COMPARATIVE STUDIES ON RESPIRATION.

XXIV. THE EFFECTS OF CHLOROFORM ON THE RESPIRATION OF DEAD AND OF LIVING TISSUE.

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(Received for publication, January 22, 1923.)

One of the most interesting problems connected with the study of respiration is the action of anesthetics on the oxidations of the living cell. In order to throw some light on this the writer has made experiments on living and dead tissue.

The organism chosen was the green marine alga, Ulva lactuca, var. latissima (sea lettuce). For a number of reasons this plant is unusually desirable. It is readily obtainable at various seasons. It consists of but two layers of cells, and in consequence the gaseous exchange is rapid, which makes it favorable for experiments on respiration. There is, however, one drawback which is the result of its habitat. The plant grows in rather stagnant water and is very much contaminated with bacteria. This difficulty was in part obviated by washing the plant in running sea water for 24 hours before use. This, of course, did not remove all of the bacteria but helped to rid the plant of most of them.

Since sea water contains buffers that tend to interfere with the results of experiments by the indicator method, van't Hoff's solution was used in place of sea water.

The first experiments were on the effect of chloroform on living Ulva. A portion of a frond was placed in the reaction chamber of the apparatus (1), together with 100 cc. of van't Hoff's solution; a current of air free from CO₂ was passed through for 1 hour, and a series of readings was made to determine the normal rate. Chloroform was then added and readings were made as often as possible to determine the changes in the rate of production of CO₂.
The results of this experiment are shown in Fig. 1. As may be seen from the chart, 0.5 per cent chloroform (by volume) causes a continuous fall in the rate of production of CO₂. At the end of

Fig. 1. Effect of chloroform 0.25 per cent (Curve A) and 0.5 per cent by volume (Curve B) on the production of CO₂ by Ulva. The normal rate is taken as 100 per cent (this is the reciprocal of the time required to make the standard change in the indicator, which varied from 2.25 to 2.75 minutes). Average of 3 experiments: probable error of the mean is less than 5 per cent of the mean.
one and one half hours the rate has dropped to 25 per cent of the normal rate. The action of 0.25 per cent is quite different. This concentration causes an increase in the rate which is followed by a decrease. These results are quite in agreement with those found by Haas (2) working with *Laminaria*.

In order to gain some insight into the mechanism of respiration, experiments were made to ascertain whether oxidation could be accelerated by the addition of substances which from a theoretical standpoint would be expected to act like the normal components of the oxidative system of the cell.

On the assumption that the first step is the formation of a peroxide in the cell, it would seem possible to influence the rate of respiration by $\text{H}_2\text{O}_2$. Accordingly, *Ulva* was placed in 100 cc. of van't Hoff's solution containing 1.0 per cent $\text{H}_2\text{O}_2$.

The result is shown in section AB of the curve given in Fig. 2. It might be expected that the $\text{H}_2\text{O}_2$ could increase the production of CO$_2$ by supplying available oxygen (3), or decrease the rate by the destruction of enzymes or other substances necessary for respiration (4). In this case the latter effect seems to predominate.

If this is the case the addition of some substance that will act like the oxidizing enzymes of the cell ought to cause *Ulva* that has been treated with $\text{H}_2\text{O}_2$ to produce CO$_2$ at an increased rate. Iron has long been known to show a remarkable resemblance to the oxidizing enzymes of the cell (5), and section BC of the curve in Fig. 2 shows that the addition of 5 cc. of 0.0005 M Fe$_2$(SO$_4$)$_3$ causes the rate to increase again.

Fig. 3 shows the effect of placing *Ulva* in van't Hoff's solution containing 5 cc. 0.0005 M Fe$_2$(SO$_4$)$_3$. There is an immediate increase in the rate of respiration, as would be expected. The subsequent fall may be due to the exhaustion of the immediately available supply of oxidizable material or to the exhaustion of the peroxide in the cell.

In order to make experiments on dead material, *Ulva* was prepared in the following manner. The material was collected, care being taken to select only the best fronds, and placed in the shade until quite dry. It was then taken into the laboratory and dried in an oven at 80°C. It was then stored in paraffined boxes until used.
Material prepared in this way was believed to be dead and when tested with gum guaiac gave no test for oxidizing enzymes, although living material gives a good test with gum guaiac.

