenberger has recently published, the author criticises the deductions that we have drawn from the preceding experiments, and combats the explanation which we have given of the phenomena of fermentation. ⁶ It is an easy matter to show the weak point of M. Schutzenberger's reasoning. We determined the power of the ferment by the relation of the weight of sugar decomposed to the weight of the yeast produced. M. Schutzenberger asserts that in doing this we lay down a doubtful hypothesis, and he thinks that this power, which he terms fermentative energy, may be estimated more correctly by the quantity of sugar decomposed by the unit-weight of yeast in unit-time; moreover, since our experiments show that yeast is very vigorous when it has a sufficient supply of oxygen, and that, in such a case, it can decompose much sugar in a little time, M. Schutzenberger concludes that it must then have great power as a ferment, even greater than when it performs its functions without the aid of air, since under this condition it decomposes sugar very slowly. In short, he is disposed to draw from our observations the very opposite conclusion to that which we arrived at.

M. Schutzenberger has failed to notice that the power of a ferment is independent of the time during which it performs its functions. We placed a trace of yeast in one litre of saccharine wort; it propagated, and all the sugar was decomposed. Now, whether the chemical action involved in this decomposition of sugar had required for its completion one day, or one month, or one year, such a factor was of no more importance in this matter than the mechanical labour required to raise a ton of materials from the ground to the top of a house would be affected by the fact that it had taken twelve hours instead of one. The notion of time has nothing to do with the definition of work. M. Schutzenberger has not perceived that in introducing the consideration of time into the definition of the power of a ferment, he must introduce at the same time, that of the vital activity of the cells which is independent of their character as a ferment. Apart from the consideration of the relation existing between the weight of fermentable substance decomposed and that of ferment produced, there is no occasion to speak of fermentations or of ferments. The phenomena of fermentation and of ferments have been placed apart from others, precisely because, in certain chemical actions, that ratio has been out of proportion; but the time that these phenomena require for their accomplishment has nothing to do with either their existence proper, or with their power. The cells of a ferment may, under some circumstances, require eight days for revival and propagation, whilst, under other conditions, only a few hours are necessary; so that, if we introduce the notion of time into our estimate of their power of decomposition, we may be led to conclude that in the first case that power was entirely wanting, and that in the second case it was considerable, although all the time we are dealing with the same organism - the identical ferment.

M. Schutzenberger is astonished that fermentation can take place in the presence of free oxygen, if, as we suppose, the decomposition of the sugar is the consequence of the nutrition of the yeast, at the expense of the combined oxygen, which yields itself to the ferment. At all events, he argues, fermentation ought to be slower in the presence of free oxygen. But why should it be slower? We have proved that in the presence of oxygen the vital activity of the cells increases, so that, as far as rapidity of action is concerned, its power cannot be diminished. It might, nevertheless, be weakened as a ferment, and this is precisely what happens. Free oxygen imparts to the yeast a vital activity, but at the same time impairs its power as yeast-qua yeast, inasmuch as under this condition it approaches the state in which it can carry on its vital processes after the manner of an ordinary fungus: the mode of life, that is, in which the ratio between the weight of sugar decomposed and the weight of the new cells produced will be the same as holds generally among organisms which are not ferments. In short, varying our form of expression a little, we may conclude with perfect truth, from the sum total of observed facts, that the yeast which lives in the presence of oxygen and can assimilate as much of that gas as is necessary to its perfect nutrition, ceases absolutely to be a ferment at all. Nevertheless, yeast formed under these conditions and subsequently brought into the presence of sugar, out of the influence of air, would decompose more in a given time than in any other of its states. The reason is that yeast which has formed in contact with air, having the maximum of free oxygen that it can assimilate is fresher and possessed of greater vital activity than that which has been formed without air or with an insufficiency of air. M. Schutzenberger would associate this activity with the notion of time in estimating the power of the ferment; but he forgets to notice that yeast can only manifest this maximum of energy under a radical change of its life conditions; by having no more air at its disposal and breathing no more free oxygen. In other words, when its respiratory power becomes null, its fermentative power is at its greatest. M. Schutzenberger asserts exactly the opposite (p. 151 of his work - Paris. 1875), ⁷ and so gratuitously places himself in opposition to facts.

In presence of abundant air supply, yeast vegetates with extraordinary activity. We see this in the weight of new yeast, comparatively large, that may be formed in the course of a few hours. The microscope still more clearly shows this activity in the rapidity of budding, and the fresh and active appearance of all the cells. Fig. 6 represents the yeast of our last experiment at the moment when we stopped the fermentation. Nothing has been taken from imagination, all the groups have been faithfully sketched as they were.⁸

In passing it is of interest to note how promptly the preceding results were turned to good account practically. In well-managed distilleries, the custom of aerating the wort and the juices to render them more adapted to fermentation, has been introduced. The molasses mixed with water is permitted to run in thin threads through the air at the moment when the yeast is added. Manufactories have been erected in which the manufacture of yeast is almost exclusively carried on. The saccharine worts, after the addition of yeast, are left to themselves, in contact with air, in shallow vats of large superficial area, realizing thus on an immense scale the conditions of the experiments which we undertook in 1861, and which we have already described in determining the rapid and easy multiplication of yeast in contact with air.

The next experiment was to determine the volume of oxygen absorbed by a known quantity of yeast, the yeast living in contact with air, and under such conditions that the absorption of air was comparatively easy and abundant.

With this object we repeated the experiment that we performed with the large-bottomed flask (Fig. 4), employing a vessel shaped like Fig. B (Fig. 7), which is, in point of fact, the flask A with its neck drawn out and closed in a flame, after the introduction of a thin layer of some saccharine juice impregnated with a trace of pure yeast. The following are the data and results of an experiment of this kind.

⁹ The weight of the yeast, in a state of dryness, was 0.035 gramme.

It follows that in the production of 35 milligrammes (0.524 grain) of yeast there was an absorption of 14 or 15 cc. (about 7/8 cub. in.) of oxygen, even supposing that the yeast was formed entirely under the influence of that gas: this is equivalent to not less than 414 cc. for 1 gramme of yeast (or about 33 cubic inches for every 20 grains). ¹⁰

Such is the large volume of oxygen necessary for the development of one gramme of yeast when the plant can assimilate this gas after the manner of an ordinary fungus.

Let us now return to the first experiment described in the paragraph on page 278 in which a flask of three litres capacity was filled with fermentable liquid, which, when caused to ferment, yielded 2.25 grammes of yeast, under circumstances where it could not obtain a greater supply of free oxygen than 16.5 cc. (about one cubic inch). According to what we have just stated, if this 2.25 grammes (34 grains) of yeast had not been able to live without oxygen, in other words, if the original cells had been unable to multiply otherwise than by absorbing free oxygen, the amount of that gas required could not have been less than 2.25×414 cc., that is, 931.5 cc. (56.85 cubic inches). The greater part of the 2.25 grammes, therefore, had evidently been produced as the growth of an anaerobian plant.

Ordinary fungi likewise require large quantities of oxygen for their development, as we may readily prove by cultivating any mould in a closed vessel full of air, and then taking the weight of plant formed and measuring the volume of oxygen absorbed. To do this, we take a flask of the shape shown in Fig. 8, capable of holding about 300 cc. (10 1/2 fluid ounces), and containing a liquid adapted to the life of moulds. We boil this liquid, and seal the drawn-out point after the steam has expelled the air wholly or in part; we then open the flask in a garden or in a room. Should a fungus-spore enter the flask, as will invariably be the case in a certain number of flasks out of several used in the experiment, except under special circumstances, it will develop there and gradually absorb all the oxygen contained in the air of the flask. Measuring the volume of this air, and weighing, after drying, the amount of plant formed, we find that for a certain quantity of oxygen absorbed we have a certain weight of mycelium, or of mycelium together with its organs of fructification. In an experiment of this kind, in which the plant was weighed a year after its development, we found for 0.008 gramme (0.123 grain) of mycelium, dried at 100 C. (212 F.), an absorption that amounted to not less than 43 cc. (2.5 cubic inches) of oxygen at 25 C. These numbers, however, must vary sensibly with the nature of the mould employed, and also with the greater or less activity of its development, because the phenomena is complicated by the presence of accessory oxidations, such as we find in the case of *mycoderma vini* and *aceti*, to which cause the large absorption of oxygen in our last experiment may doubtless be attributed. ¹¹

The conclusions to be drawn from the whole of the preceding facts can scarcely admit of doubt. As for ourselves, we have no hesitation in finding them the foundation of the true theory of fermentation. In the experiments which we have described, fermentation by yeast, that is to say, by the type of ferments properly so called, is presented to us, in a word, as the direct consequence of the processes of nutrition, assimilation and life, when these are carried on without the agency of free oxygen. The heat required in the accomplishment of that work must necessarily have been borrowed from the decomposition of the fermentable matter, that is from the saccharine substance which, like other unstable substances, liberates heat in undergoing decomposition. Fermentation by means of yeast appears, therefore, to be essentially connected with the property possessed by this minute cellular plant of performing its respiratory functions, somehow or other, with oxygen existing combined in sugar. Its fermentative power - which power must not be confounded with the fermentative activity or the intensity of decomposition in a given time - varies considerably between two limits, fixed by the greatest and least possible access to free oxygen which the plant has in the process of nutrition. If we supply it with a sufficient quantity of free oxygen for the necessities of its life, nutrition, and respiratory combustions, in other words, if we cause it to live after the manner of a mould, properly so called, it ceases to be a ferment, that is, the ratio between the weight of the plant developed and that of the sugar decomposed, which forms its principal food, is similar in amount to that in the case of fungi. ¹² On the other hand, if we deprive the yeast of air entirely, or cause it to develop in a saccharine medium deprived of free oxygen, it will multiply just as if air were present, although with less activity, and under these circumstances its fermentative character will be most marked; under these circumstances, moreover, we shall find the greatest disproportion, all other conditions being the same, between the weight of yeast formed and the weight of sugar decomposed. Lastly, if free oxygen occurs in varying quantities, the ferment-power of the yeast may pass through all the degrees comprehended between the two extreme limits of which we have just spoken. It seems to us that we could not have a better proof of the direct relation that fermentation bears to life, carried on in the absence of free oxygen, or with a quantity of that gas insufficient for all the acts of nutrition and assimilation.

Another equally striking proof of the truth of this theory is the fact previously demonstrated that the ordinary moulds assume the character of a ferment when compelled to live without air, or with quantities of air too scant to permit of their organs having around them as much of that element as is necessary for their life as aerobian plants. Ferments, therefore, only possess in a higher degree a character which belongs to many common moulds, if not to all, and which they share, probably, more or less, with all living cells, namely the power of living either an aerobian or anaerobian life, according to the conditions under which they are placed.

It may be readily understood how, in their state of aerobic life, the alcoholic ferments have failed to attract attention. These ferments are only cultivated out of contact with air, at the bottom of liquids which soon become saturated with carbonic acid gas. Air is only present in the earlier developments of their germs, and without attracting the attention of the operator, whilst in their state of anaerobic growth their life and action are of prolonged duration. We must have recourse to special experimental apparatus to enable us to demonstrate the mode of life of alcoholic ferments under the influence of free oxygen; it is their state of existence apart from air, in the depths of liquids, that attracts all our attention. The results of their action are, however, marvellous, if we regard the products resulting from them, in the important industries of which they are the life and soul. In the case of ordinary moulds, the opposite holds good. What we want to use special experimental apparatus for with them, is to enable us to demonstrate the possibility of their continuing to live for a time out of contact with air, and all our attention, in their case, is attracted by the facility with which they develop under the influence of oxygen. Thus the decomposition of saccharine liquids, which is the consequence of the life of fungi without air, is scarcely perceptible, and so is of no practical importance. Their aerial life, on the other hand, in which they respire and accomplish their process of oxidation under the influence of free oxygen is a normal phenomenon, and one of prolonged duration which cannot fail to strike the least thoughtful of observers. We are convinced that a day will come when moulds will be utilised in certain industrial operations, on account of their power in destroying organic matter. The conversion of alcohol into vinegar in the process of acetification and the production of gallic acid by the action of fungi on wet gall nuts, are already connected with this kind of phenomena. ¹³ On this last subject, the important work of M. Van Tieghem (*Annales Scientifiques de l'Ecole Normale*, vol. vi.) may be consulted.

The possibility of living without oxygen, in the case of ordinary moulds, is connected with certain morphological modifications which are more marked in proportion as this faculty is itself more developed. These changes in the vegetative forms are scarcely perceptible, in the case of penicillium and mycoderma vini, but they are very evident in the case of aspergillus, consisting of a marked tendency on the part of the submerged mycelial filaments to increase in diameter, and to develop cross partitions at short intervals, so that they sometimes bear a resemblance to chains of conidia. In mucor, again, they are very marked, the inflated filaments which, closely interwoven, present chains of cells, which fall off and bud, gradually producing a mass of cells. If we consider the matter carefully, we shall see that yeast presents the same characteristics.

It is a great presumption in favor of the truth of theoretical ideas when the results of experiments undertaken on the strength of those ideas are confirmed by various facts more recently added to science, and when those ideas force themselves more and more on our minds, in spite of a prima facie improbability. This is exactly the character of those ideas which we have just expounded. We pronounced them in 1861, and not only have they remained unshaken since, but they have served to foreshadow new facts, so that it is much easier to defend them in the present day than it was to do so fifteen years ago. We first called attention to them in various notes, which we read before the Chemical Society of Paris, notably at its meetings of April 12th and June 28th, 1861, and in papers in the *Comptes rendus de l'Academie des Sciences*. It may be of some interest to quote here, in its entirety, our communication of June 28th, 1861, entitled, "Influences of Oxygen on the Development of Yeast and on Alcoholic Fermentation," which we extract from the *Bulletin de la Societe Chimique de Paris*: -

"M. Pasteur gives the result of his researches on the fermentation of sugar and the development of yeast-cells, according as that fermentation takes place apart from the influence of free oxygen or in contact with that gas. His experiments, however, have nothing in common with those of Gay-Lussac, which were performed with the juice of grapes crushed under conditions where they would not be affected by air, and then brought into contact with oxygen.

