activity that occurs at the beginning of the fermentation under the influence of the oxygen which is dissolved in the liquid when the process begins. In addition, the author has observed that beer yeast, when seeded into an albuminous liquid, such as yeast water,* continues to multiply even when there is no trace of sugar in the liquid, but only if oxygen from the air is present in large amounts. The same experiment can be repeated with an albuminous liquid mixed with a solution of non-fermentable sugar such as crystalline lactose. The results are quite similar.

The yeast formed in this way in the absence of sugar has not changed its nature. It is able to ferment sugar if it is allowed to act on it in the absence of air. It should be remarked always that the development of the yeast is quite poor in the absence of a fermentable material.

In summary, beer yeast behaves exactly like an ordinary plant,† and the analogy may be completed if ordinary plants would have an affinity for oxygen which would permit them to respire with the aid of this element by removing it from compounds that are not too stable, in which case, following M. Pasteur, they would be considered to be ferments for these materials.

M. Pasteur stated that he hoped to achieve this result, by finding conditions in which certain lower plants could live in the absence of air but in the presence of sugar, in this way bringing about the fermentation of this substance in the same way as beer yeast.

* [A water extract of yeast cells.]

† [This presumably means that beer yeast is able to respire under aerobic conditions in the same way that green plants do.]

Comment

This is apparently the first description of a phenomenon known in biochemistry today as the Pasteur effect. It describes the different behavior of yeast when grown aerobically or anaerobically. When yeast grows anaerobically, a large amount of sugar is converted into alcohol, with some of the sugar being converted into yeast protoplasm. The efficiency of the process of anaerobic growth is poor. Under aerobic conditions, although less sugar is used, a greater proportion of the sugar is converted into yeast protoplasm, with little or no alcohol produced. A point, not stressed here by Pasteur, but described in later work, is that under aerobic conditions, the utilization of sugar actually seems to be inhibited. Therefore, although a much greater efficiency of conversion of sugar into yeast protoplasm exists aerobically, so that greater amounts of yeast cells are formed, the actual utilization of sugar is depressed. The reasons for this are quite complex, and even today are not completely explained. This latter phenomenon is the Pasteur effect and has been found in other organisms and in animal tissues, and currently has been studied considerably in relation to cancer tissue metabolism. The implications of a discovery can never be realized fully at the time the discovery is made.

Another observation made by Pasteur in this paper is that yeast seems to be able to grow anaerobically when sugar is present, but not when some other carbon and energy source replaces sugar. In the presence of other carbon sources, the yeast can grow, but only aerobically. The explanation for this observation has only been made in the last 20 years. We know that when sugar is present, yeast cells can obtain energy from this source anaerobically by oxidizing part of the sugar into carbon dioxide, while another part of the sugar is reduced to alcohol. Thus some sugar molecules are electron acceptors, while other molecules are electron donors. During this process,

called glycolysis, energy is released which is stored as high energy phosphate bonds. No free oxygen is necessary for this process. However, when other energy sources are used which are not sugars, such as amino acids or organic acids, these compounds cannot be handled in the above manner, and must be oxidized using free oxygen as the electron acceptor. Therefore yeast can only grow when these materials are the energy sources when there is oxygen present.

The discovery that yeast could grow both aerobically and anaerobically is quite important for biochemistry and biology. Pasteur could not see his discovery in the light of modern work, and therefore could only view it in terms of the fermentative process he was seeking to explain. His hope to bring about fermentation of sugar with green plants remains unfulfilled.

On the organized bodies which exist in the atmosphere; examination of the doctrine of spontaneous generation

1861 • Louis Pasteur

Pasteur, L. 1861. Mémoire sur les corpuscles organisés qui existent dans l'atmosphère. Examen de la doctrine des générations spontanées. *Annales des sciences naturelles*, 4th series, Vol. 16, pages 5–98.

Chapter I. Historical. . . .