For use in the apparatus the material was dialyzed for 12 hours to remove the buffers and then placed in van't Hoff's solution. Tests showed that tissue prepared in this way did not produce CO₂. The
addition of $\text{H}_2\text{O}_2$ also failed to cause a production of $\text{CO}_2$. The addition of $\text{Fe}_2(\text{SO}_4)_3$, however, caused a very distinct reaction. Fig. 4 shows the result of the addition of the iron solution. It may be seen that the rate of production of $\text{CO}_2$ becomes quite rapid. It was found that this rate was constant for about 6 hours and at the end of 24
hours had fallen off about 50 per cent. One might conclude from this that the peroxide and peroxidase are essential to the production of CO₂ by the living tissue.

![Graph](image)

**Fig. 4.** The production of CO₂ by dead *Ulva* in 100 cc. van't Hoff's solution containing 1 per cent H₂O₂ when 5 cc. Fe₂(SO₄)₃ 0.0005 M is added. Average of 5 experiments: probable error of the mean is less than 5 per cent of the mean.

The addition of chloroform to tissue that had been treated in this manner gave results that are very interesting as compared with those of other workers who used the indicator method. It had been found
that in the case of various organisms, just as in Ulva (Fig. 1), certain concentrations cause a decrease in the rate, while others produce a preliminary increase. The following experiment seems to throw light upon why this happens. Some dead Ulva was placed in van't Hoff's solution containing H$_2$O$_2$ and Fe$_2$(SO$_4$)$_3$ and was made free from CO$_2$. After 1 hour the rate of production of CO$_2$ became constant (this is called 100 per cent) and 1 per cent chloroform was then added.

Fig. 5 shows the result of this experiment. Curve A is the effect of 1 per cent chloroform when the mixture consists of 20 gm. of dried Ulva, 75 cc. of van't Hoff's solution, 20 cc. of H$_2$O$_2$ (4 per cent), and 5 cc. of 0.0005 M Fe$_2$(SO$_4$)$_3$; Curve B is the effect when 10 cc. of the iron solution are used; Curve C when 20 cc. are present.

It will be noted that the shape of the curve is dependent on the amount of iron present. The writer has found that the production of CO$_2$ by dead Ulva in the absence of chloroform is directly proportional to the amount of iron present. It is, therefore, probable that we have to do with two consecutive processes, the first causing a rise, the second a fall, and that if the reaction proceeds rapidly the first may be finished before the first reading is taken. It is also possible that the iron inhibits the first reaction. With smaller amounts of iron the rise is observable. Thus with the minimum amount of iron (A) there is a decided rise which is followed by a decrease below 100 per cent; B shows the same sort of an increase but has a greater decrease; while C, with the maximum amount of iron (hence the greatest velocity), shows no rise upon the addition of chloroform, although it is possible that a rise would be observed if an earlier reading could be taken.

It is evident, therefore, that dead tissue in the presence of suitable reagents, when treated with chloroform, can be made to give either an increased production of CO$_2$ (followed by a decrease) or that the rate may be made to decrease from the start. Both of these effects are found in living tissue, the result depending on the concentration of the chloroform and the kind of tissue. The nature of this reaction and its chemical significance will be taken up in a later paper.
Fig. 5. The production of CO$_2$ by dead Uloa in 75 cc. van't Hoff's solution, 20 cc. 4 per cent H$_2$O$_2$, and 5 cc. (Curve A), 10 cc. (Curve B), and 20 cc. (Curve C) 0.0005 M Fe$_2$(SO$_4$)$_3$. In each case 1 per cent chloroform was added; the rate obtained in the mixture before the addition of chloroform is taken as 100 per cent. Average of 3 experiments: probable error of the mean is less than 5 per cent of the mean.
SUMMARY.

1. Chloroform in low concentration (0.25 per cent) causes an increase in the rate of production of CO₂ in Ulva; this is followed by a decrease. In higher concentration (0.5 per cent) only a decrease is observed.

2. Assuming that the normal oxidation depends on the action of peroxide and peroxidase, experiments were made by placing Ulva in 1.0 per cent H₂O₂ and in Fe₅(SO₄)₉ (which acts like a peroxidase). The former diminishes the rate, the latter increases and subsequently decreases it.

3. When Ulva is killed in such a manner as to destroy the oxidizing enzymes, no CO₂ is produced unless H₂O₂ and Fe₅(SO₄)₉ are present. If to this mixture chloroform is added, the effect depends on the concentration of the iron. If the concentration is low there is an increase in the production of CO₂ followed by a decrease. If the concentration is high the rate appears to decrease from the start.

BIBLIOGRAPHY.

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