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The production of antibiotics by plankton algae and its effect upon bacterial activities in the sea *

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Summary—Laboratory experiments with *Chlorella* and plankton diatoms show that small concentrations of the algae produce antibiotics which highly decrease the bacterial activities, including respiration. If the concentration of organic matter is about the same as in natural sea water, the decrease in bacterial respiration is of a much higher order of magnitude than the rate of photosynthesis of the algae.

The normal light and dark bottle oxygen experiments cannot be used for measuring organic productivity in oligotrophic water. Intense bacterial activities, which occur in the dark bottles as soon as natural sea water is enclosed in the bottles, are much reduced in the light bottles: thus there is an important difference in oxygen consumption between the two kinds of bottles, a difference which has nothing to do with photosynthesis. The disagreement for tropical oceanic water between the oxygen technique and the C-14 technique is thus easy to explain.

It is suggested that the average age of the dissolved organic matter found in sea water is some thousand years. This organic matter is most likely the most important food source—although indirectly—for the bottom animals living at very great depths.

INTRODUCTION

In productive coastal areas the so called "light and dark bottle" oxygen experiments originally introduced by Gaarder and Gran (1927) have been of great value for estimating organic productivity by the phytoplankton. Riley (1939), however, published results of such experiments lasting 3 days from the Sargasso Sea, which according to all evidence is one of the regions in the oceans most scarce in organic life. According to Riley's measurements, this sea would be one of the most productive sea areas of the world—if not the most productive.

Steemann Nielsen (1954), by using the carbon-14 method, could not verify the high rate of organic production in the Sargasso Sea. On the contrary, this sea was found to be about the poorest area in all oceans regarding organic productions by the plankton algae. This result, on the other hand, is in absolute agreement with the general hydrographic conditions (minimum supply of nutrient salts), the low standing crop of phyto- and zooplankton found, and the scarcity of fish. It could further be shown that, theoretically, an organic production of the size claimed by Riley must be considered impossible in the clear water of the Sargasso Sea, where most light by far is absorbed by the water itself. The energy absorbed by the plankton algae is only sufficient for a small photosynthetic rate.

It could, however, be verified that the light and dark bottle oxygen experiments give results comparable with those obtained by Riley.

It is a well-known fact (cf. the next section) that the bacterial activities in sea water are highly intensified if the water is enclosed in glass bottles. The respiration rates

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found by using light and dark bottle experiments of long duration in tropical oceanic water must be considered almost entirely due to the increased bacterial activities. They do not tell anything about the rate of respiration under natural conditions in the sea. The smaller oxygen consumption in the light bottles as compared with the dark bottles (no oxygen production is ever observed) might of course be due to photosynthesis. A reduction of the bacterial respiration directly or indirectly due to light can, however, as well explain the fact. Originally (Steemann Nielsen, 1952) the idea of a direct bactericidal effect of sunlight was suggested. In the 1954 article it was, however, suggested instead that antibiotics produced by the plankton algae in light reduce the bacterial activity. It was shown first by Pratt *et al.* (1944) that antibiotics are produced by plankton algae.

Ryther (1954) suggests that the disagreement between the light and dark bottle technique and the 14C technique in tropical oceanic water is possibly due to (1) the supposition that plankton algae nearly exclusively respire the newly formed products of their photosynthesis and (2) the supposition that the rate of algae respiration is about equal to the photosynthetic rate in these waters.

Experiments presented by Ryther in order to give his suggestions an experimental background have now been repeated in this laboratory (Steemann Nielsen and Al Kholy, in press). No corroboration of his results could, however, be obtained. These seem obviously to be the result of an unsatisfactory experimental technique. It is further shown that his suggestions would lead to some fantastic and quite unrealistic assumptions of what is going on in the oceans.