CHEMISTS HAVE DISCOVERED DURING THE last twenty years a variety of really extraordinary phenomena which have been given the generic name of *fermentations*. All of these require the cooperation of two substances: one which is fermentable, such as sugar, and the other nitrogenous, which is always an albuminous substance. But here is the universally accepted theory for this phenomenon: the albuminous material undergoes, when it comes in contact with air, an alteration, a particular oxidation of an unknown nature, which gives it the characteristics of a *ferment*. That is, it acquires the property of being able to cause fermentation upon contact with fermentable substances.

The oldest and the most remarkable ferment which has been known to be an organized being is the yeast of beer. But in all of the fermentations discovered since the beer yeast was shown to be organized, it has not been possible to demonstrate the existence of organized beings, even after careful study. Therefore, physiologists have gradually abandoned regretfully the hypothesis of M. Cagniard de Latour concerning a probable relation between the organized nature of this ferment and its ability to cause the fermentation. Instead, to beer yeast has been applied the following general theory: "It is not the fact that it is organized that makes the beer yeast active, rather it is because it has been in contact with air. It is the dead material of the yeast, that which has lived and is in the process of change, which acts on the sugar."

My studies have lead me to entirely different conclusions. I have found that all true fermentations—viscous, lactic, butyric, or those of tartaric acid, malic acid, or urine—only occur with the presence and multiplication of an organized being. Therefore, the organized nature of the beer yeast is not a disadvantage for the theory of fermentation. Rather, this shows that it is no different than other ferments and fits the common rule. In my opinion, the albuminous materials were never the ferments, but the nutrients of the ferment. The true ferments were organized beings.

This granted, it was known that the ferments originate through the contact of albuminous materials with oxygen gas. If this is so, there are two possibilities to explain this. Since the ferments are organized, it is possible that oxygen, acting as itself, is able to induce the production of the ferments through its contact with the nitrogenous materials, and therefore the ferments have arisen spontaneously. But, if the ferments are not spontaneously generated beings, it is not the oxygen gas itself which is necessary for their formation, but the stimulation by oxygen of a germ which is either carried with it by the air, or which already exists preformed in the nitrogenous or fermentable materials. At this point in my studies on fermentations, I wanted to arrive at an opinion on the question of spontaneous generation. I would perhaps be able to uncover a powerful argument in favor of my ideas on the fermentation themselves.

The researches which I will report here are only a digression made necessary by my studies on fermentations. . . .

Chapter II. Examination under the microscope of the solid particles which are disseminated in the atmosphere.

My first problem was to develop a method which would permit me to collect in all seasons the solid particles that float in the air and examine them under the microscope. It was at first necessary to eliminate if possible the objections which the proponents of spontaneous generation have raised to the age-old hypothesis of the aerial dissemination of germs.

When the organic materials of infusions have been heated, they become populated with infusoria or molds. These organized bodies are in general neither so numerous nor so diverse as those that develop in infusions that have not been previously boiled, but they form nevertheless. But the germs of these infusoria and molds can only come from the air, if the liquid is boiled, because the boiling destroys all those that were present in the container or which had been brought there by the liquid. The first question to resolve is therefore: are there germs in the air? Are they there in sufficient numbers to explain the appearance of organized bodies in infusions which have been previously heated? Is it possible to obtain an approximate idea of the number of germs in a given volume of ordinary air? . . .

The procedure which I followed for collecting the suspended dust in the air and examining it under the microscope is very simple. A volume of the air to be examined is filtered through guncotton which is soluble in a mixture of alcohol and ether. The fibers

of the guncotton stop the solid particles. The cotton is then treated with the solvent until it is completely dissolved. All of the particles fall to the bottom of the liquid. After they have been washed several times, they are placed on the microscope stage where they are easily examined. . . .

