Life Without Oxygen—I*

The Anaerobic Beginnings of Life

By Carl Snyder

It has been suggested that the beginnings of life must have been in the absence of uncombined oxygen, that is, in Pasteur's phrase, anaerobic. At least five or six convergent lines of evidence lead to this unexpected conclusion. These are:

1. That what appear to be the simplest forms of life are anaerobic still and that the majority of lower organisms, both plant and animal, can live under anaerobic conditions more or less continuously.

2. That the fundamental chemical processes of the cell in all organisms, even the highest, are anaerobic, phenomena of oxidation being of secondary or ulterior importance.

3. That the relations of anaerobic and aerobic life are genetic and that we have clear evidence of the gradual evolution of the latter from the former, an increasing need of oxygen accompanying an increasing complexity of chemical and morphological organization.

4. That this increase in need of oxygen as complexity increases is paralleled in the growth of the individual organism, the ability to endure complete abstraction of oxygen varying inversely with age and size.

5. That the oxygen of the atmosphere appears to have been formed exclusively by plant action. In the beginning there was probably no oxygen free: if there had been any, it would have been very quickly absorbed by the unoxidized substances of the earth's crust or the quantity would have been so small as to be practically negligible.

The Facts of Anaerobiosis.—So incessant and so absolute is the need of oxygen by the human organism and by animal organisms in general, that in the half-century following the momentous work of Lavoisier the belief in oxidation or "combustion" as a primary process of life came to be an *idée fixe* among physiologists, in spite of the fact that Spallanzani, who was almost contemporary with Lavoisier, had shown that infusorial animalcules can thrive when the supply of oxygen is reduced to a minimum (Spallanzani operated with air pressures of from one to three inches) and that the production of carbonic acid gas continues, in some cases without diminution, when the supply of oxygen is entirely cut off.

But everyday familiar experience proved too strong and so it was that the proof came as a shock when the existence of beings was established which not only did not need oxygen but to which the slightest trace of oxygen is as fatal as lack of it is to us. Had the discovery been announced with anything less than the preponderant authority of Pasteur it must have long remained unrecognized. Pasteur's first communication was published in 1861 and was entitled: "Animalcules infusoires vivants sans gaz oxygène libre et déterminant des fermentations." In a footnote he remarks: "I propose, with all reserve, these new words, *aerobies* and *anaerobies*, to indicate the existence of two classes of primitive beings, one incapable of living save in the presence of oxygen; the other able to multiply indefinitely without contact with this gas."

During the half-century that has followed this announcement, this capacity of existing, for a time at least, in the complete absence of uncombined oxygen—facultative anaerobiosis—has been shown to be of wide occurrence in both the plant and animal kingdoms. On the other hand, the possibility of complete and unconditional anaerobic existence has been repeatedly denied; needless to say, it is one of the most difficult things in the world to establish the absence of oxygen. Thus Beijerinck was able to show that, in the case of certain forms supposedly anaerobic, for example the butyric acid ferments, the minutest trace of oxygen sufficed during a long series of generations. Beijerinck, therefore, proposed to divide living organisms into two classes—those which thrive best when the oxygen tension is at a maximum and those which thrive best when it is at a very low tension. The former he called *aerophile*, the latter *microaerophile*. This conception seemed to fit the facts so well that it was generally accepted for a time by the most careful observers, among them Fermi, A. Fischer, Chudjakow and others. But the discovery and investigation of the light-producing or photo-bacteria revealed a test for the presence of oxygen of such sensitiveness as to disclose the most minute trace. With the aid of this last and the development of a technique of quite extraordinary refinement, it has been possible, notably through the researches of Omelianski, Burri and latterly by the decisive work of Kürsteiner, to establish the correctness of Pasteur's early observations.

