THE MEASUREMENT OF THE OXYGEN CONSUMPTION OF DAPHNIA BY A MODIFICATION OF THE CARTESIAN DIVER TECHNIQUE

By R. J. O'CONNOR

From the Westminster Hospital School of Medicine

(Received 3 February 1948)

(With Two Text-figures)

INTRODUCTION

The use of the Cartesian diver as a micromanometer was first described by Linderstrøm-Lang (1937). Boell, Needham & Rogers (1939) adapted the method to the measurement of the respiration of fragments of amphibian embryos and, in this and subsequent papers, many details of technique were worked out (see Needham, Rogers & Shen Shih-Chang, 1939). Boell, with other collaborators, has used the technique to measure the respiration of early mammalian embryos (Boell & Nicholas, 1939), and to estimate the distribution of cholinesterase (Boell & Nachmansohn, 1940; Boell & Shen Shih-Chang, 1944). Further use of the technique in Linderstrøm-Lang's laboratory has included the measurement of the oxygen consumption of tissue cultures (Zamecnik, 1941). A detailed theoretical analysis of the method has been undertaken (Linderstrøm-Lang & Holter, 1942; Linderstrøm-Lang, 1943), and based on this further technical details have been worked out (Holter, 1943). In all these methods the total capacity of the divers has been less than 40 \( \mu l \), and this capacity has been greatly reduced in further modifications of the technique described by Zéuthen (1943, 1946).*

In order to adapt the method to the measurement of the oxygen consumption of *Daphnia* it was found necessary to design divers capable of accommodating 40–50 \( \mu l \) of fluid in which four to five *Daphnia* were suspended. Thus it became necessary to increase the total capacity to over 100 \( \mu l \), creating certain possibilities of error, which have been experimentally investigated, and the results are included in the description of the modified technique.

DESCRIPTION OF THE TECHNIQUE

Briefly to recapitulate the principles of the method, the reaction vessel, or diver, is made of capillary tubing and consists of an open neck, an expanded bulb, and solidified tail which causes the diver to float tail downwards. If the diver is floated in a suitable vessel and the pressure within the vessel altered, fluid can be made to

* Woerderman & Raven (1946) refer to a further modification by Ten Cate.
enter or leave the neck, thus altering the buoyancy of the diver so that, by suitably controlling the pressure, the diver can be brought to equilibrium at a determined level. Any change in the volume of gas within the diver will alter the pressure necessary to produce this equilibrium.

Construction of the divers

The shape and structure of the divers are indicated in Fig. 1, where it will be seen that, in addition to the neck, bulb, and solid tail there is a central cup incorporated in the tail and projecting into the bulb for about two-thirds of the diameter. This central cup contains sodium hydroxide solution for the absorption of carbon dioxide. Although there is some variation in the size of the divers used, the following are approximate average dimensions:

- Total capacity: 120 μl.
- Length of neck: 15 mm.
- Internal diameter of neck: 1.3 mm.
- Internal diameter of cup: 0.5 mm.

The dimensions are such that the bulb is half filled by 40–50 μl. of fluid.

The divers are made by blowing a bulb 2–3 cm. from the end of a suitable piece of capillary tubing, and inserting a second piece of tubing within the first. A close fit is necessary and the second piece of tubing is made to protrude the required distance into the bulb. The double layer of tubing is then solidified to form the tail, care being taken to obliterate the space between the two layers of tubing. The tail and neck are then cut off at the required lengths. The divers can be cleaned and used repeatedly.

Filling the divers

To introduce the *Daphnia* the divers are completely filled with the suspension medium, which in this case is filtered aquarium water. *Daphnia* are picked up by a platinum loop, introduced into the necks of the divers, and made to enter the bulb by means of a straight platinum wire. Excess fluid is then removed by a capillary pipette leaving about 40–50 μl. fluid in the bulb. Any fluid in the central cup is removed. The internal surface of the neck is then dried with strips of filter-paper and coated with a layer of paraffin wax introduced by a heated wire. Care is taken to see that the coating stops precisely at the junction of neck and bulb and that the amount of wax does not materially reduce the internal diameter of the neck. Next, by means of a waxed capillary pipette, 5% sodium hydroxide solution is introduced into the central cup filling it to within 2 mm. of its opening. It is convenient to colour the sodium hydroxide solution.

