A METHOD FOR DETERMINING THE OXYGEN CONSUMPTION OF A SINGLE CELL

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I

INTRODUCTION

Several methods are available for determining the O_2 consumption of a large number of cells. Only one author, Kalmus (1), has attempted to estimate the metabolism of a single cell directly. His method, as he applied it to Paramecium is briefly as follows: An animal in a small amount of culture medium is drawn up into a capillary tube. This is followed by a little paraffin oil and next a column of air. This end of the tube is now sealed in a flame. KOH 10 per cent and paraffin oil are drawn in, in that order, through the opposite end of the tube, by gently warming the enclosed air column and allowing it to contract subsequently. After the capillary has cooled, the movement of the meniscus between the oil and the KOH is followed by means of an ocular micrometer. Readings are made every 10 minutes for one hour. From these the contraction of the trapped air is determined, the decrease in volume representing the amount of oxygen consumed by the Paramecium. The method was later modified (2) slightly so that the animal was located in a bulb at one end of the capillary, but the essentials were the same as before.

Reasonably constant results were obtained on repetition of Kalmus' work (Table I). It later appeared, however, that the results were equally constant whether or not an animal were present. When capillary tubes of about the same diameter are heated in the same manner and treated similarly throughout the course of the procedure, they will cool down at the same rate for the corresponding hour, over

a period of many hours. This is illustrated in Table II. A capillary containing no animal was observed for 7 hours after a preliminary cooling in water for one half hour following the fusing of one end. Since the motion of the meniscus continued for at least 7 hours it seemed essential to devise a method which did not entail the application of heat to the capillary tube.

TABLE I

Apparent O_2 Consumption of a Single Paramecium by Method of Kalmus

Experiment	Apparent O_2 consumption in $mm.^3$ per hour
1	0.045
2	0.024
3	0.033
4	0.043
5	0.042
6	0.052
7	0.042

TABLE II

Motion of Meniscus in a Kalmus Tube Containing No Animal

Each unit is equivalent to a decrease in volume of 0.009 $mm.^3$

Elapsed time in hours	Total motion of meniscus in units
1	4.5
2	7.5
3	10.0
4	12.0
7	14.5

II

Apparatus and Method of Calculation

The respirometer consists of a glass capillary tube about 6 cm. in length and 0.3 mm. internal diameter. The diameter may be varied to suit the size of the cell under investigation. It is desirable to have the capillary of as small diameter as possible without crowding the cell. This increases the sensitivity of the respirometer since it permits maximum horizontal readings as the air column decreases.

The tube is filled by means of the injection system of a Chambers' micromanipulator. The materials are drawn in, in such order as to give the final arrangement indicated in Fig. 1. First, mineral oil, followed by the cell in a small amount of culture medium; next, air; then a small amount of 5 per cent KOH, and finally mineral oil again. Approximate dimensions of each column are shown in the diagram. It is well to have a minimum of culture medium in order to make the system as compact as possible. On the other hand, it is essential to have a moderate excess of air so that the oxygen consumption of the cell will not lower the partial pressure of the oxygen too markedly.

Once filled, the tubes are placed in a water bath so constructed that it may be held firmly by the mechanical stage of a microscope. The tubes are arranged so that oil extends out to both ends, thus diminishing the ease of access of O_2. They are held by means of rubber bands in two parallel grooved ledges cemented along the base of the

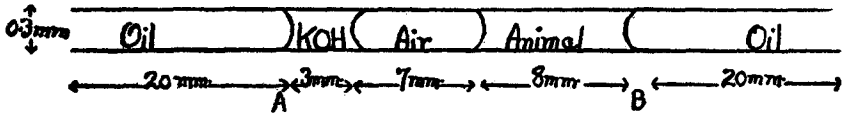

FIG. 1. Microrespirometer

bath. The direction of the capillary is such that it is parallel to the horizontal movement of the mechanical stage. A series of six or eight tubes will conveniently fit in the bath and with each group a control capillary must be run. This latter is made in a manner identical with the experimental tubes, except that no cell is included in the culture medium.

The water bath and microscope are now placed in a constant temperature air chamber. Allowing about a half hour for the system to reach thermal equilibrium, the first readings are then taken. Since it is possible to get much sharper readings at the oil-liquid meniscus than at that between air and liquid, the distance between the two oil menisci is recorded, (A–B). For all practical purposes, however, what is being followed is the change in volume of the enclosed air since that is the only variable directly measured during the course of the experiment. At the same time the internal diameter of the tube is recorded by means of a calibrated micrometer scale in the ocular

lens of the microscope. The readings on the mechanical stage can be taken to hundredths of a millimeter by interpolating on the vernier which is graduated to tenths. The approximate length of the air columns should be noted for later reference concerning thermal and barometric corrections.