To those to whom the law of continuity or curve of probabilities is a constant mental companion, this

final demonstration seemed a foregone conclusion. The fact that in passing from organisms like the higher animals to which a constant supply of oxygen is a necessity, every gradation in the need of oxygen was to be met with down to a point at which the utmost refinement of technique was required to discover that it was of use at all, left little reasonable doubt in the philosophic mind that beyond this point the absolute anaerobe must exist.

Why, now, should we regard these anaerobic bacteria as the lowest, the most primitive and, therefore, in all probability, the earliest of all living organisms known? For very much the same reason that we speak of the plant as "lower" in the scale of life than the animal. In general, at least, the higher types of animals are able to exist only by means of preformed organic products. The plant is able to derive its food directly from the soil and the air. It is evident, therefore, that a great land flora must have preceded any notable development of a land fauna.

Further, it is known that prior to the development of an extensive land flora there must come a great bacterial population, both to form and to prepare the soil. This is evident from what happened after the Krakatoa explosion in 1884. So far then as life on land is concerned, an extremely primitive microscopic life is apparently antecedent to all other forms.

The same is true of the sea. In recent work, A. Pütter has endeavored to show that the floral and primitively synthetic organisms of the sea are quite inadequate to support the astounding abundance of life which, little by little, we are coming to learn exists there—a volume so great that compared with it the total amount of land and aerial life is an almost negligible quantity. Pütter, therefore, endeavors to show that the sea is a vast, though very weak, organic solution. His results have not been confirmed and have even been directly called in question, though on the face of the evidence some such conception as his seems to be necessary to many. But whether or no the destructive metabolism of the sea be less complete than that on the land, it is still obvious that a vast floral life, directly utilizing solar energy, is a condition of a development of oceanic animal life such as we know exists; such a flora is found in the Plankton. It is evident that the algae, the desmids and their like must have preceded, in point of time, the more elaborate animal types. Side by side with these is to be found in sea water the same teeming bacterial life as in the soil; moreover, for the most part, marine bacteria are of the same types and varieties as soil bacteria.

It is amid these prototypes of life, both in the sea and in the soil, that the anaerobic bacteria are found. Like all bacteria and the most primitive types in general, they are neither plant nor animal in any clear sense of the word. Some, like the plants, derive their nutrition directly from inorganic materials; others live on the dead organic substance of plants and animals; while others are able to exist only as parasites, inhabiting the tissues or cavities of living organisms. Finally, some live now in the one way, now in the other. They contain no chlorophyll; they need no sunlight. Some are fixed but others are free swimming organisms. But whereas only a few of the bacteria and practically none of the plant forms—the case of the Schizophyceae is still rather doubtful—are able to assimilate gaseous nitrogen, it is among the anaerobic bacteria that the most typical nitrogen fixers are found.

Consider the implication. The anaerobic organism is evidence of the fact that gaseous oxygen is not essential to the synthetic changes which accompany life. It is only as we rise in the scale of life that the need of oxygen becomes obvious. On the other hand, the capacity of assimilating nitrogen exists only in the lowest forms and as we ascend the scale is very quickly lost. Considering how essential the element nitrogen is in the chemistry of life and the established non-essential character of gaseous oxygen, we seem driven to the conclusion that the most primitive and therefore the earliest form must have been a nitrogen fixer to which a supply of uncombined oxygen was immaterial.

It is true that *Bacterium clostridium* (Past.), the best studied of the organisms that assimilate nitrogen, requires for its nutrition a supply of glucose, that is to say an organic product; while forms are known, like Nostoc, among the Schizophyceae, which are able to fix at least minor quantities of nitrogen in an entirely inorganic culture medium, that is to say they do not require a ready-made supply of complex organic carbon compounds. It is for this reason that the Nostocae have been regarded by some as the more primitive form. Euler observes:

"We have herewith every reason to seek among the Schizophyceae the earliest inhabitants of the earth. Further support for this assumption is derived from repeated observations that these algae are always the first colonists on sand, bare and humus soil and the like. A celebrated example is that afforded by the Nostocae, which Treub found as the earliest germinants on the sterile soil of Krakatoa, after the famous catastrophe."