The air in the divers is then replaced by oxygen. About 100 c.c. of moist oxygen are passed through the divers. During subsequent manipulations some of the oxygen
The measurement of the oxygen consumption of Daphnia may be lost by diffusion, but by absorbing the contained gas with alkaline pyrogallol solution it has been shown that the atmosphere in the divers during the actual readings is over 95% oxygen.

In those cases where a reagent is to be added to the fluid in the bulb during the period of observation, this is introduced in solution into the neck to form a column of fluid immediately adjacent to the bulb. The concentration of the reagent should be adjusted so that the required amount is contained in a column of fluid approximately 4 mm. in length. An oil seal is next added consisting of a column of light liquid paraffin about 2 mm. in length and separated from the reagent by about the same distance. Oxygen is again passed into the open portion of the neck, and the terminal 3 mm. filled with flotation fluid (see next section), leaving a gas space between the flotation fluid and the oil seal.

At this stage it is convenient to calculate the amounts of fluid added to the neck by measuring the lengths of the fluid columns, and the internal diameter of the neck, by means of a microscope with a measuring eyepiece. The amount of fluid in the bulb is obtained by weighing the divers at the appropriate stages of the filling process.

**Flotation fluid—adjustment of diver weight**

Previous investigations (Linderstrøm-Lang & Holter, 1942; Boell et al. 1939) have shown that the properties of the flotation fluid have an important effect in limiting the amount of gaseous diffusion that occurs between the flotation fluid and the contents of the diver. In the present method the fluid used is a sodium chloride solution of a specific gravity of 1.1. Divers filled as described are transferred to a beaker filled with this fluid in which they float easily. Fragments of plasticine are dropped into the beaker, picked up by the tail of the diver, and the amount adjusted until the diver just floats in the fluid. This adjustment should be performed at approximately the temperature at which the measurements are to be made and the fluid in the beaker is, therefore, warmed when necessary.

**Flotation tubes**

When the weight of the divers has been adjusted they are transferred to the flotation tubes which are 15 cm. in length and 3 cm. internal diameter. These are four-fifths filled with the flotation fluid and midway in their length a horizontal circular mark is etched—the flotation level. After introducing the divers—one to each tube—a disk of cork is dropped in. This floats at the surface and prevents contact between the surface of the fluid and the neck of the diver avoiding displacement of fluid in the neck by surface-tension effects. Before the introduction of the divers, the fluid in the tubes is saturated with oxygen.

**Water-bath—control of pressure in flotation tubes**

Six flotation tubes, each containing a single diver, are suspended vertically in a water-bath with a glass front. The temperature is controlled to within less than 0.1° C. which is adequate if a control diver is used. Each flotation tube is connected to an open water manometer capable of measuring a pressure change of 100 cm.
water, and to an apparatus to vary the pressure in the tubes. This consists of a 20 c.c. syringe with a 5 c.c. syringe as a fine adjustment, the alteration in pressure being transmitted by the air contained in the connecting tubes. Taps are incorporated in the system so that the pressure can be varied and measured in each flotation tube independently (see Fig. 2).

**Addition of reagent**

The method of transferring the reagent from the neck to the bulb contents is derived from Boell, Koch & Needham (1939). The manometer is shut off by closing tap 13 in Fig. 2 and then sufficient pressure can then be created by the 20 c.c. syringe to drive the reagent over the internal surface of the bulb so that it mixes with the bulb contents. The pressure can be controlled so that none of the oil leaves the neck and is returned to its original position on release of the pressure. Observations with coloured fluids indicate that about 20 min. should be allowed for mixing, but this period is considerably reduced when the bulb contains actively moving *Daphnia*.

**Method of making readings—calculation of results**

In between readings it has been found most convenient to decrease the pressure in the flotation tubes so that the divers come to rest in contact with the under, submerged surface of the cork disk. When a reading is to be made, the pressure is...
The measurement of the oxygen consumption of Daphnia increased so that the diver sinks, and by adjusting the pressure, is brought to equilibrium at the flotation level, this being judged by direct vision. Equilibrium is considered to be reached when the diver remains steady for 5-10 sec. The pressure at this equilibrium—the flotation pressure—is then read and the diver returned to the top of the flotation tube. It has been found that the readings can be reproduced to within 1 mm. water.