"Yeast, when perfectly developed, is able to bud and grow in a saccharine and albuminous liquid, in the complete absence of oxygen or air. In this case but little yeast is formed, and a comparatively large quantity of sugar disappears - sixty or eighty parts for one of yeast formed. Under these conditions fermentation is very sluggish.

"If the experiment is made in contact with the air, and with a great surface of liquid, fermentation is rapid. For the same quantity of sugar decomposed much more yeast is formed. The air with which the liquid is in contact is absorbed by the yeast. The yeast develops very actively, but its fermentative character tends to disappear under these conditions; we find, in fact, that for one part of yeast formed, not more than from four to ten parts of sugar are transformed. The fermentative character of this yeast nevertheless continues, and produces even increased effects, if it is made to act on sugar apart from the influence of free oxygen.

"It seems, therefore, natural to admit that when yeast functions as a ferment by living apart from the influence of air, it derives oxygen from the sugar, and that this is the origin of its fermentative character.

"M. Pasteur explains the fact of the immense activity at the commencement of fermentations by the influence of the oxygen of the air held in solution in the liquids, at the time when the action commences. The author has found, moreover, that the yeast of beer sown in an albuminous liquid, such as yeast-water, still multiplies, even when there is not a trace of sugar in the liquid, provided always that atmospheric oxygen is present in large quantities. When deprived of air, under these conditions, yeast does not germinate at all. The same experiments may be repeated with albuminous liquid, mixed with a solution of non-fermentable sugar, such as ordinary crystallized milk-sugar. The results are precisely the same.

"Yeast formed thus in the absence of sugar does not change its nature; it is still capable of causing sugar to ferment, if brought to bear upon that substance apart from air. It must be remarked, however, that the development of yeast is effected with great difficulty when it has not a fermentable substance for its food. In short, the yeast of beer acts in exactly the same manner as an ordinary plant, and the analogy would be complete if ordinary plants had such an affinity for oxygen as permitted them to breathe by appropriating this element from unstable compounds, in which case, according to M. Pasteur, they would appear as ferments for those substances.

"M. Pasteur declares that he hopes to be able to realize this result, that is to say, to discover the conditions under which certain inferior plants may live apart from air in the presence of sugar, causing that substance to ferment as the yeast of beer would do."

This summary and the preconceived views that it set forth have lost nothing of their exactness; on the contrary, time has strengthened them. The surmises of the last two paragraphs have received valuable confirmation from recent observations made by Messrs. Lechartier and Bellamy, as well as by ourselves, an account of which we must put before our readers. It is necessary, however, before touching upon this curious feature in connection with fermentations to insist on the accuracy of a passage in the preceding summary; the statement, namely, that yeast could multiply in an albuminous liquid, in which it found a non-fermentable sugar, milk-sugar, for example. The following is an experiment on this point: - On August 15th, 1875, we sowed a trace of yeast in 150 cc. (rather more than 5 fluid ounces) of yeast-water, containing 2 1/2 per cent. of milk-sugar. The solution was prepared in one of our double-necked flasks, with the necessary precautions to secure the absence of germs, and the yeast sown was itself perfectly pure. Three months afterwards, November 15th, 1875, we examined the liquid for alcohol; it contained only the smallest trace; as for the yeast (which had sensibly developed), collected and dried on a filter paper, it weighed 0.050 gramme (0.76 grain). In this case we have the yeast multiplying without giving rise to the least fermentation, like a fungoid growth, absorbing oxygen, and evolving carbonic acid, and there is no doubt that the cessation of its development in this experiment was due to the progressive deprivation of oxygen that occurred. As soon as the gaseous mixture in the flask consisted entirely of carbonic acid and nitrogen, the vitality of the yeast was dependent on, and in proportion to, the quantity of air which entered the flask in consequence of variations of temperature. The question now arose, was this yeast, which had developed wholly as an ordinary fungus, still capable of manifesting the character of a ferment? To settle this point we had taken the precaution on August 15th, 1875, of preparing another flask, exactly similar to the preceding one in every respect, and which gave results identical with those described. We decanted this November 15th, pouring some wort on the deposit of the plant, which remained in the flask. In less than five hours from the time we placed it in the oven, the plant started fermentation in the wort, as we could see by the bubbles of gas rising to form patches on the surface of the liquid. We may add that yeast in the medium which we have been discussing will not develop at all without air.

The importance of these results can escape no one; they prove clearly that the fermentative character is not an invariable phenomenon of yeast-life, they show that yeast is a plant which does not differ from ordinary plants, and which manifests its fermentative power solely in consequence of particular conditions under which it is compelled to live. It may carry on its life as a ferment or not, and after having lived without manifesting the slightest symptom of fermentative character, it is quite ready to manifest that character when brought under suitable conditions. The fermentative property, therefore, is not a power peculiar to cells of a special nature. It is not a permanent character of a particular structure, like, for instance, the property of acidity or alkalinity. It is a peculiarity dependent on external circumstances and on the nutritive conditions of the organism.

Section II.

Fermentation in Saccharine Fruits Immersed in Carbonic Acid Gas

The theory which we have, step by step, evolved, on the subject of the cause of the chemical phenomena of fermentation, may claim a character of simplicity and generality that is well worthy of attention. Fermentation is no longer one of those isolated and mysterious phenomena which do not admit of explanation. It is the consequence of a peculiar vital process of nutrition which occurs under certain conditions, differing from those which characterize the life of all ordinary beings, animal or vegetable, but by which the latter may be affected, more or less, in a way which brings them, to some extent within the class of ferments, properly so called. We can even conceive that the fermentative character may belong to every organized form, to every animal or vegetable cell, on the sole condition that the chemico-vital acts of assimilation and excretion must be capable of taking place in that cell for a brief period, longer or shorter it may be, without necessity for recourse to supplies of atmospheric oxygen; in other words, the cell must be able to derive its needful heat from the decomposition of some body which yields a surplus of heat in the process.

As a consequence of these conclusions it should be an easy matter to show, in the majority of living beings, the manifestation of the phenomena of fermentation; for there are, probably, none in which all chemical action entirely disappears, upon the sudden cessation of life. One day, when we were expressing these views in our laboratory, in the presence of M. Dumas, who seemed inclined to admit their truth, we added: "We should like to make a wager that if we were to plunge a bunch of grapes into carbonic acid gas, there would be immediately produced alcohol and carbonic acid gas, in consequence of a renewed action starting in the interior cells of the grapes, in such a way that these cells would assume the functions of yeast cells. We will make the experiment, and when you come to-morrow" - it was our good fortune to have M. Dumas working in our laboratory at that time - "we will give you an account of the result." Our predictions were realized. We then endeavoured to find, in the presence of M. Dumas, who assisted us in our endeavour, cells of yeast in the grapes; but it was quite impossible to discover any. ¹

Encouraged by this result, we undertook fresh experiments on grapes, on a melon, on oranges, on plums, and on rhubarb leaves, gathered in the garden of the Ecole Normale, and, in every case, our substance, when immersed in carbonic acid gas, gave rise to the production of alcohol and carbonic acid. We obtained the following surprising results from some prunes de Monsieur: ² - On July 21, 1872, we placed twenty-four of these plums under a glass bell, which we immediately filled with carbonic acid gas. The plums had been gathered on the previous day. By the side of the bell we placed other twenty-four plums, which were left there uncovered. Eight days afterwards, in the course of which time there had been a considerable evolution of carbonic acid from the bell, we withdrew the plums and compared them with those which had been left exposed to the air. The difference was striking, almost incredible. Whilst the plums which had been surrounded with air (the experiments of Berard have long since taught us that, under this latter condition, fruits absorb oxygen from the air and emit carbonic acid gas in almost equal volume) had become very soft and watery and sweet, the plums taken from under the jar had remained very firm and hard, the flesh was by no means watery, but they had lost much sugar. Lastly, when submitted to distillation, after crushing, they yielded 6.5 grammes (99.7 grains) of alcohol, more than 1 per cent. of the total weight of the plums. What better proof than these facts could we have of the existence of a considerable chemical action in the interior of fruit, an action which derives the heat necessary for its manifestation from the decomposition of the sugar present in the cells? Moreover, and this circumstance is especially worthy of our attention, in all these experiments we found that there was a liberation of heat, of which the fruit and other organs were the seat, as soon as they were plunged in the carbonic acid gas. This heat is so considerable that it may at times be detected by the hand, if the two sides of the bell, one of which is in contact with the objects, are touched alternately. It also makes itself evident in the formation of little drops on those parts of the bell which are less directly exposed to the influence of the heat resulting from the decomposition of the sugar of the cells.³

In short, fermentation is a very general phenomenon. It is life without air, or life without free oxygen, or, more generally still, it is the result of a chemical process accomplished on a fermentable substance capable of producing heat by its decomposition, in which process the entire heat used up is derived from a part of the heat that the decomposition of the fermentable substance sets free. The class of fermentations properly so called, is, however, restricted by the small number of substances capable of decomposing with the production of heat, and at the same time of serving for the nourishment of lower forms of life, when deprived of the presence and action of air. This, again, is a consequence of our theory, which is well worthy of notice.

⁴ In 1821, in a very remarkable work, especially when we consider the period when it appeared, Berard demonstrated several important propositions in connection with the maturation of fruits:

I. All fruits, even those that are still green, and likewise even those that are exposed to the sun, absorb oxygen and set free an almost equal volume of carbonic acid gas. This is a condition of their proper ripening.

II. Ripe fruits placed in a limited atmosphere, after having absorbed all the oxygen and set free an almost equal volume of carbonic acid, continue to emit that gas in notable quantity, even when no bruise is to be seen - "as though by a kind of fermentation," as Berard actually observes - and lose their saccharine particles, a circumstance which causes the fruits to appear more acid, although the actual weight of their acid may undergo no augmentation whatever.

In this beautiful work, and in all subsequent ones of which the ripening of fruits has been the subject, two facts of great theoretical value have escaped the notice of the authors; these are the two facts which Messrs. Lechartier and Bellamy pointed out for the first time, namely, the production of alcohol and the absence of cells of ferments. It is worthy of remark that these two facts, as we have shown above, were actually fore-shadowed in the theory of fermentation that we advocated as far back as 1861, and we are happy to add that Messrs. Lechartier and Bellamy, who at first had prudently drawn no theoretical conclusions from their work, now entirely agree with the theory we have advanced.⁵ Their mode of reasoning is very different from that of the savants with whom we discussed the subject before the Academy, on the occasion when the communication which we addressed to the Academy in October, 1872, attracted attention once more to the remarkable observations of Messrs. Lechartier and Bellamy.⁶ M. Fremy, in particular, was desirous of finding in these observations a confirmation of his views on the subject of hemi-organism, and a condemnation of ours, notwithstanding the fact that the preceding explanations, and, more particularly our Note of 1861, quoted word for word in the preceding section, furnish the most conclusive evidence in favor of those ideas which we advocate. Indeed, as far back as 1861 we pointed out very clearly that if we could find plants able to live when deprived of air, in the presence of sugar, they would bring about a fermentation of that substance, in the same manner that yeast does. Such is the case with the fungi already studied; such, too, is the case with the fruits employed in the experiments of Messrs. Lechartier and Bellamy, and in our own experiments, the results of which not only confirm those obtained by these gentlemen, but even extend them, in so far as we have shown that fruits, when surrounded with carbonic acid gas immediately produce alcohol. When surrounded with air, they live in their aerobian state and we have no fermentation; immersed immediately afterwards in carbonic acid gas, they now assume their anaerobian state, and at once begin to act upon the sugar in the manner of ferments, and emit heat. As for seeing in these facts anything like a confirmation of the theory of hemi-organism, imagined by M. Fremy, the idea of such a thing is absurd. The following, for instance, is the theory of the fermentation of the vintage, according to M. Fremy.⁷

⁸ our author says, "I hold that in the production of wine it is the juice of the fruit itself that, in contact with air, produces grains of ferment, by the transformation of the albuminous matter; Pasteur, on the other hand, maintains that the fermentation is produced by germs existing outside of the grapes."

Now what bearing on this purely imaginary theory can the fact have, that a whole fruit, immersed in carbonic acid gas, immediately produces alcohol and carbonic acid? In the preceding passage which we have borrowed from M. Fremy, an indispensable condition of the transformation of the albuminous matter is the contact with air and the crushing of the grapes. Here, however, we are dealing with uninjured fruits in contact with carbonic acid gas. Our theory, on the other hand, which, we may repeat, we have advocated since 1861, maintains that all cells become fermentative when their vital action is protracted in the absence of air, which are precisely the conditions that hold in the experiments on fruits immersed in carbonic acid gas. The vital energy is not immediately suspended in their cells, and the latter are deprived of air. Consequently, fermentation must result. Moreover, we may add, if we destroy the fruit, or crush it before immersing it in the gas, it no longer produces alcohol or fermentation of any kind, a circumstance that may be attributed to the fact of the destruction of vital action in the crushed fruit. On the other hand, in what way ought this crushing to affect the hypothesis of hemi-organism? The crushed fruit ought to act quite as well, or even better than that which is uncrushed. In short, nothing can be more directly opposed to the theory of the mode of manifestation of that hidden force to which the name of hemi-organism has been given, than the discovery of the production of these phenomena of fermentation in fruits surrounded with carbonic acid gas; whilst the theory, which sees in fermentation a consequence of vital energy in absence of air, finds in these facts the strictest confirmation of an express prediction, which from the first formed an integral part of its statement.