But the ability to assimilate carbon from its simplest inorganic compounds, the carbonates, not only from carbon dioxide but even from the deadly monoxide, is shared by many absolute anaerobes, even though it is not by *Bacterium pasteurianum*. Nostoc is apparently better able to propagate rapidly under the atmospheric conditions which now prevail but as we shall see it is at least extremely doubtful if these same conditions obtained in the earlier epochs of the earth's history.

The conclusion that the anaerobic nitrifiers constitute the more primitive form is supported by the behavior of these and other low forms toward an oxygen supply. It is now a well-established fact that many, if not all, of the incontestably anaerobic bacteria may, under suitable conditions, be cultivated and reproduce rapidly under ordinary atmospheric conditions. On the other hand, some at least of the supposedly absolute aerobes, as Rosenthal, Tarozzi and others have shown, if passed through a series of cultures in which the supply of oxygen is more and more attenuated, may at last be cultivated freely under completely anaerobic conditions. In the ancient phrase they may be "adapted" to an anaerobic life.

It has long been known that there are intermediate forms which are indifferently oxygen users or abstainers, according as this element is present or absent; in other words, the oxygen habit is apparently one which may be readily assumed and readily put aside. On the other hand, there does not seem to be any clear evidence that a corresponding nitrogen habit may be acquired where it is non-existent. The ability to assimilate uncombined nitrogen seems to be an extremely primitive faculty, quickly put aside and finally lost as the complexity of the organism increases.

In the light of this conclusion and the fact that complete and enduring anaerobiosis is to be found only among the more primitive forms, it seems almost impossible to regard the faculty of living without oxygen as an "acquirement." We shall have decisive proof in the sequel that it is not. It is the ability to use uncombined oxygen which is the "acquirement;" and we seem, therefore, driven to the conclusion that the anaerobic was the more primitive and the earlier form.

What is true of the bacteria is equally true of the protozoa. Thus Bunge has shown that many of the more primitive forms are either actively or conditionally anaerobic to a very considerable degree. Vinegar cels (*Anguillula aceti*) lived and displayed the liveliest sort of motions in complete absence of oxygen during seven days, leeches during three days, Ascaris five days, Hæmoptis two days, Clepsina six days, Nephillis two days, snails ten to fifteen hours, etc. Apparently the observations were carried out at room temperature; Pflueger, however, has shown that the ability of the frog to resist the withdrawal of oxygen is inversely proportional to the temperature, so that it is probable that at the ordinary temperatures at which many of the slime worms live their need of oxygen is still less than Bunge's observations would lead us to suppose is the case.

Bunge's work has been continued by Weinland, Pütter and Lesser among others. Pütter kept leeches alive during ten days; *Opalina* during twenty days; he also experimented with *Paramœcium caudatum*, *Colpidium colpoda*, *Opalina ranarum*, *Balantidium eozoon*, *Nyctotheris caudiformis* and *Spirostomum ambiguum*. Pütter's conclusions were as follows:

"In their widespread independence of molecular (free) oxygen and in the dependence of the faculty of living anaerobically on the state of nutrition and the supply of nutriment, the protozoa present a close agreement with countless plants, while in the animal kingdom we have as yet few analogies. But this is doubtless due to the fact that we have as yet few experiments on the respiration of the lower animals and that these even have generally been interpreted so as to agree with the dogma of the invariable necessity of molecular oxygen."

Duclaux speaks to the same effect when he remarks upon the part which pure suggestion has played in the formation of the ancient (but still largely dominant) theories of respiration.

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The Fundamentally Anaerobic Character of the Vital Process.—The first wide breach in the ancient dogma that oxygen played an essential part in vital metabolism came from the researches of Pasteur; his work was the first which distinctly showed that the vital process was to be correlated with the process of fermentation. It was he, indeed, who defined fermentation as "life without air," that is without oxygen as such. It is among the further ironies that though the long strife between the followers of Pasteur and the followers of Liebig, over the chemical nature of fermentation, was ended finally and decisively in 1897 by Buchner's epoch-making discovery of zymase, most biologists still cling implicitly to Pasteur's ideas of life in spite of the proof that Pasteur himself supplied that life and fermentation are one and the same thing.

