

COMPARATIVE STUDIES ON RESPIRATION.

VI. INCREASED PRODUCTION OF CARBON DIOXIDE ACCOMPANIED BY DECREASE OF ACIDITY.

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(Received for publication, January 9, 1919.)

In a previous paper¹ I have shown that high concentrations of ether greatly increase the production of CO₂ by animals and that this is associated with irreversible changes ending in death. It might be thought that this increase could be explained on the ground that the process of death is often accompanied by the production of lactic acid, and this might displace CO₂ from carbonates and bicarbonates present in the tissues. The resulting evolution of CO₂ might be mistaken for increased respiration.

In order to obtain some light on this question it seemed desirable to experiment upon an organism in which death is not accompanied by an increase in acidity. Suitable material for such experiments is furnished by the petals of many flowers containing natural indicators, whose colors show that during the process of death the cell contents become less acid.²

Two species of *Salvia* were used in these experiments, *Salvia involucrata* and *Salvia splendens*. The natural indicators contained in the petals were calibrated by placing the petals in boiling water and then transferring them to buffer solutions of known pH value. They remained in the buffer solutions until complete penetration had taken place (this required only a few minutes). As the color of the indicator in various buffer solutions was known, it was a simple matter to determine the changes in acidity³ which take place in flowers immersed

¹ Irwin, M., *J. Gen. Physiol.*, 1918-19, i, 209.

² Haas, A. R., *J. Biol. Chem.*, 1916, xxvii, 233.

³ The petals contain some cells which lack the indicator; their acidity was consequently not determined.

in a 7.3 per cent (by volume) solution of ether. These changes are shown in Table I.

The CO₂ output of *Salvia involucrata* was measured by the methods used in the previous investigation. The petals⁴ were immersed in tap water and the normal respiration was determined. The petals were then immersed in a solution of 7.3 per cent ether (by volume) and new determinations were made. The results are shown in Fig. 1.

Objection might be made to submerging the petals in liquid on the ground that this condition is abnormal and that gas might be given off more rapidly through the stomata when ether is present (as the result of changes in surface tension). For this reason it seemed de-

TABLE I.

Changes in the pH Value of Salvia Petals during the Process of Death Caused by Immersion in Solution of 7.3 Per Cent Ether (by Volume).

Length of exposure to ether.	pH value.	
	<i>Salvia involucrata.</i>	<i>Salvia splendens.</i>
<i>min.</i>		
0	4.5	3
3	5.0	
5	6.0	
6.5		5
7	7 to 8	
10		7 to 8

sirable to repeat the experiments by the method described by Osterhout.⁵ As flowers of *Salvia involucrata* were no longer available *Salvia splendens* was used. The corollas were placed in a glass tube, the normal respiration was measured, and sufficient ether was then introduced into the apparatus to saturate the air with ether vapor at 20°C.⁶ The rate of respiration was then determined at frequent intervals. In respect to the per cent of increase produced by ether the results agreed closely with those shown in Fig. 1.

⁴ In all the experiments the entire corolla was used, care being taken to detach it with as little injury as possible.

⁵ Osterhout, W. J. V., *J. Gen. Physiol.*, 1918-19, i, 17.

⁶ The petals were at no time in contact with liquid.

Experiments were made to determine whether exposure to ether causes an increase in the consumption of oxygen. For this purpose the apparatus shown in Fig. 2 was employed. The petals were placed in a glass tube A, which was connected at one end (by tubes of glass