We should not be justified in devoting further time to opinions which are not supported by any serious experiment. Abroad, as well as in France, the theory of the transformation of albuminous substances into organized ferments had been advocated long before it had been taken up by M. Fremy. It no longer commands the slightest credit, nor do any observers of note any longer give it the least attention; it might even be said that it has become a subject of ridicule.

An attempt has also been made to prove that we have contradicted ourselves, inasmuch as in 1860 we published our opinion that alcoholic fermentation can never occur without a simultaneous occurrence of organization, development, and multiplication of globules; or continued life, carried on from globules already formed. ⁹ Nothing, however, can be truer than that opinion, and at the present moment, after fifteen years of study devoted to the subject since the publication to which we have referred, we need no longer say, "we think," but instead, "we affirm," that it is correct. It is, as a matter of fact, to alcoholic fermentation, properly so called, that the charge to which we have referred relates-to that fermentation which yields, besides alcohol, carbonic acid, succinic acid, glycerine, volatile acids, and other products. This fermentation undoubtedly requires the presence of yeast-cells under the conditions that we have named. Those who have contradicted us have fallen into the error of supposing that the fermentation of fruits is an ordinary alcoholic fermentation, identical with that produced by beer yeast, and that, consequently, the cells of that yeast must, according to our own theory, be always present. There is not the least authority for such a supposition. When we come to exact quantitative estimations - and these are to be found in the figures supplied by Messrs. Lechartier and Bellamy - it will be seen that the proportions of alcohol and carbonic acid gas produced in the fermentation of fruits differ widely from those that we find in alcoholic fermentations properly so called, as must necessarily be the case since in the former the fermentation is effected by the cells of a fruit, but in the latter by cells of ordinary alcoholic ferment. Indeed we have a strong conviction that each fruit would be found to give rise to special action, the chemical equation of which would be different from that in the case of other fruits. As for the circumstance that the cells of these fruits cause fermentation without multiplying, this comes under the kind of activity which we have already distinguished by the expression continuous life in cells already formed.

We will conclude this section with a few remarks on the subject of equations of fermentations, which have been suggested to us principally in attempts to explain the results derived from the fermentation of fruits immersed in carbonic acid gas.

Originally, when fermentations were put amongst the class of decompositions by contact-action, it seemed probable, and, in fact, was believed, that every fermentation has its own well-defined equation which never varied. In the present day, on the contrary, it must be borne in mind that the equation of a fermentation varies essentially with the conditions under which that fermentation is accomplished, and that a statement of this equation is a problem no less complicated than that in the case of the nutrition of a living being. To every fermentation may be assigned an equation in a general sort of way, an equation, however, which, in numerous points of detail, is liable to the thousand variations connected with the phenomena of life. Moreover, there will be as many distinct fermentations brought about by one ferment as there are fermentable substances capable of supplying the carbon element of the food of that same ferment, in the same way that the equation of the nutrition of an animal will vary with the nature of the food which it consumes. As regards fermentation producing alcohol, which may be effected by several different ferments, there will be as in the case of a given sugar, as many general equations as there are ferments, whether they be ferment-cells properly so called, or cells of the organs of living beings functioning as ferments. In the same way the equation of nutrition varies in the case of different animals nourished on the same food. And it is from the same reason that ordinary wort produces such a variety of beers when treated with the numerous alcoholic ferments which we have described. These remarks are applicable to all ferments alike; for instance, butyric ferment is capable of producing a host of distinct fermentations, in consequence of its ability to derive the carbonaceous part of its food from very different substances, from sugar, or lactic acid, or glycerine, or mannite, and many others.

When we say that every fermentation has its own peculiar ferment, it must be understood that we are speaking of the fermentation considered as a whole, including all the accessory products. We do not mean to imply that the ferment in question is not capable of acting on some other fermentable substance and giving rise to fermentation of a very different kind. Moreover, it is quite erroneous to suppose that the presence of a single one of the products of a fermentation implies the co-existence of a particular ferment. If, for example, we find alcohol among the products of a fermentation, or even alcohol and carbonic acid gas together, this does not prove that the ferment must be an alcoholic ferment, belonging to alcoholic fermentations, in the strict sense of the term. Nor, again, does the mere presence of lactic acid necessarily imply the presence of lactic ferment. As a matter of fact, different fermentations may give rise to one or even several identical products. We could not say with certainty, from a purely chemical point of view, that we were dealing, for example, with an alcoholic fermentation properly so called, and that the yeast of beer must be present in it, if we had not first determined the presence of all the numerous products of that particular fermentation under conditions similar to those under which the fermentation in question had occurred. In works on fermentation the reader will often find those confusions against which we are now attempting to guard him. It is precisely in consequence of not having had their attention drawn to such observations that some have imagined that the fermentation in fruits immersed in carbonic acid gas is in contradiction to the assertion which we originally made in our Memoir on alcoholic fermentation published in 1860, the exact words of which we may here repeat: - "The chemical phenomena of fermentation are related essentially to a vital activity, beginning and ending with the latter; we believe that alcoholic fermentation never occurs" - we were discussing the question of ordinary alcoholic fermentation produced by the yeast of beer - "without the simultaneous occurrence of organization, development, and multiplication of globules, or continued life, carried on by means of the globules already formed. The general results of the present Memoir seem to us to be in direct opposition to the opinions of MM. Liebig and Berzelius." These conclusions, we repeat, are as true now as they ever were, and are as applicable to the fermentation of fruits, of which nothing was known in 1860, as they are to the fermentation produced by the means of yeast. Only, in the case of fruits, it is the cells of the parenchyma that function as ferment, by a continuation of their activity in carbonic acid gas whilst in the other case the ferment consists of cells of yeast.

There should be nothing very surprising in the fact that fermentation can originate in fruits and form alcohol without the presence of yeast, if the fermentation of fruits were not confounded completely with alcoholic fermentation yielding the same products and in the same proportions. It is through the misuse of words that the fermentation of fruits has been termed alcoholic, in a way which has misled many persons. ¹⁰ In this fermentation, neither alcohol nor carbonic acid gas exists in those proportions in which they are found in fermentation produced by yeast; and, although we may determine in it the presence of succinic acid, glycerine, and a small quantity of volatile acids¹¹ the relative proportions of these substances will be different from what they are in the case of alcoholic fermentation.

Section III.

Reply To Certain Critical Observations Of The German Naturalists, Oscar Brefeld And Moritz Traube

The essential point of the theory of fermentation which we have been concerned in proving in the preceding paragraphs may be briefly put in the statement that ferments properly so called constitute a class of beings possessing the faculty of living out of contact with free oxygen; or, more concisely still, we may say that fermentation is a result of life without air.

If our affirmation were inexact, if ferment cells did require for their growth or for their increase in number or weight, as all other vegetable cells do, the presence of oxygen, whether gaseous or held in solution in liquids, this new theory would lose all value, its very *raison d'être* would be gone, at least as far as the most important part of fermentations is concerned. This is precisely what M. Oscar Brefeld has endeavoured to prove in a Memoir read to the Physico-Medical Society of Wurzburg on July 26th, 1873, in which, although we have ample evidence of the great experimental skill of its author, he has nevertheless, in our opinion, arrived at conclusions entirely opposed to fact.

"From the experiments which I have just described," he says, "it follows, in the most indisputable manner, that a ferment cannot increase without free oxygen. Pasteur's supposition that a ferment, unlike all other living organisms, can live and increase at the expense of oxygen held in combination is, consequently, altogether wanting in any solid basis of experimental proof. Moreover, since, according to the theory of Pasteur, it is precisely this faculty of living and increasing at the expense of the oxygen held in combination that constitutes the phenomenon of fermentation, it follows that the whole theory, commanding though it does such general assent, is shown to be untenable; it is simply inaccurate."

The experiments to which Dr. Brefeld alludes, consisted in keeping under continued study with the microscope, in a room specially prepared for the purpose, one or more cells of ferment in wort in an atmosphere of carbonic acid gas free from the least traces of free oxygen. We have, however, recognized the fact that the increase of a ferment out of contact with air is only possible in the case of a very young specimen; but our author employed brewer's yeast taken after fermentation, and to this fact we may attribute the non-success of his growths. Dr. Brefeld, without knowing it, operated on yeast in one of the states in which it requires gaseous oxygen to enable it to germinate again. A perusal of what we have previously written on the subject of the revival of yeast according to its age will show how widely the time required for such revival may vary in different cases. What may be perfectly true of the state of a yeast to-day may not be so to-morrow, since yeast is continually undergoing modifications. We have already shown the energy and activity with which a ferment can vegetate in the presence of free oxygen, and we have pointed out the great extent to which a very small quantity of oxygen held in solution in fermenting liquids can operate at the beginning of fermentation. It is this oxygen that produces revival in the cells of the ferment and enables them to resume the faculty of germinating and continuing their life, and of multiplying when deprived of air.

In our opinion, a simple reflection should have guarded Dr. Brefeld against the interpretation which he has attached to his observations. If a cell of ferment cannot bud or increase without absorbing oxygen, either free or held in solution in the liquid, the ratio between the weight of the ferment formed during fermentation and that of oxygen used up must be constant. We had, however, clearly established, as far back as 1861, the fact that this ratio is extremely variable, a fact, moreover, which is placed beyond doubt by the experiments described in the preceding section. Though but small quantities of oxygen are absorbed, a considerable weight of ferment may be generated; whilst if the ferment has abundance of oxygen at its disposal, it will absorb much, and the weight of yeast formed will be still greater. The ratio between the weight of ferment formed and that of sugar decomposed may pass through all stages within certain very wide limits, the variations depending on the greater or less absorption of free oxygen. And in this fact, we believe, lies one of the most essential supports of the theory which we advocate. In denouncing the impossibility, as he considered it, of a ferment living without air or oxygen, and so acting in defiance of that law which governs all living beings, animal or vegetable, Dr. Brefeld ought also to have borne in mind the fact which we have pointed out, that alcoholic yeast is not the only organized ferment which lives in an anaerobian state. It is really a small matter that one more ferment should be placed in a list of exceptions to the generality of living beings, for whom there is a rigid law in their vital economy which requires for continued life a continuous respiration, a continuous supply of free oxygen. Why, for instance, has Dr. Brefeld omitted the facts bearing on the life of the vibrios of butyric fermentation? Doubtless he thought we were equally mistaken in these: a few actual experiments would have put him right.

These remarks on the criticisms of Dr. Brefeld are also applicable to certain observations of M. Moritz Traube's, although, as regards the principal object of Dr. Brefeld's attack, we are indebted to M. Traube for our defence. This gentleman maintained the exactness of our results before the Chemical Society of Berlin, proving by fresh experiments that yeast is able to live and multiply without the intervention of oxygen. "My researches," he said, "confirm in an indisputable manner M. Pasteur's assertion that the multiplication of yeast can take place in media which contain no trace of free oxygen. . . . M. Brefeld's assertion to the contrary is erroneous." But immediately afterwards M. Traube adds: "Have we here a confirmation of Pasteur's theory? By no means. The results of my experiments demonstrate on the contrary that this theory has no true foundation." What were these results? Whilst proving that yeast could live without air, M. Traube, as we ourselves did, found that it had great difficulty in living under these conditions; indeed he never succeeded in obtaining more than the first stages of true fermentation. This was doubtless for the two following reasons: first, in consequence of the accidental production of secondary and diseased fermentations which frequently prevent the propagation of alcoholic ferment; and, secondly, in consequence of the original exhausted condition of the yeast employed. As long ago as 1861, we pointed out the slowness and difficulty of the vital action of yeast when deprived of air; and a little way back, in the preceding section, we have called attention to certain fermentations that cannot be completed under such conditions without going into the causes of these peculiarities. M. Traube expresses himself thus: "Pasteur's conclusion, that yeast in the absence of air is able to derive the oxygen necessary for its development from sugar, is erroneous; its increase is arrested even when the greater part of the sugar still remains undecomposed. It is in a mixture of albuminous substances that yeast, when deprived of air, finds the materials for its development." This last assertion of M. Traube's is entirely disproved by those fermentation experiments in which, after suppressing the presence of albuminous substances, the action, nevertheless, went on in a purely inorganic medium, out of contact with air, a fact, of which we shall give irrefutable proofs. ¹

Section IV.

Fermentation of Dextro-Tartrate of Lime

Tartrate of lime, in spite of its insolubility in water, is capable of complete fermentation in a mineral medium.

If we put some pure tartrate of lime, in the form of a granulated, crystalline powder, into pure water, together with some sulphate of ammonia and phosphates of potassium and magnesium, in very small proportions, a spontaneous fermentation will take place in the deposit in the course of a few days, although no germs of ferment have been added. A living, organized ferment, of the vibronic type, filiform, which tortuous motions, and often of immense length, forms spontaneously by the development of some germs derived in some way from the inevitable particles of dust floating in the air or resting on the surface of the vessels or material which we employ. The germs of the vibrios concerned in putrefaction are diffused around us on every side, and, in all probability, it is one or more of these germs that develop in the medium in question. In this way they effect the decomposition of the tartrate, from which they must necessarily obtain the carbon of their food without which they cannot exist, while the nitrogen is furnished by the ammonia of the ammoniacal salt, the mineral principles by the phosphate of potassium and magnesium, and the sulphur by the sulphate of ammonia. How strange to see organization, life, and motion originating under such conditions! Stranger still to think that this organization, life, and motion are effected without the participation of free oxygen. Once the germ gets a primary impulse on its living career by access of oxygen, it goes on reproducing indefinitely, absolutely without atmospheric air. Here then we have a fact which it is important to establish beyond the possibility of doubt, that we may prove that yeast is not the only organized ferment able to live and multiply when out of the influence of free oxygen.

