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THE LUMINOUS BACTERIA[1]

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Among the light-emitting organisms present on the earth, a form that has been very extensively studied is the luminous bacterium of the sea. These organisms constantly emit a brilliant light, and when observed in the dark growing in "colonies" on some favorable food substance, glow with a luminesence that fully credits the name, the *Photobacterium phosphorescens.*

The form of light-emitting bacterium that has been most used in experimentation is a very small bacillus with rounded ends whose body is about twice as long as its diameter. They are so small that microscopes of the highest magnification are necessary for observation.

These bacteria are present in every ocean, and when some marine animals die the luminous bacteria begin to grow on them in "colonies" of countless millions of individual cells; and, in fact, when many marine fishes are removed from the sea-water and allowed to remain in the open air, the luminous colonies of bacteria will appear on the surface of the scales of the fish and about the head and mouth within a very few hours. In the dark the colonies are observed as glowing patches of bluish-green light. These may be easily isolated in pure cultures and grown on artificial media in the manner of other bacteria. By frequent transfer to fresh nutriment, the bacteriologist may maintain a source of supply for numerous experiments.

Luminous bacteria have been known to infect fresh water animals, though probably quite accidentally through having been injested with particles of food. Certain luminous shrimps in Japan, and a squid found at Naples are luminous due to the growth of these organisms within their bodies. Frogs, when injected with luminous bacteria, may be made to glow, especially about the mouth and on the abdomen where the skin has less pigment to prevent the light from becoming visible. The luminous bacteria will not glow when injected into the living mammal, for there the temperature is much too high for them to live and luminesce.

[1]Read before the Tennessee Academy of Science at its Nashville meeting, November 29, 1929.

Some animals have a symbiotic relation with luminous bacteria. An example of such a condition is found in the case of the small marine fish Photoplepharon, which has a modified organ for the growth of luminous bacteria peculiar to the fish itself. This organ is fitted with an opaque membrane similar to an eyelid, which may be raised or lowered, allowing the glow of the bacteria to illuminate the surrounding sea-water or shutting off the glow as one might pull down a curtain before a lighted lamp.

Luminous bacteria may not be stimulated to a greater brightness of glow excepting when they are shaken with air after being in the absence of oxygen so long as to cause their luminescence to disappear. It may be simply demonstrated that luminous bacteria are absolutely dependent on oxygen for their luminescence. Make a suspension of a culture of bacteria brushed from the surface of a nutrient medium into sea-water. Such an emulsion will glow brilliantly in the dark. Allow it to stand undisturbed for a few minutes in a tall test-tube and soon the living bacteria will use up all the oxygen in the suspension before a sufficient amount can diffuse down into the liquid from the air, and the entire container will become dark excepting for a glowing ring at the surface of the suspension, where the bacteria are in contact with the air containing sufficient oxygen for luminescence. However, if this tube is given a single shake to admit air to the whole suspension the glow will immediately return uniformly throughout the whole of the liquid.

It has been shown that luminous bacteria will glow even when extremely small amounts of oxygen are present. Consequently, they may serve as very delicate indicators of the presence of oxygen. Luminous bacteria suspended in sea-water and under the surface of several inches of paraffin oil will glow at the surface of contact between the emulsion of bacteria and the oil, indicating that there is actually enough air diffusing down through the oil and along the side of the tube to permit luminescence of the bacteria. We know that when under such circumstances no glow appears at the surface of the bacterial suspension, there is very little oxygen present indeed.

Most other luminous organisms with which we are familiar, the fireflies, worms living in the sea, beetles, et cetera, glow only intermittently or else when they are disturbed. But like the luminous fungi that one sometimes finds on forest trees and rotten logs, the Photobacterium glows constantly so long as enough oxygen is present to permit luminescence. We must remember, however, that luminous bacteria are so widely distributed in the open ocean that we cannot see their glow there until they are aggregated in millions on some favorable food substance. The phosphorescence of the sea is due to other animals. Take a canoe paddle or a net and brush through the sea-water at night. One can probably trace the path of the net through the water by the train of fiery light behind it. This train of light quickly disappears. It is due to tiny protozoa, worms, and jelly-fishes quite different from the luminous bacteria.