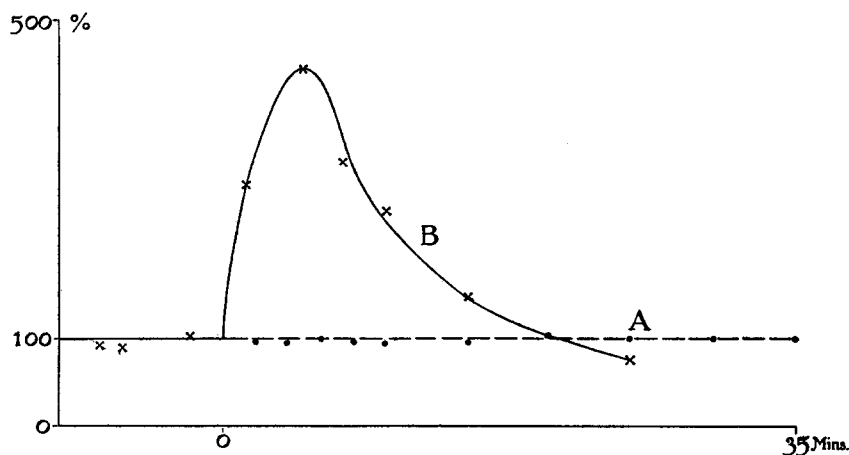


FIG. 1. Curves showing the respiration of *Salvia* petals first in tap water (horizontal line) and then in 7.3 per cent ether. Time is reckoned from the beginning of exposure to ether. The respiration of a control in tap water is shown by the broken line. The normal rate (which is taken as 100 per cent) corresponds to a change in pH value from 8.0 to 7.7 in 193 seconds. Temperature, $20^{\circ} \pm 1^{\circ}\text{C}$. Probable error less than 1.7 per cent of the mean. Curve A, average of six experiments; Curve B, average of four experiments.

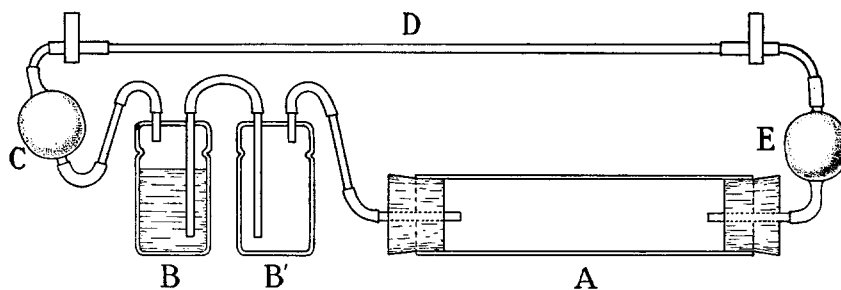


FIG. 2. Apparatus for the determination of oxygen consumption. Petals were placed in A; by alternate compression of the bulbs C and E the air in the apparatus was made to circulate. At the close of each experiment the tube D was removed and its contents were analyzed for oxygen.

and rubber)⁷ to a bottle B, and a safety bottle B'. B contained strong H₂SO₄ and was connected to the rubber syringe bulb C. A was connected at the other end to the rubber syringe bulb E and to the long narrow glass tube D. When the bulbs C and E⁸ were compressed, the air passed through A, B', and B into C, and thence through D and E back to A.

Petals were placed in A and by alternately compressing the bulbs C and E a constant circulation of air was maintained for 10 minutes, after which the tube D was clamped off at both ends and the contained gas was analyzed for oxygen.⁹ After making determinations of the normal oxygen consumption in this manner the petals were removed and exposed for 3 minutes to air saturated with ether vapor at 18° ± 1°C. They were then placed in A and a new determination was made. The H₂SO₄ removed the ether so that it could not interfere with the subsequent analysis.

Since the concentration of ether¹⁰ was less than in the experiments previously described, the oxygen consumption could not be expected to equal the production of CO₂ in these experiments. Nevertheless the average of four experiments showed that ether increased the consumption of oxygen to 2.3 times the normal. The CO₂ production during the same time (in a saturated solution of ether), as measured by the methods previously discussed, amounted to three times the normal.

It is therefore evident that in this instance a high concentration of ether causes an increase in the consumption of oxygen as well as in the production of CO₂ and that this is accompanied by a decrease in the acidity of the cells. It is therefore possible that in other cases where the acidity of the cell cannot be directly measured, the evolution of CO₂ observed under the influence of ether is not wholly due to the displacement of CO₂ from carbonates and bicarbonates stored in the tissues.

⁷ All connections were made with as little rubber tubing as possible, and care was taken to make all joints air-tight.

⁸ These are provided with valves.

⁹ The method employed was that described by Osterhout, W. J. V., *Am. J. Bot.*, 1918, v, 105.

¹⁰ The concentration of ether constantly diminished during the 10 minutes.

SUMMARY.

In petals of *Salvia* high concentrations of ether cause an increase in oxygen consumption and in the production of CO₂, while at the same time a decrease occurs in the acidity of the cell contents.