Into a flask, like that represented in Fig. 9, of 2.5 litres (about four pints) in capacity, we put:

Pure, crystallized, neutral tartrate of lime	100 grammes
Phosphate of ammonia	1 "
" magnesium	1 "
" potassium	0.5 "
Sulphate of ammonia	0.5 "

(1 gramme = 15.43 grains)

To this we added pure distilled water, so as entirely to fill the flask.

In order to expel all the air dissolved in the water and adhering to the solid substances, we first placed our flask in a bath of chloride of calcium in a large cylindrical white iron pot set over a flame. The exit-tube of the flask was plunged in a test tube of Bohemian glass three-quarters full of distilled water, and also heated by a flame. We boiled the liquids in the flask and test-tube for a sufficient time to expel all the air contained in them. We then withdrew the heat from under the test-tube, and immediately afterwards covered the water which it contained with a layer of oil and then permitted the whole apparatus to cool down.

Next day we applied a finger to the open extremity of the exit-tube, which we then plunged in a vessel of mercury. In this particular experiment which we are describing, we permitted the flask to remain in this state for a fortnight. It might have remained there for a century without ever manifesting the least sign of fermentation, the fermentation of the tartrate being a consequence of life, and life after boiling no longer existed in the flask. When it was evident that the contents of the flask were perfectly inert, we impregnated them rapidly, as follows: all the liquid contained in the exit-tube was removed by means of a fine caoutchouc tube, and replaced by about 1 c. (about 17 minims) of liquid and deposit from another flask, similar to the one we have just described, but which had been fermenting spontaneously for twelve days; we lost no time in refilling completely the exit-tube with water which had been first boiled and then cooled down in carbonic acid gas. This operation lasted only a few minutes. The exit-tube was again plunged under mercury. Subsequently the tube was not moved from under the mercury, and as it formed part of the flask, and there was neither cork nor india-rubber, any introduction of air was consequently impossible. The small quantity of air introduced during the impregnation was insignificant and it might even be shown that it injured rather than assisted the growth of the organisms, inasmuch as these consisted of adult individuals which had lived without air and might be liable to be damaged or even destroyed by it. Be this as it may, in a subsequent experiment we shall find the possibility removed of any aeration taking place in this way, however infinitesimal, so that no doubts may linger on this subject.

The following days the organisms multiplied, the deposit of tartrate gradually disappeared, and a sensible ferment action was manifest on the surface, and throughout the bulk of the liquid. The deposit seemed lifted up in places, and was covered with a layer of dark-grey colour, puffed up, and having an organic and gelatinous appearance. For several days, in spite of this action in the deposit, we detected no disengagement of gas, except when the flask was slightly shaken, in which case rather large bubbles adhering to the deposit rose, carrying with them some solid particles, which quickly fell back again, whilst the bubbles diminished in size as they rose, from being partially taken into solution, in consequence of the liquid not being saturated. The smallest bubbles had even time to dissolve completely before they could reach the surface of the liquid. In course of time the liquid was saturated, and the tartrate was gradually displaced by mammillated crusts, or clear, transparent crystals of carbonate of lime at the bottom and on the sides of the vessel.

The impregnation took place on February 10th, and on March 15th the liquid was nearly saturated. The bubbles then began to lodge in the bent part of the exit-tube, at the top of the flask. A glass measuring-tube containing mercury was now placed with its open end over the point of the exit-tube under the mercury in the trough, so that no bubble might escape. A steady evolution of gas went on from the 17th to the 18th, 17.4 cc. (1.06 cubic inches) having been collected. This was proved to be nearly absolutely pure carbonic acid, as indeed might have been suspected from the fact that the evolution did not begin before a distinct saturation of the liquid was observed. ¹

² This smaller flask had been previously filled with carbonic acid. The carbonic acid of the fermented liquid was then expelled by means of heat, and collected over mercury. In this way we found a volume of 8.322 litres (508 cubic inches) of gas in solution, which, added to the 2.135 litres, gave a total of 10.457 litres (638.2 cubic inches) at 20 degrees and 760 mm., which, calculated to 0 C. and 760 mm. atmospheric pressure (32 F. and 30 inches) gave a weight of 19.700 grammes (302.2 grains) of carbonic acid.

Exactly half of the lime in the tartrate employed got used up in the soluble salts formed during fermentation; the other half was partly precipitated in the form of carbonate of lime, partly dissolved in the liquid by the carbonic acid. The soluble salts seemed to us to be a mixture or combination of 1 equivalent of metacetate of lime, with 2 equivalents of the acetate, for every 10 equivalents of carbonic acid produced, the whole corresponding to the fermentation of 3 equivalents of neutral tartrate of lime.³ This point, however, is worthy of being studied with greater care: the present statement of the nature of the products formed is given with all reserve. For our point, indeed, the matter is of little importance, since the equation of the fermentation does not concern us.

After the completion of fermentation there was not a trace of tartrate of lime remaining at the bottom of the vessel: it had disappeared gradually as it got broken up into the different products of fermentation, and its place was taken by some crystallized carbonate of lime - the excess, namely, which had been unable to dissolve by the action of the carbonic acid. Associated, moreover, with this carbonate of lime there was a quantity of some kind of animal matter, which, under the microscope, appeared to be composed of masses of granules mixed with very fine filaments of varying lengths, studded with minute dots, and presenting all the characteristics of a nitrogenous organic substance. ⁴ That this was really the ferment is evident enough from all that we have already said. To convince ourselves more thoroughly of the fact, and at the same time to enable us to observe the mode of activity of the organism, we instituted the following supplementary observation. Side by side with the experiment just described, we conducted a similar one, which we intermitted after the fermentation was somewhat advanced, and about half of the tartrate dissolved. Breaking off with a file the exit-tube at the point where the neck began to narrow off, we took some of the deposit from the bottom by means of a long straight piece of tubing, in order to bring it under microscopical examination. We found it to consist of a host of long filaments of extreme tenuity, their diameter being about 1/1000th of a millimetre (0.000039 in.); their length varied, in some cases being as much as 1/20th of a millimetre (0.0019 in.). A crowd of these long vibrios were to be seen creeping slowly along, with a sinuous movement, showing three, four, or even five flexures. The filaments that were at rest had the same aspect as these last, with the exception that they appeared punctuate, as though composed of a series of granules arranged in irregular order. No doubt these were virious in which vital action had ceased, exhausted specimens which we may compare with the old granular ferment of beer, whilst those in motion may be compared with young and vigorous yeast. The absence of movement in the former seems to prove that this view is correct. Both kinds showed a tendency to form clusters, the compactness of which impeded the movements of those which were in motion. Moreover, it was noticeable that the masses of these latter rested on tartrate not yet dissolved, whilst the granular clusters of the others rested directly on the glass, at the bottom of the flask, as if, having decomposed the tartrate, the only carbonaceous food at their disposal, they had then died on the spot where we captured them, from inability to escape, precisely in consequence of that state of entanglement which they combined to form, during the period of their active development. Besides these we observed vibrios of the same diameter, but of much smaller length, whirling round with great rapidity, and darting backwards and forwards; these were probably identical with the longer ones, and possessed greater freedom of movement, no doubt in consequence of their shortness. Not one of these vibrios could be found throughout the mass of the liquid.

We may remark that as there was a somewhat putrid odour from the deposit in which the vibrios swarmed, the action must have been one of reduction, and no doubt to this fact was due the greyish coloration of the deposit. We suppose that the substances employed, however pure, always contain some trace of iron, which becomes converted into the sulphide, the black colour of which would modify the originally white deposit of insoluble tartrate and phosphate.

But what is the nature of these vibrios? We have already said that we believe that they are nothing but the ordinary vibrios of putrefaction, reduced to a state of extreme tenuity by the special conditions of nutrition involved in the fermentable medium used; in a word, we think that the fermentation in question might be called putrefaction of tartrate of lime. It would be easy enough to determine this point by growing the vibrios of such fermentation in media adapted to the production of the ordinary forms of vibrio; but this is an experiment which we have not ourselves tried.

One word more on the subject of these curious beings. In a great many of them there appears to be something like a clear spot, a kind of bed, at one of their extremities. This is an illusion arising from the fact that the extremity of these vibrios is curved, hanging downwards, thus causing a greater refraction at that particular point, and leading us to think that the diameter is greater at that extremity. We may easily undeceive ourselves if we watch the movements of the vibrio, when we will readily recognize the bend, especially as it is brought into the vertical plane passing over the rest of the filament. In this way we will see the bright spot, the head, disappear, and then reappear.

The chief inference that it concerns us to draw from the preceding facts is one which cannot admit of doubt, and which we need not insist on any further - namely that vibrios, as met with in the fermentation of neutral tartrate of lime, are able to live and multiply when entirely deprived of air.

Section V.

Another Example of Life Without Air-Fermentation of Lactate of Lime

As another example of life without air, accompanied by fermentation properly so called, we may lastly cite the fermentation of lactate of lime in a mineral medium.

In the experiment described in the last paragraph, it will be remembered that the ferment liquid and the germs employed in its impregnation came in contact with air, although only for a very brief time. Now, notwithstanding that we possess exact observations which prove that the diffusion of oxygen and nitrogen in a liquid absolutely deprived of air, so far from taking place rapidly, is, on the contrary, a very slow process indeed; yet we were anxious to guard the experiment that we are about to describe from the slightest possible trace of oxygen at the moment of impregnation.

¹ were introduced successively, viz:

Pure lactate of lime	225 grammes
Phosphate of ammonia	0.75 "
Phosphate of potassium	0.4 "
Sulphate of magnesium	0.4 "
Sulphate of ammonia	0.2 "

(1 gramme = 15.43 grains)

On March 23rd, 1875, we filled a 6 litre (about 11 pints) flask, of the shape represented in Fig. 11, and placed it over a heater. Another flame was placed below a vessel containing the same liquid, into which the curved tube of the flask plunged. The liquids in the flask and in the basin were raised to boiling together, and kept in this condition for more than half-an-hour, so as to expel all the air held in solution. The liquid was several times forced out of the flask by the steam, and sucked back again; but the portion which re-entered the flask was always boiling. On the following day when the flask had cooled, we transferred the end of the delivery tube to a vessel full of mercury and placed the whole apparatus in an oven at a temperature varying between 25 C. and 30 C. (77 F. and 86 F.); then, after having refilled the small cylindrical tap-funnel with carbonic acid, we passed into it with all necessary precautions 10 cc. (0.35 fl. oz.) of a liquid similar to that described, which had been already in active fermentation for several days out of contact with air and now swarmed with vibrios. We then turned the tap of the funnel, until only a small quantity of liquid was left, just enough to prevent the access of air. In this way the impregnation was accomplished without either the ferment-liquid or the ferment-germs having been brought in contact, even for the shortest space, with the external air. The fermentation, the occurrence of which at an earlier or later period depends for the most part on the condition of the impregnating germs, and the number introduced in the act, in this case began to manifest itself by the appearance of minute bubbles from March 29th. But not until April 9th did we observe bubbles of larger size rise to the surface. From that date onward they continued to come in increasing number, from certain points at the bottom of the flask, where a deposit of earthy phosphates existed; and at the same time the liquid, which for the first few days remained perfectly clear, began to grow turbid in consequence of the development of vibrios. It was on the same day that we first observed a deposit on the sides of carbonate of lime in crystals.

It is a matter of some interest to notice here that, in the mode of procedure adopted, everything combined to prevent the interference of air. A portion of the liquid expelled at the beginning of the experiment, partly because of the increased temperature in the oven and partly also by the force of the gas, as it began to be evolved from the fermentative action, reached the surface of the mercury, where, being the most suitable medium we know for the growth of bacteria, it speedily swarmed with these organisms. ² In this way any passage of air, if such a thing were possible, between the mercury and the sides of the delivery-tube was altogether prevented, since the bacteria would consume every trace of oxygen which might be dissolved in the liquid lying on the surface of the mercury. Hence it is impossible to imagine that the slightest trace of oxygen could have got into the liquid in the flask.

Before passing on we may remark that in this ready absorption of oxygen by bacteria we have a means of depriving fermentable liquids of every trace of that gas with a facility and success equal or even greater than by the preliminary method of boiling. Such a solution as we have described, if kept at summer heat, without any previous boiling, becomes turbid in the course of twenty-four hours from a spontaneous development of bacteria; and it is easy to prove that they absorb all the oxygen held in solution. ³ If we completely fill a flask of a few litres capacity (about a gallon) (Fig. 9) with the liquid described, taking care to have the delivery-tube also filled, and its opening plunged under mercury, and, forty-eight hours afterwards by means of a chloride of calcium bath, expel from the liquid on the surface of the mercury all the gas which it holds in solution, this gas, when analyzed, will be found to be composed of a mixture of nitrogen and carbonic acid gas, without the least trace of oxygen. Here, then, we have an excellent means of depriving the fermentable liquid of air; we simply have completely to fill a flask with the liquid, and place it in the oven, merely avoiding any addition of butyric vibrios, before the lapse of two or three days. We may wait even longer; and then, if the liquid does become impregnated spontaneously with vibrio germs, the liquid, which at first was turbid from the presence of bacteria, will become bright again, since the bacteria, when deprived of life, or, at least, of the power of moving, after they have exhausted all the oxygen in solution, will fall inert to the bottom of the vessel. On several occasions we have determined this interesting fact, which tends to prove that the butyric vibrios cannot be regarded as another form of bacteria, inasmuch as, on the hypothesis of an original relation between the two productions, butyric fermentation ought in every case to follow the growth of bacteria.

Before we go any further, let us devote some attention to the vibrios of the preceding fermentations.