Each time the diver is brought to equilibrium the volume of the contained gas is brought to a constant volume, so that the diver acts as a constant volume manometer and the relationship between change in volume and change in flotation pressure is given by the equation of Boell et al. (1939), which contains a term to allow for the gas dissolved in the fluid within the diver. In the present case the gas is oxygen and the low coefficient of solubility reduces this term to less than 3% of the oxygen uptake. It can, therefore, be ignored in most calculations so that when measurements are made at 27°C the oxygen uptake is given by

\[ V = p \frac{V_g 273}{A 300}, \]

where \( V \) is the amount of oxygen taken up in microlitres, \( V_g \) is the volume of the gas space in the diver in microlitres, \( p \) is the change in flotation pressure in millimetres water, and \( A \) is the atmospheric pressure in millimetres water. \( V_g \) is obtained by subtracting the volume of fluid in the diver from the total volume—the latter being obtained by weighing when filled with mercury. The calculations are performed for each experiment, but dimensions of the divers are such that 1 cm. change in flotation pressure corresponds to 0.05-0.08 \( \mu \)l. oxygen.

INVESTIGATION OF THE MODIFIED TECHNIQUE

A comparison with other techniques will show that many precautions suggested have not been adopted, and this, in addition to the increased size of the diver, leads to certain possibilities of error which have been investigated by experiments described below.

Performance of control divers

Here divers have been filled as described, except that the bulb contained a non-reacting fluid so that any changes in the flotation pressure would be due to physical conditions, such as fluctuations in the temperature of the water-bath, changes in atmospheric pressure, and interchange of gas between the contents of the diver and the flotation fluid. Two oxygen-filled control divers were observed at frequent intervals for a period of 5 hr. In one case the flotation fluid was saturated with air, in the other with oxygen. In neither case was there any tendency for the flotation pressure to alter in a constant direction, and the maximum change in the flotation pressure in any one hour was 2 mm. water. Thus it is taken that the fluctuations are due to minor alterations in the physical conditions and that these, in actual experiments, would be adequately controlled by the use of a control diver.
Accuracy of the addition of reagents—accuracy of measurements

The possibility was considered that some of the reagent added to the bulb from the neck might adhere either to the neck or the bulb and not mix with the bulb fluid. To test this \( \text{N/10 HCl} \) was placed in the bulb and \( 0.1\% \) sodium bicarbonate added from the neck. In this case there was no sodium hydroxide solution in the central cup, so that the carbon dioxide evolved caused a change in the flotation pressure of the diver. From this the amount of carbon dioxide evolved could be calculated by appropriately modifying the formula given on page 317 so as to include the carbon dioxide dissolved in the fluid in the diver. This was then compared with the amount of carbon dioxide to be expected from the amount of sodium bicarbonate added. The results of five such experiments are given in Table 1. It should be noted that the altered flotation pressure was reached in less than 15 min.

### Table 1. Evolution of carbon dioxide from sodium bicarbonate solution

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Calculated</th>
<th>Measured by Cartesian diver</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.62</td>
<td>1.71</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>1.79</td>
<td>1.86</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>2.01</td>
<td>1.97</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>2.35</td>
<td>2.34</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>2.06</td>
<td>2.05</td>
<td>0.5</td>
</tr>
</tbody>
</table>

### Table 2. Rapidity of absorption of carbon dioxide

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Amount of CO(_2) evolved ((\mu l).)</th>
<th>Time to regain original flotation pressure ± 2 mm. water (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.8</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>3.7</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>3.9</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>3.9</td>
<td>10</td>
</tr>
<tr>
<td>Average</td>
<td>3.9</td>
<td>11</td>
</tr>
</tbody>
</table>

Speed of absorption of carbon dioxide

In the Barcroft technique Dixon & Elliot (1930) have shown that the rate of absorption of carbon dioxide by alkali in a central cup may be inadequate. Since, on a smaller scale, the conditions here are comparable, this point was tested by direct experiment. As in the previous section, sodium bicarbonate solution was added to hydrochloric acid in the bulb, but in this case the cup of the diver contained sodium hydroxide solution. When the sodium bicarbonate was added to the acid there was a slight increase of buoyancy, followed by a return to the original flotation pressure. In Table 2 are set forth the amounts of carbon dioxide evolved in five experiments, and the time required for the divers to return to within 2 mm. of the original flotation pressure. From the results it is seen that an average of
The measurement of the oxygen consumption of Daphnia 319

3.9 μl. carbon dioxide was absorbed in an average of 11 min. This amount of carbon dioxide is greater than that evolved in 2 or 3 hr. when the oxygen uptake of Daphnia was measured.