If injurious substances are added to sea-water containing luminous bacteria, the light will dim as more and more bacteria are destroyed in the suspension, thus serving as an index to the toxic qualities of a substance and indicating the relative time for the effects to occur. Sometimes these cells may be subjected to anaesthesia, their glow may be greatly reduced and may recover on the removal of the narcotizing substance. Potassium cyanide will reduce the luminescence and slow the respiration. Carbon dioxide added to the sea-water combines with it and acts as a narcotic to dim the glow of the bacteria suspended within.

Because luminous bacteria are such delicate indicators for oxygen, they may be utilized to visibly demonstrate oxygen production during the process of photosynthesis by the green chlorophyll of plants when in the presence of light. Beijerinck in 1902 placed an emulsion of clover leaves in sea-water containing luminous bacteria. When in the dark no photosynthetic action and production of oxygen occurred, the bacteria soon used up all the available oxygen contained in the suspension and the material began to dim. When the suspension had become quite dark a beam of light was thrown on the tube and quickly shut off. It was then found that the bacteria had begun to glow again, indicating that the plant substance had been stimulated to photosynthetic production of oxygen by the brief exposure to the light, while no oxygen was produced by the chlorophyll in the absence of the light.

It has been found through the agency of luminous bacteria as indicators, that clover leaves may retain this power of photosynthesis after three months of drying, but will recover to no appreciable degree after five months in a dessicator. Frozen leaves will produce oxygen when warmed and immediately tested, but they soon lose this ability. Numerous experiments have been performed since the time of Beijerinck by various investigators using luminous bacteria as delicate indicators of the presence of oxygen. Molisch in Germany and Harvey in this country have contributed to these studies. The fact that luminous bacteria are so sensitive to the presence of oxygen greatly increases their importance as instruments of investigation. The amount of oxygen present in a solution when the luminous bacteria just begin to dim has been already estimated as well as the amount present when visible luminescence disappears. Quite definite knowledge can be obtained as to the amount of oxygen present in a dimming suspension of the organisms. The curve given in the figure indicates the point of lessened oxygen concentration at which dimming occurs and also the point at which the partial pressure of the oxygen in the atmosphere in contact with the bacterial cell has become sufficient to allow complete respiration of the cell. Luminous bacteria are most favorably adapted to such experiments as these.

Luminous bacteria are quite easily killed by slightly increasing temperatures. They require alkaline media for favorable growth and must never be placed in the incubator, for temperatures above twenty-eight degrees Centigrade will produce rapid degeneration of the culture and a disappearance of the luminosity.

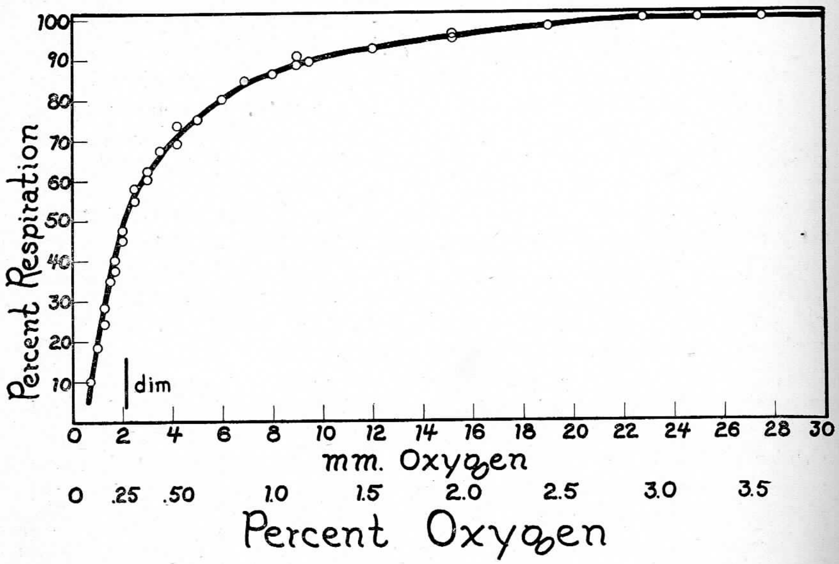

Fig. 1.—Respiration of Luminous Bacteria at Various Partial Pressures of Oxygen.

One can only speculate on the purpose of this luminous characteristic in such an organism as a bacterium. We are chiefly interested in the chemical nature of the processes of growth and luminescence of the cells. Nevertheless, as an object of nature study and as an example of the diversity of living matter, the luminous bacteria indicate to us that although we may regard them as simple cells on the very borderland of life, they are really quite complex and present a challenge to the biologist who desires to inquire into the chemical fundamentals of living material.