On May 27th, 1862, we completely filled a flask capable of holding 2.780 litres (about five pints) with the solution of lactate and phosphates.⁵ We refrained from impregnating it with any germs. The liquid became turbid from a development of bacteria and then underwent butyric fermentation. By June 9th the fermentation had become sufficiently active to enable us to collect in the course of twenty-four hours, over mercury, as in all our experiments, about 100 cc. (about 6 cubic inches) of gas. By June 11th, judging from the volume of gas liberated in the course of twenty-four hours, the activity of the fermentation had doubled. We examined a drop of the turbid liquid. Here are the notes accompanying the sketch (Fig. 12) as they stand in our note-book: "A swarm of vibrios, so active in their movements that the eye has great difficulty in following them. They may be seen in pairs throughout the field, apparently making efforts to separate from each other. The connection would seem to be by some invisible, gelatinous thread, which yields so far to their efforts that they succeed in breaking away from actual contact, but yet are, for a while, so far restrained that the movements of one have a visible effect on those of the other. By and by, however, we see a complete separation effected, and each moves on its separate way with an activity greater than it ever had before."x

One of the best methods that can be employed for the microscopical examination of these vibrios, quite out of contact with air, is the following. After butyric fermentation has been going on for several days in a flask (Fig. 13), we connect this flask by an indiarubber tube with one of the flattened bulbs previously described, which we then place on the stage of the microscope (Fig. 13). When we wish to make an observation we close, under the mercury, at the point b, the end of the drawn-out and bent delivery-tube. The continued evolution of gas soon exerts such a pressure within the flask, that when we open the tap r, the liquid is driven into the bulb II, until it becomes quite full and the liquid flows over into the glass V. In this manner we may bring the vibrios under observation without their coming into contact with the least trace of air, and with as much success as if the bulb, which takes the place of an object glass, had been plunged into the very centre of the flask. The movements and fissiparous multiplication of the vibrios may thus be seen in all their beauty, and it is indeed a most interesting sight. The movements do not immediately cease when the temperature is suddenly lowered, even to a considerable extent, 15 C. (59 F) for example; they are only slackened. Nevertheless, it is better to observe them at the temperatures most favourable to fermentation, even in the oven where the vessels employed in the experiment are kept at a temperature between 25 C. and 30 C. (77 F. and 86 F.).

We may now continue our account of the fermentation which we were studying when we made this last digression. On June 17th that fermentation produced three times as much gas as it did on June 11th, when the residue of hydrogen, after absorption by potash, was 72.6 per cent.; whilst on the 17th it was only 49.2 per cent. Let us again discuss the microscopic aspect of the turbid liquid at this stage. Appended is the sketch we made (Fig. 14) and our notes on it: "A most beautiful object: vibrios all in motion, advancing or undulating. They have grown considerably in bulk and length since the 11th; many of them are joined together in long sinuous chains, very mobile at the articulations, visibly less active and more wavering in proportion to the number that go to form the chain, of the length of the individuals." This description is applicable to the majority of the vibrios which occur in cylindrical rods and are homogeneous in aspect. There are others, of rare occurrence in chains, which have a clear corpuscle, that is to say, a portion more refractive than other parts of the segments, at one of their extremities. Sometimes the foremost segment has the corpuscle at one end, sometimes the other. The long segments of the commoner kind attain a length of from 10 to 30 and even 45 thousandths of a millimetre. Their diameter is from 1 1/2 to 2, very rarely 3, thousandths of a millimetre. ⁶

On June 28th, fermentation was quite finished; there was now longer any trace of gas, nor any lactate in solution. All the infusoria were lying motionless at the bottom of the flask. The liquid clarified by degrees, and in the course of a few days became quite bright. Here we may inquire, were these motionless infusoria, which from complete exhaustion of the lactate, the source of the carbonaceous part of their food, were no lying inert at the bottom of the fermenting vessel - were they dead beyond the power of revival? ⁷ The following experiment leads us to believe that they were not perfectly lifeless, and that they might behave in the same manner as the yeast of beer, which, after it has decomposed all the sugar in a fermentable liquid, is ready to revive and multiply in a fresh saccharine medium. On April 22nd, 1875, we left in the oven at a temperature of 25C. (77F.) a fermentation of lactate of lime that had been completed. The delivery tube of the flask A, (Fig. 15), in which it had taken place, had never been withdrawn from under the mercury. We kept the liquid under observation daily, and saw it gradually become brighter; this went on for fifteen days. We then filled a similar flask, B, with the solution of lactate, which we boiled, not only to kill the germs of vibrios which the liquid might contain, but also to expel the air that it held in solution. When the flask, B, had cooled, we connected the two flasks, avoiding the introduction of air, ⁸ after having slightly shaken the flask, A, to stir up the deposit at the bottom. There was then a pressure due to carbonic acid at the end of the delivery tube of this latter flask, at the point a, so that on opening the taps r and s, the deposit at the bottom of flask A was driven over into flask B, which in consequence was impregnated with the deposit of a fermentation that had been completed fifteen days before. Two days after impregnation the flask B began to show signs of fermentation. It follows that the deposit of vibrios of a completed butyric fermentation may be kept, at least for a certain time, without losing the power of causing fermentation. It furnishes a butyric ferment, capable of revival and action in a suitable fresh fermentable medium.

The reader who has attentively studied the facts which we have placed before him cannot, in our opinion, entertain the least doubt on the subject of the possible multiplication of the vibrios of a fermentation of lactate of lime out of contact with atmospheric oxygen. If fresh proofs of this important proposition were necessary, they might be found in the following observations, from which it may be inferred that atmospheric oxygen is capable of suddenly checking a fermentation produced by butyric vibrios, and rendering them absolutely motionless, so that it cannot be necessary to enable them to live. On May 7th, 1862, we placed in the oven a flask holding 2.580 litres (4 1/2 pints), and filled with the solution of lactate of lime and phosphates, which we had impregnated on the 9th with two drops of a liquid in butyric fermentation. In the course of a few days fermentation declared itself: on the 18th it was active; on the 30th it was very active. On June 1st it yielded hourly 35 cc. (2.3 cubic inches) of gas, containing ten per cent. of hydrogen. On the 2nd we began the study of the action of air on the vibrios of this fermentation. To do this we cut off the delivery-tube on a level with its point of junction to the flask, then with a 50 cc. pipette we took out that quantity (1 3/4 fl. oz.) of liquid which was, of course, replaced at once by air. We then reversed the flask with the opening under the mercury, and shook it every ten minutes for more than an hour. Wishing to make sure, to begin with, that the oxygen had been absorbed we connected under the mercury the beak of the flask by means of a thin india-rubber tube filled with water, with a small flask, the neck of which had been drawn out and was filled with water; we then raised the large flask with the smaller kept above it. A Mohr's clip, which closed the india-rubber tube, and which we then opened, permitted the water contained in the small flask to pass into the large one, whilst the gas, on the contrary, passed upwards from the large flask into the small one. We analyzed the gas immediately, and found that, allowing for the carbonic acid and hydrogen, it did not contain more than 14.2 per cent. of oxygen, which corresponds to an absorption of 6.6 cc., or of 3.3 cc. (0.2 cubic inch) of oxygen for the 50 cc. (3.05 cubic inches) of air employed. Lastly, we again established connection by an india-rubber tube between the flasks, after having seen by microscopical examination that the movements of the vibrios were very languid. Fermentation had become less vigorous without having actually ceased, no doubt because some portions of the liquid had not been brought into contact with the atmospheric oxygen, in spite of the prolonged shaking that the flask had undergone after the introduction of the air. Whatever the cause might have been, the significance of the phenomenon is not doubtful. To assure ourselves further of the effect of air on the vibrios, we half filled two test tubes with the fermenting liquid taken from another fermentation which had also attained its maximum of intensity, into one of which we passed a current of air, into the other carbonic acid gas. In the course of half an hour, all the vibrios in the aerated tube were dead, or at least motionless, and fermentation had ceased. In the other tube, after three hours' exposure to the effects of the carbonic acid gas, the vibrios were still very active, and fermentation was going on.

There is a most simple method of observing the deadly effect of atmospheric air upon vibrios. We have seen in the microscopical examination made by means of the apparatus represented in Fig. 13, how remarkable were the movements of the vibrios when absolutely deprived of air, and how easy it was to discern them. We will repeat this observation, and at the same time make a comparative study of the same liquid under the microscope in the ordinary way, that is to say, by placing a drop of the liquid on an object-glass, and covering it with a thin glass slip, a method which must necessarily bring the drop into contact with air, if only for a moment. It is surprising what a remarkable difference is observed immediately between the movements of the vibrios in the bulb and those under the glass. In the case of the latter, we generally see all movement at once cease near the edges of the glass, where the drop of liquid is in direct contact with the air; the movements continue for a longer or shorter time about the centre, in proportion as the air is more or less intercepted by the vibrios at the circumference of the liquid. It does not require much skill in experiments of this kind to enable one to see plainly that immediately after the glass has been placed on the drop, which has been affected all over by atmospheric air, the whole of the vibrios seem to languish and to manifest symptoms of illness - we can think of no better expression to explain what we see taking place - and that they gradually recover their activity about the centre, in proportion as they find themselves in a part of the medium that is less affected by the presence of oxygen.

Some of the most curious facts are to be found in connection with an observation, the correlative and inverse of the foregoing, on the ordinary aerobian bacteria. If we examine below the microscope a drop of liquid full of these organisms under a coverslip, we very soon observe a cessation of motion in all the bacteria which lie in the central portion of the liquid, where the oxygen rapidly disappears to supply the necessities of the bacteria existing there; whilst, on the other hand, near the edges of the cover-glass the movements are very active, in consequence of the constant supply of air. In spite of the speedy death of the bacteria beneath the centre of the glass, we see life prolonged there if by chance a bubble of air has been enclosed. All round this bubble a vast number of bacteria collect in a thick, moving circle, but as soon as all the oxygen of the bubble has been absorbed they fall apparently lifeless, and are scattered by the movement of the liquid. ⁹

We may here be permitted to add, as a purely historical matter, that it was these two observations just described, made successively one day in 1861, on vibrios and bacteria, that first suggested to us the idea of the possibility of life without air, and caused us to think that the vibrios which we met so frequently in our lactic fermentations must be the true butyric ferment.

We may pause to consider an interesting question in reference to the two characters under which vibrios appear in butyric fermentations. What is the reason that some vibrios exhibit refractive corpuscles, generally of a lenticular form, such as we see in Fig. 14. We are strongly inclined to believe that these corpuscles have to do with a special mode of reproduction in the vibrios, common alike to the anaerobian forms which we are studying, and the ordinary aerobian forms in which also the corpuscles of which we are speaking may occur. The explanation of the phenomenon, from our point of view, would be that, after a certain number of fissiparous generations, and under the influence of variations in the composition of the medium, which is constantly changing through fermentation as well as through the active life of the vibrios themselves, cysts, which are simply the refractive corpuscles, form along them at different points. From these gemmules we have ultimately produced vibrios, ready to reproduce others by the process of transverse division for a certain time, to be themselves encysted, later on. Various observations incline us to believe that, in their ordinary form of minute, soft, exuberant rods, the vibrios perish when submitted to desiccation, but when they occur in corpuscular or encysted form they possess unusual powers of resistance and may be brought to the state of dry dust and be wafted about by winds. None of the matter which surrounds the corpuscle or cyst seems to take part in the preservation of the germ, when the cyst is formed, for it is all re-absorbed, gradually leaving the cyst bare. The cysts appear as masses of corpuscles, in which the most practiced eye cannot detect anything of an organic nature, or anything to remind one of the vibrios which produced them; nevertheless, these minute bodies are endowed with a latent vital action, and only await favourable conditions to develop long rods of vibrios. We are not, it is true, in a position to adduce any very forcible proofs in support of these opinions. They have been suggested to us by experiments, none of which, however, have been absolutely decisive in their favour. We may cite one of our observations on this subject.

In a fermentation of glycerine in a mineral medium - the glycerine was fermenting under the influence of butyric vibrios - after we had determined the, we may say, exclusive presence of lenticular vibrios, with refractive corpuscles, we observed the fermentation, which for some unknown reason had been very languid, suddenly become extremely active, but now through the influence of the ordinary vibrios. The gemmules with brilliant corpuscles had almost disappeared; we could see but very few, and those now consisted of the refractive bodies alone, the bulk of the vibrios accompanying them having undergone some process of re-absorption.

Another observation which still more closely accords with this hypothesis is given in our work on silk-worm disease (vol. 1, p.256). We there demonstrated that, when we place in water some of the dust formed of desiccated vibrios, containing a host of these refractive corpuscles, in the course of a very few hours large vibrios appear, well-developed rods fully grown, in which the brilliant points are absent; whilst in the water no process of development from smaller vibrios is to be discerned, a fact which seems to show that the former had issued fully grown from the refractive corpuscles, just as we see colpoda issue with their adult aspect from the dust of their cysts. This observation, we may remark, furnishes one of the best proofs that can be adduced against the spontaneous generation of vibrios or bacteria, since it is probable that the same observation applies to bacteria. It is true that we cannot say of mere points of dust examined under the microscope, that one particular germ belongs to vibrio, another to bacterium; but how is it possible to doubt that the vibrios issue, as we see them, from an ovum of some kind, a cyst, or germ, of determinate character, when, after having placed some of those indeterminate motes of dust into clean water, we suddenly see, after an interval of not more than one or two hours, an adult vibrio crossing the field of the microscope, without our having been able to detect any intermediate state between its birth and adolescence?

It is a question whether differences in the aspect and nature of vibrios, which depend upon their more or less advanced age, or are occasioned by the influence of certain conditions on the medium in which they propagate, do not bring about corresponding changes in the course of the fermentation and the nature of its products. Judging at least from the variations in the proportions of hydrogen and carbonic acid gas produced in butyric fermentations, we are inclined to think that this must be the case; nay, more, we find that hydrogen is not even a constant product in these fermentations. We have met with butyric fermentations of lactate of lime which did not yield the minutest trace of hydrogen, or anything besides carbonic acid. Fig. 16 represents the vibrios which we observed in a fermentation of this kind. They present no special features. Butyl alcohol is, according to our observations, an ordinary product, although it varies and is by no means a necessary concomitant of these fermentations. It might be supposed, since butylic alcohol may be produced, and hydrogen be in deficit, that the proportion of the former of these products would attain its maximum when the latter assumed a minimum. This, however, is by no means the case; even in those few fermentations that we have met with in which hydrogen was absent, there was no formation of butylic alcohol.

