

## MEASUREMENTS OF THE METABOLISM OF TWO PROTOZOANS

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Dr. J. A. Dawson of the Department of Zoology, Harvard University, has been kind enough to place at the writer's disposal relatively large quantities of two protozoans ("*Amoeba proteus*" according to Schaeffer, 1916; and *Blepharisma undulans*) in sufficiently pure condition to make possible some experiments on their metabolism. The results of these experiments, together with some observations on the red pigment of *Blepharisma* are recorded here. Dr. Dawson describes in a separate paper his methods of culturing the organisms.

### I

#### *Experiments on Amoeba proteus*

The amebae obtained were always accompanied by large numbers of *Chilomonas*. In order to be sure that any metabolism found should refer to *Amoeba proteus* alone, the *Chilomonas* cells, which are much smaller in size, were washed out on a 200-mesh phosphor-bronze sieve, which retained all amebae. After two washings, a microscopical examination of the amebae showed them to be practically free of *Chilomonas*. The former were then concentrated by allowing them to settle in shallow dishes, and decanting. They were washed once or twice on the centrifuge to remove bacteria, and a suspension of them was made up and pipetted into the vessels of Barcroft-Warburg manometers.

Various suspending solutions were tried, similar in composition to Ringer, but much more dilute. None proved successful, however, and metabolism was demonstrable only in distilled water. The water used was commercial distilled water redistilled from a Pyrex still.

Table I gives complete data for one experiment. The temperature was 20°C., and a mixture of 5 per cent CO<sub>2</sub> in air was used. The manometer vessels were the rectangular type, illustrated in a paper by

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TABLE I  
*Respiration of Amoeba proteus*

Vessel 6	Vessel 3
$V_F = 7$ cc., 14 mm. <sup>3</sup> cells	$v_f = 3$ cc., 6 mm. <sup>3</sup> cells
$K_{O_2} = 0.56$	$k_{O_2} = 0.96$
$K_{CO_2} = 1.15$	$k_{CO_2} = 1.22$
$\Delta H = 09.9$ mm. in 130 minutes	$\Delta h = -1.7$ mm. in 130 minutes
$X_{O_2} = -4.5$ mm. <sup>3</sup>	
$X_{CO_2} = +4.35$ mm. <sup>3</sup>	
$Q_{O_2} = -1.6$	

the writer (1929), without alkali well. For a detailed explanation of the technique, see Warburg (1926). The notation of Table I is as follows:

- $V_F$  = volume of cell suspension.  
 $K_{O_2}$  = constant of the vessel for oxygen.  
 $K_{CO_2}$  = constant of the vessel for carbon dioxide.  
 $\Delta h$  = pressure change on the manometer.  
 $X_{O_2}$  = oxygen absorbed.  
 $X_{CO_2}$  = carbon dioxide evolved.

Capital letters refer to the vessel with the large volume of cell suspension, small letters to the one with the small volume of cell suspension. The vessels contained suspensions of the same density, *i.e.*, the same volume of cells per cc.  $X_{O_2}$  and  $X_{CO_2}$  were calculated from these formulae:

$$X_{O_2} = \frac{\Delta H K_{CO_2} - \frac{V_F}{v_f} \Delta h k_{CO_2}}{\frac{K_{CO_2}}{K_{O_2}} - \frac{k_{CO_2}}{k_{O_2}}}$$

$$X_{CO_2} = \frac{\Delta H K_{O_2} - \frac{V_F}{v_f} \Delta h k_{O_2}}{\frac{K_{O_2}}{K_{CO_2}} - \frac{k_{O_2}}{k_{CO_2}}}$$

$X_{O_2}$  and  $X_{CO_2}$  refer to the gas exchange in Vessel number 6, which contained 14 mm.<sup>3</sup> cells, over a period of 130 minutes, the time over

which the observation of the values of  $\Delta H$  and  $\Delta h$  were read. For convenience in comparing with other figures, the value of  $X_{O_2}$  is reduced to the oxygen consumption in cubic millimeters per hour per 10 mm.<sup>3</sup> cells, or  $Q_{O_2}$ , equal in this case to  $-1.6$ . This figure is much lower than figures published by the writer for green algae, where the value of  $Q_{O_2}$  ranged from about  $-5$  to  $-10$ . Adolph (1929, p. 313) gives an average value for the oxygen consumption of freshly isolated frog skin, of 133 mm.<sup>3</sup> O<sub>2</sub> per gm. of fresh weight. Assuming the density of the tissue to be close to 1, frog skin has, according to Adolph's figures, a  $Q_{O_2}$  of about  $-1.33$ , very close to the writer's figure of  $-1.6$  for *Amoeba*.

Attempts were made to demonstrate anaerobic metabolism, by suspending the cells in media containing glucose or bicarbonate or both, and with an atmosphere of nitrogen which had been passed over red-hot copper to remove oxygen. No anaerobic metabolism was found.

Although it would undoubtedly be of interest to study the characteristics of *Amoeba* respiration, the writer did not undertake further experiments. Owing to the small gas exchange, much larger amounts of cells would be necessary than were available at one time.