Ryther's suggestion that the algae nearly exclusively respire the newly formed products of their photosynthesis seems to be contradicted by the work of the Berkeley Group. According to Calvin and Massini (1952), algae in light respire, to a slight degree only, newly formed products. There is, however, as will be shown in a subsequent paper, really a connection between photosynthesis and respiration, although not of the extent supposed by Ryther. It has some influence on the measurement of the organic production by the 14C technique. Under very unfortunate conditions in nature the results from using this technique may possibly be up to about 15% too low. Normally, however, the influence is negligible.

DISSOLVED ORGANIC MATTER IN SEA WATER AND ITS DECOMPOSITION

One of the most amazing statements about the sea is that by far the largest amount of the organic matter existing anywhere on this globe is found dissolved in the oceans. According to Keys, Christensen and Krogh (1935) the dissolved organic matter in water from all depths of the oceans corresponds to about 1·2–2·0 mg C and 0·2 mg N per litre. About 15 kg organic matter is thus found below every m^2 ocean surface. In comparison, the average annual net production per m^2 by the phytoplankton is only about 1% of this (Steemann Nielsen, 1952).

Most of the organic matter produced by the plankton algae is presumably used rather soon for respiration in some organism or other. The magnitude of animal life in the sea could otherwise scarcely be explained. The organic matter dissolved in ocean water must therefore on an average be rather old, presumably several thousand years.

The nature of the organic matter in question has not yet been investigated. If the water is *in situ*, an extremely slow decomposition of the organic matter takes place. About 25–50% is, however, readily decomposed by bacteria, if the water is stored in glass bottles (Keys, Christensen and Krogh, 1935; Waksman and Carey, 1935).

It has been shown by ZoBell and Anderson (1936) that the presence of solid surfaces is the factor which makes the bacterial decomposition of dissolved organic matter in ocean water possible. Small bottles in which the ratio—inner surface of

the bottle : volume of the water—is high, give rise to a faster bacterial decomposition than bigger bottles, in which the ratio is lower. The addition of glass beads in the same way increases the bacterial activity. According to ZoBell (1946) the influence of the surfaces may be explained (1) through accumulation of the organic matter due to surface activities, (2) by assuming that exoenzymes necessary for attacking the organic matter can only be maintained in sufficient concentration if solid surfaces are present.

In natural sea water, solid surfaces are of course found. The phytoplankton, zooplankton, and particulate dead material all have solid surfaces. In oceanic surface water these surfaces are, however, scarcely more than about 10 mm^2 per litre. In water from deeper layers they are much smaller. The inner surface of a one-litre bottle is about 10^5 as large.

The surfaces of plankton algae seem normally to be rather unsuitable for bacterial growth. If plankton algae are growing well, very few bacteria—if any—can be observed to be attached to their surfaces. If the surfaces of the algae are densely covered with bacteria, this indicates that they are not in a healthy state. The whole procedure of growing algae in a pilot plant, where it is impossible to keep the culture sterile, is based upon the fact that the culture itself is able to keep the bacterial activities at a minimum. This has been known for a long time. Thus Waksman *et al.* (1937) state that " The result . . . confirmed the previous observation that, in the presence of a living culture of *Nitzschia* (a plankton algae), the bacterial activities were very limited."

The statement above does not contradict the fact, often observed, that bacteria mostly are more abundant where a high phytoplankton population is found (e.g. Gran, 1933). The heterotrophic micro-organisms are first of all numerous at the end of a phytoplankton bloom. Beside bacteria, heterotrophic flagellates are also found. Due to the bloom of autotrophic organisms, relatively large quantities of easily accessible organic matter first of all originating from dying and half-digested cells, are contributed to the water. Solid surfaces apparently are not necessary for the bacteria attacking this easily accessible organic matter (no exoenzymes necessary?). They can thus also be in a real planktonic state.

It can be stated that the dissolved organic matter in sea water consists of the following components:

> (1) matter very easily accessible for bacteria, (2) matter only accessible for bacteria if solid surfaces are present, (3) matter which apparently cannot be decomposed by bacteria at all. In oligotrophic oceanic water, practically only the two last-mentioned components are found.