From a consideration of all the facts detailed in this section we can have no hesitation in concluding that, on the one hand, in cases of butyric fermentation, the vibrios which abound in them and constitute their ferment, live without air or free oxygen; and that, on the other hand, the presence of gaseous oxygen operates prejudicially against the movements and activity of those vibrios. But now does it follow that the presence of minute quantities of air brought into contact with a liquid undergoing butyric fermentation would prevent the continuance of that fermentation or even exercise any check upon it? We have not made any direct experiments upon this subject; but we should not be surprised to find that, so far from hindering, air may, under such circumstances, facilitate the propagation of the vibrios and accelerate fermentation. This is exactly what happens in the case of yeast. But how could we reconcile this, supposing it were proved to be the case, with the fact just insisted on as to the danger of bringing the butyric vibrios into contact with air? It may be possible that life without air results from habit, whilst death through air may be brought about by a sudden change in the conditions of the existence of the vibrios. The following remarkable experiment is well-known: A bird is placed in a glass jar of one or two litres (60 to 120 cubic inches) in capacity which is then closed. After a time the creature shows every sign of intense uneasiness and asphyxia long before it dies; a similar bird of the same size is introduced into the jar; the death of the latter takes place instantaneously, whilst the life of the former may still be prolonged under these conditions for a considerable time, and there is no difficulty even in restoring the bird to perfect health by taking it out of the jar. It seems impossible to deny that we have here a case of the adaptation of an organism to the gradual contamination of the medium; and so it may likewise happen that the anaerobian vibrios of a butyric fermentation, which develop and multiply absolutely without free oxygen, perish immediately when suddenly taken out of their airless medium, and that the result might be different if they had been gradually brought under the action of air in small quantities at a time.

We are compelled here to admit that vibrios frequently abound in liquids exposed to the air, and that they appropriate the atmospheric oxygen, and could not withstand a sudden removal from its influence. Must we, then, believe that such vibrios are absolutely different from those of butyric fermentations? It would, perhaps, be more natural to admit that in the one case there is an adaptation to life with air, and in the other case an adaptation to life without air; each of the varieties perishing when suddenly transferred from its habitual condition to that of the other, whilst by a series of progressive changes one might be modified into the other. ¹⁰ We know that in the case of alcoholic ferments, although these can actually live without air, propagation is wonderfully assisted by the presence of minute quantities of air; and certain experiments which we have not yet published lead us to believe that, after having lived without air, they cannot be suddenly exposed with impunity to the influence of large quantities of oxygen.

We must not forget, however, that aerobic torulae and anaerobian ferments present an example of organisms apparently identical, in which, however, we have not yet been able to discover any ties of a common origin. Hence we are forced to regard them as a distinct species; and so it is possible that there may likewise be aerobic and anaerobian vibrios without any transformation of the one into the other.

The question has been raised whether vibrios, especially those which we have shown to be the ferment of butyric and many other fermentations, are in their nature, animal or vegetable. M. Ch. Robin attaches great importance to the solution of this question, of which he speaks as follows: ¹¹ "The determination of the nature, whether animal or vegetable, of organisms, either as a whole or in respect to their anatomical parts, assimilative or reproductive, is a problem which has been capable of solution for a quarter of a century. The method has been brought to a state of remarkable precision, experimentally, as well as in its theoretical aspects, since those who devote their attention to the organic sciences consider it indispensable in every observation and experiment to determine accurately, before anything else, whether the object of their study is animal or vegetable in its nature, whether adult or otherwise. To neglect this is as serious an omission for such students as for chemists would be the neglecting to determine whether it is nitrogen or hydrogen, urea or stearine, that has been extracted from a tissue, or which it is whose combinations they are studying in this or that chemical operation. Now, scarcely any one of those who study fermentations, properly so-called, and putrefactions, ever pay any attention to the preceding data. . . . Among the observers to whom I allude, even M. Pasteur is to be found, who, even in his most recent communications, omits to state definitely what is the nature of many of the ferments which he has studied, with the exception, however, of those which belong to the cryptogamic group called torulaceae. Various passages in his work seem to show that he considers the cryptogamic organisms called bacteria, as well as those known as vibrios, as belonging to the animal kingdom (see Bulletin de l' Academie de Medecine, Paris 1875, pp. 249, 251, especially 256, 266, 267, 289, and 290). These would be very different, at least physiologically, the former being anaerobian, that is to say, requiring no air to enable them to live, and being killed by oxygen, should it be dissolved in the liquid to any considerable extent."

We are unable to see the matter in the same light as our learned colleague does; to our thinking, we should be labouring under a great delusion were we to suppose "that it is quite as serious an omission not to determine the animal or vegetable nature of a ferment as it would be to confound nitrogen with hydrogen or urea with stearine." The importance of the solutions of disputed questions often depends on the point of view from which these are regarded. As far as the result of our labours is concerned, we devoted our attention to these two questions exclusively: 1. Is the ferment, in every fermentation properly so called, an organized being? 2. Can this organized being live without air? Now, what bearing can the question of the animal or vegetable nature of the ferment, of the organized being, have upon the investigation of these two problems? In studying butyric fermentation, for example, we endeavoured to establish these two fundamental points; 1. The butyric ferment is a vibrio. 2. This vibrio may dispense with air in its life, and, as a matter of fact, does dispense with it in the act of producing butyric fermentation. We did not consider it at all necessary to pronounce any opinion as to the animal or vegetable nature of this organism, and, even up to the present moment, the idea that vibrio is an animal and not a plant is in our minds, a matter of sentiment rather than of conviction.

M. Robin, however, would have no difficulty in determining the limits of the two kingdoms. According to him, "every variety of cellulose is, we may say, insoluble in ammonia, as also are the reproductive elements of plants, whether male or female. Whatever phase of evolution the elements which reproduce a new individual may have reached, treatment with this reagent, either cold or raised to boiling, leaves them absolutely intact under the eyes of the observer, except that their contents, from being partially dissolved, become more transparent. Every vegetable whether microscopic or not, every mycelium and every spore, thus preserves in its entirety its special characteristics of form, volume and structural arrangements; whilst in the case of microscopic animals, or the ova and microscopic embryos of different members of the animal kingdom, the very opposite is the case."

We should be glad to learn that the employment of a drop of ammonia would enable us to pronounce an opinion with this degree of confidence on the nature of the lowest microscopic beings; but is M. Robin absolutely correct in his assumptions? That gentleman himself remarks that spermatozoa, which belong to animal organisms, are insoluble in ammonia, the effect of which is merely to make them paler. If a difference of action in certain reagents, in ammonia, for example, were sufficient to determine the limits of the animal and vegetable kingdoms, might we not argue that there must be a very great and natural difference between moulds and bacteria, inasmuch as the presence of a small quantity of acid in the nutritive medium facilitates the growth and propagation of the former, whilst it is able to prevent the life of bacteria and vibrios? Although as is well known, movement is not an exclusive characteristic of animals, yet we have always been inclined to regard vibrios as animals, on account of the peculiar character of their movements. How greatly they differ in this respect from the diatomaceae, for example! When the vibrio encounters an obstacle it turns, or after assuring itself by some visual effort or other that it cannot overcome it, it retraces its steps. The colpoda - undoubted infusoria - behave in an exactly similar manner. It is true one may argue that the zoospores of certain cryptogamia exhibit similar movements; but do not these zoospores possess as much of an animal nature as do the spermatozoa? As far as bacteria are concerned, when, as already remarked, we see them crowd round a bubble of air in a liquid to prolong their life, oxygen having failed them everywhere else, how can we avoid believing that they are animated by an instinct for life, of the same kind that we find in animals? M. Robin seems to us to be wrong in supposing that it is possible to draw any absolute line of separation between the animal and vegetable kingdoms. The settlement of this line however, we repeat again, no matter what it may be, has no serious bearing upon the questions that have been the subject of our researches.

In like manner the difficulty which M. Robin has raised in objecting to the employment of the word germ, when we cannot specify whether the nature of that germ is animal or vegetable, is in many respects an unnecessary one. In all the questions which we have discussed, whether we were speaking of fermentation of spontaneous generation, the word germ has been used in the sense or origin of living organism. If Liebig, for example, said of an albuminous substance that it gave birth to ferment, could we contradict him more plainly than by replying "No; ferment is an organized being, the germ of which is always present, and the albuminous substance merely serves by its occurrence to nourish the germ and its successive generations"?

In our Memoir of 1862, on so called spontaneous generations, would it not have been an entire mistake to have attempted to assign specific names to the microscopic organisms which we met with in the course of our observations? Not only would we have met with extreme difficulty in the attempt, arising from the state of extreme confusion which even in the present day exists in the classification and nomenclature of these microscopic organisms, but we should have been forced to sacrifice clearness in our work besides; at all events, we should have wandered from our principal object, which was the determination of the presence or absence of life in general, and had nothing to do with the manifestation of a particular kind of life in this or that species, animal or vegetable. Thus we have systematically employed the vaguest nomenclature, such as mucors, torulae, bacteria, and vibrios. There was nothing arbitrary in our doing this, whereas there is much that is arbitrary in adopting a definite system of nomenclature, and applying it to organisms but imperfectly known, the differences or resemblances between which are only recognizable through certain characteristics, the true signification of which is obscure. Take, for example, the extensive array of widely different systems which have been invented during the last few years for the species of the genera bacterium and vibrio in the works of Cohn, H. Hoffmann, Hallier, and Billroth. The confusion which prevails here is very great, although we do not of course by any means place these different works on the same footing as regards their respective merits.

M. Robin is, however, right in recognizing the impossibility of maintaining in the present day, as he formerly did, "That fermentation is an exterior phenomenon, going on outside cryptogamic cells, a phenomenon of contact. It is probably," he adds, "an interior and molecular action at work in the innermost recesses of the substance of each cell." From the day when we first proved that it is possible for all organized ferments, properly so called, to spring up and multiply from their respective germs, sown, whether consciously or by accident, in a mineral medium free from organic and nitrogenous matters other than ammonia, in which medium the fermentable matter alone is adapted to provide the ferment with whatever carbon enters into its composition, from that time forward the theories of Liebig, as well as Berzelius, which M. Robin formerly defended, have had to give place to others more in harmony with facts. We trust that the day will come when M. Robin will likewise acknowledge that he has been in error on the subject of the doctrine of spontaneous generation, which he continues to affirm, without adducing any direct proofs in support of it, at the end of the article to which we have been here replying.

We have devoted the greater part of this chapter to the establishing with all possible exactness the extremely important physiological fact of life without air, and its correlation to the phenomena of fermentations properly so called - that is to say, of those which are due to the presence of microscopic cellular organisms. This is the chief basis of the new theory that we propose for the explanation of these phenomena. The details into which we have entered were indispensable on account of the novelty of the subject no less than on account of the necessity we were under of combating the criticisms of the two German naturalists, Drs. Oscar Brefeld and Traube, whose works had cast some doubts on the correctness of the facts upon which we had based the preceding propositions. We have much pleasure in adding that at the very moment we were revising the proofs of this chapter, we received from M. Brefeld an essay, dated Berlin, January, 1876, in which, after describing his later experimental researches, he owns with praiseworthy frankness that Dr. Traube and he were both of them mistaken. Life without air is now a proposition which he accepts as perfectly demonstrated. He has witnessed it in the case of *mucor racemosus* and has also verified it in the case of yeast. "If," he says, "after the results of my previous researches, which I conducted with all possible exactness, I was inclined to consider Pasteur's assertion as inaccurate and to attack them, I have no hesitation now in recognizing them as true, and in proclaiming the service which Pasteur has rendered to science in being the first to indicate the exact relation of things in the phenomenon of fermentation." In his later researches, Dr. Brefeld has adopted the method which we have long employed for demonstrating the life and multiplication of butyric vibrios in the entire absence of air, as well as the method of conducting growths in mineral media associated with fermentable substance. We need not pause to consider certain other secondary criticisms of Dr. Brefeld. A perusal of the present work will, we trust, convince him that they are based on no surer foundation than were his former criticisms.

To bring one's self to believe in a truth that has just dawned upon one is the first step towards progress; to persuade others is the second. There is a third step, less useful perhaps, but highly gratifying nevertheless, which is, to convince one's opponents.

We therefore, have experienced great satisfaction in learning that we have won over to our ideas an observer of singular ability, on a subject which is of the utmost importance to the physiology of cells.

Section VI.

Part I.

Reply to the Critical Observations of Liebig, Published in 1870.

In the Memoir which we published, in 1860, on alcoholic fermentation, and in several subsequent works, we were led to a different conclusion on the causes of this very remarkable phenomenon from that which Liebig had adopted. The opinions of Mitscherlich and Berzelius had ceased to be tenable in the presence of the new facts which we had brought to light. From that time we felt sure that the celebrated chemist of Munich had adopted our conclusions, from the fact that he remained silent on this question for a long time, although it had been until then the constant subject of his study, as is shown by all his works. Suddenly there appeared in the *Annales de Chimie et de Physique* a long essay, reproduced from a lecture delivered by him before the Academy of Bavaria in 1868 and 1869. In this Liebig again maintained, not, however, without certain modifications, the views which he had expressed in his former publications, and disputed the correctness of the principal facts enunciated in our Memoir of 1860, on which were based the arguments against his theory.