The duration of an experiment varies with the oxygen consumption of the cell being investigated. In general, it is advisable to allow a minimum time necessary to give a change in length of the air column equal to 0.4 mm. Under these conditions, the possible error from reading will not exceed 10 per cent.

To illustrate this discussion, data and calculations from a single experiment are given. The cell under investigation was *Actinosphaerium eichhornii.*

		Experimental			Control		
	Time	A (mm.)	B (mm.)	Difference (mm.)	A (mm.)	B (mm.)	Difference (mm.)
1930							
Aug. 23	9:20 a.m.	55.15	77.68	22.53	72.84	56.23	16.61
	2:20 p.m.	55.21	77.72	22.51	72.89	56.26	16.63
	5:35 p.m.	55.25	77.72	22.47	72.91	56.26	16.65
Aug. 24	8:30 a.m.	55.47	77.69	22.22	72.93	56.37	16.56

	Experimental	Control
Diameter tube.....	21.5 units	20.5 units
Length air column.....	6.3 mm.	7.35 mm.

In 23.2 hours the air column in the experimental tube decreased 0.31 mm. in length. The control column decreased 0.05 mm. From these figures and the volumes of the respective air columns the thermobarometric correction is −0.05 mm. The corrected shortening of the air column was 0.26 mm. The diameter of the tube was 21.5 units on the micrometer scale. (1 unit = 0.0177 mm.) Thence the O_2 consumed $= \frac{0.26 \times 0.1138}{23.2} = 0.00128$ mm.^{3} per hour, where 0.1138 mm.^{2} is the cross-sectional area of the capillary with a diameter 0.38 mm.

The control also corrects for any volume changes referable to the differing osmotic pressures of the KOH and the culture medium with a consequent water vapor exchange. This is proved by running several control tubes simultaneously and finding identical corrections from them.

III

Discussion of Errors

In this method there are several possible sources of error which must be considered. The diffusion of a gas through a capillary tube is slow. If there is a lag in the absorption of CO_2, the subsequent O_2 consumption readings will be too small. It seems, however, that while the CO_2 is probably not immediately taken up by the KOH, the system rapidly assumes a state of equilibrium, as evidenced by the linear character of the curve obtained by plotting O_2 consumption against time (Fig. 2). The ease of solubility of CO_2 in the culture me-

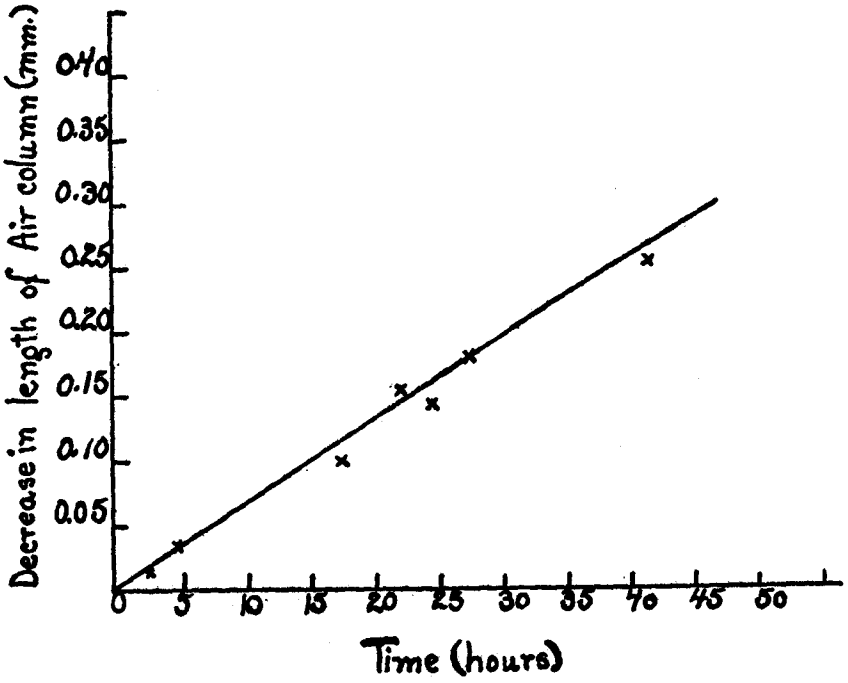

FIG. 2. O_2 consumption curve

dium further promotes the quick arrival at a steady state as does the presence of a motile cell such as a Paramecium. For all practical purposes, then, the readings obtained are as satisfactory as if all the CO_2 were immediately absorbed by the alkali.