These very simple manipulations provide a means of demonstrating that there exists in ordinary air continually a variable number of bodies. Their sizes range from extremely small up to 0.01 or more of a millimeter. Some are perfect spheres, while others are oval. Their shapes are more or less distinctly outlined. Many are completely translucent, but others are opaque with granules inside. Those which are translucent with distinct shapes resemble the spores of common molds, and could not be told from these by the most skillful microscopist. Among the other forms present, there are those which resemble spherical infusoria and may be their cysts or the globules which are generally regarded as the eggs of these small organisms. But I do not believe it is possible to state with certainty that a particular object is a spore, or more especially the spore of a particular species, or that another object is an egg, and the egg of a certain microzoan. I will limit myself to the statement that these bodies are obviously organized, resembling in all points the germs of the lowest organisms, and so diverse in size and structure that they obviously belong to a large number of species.

By using a solution of iodine, it is possible to show unequivocally that amidst the bodies there are always starch granules. But it is easy to remove all globules of this sort by diluting the dust in ordinary sulfuric acid, which dissolves immediately all of the starch. Without a doubt the sulfuric acid alters and perhaps dissolves other globules, but there still remain a large number. Sometimes it is possible to distinguish more after treatment with sulfuric acid, because the acid dissolves the calcium carbonate and dilutes the other dust particles, so that many of the organized particles are freed from the amorphous debris which had prevented them from being seen well. It is well to make observations immediately after the small bubbles of carbonic acid have been dissipated, and before needles of calcium sulfate precipitate.

A wad of guncotton 1 centimeter long by ½ centimeter in diameter was exposed to a current of air flowing at one liter per minute for 24 hours. After this time, examination revealed 20 to 30 organized bodies deposited per quarter of an hour (15 minutes). There are ordinarily several bodies in the microscope field. It should be noted that the drop of dust mixture placed on the microscope slide is only a fraction of the total mixture obtained. . . .

Chapter III. Experiments with heated air.

We may conclude that there are always in suspension in the air, organized bodies which, from their shape, size and structure, cannot be distinguished from the germs of lower organisms, and without exaggeration, the number of these is quite large. Are there amongst these bodies really those which are capable of germinating? This is a very interesting question which I believe I have been able to solve with certainty. But before presenting the results of experiments which support in particular this argument, it is necessary to determine first if the results of Dr. Schwann on the inactivity of air which has been heated red hot are correct. MM. Pouchet, Mantegazza, Joly and Musset contest this. Let us see which side is correct, especially since this will be fundamental to our later researches.

In a flask of 250–300 cc., I introduced 100–150 cc. of solution of the following composition: water, 100; sugar, 10; albuminous material and minerals from beer yeast, 0.2–0.7.

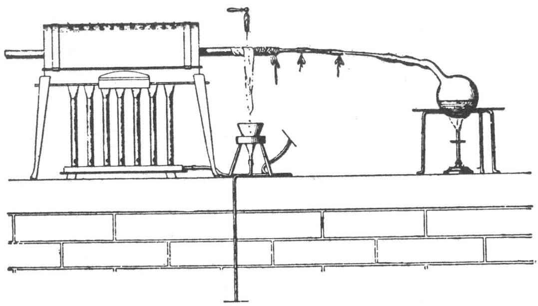

FIG. 10.

The neck of the flask is drawn out and connects with a platinum tube which is heated red hot, as shown in Figure 10. The liquid is boiled for two or three minutes, then it is allowed to cool completely. The flask is filled with ordinary air at atmospheric pressure, but all the air which has entered has been heated red hot first. Then the neck of the flask is sealed under a flame.

The flask so prepared is placed in a constant temperature chamber at 30°. It can be kept this way indefinitely without the liquid within showing the slightest alteration. Its clarity, odor, and slightly acidic character show not the slightest change. Its color darkens slightly after a time, which is undoubtedly due to a direct oxidation of the albuminous material or the sugar.

I may say with the utmost sincerity that I have never had a single experiment which has given me the slightest doubt. Sugared yeast water heated to boiling for two or three minutes, then placed in contact with heated air, does not change in the slightest, even after 18 months at 25° to 30°. At the same time, if the flask is filled with ordinary air, it undergoes an extensive change within a day or two, and is full of bacteria, vibrios, or is covered with mucors.