Pasteur showed that in the case of certain of the lower organisms the life process could be carried on in the absence of oxygen. But the generalization of this fact, that anaerobiosis is fundamentally true of the vital processes in all organisms, was first clearly set forth, apparently, by Hoppe-Seyler. This conclusion was implied in the work of Spallanzani and indeed was distinctly stated by him in the latter part of the eighteenth century. This amazingly fertile and pioneering man had studied the production of carbonic acid in the case of living and dead snails "in azotic (nitrogen) and hydrogen gas and in common air." Summing up, he said:

"I shall only conclude from these experiments that it is clearly proved that the carbonic acid gas produced by living and dead snails in common air did not result from the atmospheric oxygen, since an equal and even greater quantity was obtained in azotic and hydrogen gas."

Spallanzani was a full three-quarters of a century ahead of his time. It is instances like this that serve to remind us at times of the large amount of clear-sighted work which remains unrecognized.

Hoppe-Seyler's investigations were formulated in the first volume of his *Physiological Chemistry*, published in 1877. His cautious conclusion was stated in the following words:

"Although the putrefactive processes have been set forth in complete parallel to the phenomena of life, it should not be understood thereby that the two are identical, only that any difference is as yet unknown."

Hoppe-Seyler based his view of the fermentative nature of respiration on the following facts:

1. In the animal organism, reduced substances such as urobilin, succinic acid, hippuric acid and so forth result from the introduction of quinolinic acid and the like and appear in the urine in company with unquestionable products of oxidation.

2. Many easily oxidizable substances may pass through the organism unoxidized.

3. Between the production of carbon dioxide and the consumption of oxygen there is no constant relation.

4. Although ozone is not and can not be demonstrated in the organism, nevertheless a complete destruction of complicated organic compounds to carbon dioxide and water takes place, while we are able to bring about these same oxidations outside the body only very slowly and incompletely even with the aid of the strongest oxidizing materials.

Similar ideas were independently advanced and the anaerobic nature of the fundamental chemical changes in the cell still more definitely insisted upon by A. Gautier. His ideas are summed up in his admirable little *Chimie de la Cellule Vivante* (2nd edition, Paris, 1898). After describing his experiments, he concludes:

"Anaerobic life which up till then had been thought to be characteristic only of certain primitive microorganisms, in particular of the schizomycetes, is the essential and fundamental mode in which most protoplasm functions. This view is founded on three orders of proof. First, that the animal organism produces a number of reduced substances, among others the ptomaines and leucomaines, that I had just discovered; second, that in the humours of the economy are found the identical substances that I had shown are formed in the course of bacterial decomposition of the albumens, lactic acid, different fatty acids, amidatid compounds, phenols, carbonic acid and ammonia; third, that the quantity of oxygen found in the totality of our excretions exceeds by about one-fifth the quantity of oxygen taken from the inspired air" (p. 99).

Meanwhile, in 1875, Pflueger had repeated Spallanzani's experiments on frogs and confirmed the fact that when put into a vacuum they continue to give off carbonic acid. A little later the same phenomenon was observed by Pfeffer in plants. Pflueger gave to this process the term "intra-molecular respiration." "So firmly had the conception of combustion been impressed upon physiologists that when this anaerobic respiration came to be explained, it was supposed that certain molecules of organic matter within the cell gave up their oxygen to others that they might thus be burned in the body furnace to yield energy."