A second question arises in connection with the possible influx of oxygen, from without, to the cell.

In earlier experiments mercury was used as the seal, but it was found that the purest mercury available at that time was toxic to the animals. Although the cells did not touch the liquid directly, the coming in contact with that part of the tube over which mercury had already been drawn was sufficient to kill an *Actinosphaerium* rapidly. At the present time further investigations are in progress using mercury distilled *in vacuo*, with the idea that impurities rather than the mercury itself were the toxic agents.

The fact that oxygen is soluble in mineral oil must also be considered. The viscosity of the oil, however, will tend to slow down gaseous diffusion through it. With that in view, the oil columns are made as long as is conveniently possible. But it is at the oil-liquid interface that the most effective barrier to gaseous diffusion exists. In order to establish the magnitude of the gaseous inflow, sets of tubes each with several cells were run. The more rapid O_2 consumption of several cells together would lead to a higher gradient of O_2 pressure between the air inside and outside the capillary so that there would be an increased rate of O_2 inflow. With a doubled O_2 consumption, for example, the rate of diffusion might be doubled. This would result in the average O_2 consumption per single cell, calculated from experiments which used several cells in one tube, having a lower value than the result with the single cell. Actually such is found to be the case. The values from the multiple-cell experiments are definitely lower than those from the single-cell ones. For example, with *Paramecium caudatum*, the results for which average 0.00049 mm.^{3} of O_2 per hour by the single-cell method, a run with three animals in a respirometer gave an average of 0.00033 mm.^{3} per individual. For *Actinosphaerium eichhornii*, where the O_2 consumed is somewhat greater (0.00113 mm.^{3} per hour by the single-cell method), the decrease with several animals is correspondingly greater; so that, with two individuals in a tube, the value per animal is half the first figure. Under constant conditions, the results are remarkably consistent, as will be shown later.

Readings on the mechanical stage are accurate to at least 0.02 mm. To check the method used for determining the volume of the tube, and especially to test the accuracy of this method in reading diameters,

the following procedure was employed. A small amount of mercury was drawn into a capillary. Its volume was determined by the method described above, and its weight calculated from the specific gravity. The mercury was then carefully poured out and weighed, and the actual weight compared with that experimentally obtained. The diameter of a capillary is most readily determined at the point where the oil meniscus approaches the internal surface of the tube tangentially. With moderate illumination, this appears as a sharp white line. Needless to say, the capillary tube should be so drawn that its internal diameter does not vary within measureable limits throughout its length. An error may be introduced by failure to correct for the refraction of light by the glass tube. This error is a function of the thickness of the capillary wall and of the difference between the refractive indices of glass and water. Since the wall is thin and the difference between the refractive indices is small (0.22), the error involved is of the second order, and negligible. The experiments involving the actual weighing of a measured volume of mercury substantiate this conclusion.

That the solutions employed may not be in equilibrium with the air at the start, is a possible source of error. For that reason the KOH solution, culture medium, and mineral oil should be exposed to the air before being used.

Large errors may be introduced if the culture medium is not sterile. For example, in an experiment with *Colpidium colpoda* grown in a medium heavy with bacteria, the culture medium was not filtered. All of these tubes, including the control, consumed an amount of oxygen far beyond reasonable limits. Whenever the control readings drop markedly in the absence of any temperature change, an error of this nature is indicated.

A simple calculation shows that the magnitude of surface tension forces at the air—culture medium and culture medium-oil interfaces is of the order of 0.1 per cent of atmospheric pressure. From this point of view, then, any surface-active substances liberated by the cell will produce negligible effects.

It might be suspected that adhesive forces would restrain the liquids in the capillary from free motion. That such is not the case is evidenced by the slight but definite shift to-and-fro of points *A* and *B* (Fig. 1) from reading to reading.

IV

RESULTS

The first organism investigated was *Actinosphaerium eichhornii*. The animals were all taken from a single culture. Each one was washed

TABLE III

Rate of O_2 Consumption of a Single Actinosphaerium

Number	Duration in hours	Mm.³ of O_2 consumed per hour
1	19.00	0.00126
2	8.00	0.00090
3	23.00	0.00124
4	23.00	0.00124
5	23.00	0.00088
6	20.75	0.00123
7	20.75	0.00107
8	20.75	0.00104
9	20.75	0.00133
Average		0.00113

Temp. range: 21.8 ± 1°C.

