COMPARATIVE STUDIES ON RESPIRATION

XX. THE CAUSE OF PARTIAL RECOVERY.

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New questions regarding the mechanism of injury and recovery have recently arisen. It has become apparent that, contrary to the accepted view, recovery from injury may be either partial or complete, according to circumstances. This conclusion was drawn from investigations on electrical conductivity by Osterhout and has been confirmed by the experiments on respiration carried out by the writer.

A question of especial interest is whether partial recovery is due to the death of certain cells or to a lowered rate of metabolism of all the cells which make up the tissue. The writer has endeavored to throw light on this question by determining the number of cells that survive after exposure to a toxic agent and by comparing the rate of respiration before and after such exposure.

The material used in these experiments was a unicellular alga, Chlorella. It was isolated from the soil by Dr. Schramm and grown on agar, free from bacteria and other organisms. The agar was prepared in the following manner.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar</td>
<td>10 gm.</td>
</tr>
<tr>
<td>Calcium nitrate</td>
<td>1.026 gm.</td>
</tr>
<tr>
<td>Magnesium sulfate (hydrated)</td>
<td>0.2 gm.</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>0.2 gm.</td>
</tr>
<tr>
<td>Calcium chloride (anhydrous)</td>
<td>0.1 gm.</td>
</tr>
<tr>
<td>Ferrous sulfate</td>
<td>trace</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 cc.</td>
</tr>
</tbody>
</table>

2 Inman, O. L., J. Gen. Physiol., 1920–21, iii, 663.
These salts were dissolved in water; the agar was then added and the whole mixture sterilized in an autoclave.

From agar the alga was transferred to a liquid medium prepared in the same manner with the exception that 10 gm. of dextrose were used in place of 10 gm. of agar.

The cultures used in these experiments were grown for a period of 30 days in this liquid medium. The medium has a very slight alkaline reaction and when tested showed no detectable buffer action.

The algae were transferred to the respiration chamber along with enough of the medium to make a volume of about 20 cc. The normal rate of respiration was determined by taking the time necessary for a change from pH 7.78 to pH 7.36. (This usually took about 2 minutes). When the normal rate of respiration was practically constant the algae were separated from the medium by means of a centrifuge, which was run at a moderate speed. (Tests showed that this centrifugation caused no injury.) The reagent was then added and measurements of the rate of respiration were made at frequent intervals until the desired point below the normal rate of respiration was reached; the algae were then returned to the normal solution and at intervals the rate of respiration was measured.

The experiments were performed at 18 ± 3°C.

Fig. 1 shows the result of exposing the cells to hypertonic balanced solutions. If the respiration does not fall below 60 per cent of the normal, recovery is complete; if it falls lower recovery may be incomplete, while if the rate drops too low there is no recovery.

It was found in these experiments that there is no tendency for recovery to complete itself even when the organism is kept for days under the most favorable conditions.

Fig. 2 shows recovery after exposure to solutions of chloroform. In this, as in the previous case, no attempt was made to follow the respiration curve during the exposure since the interesting question was the amount of injury produced and the subsequent behavior. Curves A, B, and C show that if the injury is stopped when respiration is from 58 to 70 per cent of normal, there is in every case complete recovery within a period of about 4 hours and that the organism

This is the apparatus described by Osterhout. Osterhout, W. J. V., J. Gen. Physiol., 1918–19, i, 17.
is found at the end of 20 hours to be still normal. Where complete recovery was observed the algae were planted upon agar plates and they grew and formed colonies in the normal manner.

Curves $G$ and $F$ show that there is no partial recovery but a continued state of diminished metabolism which lasts over a considerable number of hours as shown by the curves.

**Fig. 1.** Curves showing recovery of *Chlorella* from the effects of a hypertonic balanced solution of NaCl plus CaCl$_2$. Curve $A$ shows the degree of recovery after an exposure of 60 minutes to a solution containing 50 cc. NaCl 1 m to each cc. of CaCl$_2$ 1 m; Curve $D$, after an exposure of 78 minutes to the same solution; Curve $C$, after an exposure of 48 minutes to a solution containing 50 cc. NaCl 1.25 m to each cc. of CaCl$_2$ 1.25 m; Curve $B$, after an exposure of 96 minutes to the last mentioned solution. The first point on each curve denotes the per cent to which the respiration had fallen when the algae were transferred from the solution to the normal medium. The ordinates represent the rate of production of CO$_2$ expressed as per cent of the normal; the abscissae, time in hours. Each curve represents a typical experiment.

Curves $D$ and $E$ show that when the respiration has fallen to about 38 per cent of the normal, as the result of the exposure to chloroform, there is no recovery after removal from the chloroform, but, on the contrary, a gradual decrease in the rate.

Experiments were next undertaken to decide whether partial recovery meant that some of the cells had been killed and others had remained uninjured, or whether all the cells remained alive but had
a lowered rate of metabolism because of the treatment. In order to throw light upon this point it was decided to resort to staining methods. Methylene blue was found to be convenient since it would quickly stain the algae that had been killed by the chloroform or the hypertonic salt solutions, but did not stain the living cells during a short exposure to the dye. In the normal culture some of the cells (from 5 to 8 per cent) were stained.

Fig. 2. Curves showing the rate of respiration of *Chlorella* after treatment with chloroform. Curves A, B, and C were obtained by using 0.225 per cent chloroform (by volume); Curves G and F with 0.27 per cent; Curve B, with 0.36 per cent. The first point on each curve denotes the per cent to which the respiration had fallen when the algae were transferred from the solution of chloroform to the normal medium. The ordinates denote the rate of production of CO₂ expressed as per cent of the normal; the abscissae, time in hours. Each curve represents a typical experiment.

Cultures of the same age were selected and the normal respiration was measured in the usual manner. Each culture was then treated with chloroform of sufficient strength to decrease the respiration of the algae. The algae were then subjected to the stain (for 3 minutes) and it was found that not more than 8 per cent of the cells were ever stained, no matter whether the respiration had fallen to 70 per cent or to 10 per cent of the normal rate. The time of exposure of the

* The cells were counted by means of a hemocytometer.
algae to the chloroform was never more than 96 minutes. After an excessively long exposure to the chloroform (10 to 15 hours) about 95 per cent of the cells stained; in this case there is little doubt that most of the cells were killed, but such long exposures were never used in the ordinary experiments.

Staining was also tried after treatment with hypertonic salt solutions. Here partial recovery was found and yet not more than 5 to 8 per cent of the cells stained. In these experiments the longest time of exposure to the salt solution was about an hour. If the cells were treated for from 5 to 8 days with hypertonic NaCl about 95 per cent stained.

It would therefore appear that a treatment with hypertonic salt solutions which lowers the rate of respiration so much that recovery is incomplete kills few or none of the cells. If this be true then it cannot be said that partial recovery is due to the fact that some of the cells are killed by the toxic agents while others recover completely. The fact that the recovery is not complete must be due to the diminished metabolism of practically all the cells.

SUMMARY.

The respiration of Chlorella is diminished by exposure to hypertonic salt solutions. After a short exposure there is complete recovery when the algae are removed to the normal medium. After a longer exposure recovery may be incomplete, as shown by the fact that the rate of respiration fails to rise to the normal level. Staining with methylene blue indicates that few, if any, of the cells are killed as the result of the exposure. It would therefore seem that the treatment produces a persistent lowering of the rate of metabolism. Such a condition of metabolism is also found after exposure to chloroform.