"I had admitted," he says, "that the resolution of fermentable matter into compounds of a simpler kind must be traced to some process of decomposition taking place in the ferment, and that the action of this same ferment on the fermentable matter must continue or cease according to the prolongation or cessation of the alteration produced in the ferment. The molecular change in the sugar, would, consequently, be brought about by the destruction or modification of one or more of the component parts of the ferment, and could only take place through the contact of the two substances. M. Pasteur regards fermentation in the following light: The chemical action of fermentation is essentially a phenomenon correlative with a vital action, beginning and ending with it. He believes that alcoholic fermentation can never occur without the simultaneous occurrence of organization, development, and multiplication of globules, or continuous life, carried on from globules already formed. But the idea that the decomposition of sugar during fermentation is due to the development of the cellules of the ferment, is in contradiction with the fact that the ferment is able to bring about the fermentation of a pure solution of sugar. The greater part of the ferment is composed of a substance that is rich in nitrogen and contains sulphur. It contains, moreover, an appreciable quantity of phosphates, hence it is difficult to conceive how, in the absence of these elements in a pure solution of sugar undergoing fermentation, the number of cells is capable of any increase."

Notwithstanding Liebig's belief to the contrary, the idea that the decomposition of sugar during fermentation is intimately connected with a development of the cellules of the ferment, or a prolongation of the life of cellules already formed, is in no way opposed to the fact that the ferment is capable of bringing about the fermentation of a pure solution of sugar. It is manifest to any one who has studied such fermentation with the microscope, even in those cases where the sweetened water has been absolutely pure, that ferment-cells do multiply, the reason being that the cells carry with them all the food-supplies necessary for the life of the ferment. They may be observed budding, at least many of them, and there can be no doubt that those which do not bud still continue to live; life has other ways of manifesting itself besides development and cell-proliferation.

If we refer to the figures on page 81 of our Memoir of 1860, Experiments D, E, F, H, I, we shall see that the weight of yeast, in the case of the fermentation of a pure solution of sugar, undergoes a considerable increase, even without taking into account the fact that the sugared water gains from the yeast certain soluble parts, since in the experiments just mentioned, the weights of solid yeast, washed and dried at 100 C. (212 F.), are much greater than those of the raw yeast employed, dried at the same temperature.

In these experiments we employed the following weights of yeast, expressed in grammes (1 gramme = 15.43 grains):

- (1) 2.313
- (2) 2.626
- (3) 1.198
- (4) 0.699
- (5) 0.326
- (6) 0.476

which became, after fermentation, we repeat, without taking into account the matters which the sugared water gained from the yeast:

		grammes.	grains.
(1) 2.486	Increase	0.173	= 2.65
(2) 2.963	"	0.337	= 5.16
(3) 1.700	"	0.502	= 7.7
(4) 0.712	"	0.013	= 0.2
(5) 0.335	"	0.009	= 0.14
(6) 0.590	"	0.114	= 1.75

Have we not in this marked increase in weight a proof of life, or, to adopt an expression which may be preferred, a proof of a profound chemical work of nutrition and assimilation?

We may cite on this subject one of our earlier experiments, which is to be found in the Comptes rendus de l'Academie for the year 1857, and which clearly shows the great influence exerted on fermentation by the soluble portion that the sugared water takes up from the globules of ferment:

"We take two equal quantities of fresh yeast that have been washed very freely. One of these we cause to ferment in water containing nothing but sugar, and, after removing from the other all its soluble particles - by boiling it in an excess of water and then filtering it to separate the globules - we add to the filtered liquid as much sugar as was used in the first case along with a mere trace of fresh yeast insufficient, as far as its weight is concerned, to affect the results of our experiment. The globules which we have sown bud, the liquid becomes turbid, a deposit of yeast gradually forms, and, side by side with these appearances, the decomposition of the sugar is effected, and in the course of a few hours manifests itself clearly. These results are such as we might have anticipated. The following fact, however, is of importance. In effecting by these means the organization into globules of the soluble part of the yeast that we used in the second case, we find that a considerable quantity of sugar is decomposed. The following are the results of our experiment; 5 grammes of yeast caused the fermentation of 12.9 grammes of sugar in six days, at the end of which time it was exhausted. The soluble portion of a like quantity of 5 grammes of the same yeast caused the fermentation of 10 grammes of sugar in nine days, after which the yeast developed by the sowing was likewise exhausted."

How is it possible to maintain that, in the fermentation of water containing nothing but sugar, the soluble portion of the yeast does not act, either in the production of new globules or the perfection of old ones, when we see, in the preceding experiment, that after this nitrogenous and mineral portion has been removed by boiling, it immediately serves for the production of new globules, which, under the influence of the sowing of a mere trace of globules, causes the fermentation of so much sugar?
1

In short, Liebig is not justified in saying that the solution of pure sugar, caused to ferment by means of yeast, contains none of the elements needed for the growth of yeast, neither nitrogen, sulphur, nor phosphorus, and that, consequently, it should not be possible, by our theory, for the sugar to ferment. On the contrary, the solution does contain all these elements, as a consequence of the introduction and presence of the yeast.

Let us proceed without examination of Liebig's criticisms:

"To this," he goes on to say, "must be added the decomposing action which yeast exercises on a great number of substances, and which resembles that which sugar undergoes. I have shown that malate of lime ferments readily enough through the action of yeast, and that it splits up into three other calcareous salts, namely, the acetate, the carbonate and the succinate. If the action of yeast consists in its increase and multiplication, it is difficult to conceive this action in the case of malate of lime and other calcareous salts of vegetable acids."

This statement, with all due deference to the opinion of our illustrious critic, is by no means correct. Yeast has no action on malate of lime, or on other calcareous salts formed by vegetable acids. Liebig had previously, much to his own satisfaction, brought forward urea as being capable of transformation into carbonate of ammonia during alcoholic fermentation in contact with yeast. This has been proved to be erroneous. It is an error of the same kind that Liebig again brings forward here. In the fermentation of which he speaks (that of malate of lime), certain spontaneous ferments are produced, the germs of which are associated with the yeast, and develop in the mixture of yeast and malate. The yeast merely serves as a source of food for these new ferments without taking any direct part in the fermentations of which we are speaking. Our researches leave no doubt on this point, as is evident from the observations on the fermentation of tartrate of lime previously given.

It is true that there are circumstances under which yeast brings about modifications in different substances. Doebereiner and Mitscherlich, more especially, have shown that yeast imparts to water a soluble material, which liquefies cane-sugar and produces inversion in it by causing it to take up the elements of water, just as diastase behaves to starch or emulsin to amygdalin.

M. Berthelot also has shown that this substance may be isolated by precipitating it with alcohol, in the same way as diastase is precipitated from its solutions.² These are remarkable facts, which are, however, at present but vaguely connected with the alcoholic fermentation of sugar by means of yeast. The researches in which we have proved the existence of special forms of living ferments in many fermentations, which one might have supposed to have been produced by simple contact action, had established beyond doubt the existence of profound differences between those fermentations, which we have distinguished as fermentations proper, and the phenomena connected with soluble substances. The more we advance, the more clearly we are able to detect these differences. M. Dumas has insisted on the fact that the ferments of fermentation proper multiply and reproduce themselves in the process whilst the others are destroyed.³ Still more recently M. Muntz has shown that chloroform prevents fermentations proper, but does not interfere with the action of diastase (*Comptes rendus*, 1875). M. Bouchardat had already established the fact that hydrocyanic acids, salts of mercury, ether, alcohol, creosote, and the oils of turpentine, lemon, cloves, and mustard destroy or check alcoholic fermentations, whilst in no way interfering with the glucoside fermentations (*Annales de Chimie et de Physique*, 3rd series, vol. xiv., 1845). We may add in praise of M. Bouchardat's sagacity, that that skilful observer has always considered these results as a proof that alcoholic fermentation is dependent on the life of the yeast-cell, and that a distinction should be made between the two orders of fermentation.

M. Paul Bert, in his remarkable studies on the influence of barometric pressure on the phenomena of life, has recognized the fact that compressed oxygen is fatal to certain ferments, whilst under similar conditions it does not interfere with the action of those substances classed under the name of soluble ferments, such as diastase (the ferment which inverts cane sugar), emulsin and others. During their stay in compressed air, ferments proper ceased their activity, nor did they resume it, even after exposure to air at ordinary pressures, provided the access of germs was prevented.

We now come to Liebig's principal objection, with which he concludes his ingenious argument, and to which no less than eight or nine pages of the *Annales* are devoted.

Our author takes up the question of the possibility of causing yeast to grow in sweetened water, to which a salt of ammonia and some yeast-ash have been added - a fact which is evidently incompatible with his theory that a ferment is always an albuminous substance on its way to decomposition. In this case the albuminous substance does not exist; we have only the mineral substances which will serve to produce it. We know that Liebig regarded yeast, and, generally speaking, any ferment whatever, as being a nitrogenous, albuminous substance which, in the same way as emulsin, for example, possesses the power of bringing about certain chemical decompositions. He connected fermentation with the easy decomposition of that albuminous substance, and imagined that the phenomenon occurred in the following manner: "The albuminous substance on its way to decomposition possesses the power of communicating to certain other bodies that same state of mobility by which its own atoms are already affected; and through its contact with other bodies it imparts to them the power of decomposing or of entering into other combinations." Here Liebig failed to perceive that the ferment, in its capacity of a living organism, had anything to do with the fermentation.

This theory dates back as far as 1843. In 1846 Messrs. Boutron and Fremy, in a Memoir on lactic fermentation, published in the *Annales de Chimie et de Physique*, strained the conclusions deducible from it to a most unjustifiable extent. They asserted that one and the same nitrogenous substance might undergo various modifications in contact with air, so as to become successively alcoholic, lactic, butyric, and other ferments. There is nothing more convenient than purely hypothetical theories, theories which are not the necessary consequences of facts; when fresh facts which cannot be reconciled with the original hypothesis are discovered, new hypotheses can be tacked on to the old ones. This is exactly what Liebig and Fremy have done, each in his turn, under the pressure of our studies, commenced in 1857. In 1864 Fremy devised the theory of hemi-organism, which meant nothing more than that he gave up Liebig's theory of 1843, together with the additions which Boutron and he had made to it in 1846; in other words, he abandoned the idea of albuminous substances being ferments, to take up another idea, that albuminous substances in contact with air are peculiarly adapted to undergo organization into new beings - that is, the living ferments which we had discovered - and that the ferments of beer and of the grape have a common origin.

Part II.

This theory of hemi-organism was word for word the antiquated opinion of Turpin. The public, especially a certain section of the public, did not go very deeply into an examination of the subject. It was the period when the doctrine of spontaneous generation was being discussed with much warmth. The new word hemi-organism, which was the only novelty in M. Fremy's theory, deceived people. It was thought that M. Fremy had really discovered the solution of the question of the day. It is true that it was rather difficult to understand the process by which an albuminous substance could become all at once a living and budding cell. This difficulty was solved by M. Fremy, who declared that it was the result of some power that was not yet understood, the power of "organic impulse."⁴

Liebig, who, as well as M. Fremy, was compelled to renounce his original opinions concerning the nature of ferments, devised the following obscure theory (Memoir by Liebig, 1870, already cited):

"There seems to be no doubt as to the part which the vegetable organism plays in the phenomenon of fermentation. It is through it alone that an albuminous substance and sugar are enabled to unite and form this particular combination, this unstable form under which alone, as a component part of the mycoderme, they manifest an action on sugar. Should the mycoderme cease to grow, the bond which unites the constituent parts of the cellular contents is loosened, and it is through the motion produced therein that the cells of yeast bring about a disarrangement or separation of the elements of the sugar into molecules."

One might easily believe that the translator for the *Annales* has made some mistake, so great is the obscurity of this passage.

Whether we take this new form of the theory or the old one, neither can be reconciled at all with the development of yeast and fermentation in a saccharine mineral medium, for in the latter experiment fermentation is correlative to the life of the ferment and to its nutrition, a constant change going on between the ferment and its food-matters, since all the carbon assimilated by the ferment is derived from sugar, its nitrogen from ammonia and phosphorus from the phosphates in solution. And even all said, what purpose can be served by the gratuitous hypothesis of contact-action or communicated motion? The experiment of which we are speaking is thus a fundamental one; indeed, it is its possibility that constitutes the most effective point in the controversy. No doubt Liebig might say, "but it is the motion of life and of nutrition which constitutes your experiment, and this is the communicated motion that my theory requires." Curiously enough, Liebig does endeavour, as a matter of fact, to say this, but he does so timidly and incidentally: "From a chemical point of view, which point of view I would not willingly abandon, a vital action is a phenomenon of motion, and, in this double sense of life M. Pasteur's theory agrees with my own, and is not in contradiction with it (page 6)." This is true. Elsewhere Liebig says:

"It is possible that the only correlation between the physiological act and the phenomenon of fermentation is the production, in the living cell, of the substance which, by some special property analogous to that by which emulsin exerts a decomposing action on salicin and amygdalin, may bring about the decomposition of sugar into other organic molecules; the physiological act, in this view, would be necessary for the production of this substance, but would have nothing else to do with the fermentation (page 10)." To this, again, we have no objection to raise.

Liebig, however, does not dwell upon these considerations, which he merely notices in passing, because he is well aware that, as far as the defence of his theory is concerned, they would be mere evasions. If he had insisted on them, or based his opposition solely upon them, our answer would have been simply this: "If you do not admit with us that fermentation is correlated with the life and nutrition of the ferment, we agree upon the principal point. So agreeing, let us examine, if you will, the actual cause of fermentation; this is a second question, quite distinct from the first. Science is built up of successive solutions given to questions of ever increasing subtlety, approaching nearer and nearer towards the very essence of phenomena. If we proceed to discuss together the question of how living, organized beings act in decomposing fermentable substances, we will be found to fall out once more on your hypothesis of communicated motion, since according to our ideas, the actual cause of fermentation is to be sought, in most cases, in the fact of life without air, which is the characteristic of many ferments."