If the hypothesis about the importance of solid surfaces for the bacteria to attack the normal stock of " old " organic matter is correct, a dense bacterial flora should always be found on the surface of plankton algae. As such a flora normally is not found, the algae must be able to prevent the development.

Pratt *et al.* (1944) showed that the plankton alga *Chlorella* produces antibiotics with a high effect on bacterial growth. The normal absence of bacteria in any amount from the surfaces of healthy plankton algae of all kinds indicates that all these species produce antibiotics. The ability to avoid bacterial settlement to any higher degree by producing antibiotics may be considered to be a rather important condition, the lack of which would make these species fairly unsuitable for aquatic life.

As mentioned above, PRATT *et al.* (1944) showed that the antibiotics are given off by the alga to the water. In a sample of natural sea water containing phytoplankton, it must therefore be expected that the antibiotics produced are not only effective for the prevention of bacterial growth on the surface of the algae themselves, but also on other surfaces, thus on the inner walls of the experimental bottles.

EXPERIMENTS

Photosynthesis may be written $CO_2 + H_2O \rightarrow (H_2CO) + O_2$; respiration may be written $(H_2CO) + O_2 \rightarrow CO_2 + H_2O$. Respiration by the plankton algae, and by all other organisms present, is going on both in the light and the dark, photosynthesis by the plankton algae is going on only in the light.

When making experiments in which the amount of dissolved oxygen in the water is measured before the experiment, and in light and dark bottles at the end of the experiment, the difference in the O_2 content between the initial bottles and the dark bottles represents the rate of respiration by all organisms. The rate of photosynthesis should be measured by the O_2-difference in the light and in the dark bottles. A necessary condition is, however, that light neither directly nor indirectly decreases the rate of respiration, a condition which is not fulfilled.

Experiments were made in which bacteria were growing together with very few algae (the freshwater green alga, *Chlorella pyrenoidosa* or the marine diatom, *Thalassiosira nana*). The rate of photosynthesis per number of cells was first measured in a special experiment using a sufficient concentration of the algae. The method was the same as used in the main experiment. There the concentration of the algae was made so low that it would have been impossible to measure the rate of photosynthesis by the O_2-production according to the normal Winkler technique. By counting the number of algae cells at the start and at the end it was possible to determine growth during the experiments, and thus the total rate of photosynthesis.

In the experiments with the marine diatom, sea water from the Sound enriched with some nutrient salts was employed. In the *Chlorella* experiments the culture solution D (ÖSTERLIND, 1949) was used in a slightly modified form. In both cases 7–8 mg glucose and 2–4 mg peptone was added per l. The light intensity was 8,000 lux, the temperature 22° C. In every experiment 3 bottles of each kind was employed. Two Winkler titrations were made with water from each bottle.

In order to get an incubation by bacteria, the culture solution used for the main experiments was incubated with 0·5 ml/litre of freshly collected surface water either from a lake near Woods Hole (*Chlorella* experiments) or from the Sound (the marine diatom). The water was filtered through a coarse paper filter before use.

In Table 1 is given a typical experiment.

Table I. Chlorella pyrenoidosa

Rate of photosynthesis (real assimilation) in preliminary experiment 1·46 ± 0·01 ml O_2/l per hour.
Dilution of the algae used in the main experiment 1 : 10·000.
Duration of main experiment 72 hours—3 light periods (44 hours together) alternating with 3 dark periods.
Rate of photosynthesis (real assimilation) by *Chlorella* in light bottles (corrected for growth) per 72 hours 0·04 ml O_2/l.
O_2-consumption in dark bottles per 72 hours 3·59 ± 0·02 ml. O_2/l.
O_2-consumption in light bottles per 72 hours 2·92 ± 0·03 ml O_2/l.
Reduction in O_2-consumption in light bottles as compared with dark bottles 0·67 ± 0·04 ml O_2/l.