TABLE IV

Rate of O_2 Consumption of a Single Paramecium

Number	Duration in hours	Mm.³ of O_2 consumed per hour
1	21.75	0.00045
2	21.75	0.00050
3	43.75	0.00044
4	19.00	0.00061
5	43.75	0.00052
6	21.50	0.00044
Average		0.00049

Temp. range: 21.2 ± 1°C.

twice with double distilled water and the third wash water was used as medium for the experiment. In diameter the individuals varied slightly, averaging 0.3 mm.

The results from four successive series are incorporated in Table III. The O_2 consumed per hour per animal ranged from 0.00088 mm.^{3} to 0.00133 mm.^{3} with an average result of 0.00113 mm.^{3}

A series of *Paramecium caudatum* in culture medium gave the results shown in Table IV. The O_2 consumed per hour per animal ranged from 0.00044 mm.^{3} to 0.00061 mm ^{3} with an average of 0.00049 mm ^{3}

V

DISCUSSION

Considerable work has been done in the field of protozoan metabolism. Practically all of this has been of a qualitative nature so that only in a few cases are there data from which the oxygen consumption of a single protozoan can be calculated. The papers of Kalmus have already been mentioned. For *Paramecium caudatum* he found the oxygen consumption per hour per individual to be about 0.0052 mm.^{3} In some unpublished experiments Necheles (3) using the Warburg apparatus, concluded that a single *Paramecium caudatum* used 0.00385 mm.^{3} of O_2 per hour at 19° C. Lund (4) in a study of the respiration of *Paramecium caudatum* gives several references to experiments in which he counted the actual numbers of organisms being used. From these it can be determined that a single paramecium, starved for 48 hours previous to the run, consumes 0.00004 mm.^{3} of O_2 per hour, while in one fed on dead yeast the O_2 consumption increases over three-fold, *i.e.*, to 0.00014 mm.^{3} Zweibaum (5) with the aid of the Thunberg apparatus, followed the metabolism of *Paramecium caudatum* through its reproductive cycle. Immediately before conjugation an individual uses 0.00074 mm.^{3} of O_2 per hour. During conjugation there is a sharp increase to 0.00348 mm.^{3} which quickly drops off to 0.00068 mm.^{3}; the O_2 consumption then gradually rising to 0.00225 mm.^{3} within a week after conjugation. For *Paramecium aurelia*, Barratt (6) found the CO_2 production of 200,000 individuals at 19–21° C. to be 1.2 mg. over a period of 24 hours. The CO_2 given off by a single animal would then be 0.00012 mm.^{3} per hour. Assuming an R. Q. between 0.7 and and 1.0, the O_2 consumption per hour would be of the order of 0.00015 mm.^{3} per hour. Of course it must be remembered that the different species of Paramecium may show considerable metabolic variation. From the above, however, it is apparent that the hundred-fold variation of results cited in the literature makes any comparison of results unprofitable.

A point of interest in our experiments was the question of whether the metabolic activity of the cells varied over the long course of the measuring period. The possibility of toxic metabolic products exerting an effect on the cell as the experiment progressed was ruled out by recording data over two successive 24 hour periods with the same cell. The O_2 consumed in the second half of the run always agreed with the figure for the first half, within the 20 per cent allowed for experimental errors (Fig. 2).

By the method described it is possible to obtain data on the O_2 consumption of a single cell. In view of the various factors such as physiological state, phase in reproductive cycle, *etc.*, involved, no general significance is attached to the specific results quoted. The consistency of these results, however, under one set of definitely controlled conditions indicates that the method is valid.

SUMMARY

1. A method for measuring the O_2 consumption of a single cell is described. The cell is placed in a capillary tube adjacent to a bubble of air. KOH (5 per cent) is drawn in on the opposite side of the air and both ends of the tube are sealed with mineral oil. The decrease in the volume of the gas, representing the O_2 consumed, is followed.

2. The possible errors of the technique are appraised.

3. A single *Actinosphaerium eichhornii* consumes 0.00113 mm.^{3} of O_2 per hour. A single *Paramecium caudatum* consumes 0.00049 mm.^{3} of O_2 per hour.

4. The significance of the results and the limitations of the method are discussed.

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BIBLIOGRAPHY

1 Kalmus, H., *Biol. Zentr.*, 1927, **47**, 595.
2. Kalmus, H., *Z. vergl. Physiol.*, 1928, **7**, 304.
3. Necheles, H., Cited in Winterstein's, Handbuch der vergleichenden Physiologie, Gustav Fischer, Jena, 1924, **2**, 2. Hälfte, 931.
4. Lund, E. J., *Am. J. Physiol.*, 1918–19, **47**, 167.
5. Zweibaum, J., *Arch. Protistenkunde*, 1921–22, **44**, 99.
6. Barratt, J. O. W., *Z. allg. Physiol.*, 1905, **5**, 66.