Let us briefly see what Liebig thinks of the experiment in which fermentation is produced by the impregnation of a saccharine mineral medium, a result so greatly at variance with his mode of viewing the question.⁵ After deep consideration he pronounces this experiment to be inexact, and the result ill-founded. Liebig, however, was not one to reject a fact without grave reasons for doing so, or with the sole object of evading a troublesome discussion. "I have repeated this experiment," he says, "a great number of times, with the greatest possible care, and have obtained the same results as M. Pasteur, excepting as regards the formation and increase of the ferment." It was, however, the formation and increase of the ferment that constituted the point of the experiment. Our discussion was, therefore, distinctly limited to this: Liebig denied that the ferment was capable of development in a saccharine mineral medium, whilst we asserted that this development did actually take place, and was comparatively easy to prove. In 1871 we replied to M. Liebig before the Paris Academy of Sciences in a Note, in which we offered to prepare in a mineral medium, in the presence of a commission to be chosen for the purpose, as great a weight of ferment as Liebig could reasonably demand.⁶ We were bolder than we should, perhaps, have been in 1860; the reason was that our knowledge of the subject had been strengthened by ten years of renewed research. Liebig did not accept our proposal, nor did he even reply to our Note. Up to the time of his death, which took place on April 18th, 1873, he wrote nothing more on the subject.⁷

When we published, in 1860, the details of the experiment in question, we pointed out at some length the difficulties of conducting it successfully, and the possible causes of failure. We called attention particularly to the fact that saccharine mineral media are much more suited for the nutrition of bacteria, lactic ferment, and other lowly forms, than they are to that of yeast, and in consequence readily become filled with various organisms from the spontaneous growth of germs derived from the particles of dust floating in the atmosphere. The reason why we do not observe the growth of alcoholic ferments, especially at the commencement of the experiments, is because of the unsuitableness of those media for the life of yeast. The latter may, nevertheless, form in them subsequent to this development of other organized forms, by reason of the modification produced in the original mineral medium by the albuminous matters that they introduce into it. It is interesting to peruse, in our Memoir of 1860, certain facts of the same kind relating to fermentation by means of albumens - that of the blood for example, from which, we may mention incidentally, we were led to infer the existence of several distinct albumens in the serum, a conclusion which, since then, has been confirmed by various observers, notably by M. Bechamp. Now, in his experiments on fermentation in sweetened water, with yeast-ash and a salt of ammonia, there is no doubt that Liebig had failed to avoid those difficulties which are entailed by the spontaneous growth of other organisms than yeast. Moreover, it is possible that, to have established the certainty of this result, Liebig should have had recourse to a closer microscopical observation than from certain passages in his Memoir he seems to have adopted. We have little doubt that his pupils could tell us that Liebig did not even employ that instrument without which any exact study of fermentation is not merely difficult but well-nigh impossible. We ourselves, for the reasons mentioned, did not obtain a simple alcoholic fermentation any more than Liebig did. In that particular experiment, the details of which we gave in our Memoir of 1860, we obtained lactic and alcoholic fermentation together; an appreciable quantity of lactic acid formed and arrested the propagation of the lactic and alcoholic ferments, so that more than half of the sugar remained in the liquid without fermenting. This, however, in no way detracted from the correctness of the conclusion which we deduced from the experiment, and from other similar ones; it might even be said that, from a general and philosophical point of view - which is the only one of interest here - the result was doubly satisfactory, inasmuch as we demonstrated that mineral media were adapted to the simultaneous development of several organized ferments instead of only one. The fortuitous association of different ferments could not invalidate the conclusion that all the nitrogen of the cells of the alcoholic and lactic ferments was derived from the nitrogen in the ammoniacal salts, and that all the carbon of those ferments was taken from the sugar, since, in the medium employed in our experiment, the sugar was the only substance that contained carbon. Liebig carefully abstained from noticing this fact, which would have been fatal to the very groundwork of his criticisms, and thought that he was keeping up the appearance of a grave contradiction by arguing that we had never obtained a simple alcoholic fermentation. It would be unprofitable to dwell longer upon the subject of the difficulties which the propagation of yeast in a saccharine mineral medium formerly presented. As a matter of fact, the progress of our studies has imparted to the question an aspect very different from that which it formerly wore; it was this circumstance which emboldened us to offer, in our reply to Liebig before the Academy of Sciences in 1871, to prepare, in a saccharine mineral medium, in the presence of a commission to be appointed by our opponent, any quantity of ferment that he might require, and to effect the fermentation of any weight of sugar whatsoever.

Our knowledge of the facts detailed in the preceding chapter concerning pure ferments, and their manipulation in the presence of pure air, enables us completely to disregard those causes of embarrassment that result from the fortuitous occurrence of the germs of organisms different in character from the ferments introduced by the air or from the sides of vessels, or even by the ferment itself.

Let us once more take one of our double-necked flasks, which we will suppose is capable of containing three or four litres (six to eight pints).

Let us put into it the following:

Pure distilled water.

Sugar	200 grammes
candy	
Bitartrate of potassium.....	1.0 "
" " ammonia	0.5 "
Sulphate of ammonia	1.5 "
Ash of	1.5 "
yeast	

(1 gramme = 15.43 grains)

Let us boil the mixture, to destroy all germs of organisms that may exist in the air or liquid or on the sides of the flask, and then permit it to cool, after having placed, by way of extra precaution, a small quantity of asbestos in the end of the fine curved tube. Let us next introduce a trace of ferment into the liquid, through the other neck, which, as we have described, is terminated by a small piece of india-rubber tube closed with a glass stopper.

Here are the details of such an experiment: -

On December 9th, 1873, we sowed some pure ferment - *saccharomyces pastorianus*. From December 11, that is, within so short a time as forty-eight hours after impregnation, we saw a multitude of extremely minute bubbles rising almost continuously from the bottom, indication that at this point the fermentation had commenced. On the following days, several patches of froth appeared on the surface of the liquid. We left the flask undisturbed in the oven, at a temperature of 25 C. (77 F.) On April 24, 1874, we tested some of the liquid, obtained by means of the straight tube, to see if it still contained any sugar. We found that it contained less than two grammes, so that 198 grammes (4.2 oz. Troy) had already disappeared. Some time afterwards the fermentation came to an end; we carried on the experiment, nevertheless, until April 18, 1875.

There was no development of any organism absolutely foreign to the ferment, which was itself abundant, a circumstance that, added to the persistent vitality of the ferment, in spite of the unsuitableness of the medium for its nutrition, permitted the perfect completion of fermentation. There was not the minutest quantity of sugar remaining. The total weight of ferment, after washing and drying at 100 C. (212 F.), was 2.563 grammes (39.5 grains).

In experiments of this kind, in which the ferment has to be weighed, it is better not to use any yeast-ash that cannot be dissolved completely, so as to be capable of easy separation from the ferment formed. Raulin's liquid ⁸ may be used in such cases with success.

All the alcoholic ferments are not capable to the same extent of development by means of phosphates, ammoniacal salts, and sugar. There are some whose development is arrested a longer or shorter time before the transformation of all the sugar. In a series of comparative experiments, 200 grammes of sugar-candy being used in each case, we found that whilst *saccharomyces pastorianus* effected a complete fermentation of the sugar, the caseous ferment did not decompose more than two-thirds, and the ferment we have designated new "high" ferment not more than one-fifth: and keeping the flasks for a longer time in the oven had no effect in increasing the proportions of sugar fermented in these two last cases.

Water	1,500
Sugar candy	70
Tartaric acid	4
Nitrate of ammonia	4
Phosphate of ammonia	0.6
Carbonate of potassium	0.6
Carbonate of magnesia	0.4
Sulphate of ammonia	0.25
Sulphate of zinc	0.07
Sulphate of iron	0.07
Silicate of potassium	0.07

- J. Raulin, Paris, Victor Masson, 1870, These pour le doctorat.

We conducted a great number of fermentations in mineral media, in consequence of a circumstance which it may be interesting to mention here. A person who was working in our laboratory asserted that the success of our experiments depended upon the impurity of the sugar-candy which we employed, and that if this sugar had been pure - much purer than was the ordinary, white, commercial sugar-candy, which up to that time we had always used - the ferment could not have multiplied. The persistent objections of our friend, and our desire to convince him, caused us to repeat all our previous experiments on the subject, using sugar of great purity, which had been specially prepared for us, with the utmost care, by a skilful confectioner, Seugnot. The result only confirmed our former conclusions. Even this did not satisfy our obstinate friend, who went to the trouble of preparing some pure sugar for himself, in little crystals, by repeated crystallizations of carefully selected commercial sugar-candy; he then repeated our experiments himself. This time his doubts were overcome. It even happened that the fermentations with the perfectly pure sugar instead of being slow were very active, when compared with those which we had conducted with the commercial sugar-candy.

We may here add a few words on the non-transformation of yeast into penicillium glaucum.

If at any time during fermentation we pour off the fermenting liquid, the deposit of yeast remaining in the vessel may continue there, in contact with air, without our ever being able to discover the least formation of penicillium glaucum in it. We may keep a current of pure air constantly passing through the flask; the experiment will give the same result. Nevertheless, this is a medium peculiarly adapted to the development of this mould, inasmuch as if we were to introduce merely a few spores of penicillium an abundant vegetation of that growth will afterwards appear on the deposit. The descriptions of Messrs. Turpin, Hoffmann, and Trecul have, therefore, been based on one of these illusions which we meet with so frequently in microscopical observations.

When we laid these facts before the Academy, ⁹ M. Trecul professed his inability to comprehend them:¹⁰ "According to M. Pasteur," he said, "the yeast of beer is anaerobian, that is to say, it lives in a liquid deprived of free oxygen; and to become mycoderma or penicillium it is above all things necessary that it should be placed in air, since, without this, as the name signifies, an aerobian being cannot exist. To bring about the transformation of the yeast of beer into mycoderma cerevisiae or into penicillium glaucum we must accept the conditions under which these two forms are obtained. If M. Pasteur will persist in keeping his yeast in media which are incompatible with the desired modification, it is clear that the results which he obtains must always be negative."

Contrary to this perfectly gratuitous assertion of M. Trecul's we do not keep our yeast in media which are calculated to prevent its transformation into penicillium. As we have just seen, the principal aim and object of our experiment was to bring this minute plant into contact with air, and under conditions that would allow the penicillium to develop with perfect freedom. We conducted our experiments exactly as Turpin and Hoffmann conducted theirs, and exactly as they stipulate that such experiments should be conducted - with the one sole difference, indispensable to the correctness of our observations, that we carefully guarded ourselves against those causes of error which they did not take the least trouble to avoid. It is possible to produce a ready entrance and escape of pure air in the case of the double-necked flasks which we have so often employed in the course of this work, without having recourse to the continuous passage of a current of air. Having made a file-mark on the thin curved neck at a distance of two or three centimetres (an inch) from the flask, we must cut round the neck at this point with a glazier's diamond, and then remove it, taking care to cover the opening immediately with a sheet of paper which has been passed through the flame, and which we must fasten with a thread round the part of the neck still left. In this manner we may increase or prolong the fructification of fungoid growths, or the life of the aerobian ferments in our flasks.

What we have said of penicillium glaucum will apply equally to mycoderma cerevisiae. Notwithstanding that Turpin and Trecul may assert to the contrary, yeast, in contact with air as it was under the conditions of the experiment just described, will not yield mycoderma vini or mycoderma cerevisiae any more than it will penicillium.

The experiments described in the preceding paragraphs on the increase of organized ferments in mineral media of the composition described, are of the greatest physiological interest. Amongst other results, they show that all the proteic matter of ferments may be produced by the vital activity of the cells, which, apart altogether from the influence of light or free oxygen (unless indeed, we are dealing with aerobian moulds which require free oxygen), have the power of developing a chemical activity between carbo-hydrates, ammoniacal salts, phosphates, and sulphates of potassium and magnesium. It may be admitted with truth that a similar effect obtains in the case of the higher plants, so that in the existing state of science we fail to conceive what serious reason can be urged against our considering this effect as general. It would be perfectly logical to extend the results of which we are speaking to all plants, and to believe that the proteic matter of vegetables, and perhaps of animals also, is formed exclusively by the activity of the cells operating upon the ammoniacal and other mineral salts of the sap or plasma of the blood, and the carbo-hydrates, the formation of which, in the case of the higher plants, requires only the concurrence of the chemical impulse of green light.

Viewed in this manner, the formation of the proteic substances, would be independent of the great act of reduction of carbonic acid gas under the influence of light. These substances would not be built up from the elements of water, ammonia, and carbonic acid gas, after the decomposition of this last; they would be formed where they are found in the cells themselves, by some process of union between the carbo-hydrates imported by the sap, and the phosphates of potassium and magnesium and salts of ammonia. Lastly, in vegetable growth, by means of a carbo-hydrate and a mineral medium, since the carbo-hydrate is capable of many variations, and it would be difficult to understand how it could be split up into its elements before serving to constitute the proteic substances, and even cellulose substances, as these are carbo-hydrates. We have commenced certain studies in this direction.

If solar radiation is indispensable to the decomposition of carbonic acid and the building up of the primary substances in the case of higher vegetable life, it is still possible that certain inferior organisms may do without it and nevertheless yield the most complex substances, fatty or carbo-hydrate, such as cellulose, various organic acids, and proteic matter; not, however, by borrowing their carbon from the carbonic acid which is saturated with oxygen, but from other matters still capable of acquiring oxygen, and so of yielding heat in the process, such as alcohol and acetic acid, for example, to cite merely carbon compounds most removed from organization. As these last compounds, and a host of others equally adapted to serve as the carbonaceous food of mycodermis and the mucedines, may be produced synthetically by means of carbon and the vapour of water, after the methods that science owes to Berthelot, it follows that, in the case of certain inferior beings, life would be possible even if it should be that the solar light was extinguished. ¹¹