It is thus evident that the difference between the light and the dark bottles is not due to *Chlorella* photosynthesis. In a corresponding series made simultaneously but without *Chlorella* added the O_2-consumption was the same in the light and in the dark bottles. The difference was 0·03 ± 0·08. This indicates that no photosynthesizing cells in any important number were introduced together with the bacteria. It further shows that light has no direct effect on the activity of the bacteria developing in the bottles. This is in accordance with the results published by VACCARO and RYTHER (1954).

There is no reason to give any details about the other similar experiments made, using either *Chlorella* or the marine diatom. The results are similar. The difference in oxygen consumption between light and dark bottles was between 12 and 30 times higher than the oxygen production due to the photosynthesis of the algae.

It turned out to be impossible in these experiments to use unsterilized, stored sea water. Products produced by the special bacterial flora developing made the use of Winkler titrations rather impossible. The water was therefore collected immediately before an experiment.

Experiments were made to investigate the influence of an addition of a substratum from which a rather dense, vigorously growing *Chlorella* population had been filtered off. Although a pronounced influence was found, the direct influence of a dilute *Chlorella* population was pronouncedly higher. By adding 4% of such a substratum at the start of an experiment lasting 2 days, only a decrease in O_2-consumption of 0·09 ml/l was found. In the corresponding experiment the influence of a very dilute *Chlorella* population in the light bottles was 5 times as high. This is, however, in perfect agreement with the statements by PRATT (1948) according to which the concentration of the antobiotics produced by algae seems to be higher at a low concentration of growing *Chlorella* than at a higher concentration.

The experiments presented in this article show that the presence of algae, presumably due to the production of antibiotics, effects a reduction in the O_2-consumption of the bacteria in the light bottles. This reduction was of a higher order of magnitude than the O_2-production due to the photosynthesis of the algae. There is no reason to believe that the plankton algae living in oligotrophic oceanic water should not behave in just the same way. Thus it is not possible to use the light and dark bottle oxygen technique here. In eutrophic areas the technique is fully applicable.

In the culture medium used in the present experiments the organic matter present was easily accessible for the decomposing bacteria. It must be assumed that a number of different species took part in this decomposition. In oceanic water the dissolved organic matter presumably is only accessible for some few bacterium species able to excrete the necessary exoenzymes. It is therefore rather probable that the effect of the antibiotics produced can be still higher here. In the experiment shown in Table 1, oxygen consumption was reduced by 19% in the light bottles. In light and dark bottle experiments in oligotrophic oceanic water the reduction is mostly more than 50%.

DISCUSSION

By looking at a series of light and dark bottle experiments made in the open ocean, it is evident that the rate of respiration in the dark bottles varies from one station to another. Thus the values published by RILEY (1939) for 3 days' experiments with

surface water from the Sargasso Sea varies from 0·12 to 0·51 ml O_2 consumed per l. There is no reason to believe that the content of organic matter in the surface water of this rather uniform sea should vary much from place to place. It is more likely that the amount of antibiotics present in the water at the time it is collected varies somewhat from place to place. The time of the day for the collection may be rather important, too. Near sunset the concentration of antibiotics is possibly higher than near sunrise.

It is at present impossible to state if non-autotrophic organisms independent of light secrete antibiotics. This is by no means unbelievable. Some observations on the *Galathea* expedition support this possibility. A verification is, however, needed.

KROGH (1934)—as he explained it himself—offered a vague suggestion that the animal life at great depths in the oceans depends on bacteria. At that time he knew the concentration of soluble organic matter in ocean water. He knew, too, that this organic matter normally was not decomposed by bacteria. He did not, however, know that a material part of this organic matter is easily decomposed if solid surfaces are present. At the sea bottom such solid surfaces are present. A rather considerable decomposition of this organic matter is apparently going on here constantly. Most of the " old " organic matter diluted in sea water is most likely ultimately decomposed at the sea bottom. The bacteria living on this matter present most likely a very important, although not the only food source for the bottom animals at very great depths.

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