## II

### *Experiments with Blepharisma*

*Blepharisma* was available in larger quantities, and consequently was better adapted for these experiments. The cells were free from other protozoans, and bacteria were removed by washing on the centrifuge. Measurements were made with cells suspended in distilled water from a Pyrex still, and in dilute salt solutions. Although the rate of respiration was about the same in either case, it remained constant in salt solution and fell off rapidly in distilled water. Only those experiments made with cells suspended in salt solution are recorded here.

The salt solution was prepared freshly from stock solutions for each experiment. When mixed, its composition was as follows:

Redistilled Water.....	1 liter
NaCl.....	500 mg.
KH <sub>2</sub> PO <sub>4</sub> .....	25 mg.
CaCl <sub>2</sub> 6H <sub>2</sub> O.....	100 mg.

In this solution *Blepharisma* cells will live and remain active for several hours without showing any signs of injury. Nothing was found which when added to this solution would cause any substantial increase in respiration. The addition of 1 per cent glucose caused a 10 to 20 per cent increase in oxygen consumption. Peptone in a concentration of 1 mg. per cc. caused a slightly greater increase.

TABLE II  
*Respiration of Blepharisma*

Vessel 6, 87 mm. <sup>3</sup> cells	Vessel 3, 37.5 mm. <sup>3</sup> cells.
$V_F = 7$ cc.	$v_f = 3$ cc.
$K_{O_2} = 0.56$	$k_{O_2} = 0.96$
$K_{CO_2} = 1.15$	$k_{CO_2} = 1.22$
$\Delta H = -13$ mm. in 25'	$\Delta h = -1.7$ mm. in 25 min.
$X_{O_2} = -13.1$	
$X_{CO_2} = +11.7$	
$Q_{O_2} = -3.6$	

Table II records the details of an experiment to determine the oxygen consumption and carbon dioxide production of a sample of *Blepharisma* cells. The notation and method of calculation is the same as for Table I. The temperature was 20°C.

The rate of gas exchange indicated in Table II remained constant for a period of three hours. As in the case of *Amoeba*, the respiratory quotient,  $-\frac{X_{O_2}}{X_{CO_2}}$ , is nearly equal to unity. The value given for  $Q_{O_2}$ ,  $-3.6$ , is much greater than the figure for *Amoeba*.  $-3.6$  was the lowest value obtained for *Blepharisma*, the average being around  $-5$ . With some samples of cells,  $Q_{O_2}$  was found to be as high as  $-7$  or  $-8$ . These values compare favorably with figures of the writer, for green algae cited above. But they are much smaller than the figures given by Adolph (1929, p. 269) for *Colpoda*. He states that a unit volume of cells uses in an hour four times its volume of oxygen. This means a value of  $-40$  for  $Q_{O_2}$ . Adolph says that his figures are more or less in agreement with the values found by other workers for protozoan respiration.

*Blepharisma* shows a measurable anaerobic metabolism. This is manifest only in the presence of bicarbonate, showing that an acid is evolved under anaerobic conditions, displacing CO<sub>2</sub> from the bicarbonate.

The demonstration of anaerobic metabolism was made as follows:

To 50 cc. salt solution were added 2 cc. of  $\frac{M}{10}$  NaHCO<sub>3</sub>. 80 mm.<sup>3</sup> of fresh *Blepharisma* cells were washed in this solution and pipetted into Vessel 6.  $V_F$  was equal to 7 cc. The gas-space was swept out with nitrogen which had been passed over hot copper to remove oxygen. Nitrogen was passed through for about 5 minutes. This proved long enough to wash out the oxygen dissolved in the cell suspension without removing the CO<sub>2</sub> from the bicarbonate. Controls were made by adding the bicarbonate after saturation with nitrogen.

In the experiment referred to, 80 mm.<sup>3</sup> cells in an atmosphere of nitrogen at 20°C. showed an increase of pressure of 11 mm. per hour. Assuming this to be due entirely to evolved CO<sub>2</sub>, *i. e.*, assuming that nitrogen does not enter into the metabolism, this indicated an evolution of 12.5 mm.<sup>3</sup> of CO<sub>2</sub> from the bicarbonate per hour. Upon returning the cells to an atmosphere of air, they showed normal respiration. Glucose did not affect the anaerobic metabolism.

*Blepharisma* cells are colored bright red, and it was thought they might show some special behavior toward light. However, no change in oxygen consumption could be detected when cell suspensions were illuminated by an incandescent lamp or by the light of a Pyrex mercury arc.

#### SUMMARY

1. The respiration of *Amoeba proteus* was measured. 10 c. mm. of cells were found to use about 1.6 mm.<sup>3</sup> of oxygen per hour at 20°C. The respiratory quotient was found to be nearly unity.
2. No anaerobic metabolism was found for *Amoeba*.
3. The respiration of *Blepharisma* was found to be from 3 to 7 mm.<sup>3</sup> oxygen per hour for 10 mm.<sup>3</sup> cells. The respiratory quotient was about 1.
4. *Blepharisma* was shown to have a definite anaerobic metabolism. 80 mm.<sup>3</sup> cells caused the evolution of 12.5 mm.<sup>3</sup> carbon dioxide per hour at 20°C. in the presence of bicarbonate.

## CITATIONS

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