The experiment of Dr. Schwann is therefore of irreproachable exactitude. . . .

Chapter VI. Another very simple method to demonstrate that all of the organized bodies in infusions previously heated originate from bodies which exist in suspension in the atmospheric air.

I believe I have rigorously established in the preceding chapters that all organized bodies in infusions which have been previously heated originate only from the solid particles carried by the air and which are constantly being deposited on all objects. In order to remove the slightest doubt in the reader, may I present the results of the following experiments.

In a glass flask I placed one of the following liquids which are extremely alterable through contact with ordinary air: yeast water, sugared yeast water, urine, sugar beet juice, pepper water. Then I drew out the neck of the flask under a flame, so that a number of curves were produced in it, as can be seen in Figure 25. I then boiled

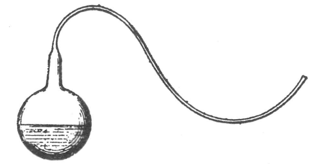

FIG. 25 A.

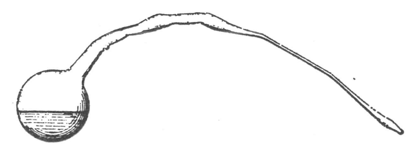

FIG. 25 B.

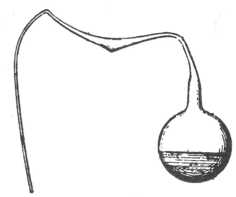

FIG. 25 C.

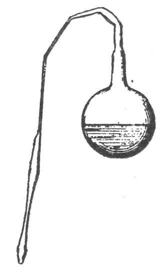

FIG. 25 D.

the liquid for several minutes until steam issued freely through the extremity of the neck. This end remained open without any other precautions. The flasks were then allowed to cool. Any one who is familiar with the delicacy of experiments concerning the so-called "spontaneous" generation will be astounded to observe that the liquid treated in this casual manner remains indefinitely without alteration. The flasks can be handled in any manner, can be transported from one place to another, can be allowed to undergo all the variations in temperature of the different seasons, the liquid does not undergo the slightest alteration. It retains its odor and flavor, and only, in certain cases, undergoes a direct oxidation, purely chemical in nature. *In no case is there the development of organized bodies in the liquid.*

It might seem that atmospheric air, entering with force during the first moments, might come in contact with the liquid in its original crude state. This is true, but it meets a liquid which is still close to the boiling point. The further entrance of air occurs much slower, and when the liquid has cooled to the point where it will not kill the germs, the entrance of air has slowed down enough so that the dust it carries which is able to act on the infusion and cause the development of organized bodies is deposited on the moist walls of the curved tube. At least, I can see no other explanation for these curious results. For, after one or more months in the incubator, if the neck of the flask is removed by a stroke of a file, without otherwise touching the flask, molds and infusoria begin to appear after 24, 36, or 48 hours, just as usual, or as if dust from the air had been inoculated into the flask.

The same experiment can be repeated with milk, except here one must take the precaution of boiling the liquid under pressure at a temperature above 100° and allowing the flask to cool with the reentry of heated air. The flask can be allowed to stand in the open, just as before. The milk undergoes no alteration. I have allowed milk prepared in this manner to incubate for many months at 25 to 30°, without alteration. One notices only a slight thickening of the cream and a direct chemical oxidation.

I do not know any more convincing experiments than these, which can be easily repeated and varied in a thousand ways. . . .

At this moment I have in my laboratory many highly alterable liquids which have remained unchanged for 18 months in open vessels with curved or inclined necks. A number of these were deposited in the office of the Academy of Sciences during the meeting of 6 February 1860, when I had the honor of communicating to them these new results.

The great interest of this method is that it proves without doubt that the origin of life, in infusions which have been boiled, arises uniquely from the solid particles which are suspended in the air. Gas, various fluids, electricity, magnetism, ozone, things known or things unknown—there is absolutely nothing in ordinary atmospheric air which brings about the phenomenon of putrefaction or fermentation in the liquids which we have studied except these solid particles. . . .