The name, however, was but a mask for mystery. Thanks to the work of the physiological botanists and

especially to E. Godlewski, to W. Palladin and their respective schools, to Kostytschew, Stoklasa, Mazé and others, the matter has now been cleared up. In the absence of oxygen, the respiration of plants we may surmise, as that of animals also, for the most part is a process strictly comparable with alcoholic fermentation. In order to exclude the intervention of any mystic "vital agencies" Palladin conducted his experiments upon plants killed by freezing. But latterly Kostytschew has been able to show a case of anaerobic respiration in the living plant in which there is no simultaneous production of alcohol. If *Agaricus campestris* be cultivated under aseptic and anaerobic conditions, no alcohol is formed; moreover an increased supply of sugar brought no increase in the production of carbon dioxide.

An endeavor to identify respiration as essentially a process of hydroxylation has been made by A. P. Matthews and by Barnes. In his paper on "The Nature of Protoplasmic Respiration and Growth," Matthews notes, among others, the following facts which must be explained by any adequate theory:

1. "Even though surrounded by oxygen all protoplasm maintains itself while alive in a reduced state and acts as an intense reducing agent. Its reducing powers are comparable with those of nascent hydrogen.

2. "For all forms of protoplasm oxygen above a certain tension is an intense poison. For some forms this tension may be no more than a very small fraction of an atmosphere; for others it is from three to four atmospheres.

3. "Atmospheric oxygen has little oxidizing power; whereas protoplasm brings about oxidation of the most radical nature and is comparable in its oxidizing powers to the most intense chemical oxidizing agents.

4. "Hydrogen is evolved as a gas by a great variety of bacteria and molds.

5. "Many forms of protoplasm such as the anaerobic bacteria are able to bring about intense oxidations in the absence of atmospheric oxygen."

Matthews adds:

"The studies of Armstrong, Dixon and others on the processes of slow and explosive oxidations and combustions throw, in my opinion, a remarkably clear light on protoplasmic respiration. These authors have shown that in ordinary oxidation the presence of some water is necessary to the oxidation. Phosphorus in a perfectly dry state will not ignite in dry air. Armstrong concludes that the primary oxidation in all these cases is not brought about by the gaseous oxygen but by the water. The atmospheric oxygen acts the part only of a depolarizer to take care of the nascent hydrogen formed from the water.

"The same hypothesis will explain protoplasmic respiration and at once makes clear the identity of anaerobic and aerobic respiration. The following theory of respiration is founded in part on Armstrong's work:

"The real respiration of all forms of protoplasm, both aerobic and anaerobic, is brought about not by the oxygen of the air but by that of the water. The hydrogen set free from the water combines with other elements of the protoplasm, thus keeping it reduced; it also combines with the oxygen of the air if this is present to form water; and in the absence of oxygen it may escape as free hydrogen.

"The only difference between anaerobic and aerobic respiration is that the anaerobic protoplasm is so powerful a reducing agent that it is able to drive hydrogen out of the water, thus oxidizing itself without the aid of atmospheric oxygen to act as a depolarizer."

Primarily then, in Matthews' view, "respiration is the dissociation of water accompanied by the liberation of hydrogen."

Practically the same view is sustained by Barnes from the botanical side. In view of the fact that the word "respiration" now connotes ideas no longer consistent with the facts, Barnes proposed the terms aerobic and anaerobic *energesis*, to which fermentative *energesis* might be added if necessary.

Still further proof that the characteristics of living protoplasm are those of a reducing agent have been brought forward by Loew and Bokorny in their well-known endeavor to establish a chemical difference between the living proteins and "dead proteins." The active or living protein behaves as an aldehyde and displays marked reducing powers. This latter capacity is not, according to these authors, displayed by dead protein.

Finally, as nearly conclusive proof that, in the plant at least, uncombined oxygen has little or nothing to do with respiration, it is to be noted that in plant respiration a direct relationship between the concentration of the oxygen and of the carbonic acid is directly demonstrable. In agreement with the law of mass action, a high concentration (four to eight per cent) markedly lowers the respiration rate.

On the other hand, any influence arising from a variation in the partial pressure in oxygen is extremely difficult to demonstrate. "Even in the higher plants," says Euler, "in which chemical differentiation has been carried to the farthest degree, a widespread indif-

ference is to be found towards such a variation, so that in many species respiration continues without interruption even when the air contains only two per cent of oxygen (according to Stieh) or indeed only one per cent (according to Johannsen). Equally indifferent are most plants towards a notable increase of the oxygen concentration, so that the latter may be increased by at least four or five times the normal amount without producing any effect. And as Bert has shown bacteria may endure simply enormous pressures."

To be continued

Research

ONE of the latest additions to curricula of technical schools is research work. Research may be regarded as the marshal's baton which every student carries in his satchel; it represents the highest ambitions of the student's breast, and it is held to confer more honor than the university degree. To be a research scholar means to the student's mind to be engaged on new and original investigations, and it places him on the same platform as Faraday, Joule, Ohm, Boyle, and the scores of other great people who have discovered new laws and added new definitions to our dictionaries.

Of the educational value of research no one can be in doubt. Properly and honestly conducted, there is probably no higher training for the mind. It leads to great precision of thought and to much experimental dexterity. Moreover, though narrow in itself, it is but the opening to a large field, and in the pursuit of it the area of knowledge opens wider and wider. It calls out, too, all the student's powers of observation, the most useful of all the faculties he acquires in the laboratory. But when we have said all this for technical college research work we have summed up nearly all that can be rightly claimed for it. Too often the student gets a wrong idea of the value of his research work. He forgets that its object is to train him, and that any other value it may have is, in ninety-nine cases out of a hundred, practically nil. Too often it is undertaken with the hope that it may bring honors rather than knowledge. The student imagines that he is doing something great for the world, and starting with an exaggerated opinion of his own importance, nourishes bitterness in his heart against those who estimate the value of his work by the accepted commercial standards. We should be the last to discourage the ardor of youth, but we venture to suggest that it would be better for his own happiness if his professors insisted constantly on the fact that college research work is only a form of training, and that its value, from any other point of view, is generally insignificant. When the student in the art school gives up drawing from the antique and begins to study from the life, he is in a parallel position to the student who leaves the ordinary cut-and-dried laboratory work on what he has trained his 'prentice hand, and takes up research work. But the art student never supposes he is making pictures, and he is careful enough, as a rule, not to let the world see what he does. He recognizes that he is only studying, only making preparation for pictures he may paint some day. The research student does not take this view; he believes that at great sacrifices on his part he is conferring a benefit on the world, and as soon as he has amassed a quantity of figures, he takes—alas too often with the encouragement of his professor—the first opportunity of rushing into print. It is there that he makes the mistake. "In most cases," said Prof. Marchant some time ago, "it is the carrying out of the work that is of the greatest value, and not the result. A great many papers are published not, mainly, in engineering subjects, of very problematical value." Now and then a student does do some really useful work, but as a rule the undeveloped state of the mind at college age and the limitations of the plant at his disposal render work that should be dignified with the name of research out of the question. Indeed, it is a pity that the work should be put to such a use. That we should pair, by applying to them the same name, an inquiry into the nature, cause, and prevention of, say, cancer and the leakage past the regulator valve of locomotive is absurd. All day long and every day engineers, chemists, physicists, etc., are engaged in testing, experimenting, and inquiring, but they look upon it as a bit of the daily routine, and do not exalt it by calling it research. The student replies, "Yes, they are doing it for business, I am doing it in the cause of science. They look for an immediate return; I am content to leave the return to chance." Precisely, the student who measures the resistance of ship-shape models in the college tank calls it research, while the men who are settling the forms of our battleships and merchantmen call it daily routine. But that is only a single example of what is happening in all directions. A youth makes a number of tests of different steels, and is honored with the name of research student, while another on 30 shillings a week is doing the same thing day after day with higher accuracy and equal intelligence, and is known as the works chemist.—*The Engineer*.