The experiments of the preceding chapters can be summarized in the following double proposition:

1. There exist continually in the air organized bodies which cannot be distinguished from true germs of the organisms of infusions.

2. In the case that these bodies, and the amorphous debris associated with them, are inseminated into a liquid which has been subjected to boiling and which would have remained unaltered in previously heated air if the insemination had not taken place, the same beings appear in the liquid as those which develop when the liquid is exposed to the open air.

If we grant this, will a proponent of spontaneous generation continue to maintain his principles, even in the presence of this double proposition? This he can do, but he is forced to reason as follows, and I let the reader be the judge of it:

"There are solid particles present in the air," he will say, "such as calcium carbonate, silica, soot, wool and cotton fibers, starch grains, etc., and at the same time there are organized bodies with a perfect resemblance to the spores of molds and the eggs of infusoria. Well, I prefer to place the origin of the molds and infusoria in the first group of amorphous bodies, rather than in the second."

In my opinion, the inconsistency of such reasoning is self-evident. The entire body of my research has placed the proponents of spontaneous generation in this predicament.

Comment

We should marvel at the logic, the clarity, and above all the simplicity of these experiments. In a few incisive blows Pasteur has ended the controversy concerning spontaneous generation. His experiments were easily reproducible, and this made it simple for others to accept his conclusions. As we shall see from Tyndall's paper, the controversy did not end completely, since Pasteur was working with nutrient fluids which did not allow for the development of heat-resistant bacterial spores. But from this time on, proponents of spontaneous generation found themselves fighting a losing battle.

At the same time that he solved the problem of spontaneous generation, Pasteur founded the science of microbiology. He showed how to sterilize a liquid, and how to keep it sterile. Only when it is possible to culture one organism in the complete absence of other organisms is it possible to determine the unique properties of the one organism under study. We shall see the importance of this requirement later, when reading of the work on infectious disease.

Studies on the biology of the bacilli

1876 · Ferdinand Cohn

Cohn, Ferdinand. 1876. Untersuchungen über Ba terien. IV. Beiträge zur Biologie der Bacille *Beiträge zur Biologie der Pflanzen*, Vol. 2, pag 249–276.

1. BACTERIA AND SPONTANEOUS GENERATION

Among the problems remaining to be solved by modern science, there is none of more significance than the question as to whether living beings develop exclusively from germs which have been produced by other beings of the same type, or whether living beings can develop from non-living material (by abiogenesis, archigenesis, spontaneous generation, *generatio spontanea, Urzeugung*). Unjustly most workers in Germany have long considered that this question has been solved in favor of the former alternative. Although since the time of Redi a large number of experiments and observations have been reported showing that a large number of types of animals and plants cannot develop unless their germs (eggs, seeds, or spores) are present, it does not necessarily follow that the development without germs is impossible for all living beings, even those that are the simplest and lowest. Those workers who deny an absolute boundary between inorganic and organic compounds, between living and non-living bodies, and who view life as a function of the identical forces which are operating in the non-living world, d not have the slightest grounds f doubting the possibility that und certain conditions protoplasm cou be formed from the atoms of carbo oxygen, hydrogen, and nitrog through a certain combination chemical and physical forces, in t same way that urea can be forme from ammonium carbonate, and th this artificially or spontaneous formed protoplasm could enter in the cycle of nature and become a li ing Monera, capable of feeding an reproducing. Therefore Pouchet an especially Ch. Bastian have recent performed us a worthy service whe they performed experiments, withou hypothesizing the impossibility spontaneous generation, to discov the conditions under which it migh be possible for living beings to develo from organic but non-living mat rial. . . .

Although the studies of the adhe ents of spontaneous generation hav followed scientific procedures, the is a criticism of these experimen which the opponents of this doctri believe has refuted spontaneous ge eration. The latter have assumed th it is an incontestable fact that livin