Adequacy of oxygen supply

When the bulb contains actively moving Daphnia no deficiency of oxygen supply would be expected because the movement of the water fleas keeps them near the surface and imparts an appreciable amount of movement to the fluid. This expectation is borne out by the fact that replacement of the oxygen by air does not diminish the rate of oxygen uptake. However, experiments were designed when the oxygen uptake of paralysed Daphnia was to be measured. In this case the Daphnia sank to the bottom of the bulb and were separated from the atmosphere of the diver by a layer of fluid 2-3 mm. in depth, through which the oxygen used would have to diffuse.

It was thus necessary to ascertain that this rate of diffusion was adequate to meet the oxygen needs of the paralysed Daphnia. The filling conditions of the divers were, therefore, reproduced with a suspension of baker's yeast, the respiratory rate of Table 3. Oxygen consumption of normal Daphnia

<table>
<thead>
<tr>
<th>Diver</th>
<th>Day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>0.23</td>
<td>0.16</td>
<td>0.40</td>
<td>0.28</td>
<td>0.25</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.24</td>
<td>0.16</td>
<td>0.40</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.25</td>
<td>0.19</td>
<td>0.35</td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>0.22</td>
<td>0.19</td>
<td>0.36</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>0.22</td>
<td>0.17</td>
<td>0.34</td>
<td>0.31</td>
<td>0.28</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>0.23</td>
<td>0.17</td>
<td>0.37</td>
<td>0.27</td>
<td>0.27</td>
</tr>
</tbody>
</table>

which was certain to exceed the rate of diffusion of oxygen into the fluid in the bulb of the diver. During the process of filling the divers these were centrifuged to pack the yeast cells at the bottom of the bulb. When measurements of oxygen uptake were made, the results obtained represented, not the respiratory rate of the yeast, but the rate of diffusion of oxygen into the bulb fluid. All divers were tested in this way before being put into use, and none were used unless the rate of diffusion under these conditions was 2 μl./hr. or more. In experiments involving measurements on paralysed Daphnia the rates of oxygen uptake were considerably less than this.

THE OXYGEN CONSUMPTION OF NORMAL DAPHNIA

Both D. pulex and D. obtusa were used. These were gathered from ponds and stored in aquaria until required for use. Each measurement was made on four or five individuals between 1.5 and 2.0 mm. length suspended in 40-50 μl. of fluid in a single diver. No differences were noticed in the two species so they are not distinguished in the results given. Measurements were made at 27° C. and extended over an hour or more, resulting in a change of flotation pressure of more than 150 mm. water. In Table 3 are set forth the results of a series of measurements performed on different days and on different batches of Daphnia, each measurement
consisting of five divers each containing five Daphnia. The results are expressed in μl./flea/hr. It will be seen that although there is a considerable variation in the average results obtained on different days, with different batches of Daphnia, the results on any one day are considerably more constant.

DISCUSSION
In order to accommodate the Daphnia the size of the divers has been considerably increased as compared with other techniques. Although this allows the divers to be filled without special pipettes there is a decrease in sensitivity. In the present technique a change of 1 cm. water in flotation pressure represents a gas change of 0.05–0.08 μl., whereas in the technique of Boell et al. (1939) a similar change of pressure corresponds to 0.003–0.022 μl. and in the smaller diver, as described by Zeuthen (1943), this figure would be still smaller.

In the case of Daphnia with a high and variable rate of oxygen consumption, the resulting loss of accuracy is not of consequence, but since the technique could be applied to other material, the degree of accuracy is worthy of consideration. From Table 1 it is seen that measurements of 2 μl. of a change can be measured to within an accuracy of 10%, and although the gas concerned was carbon dioxide, the error in the case of oxygen would not be expected to be any greater. This gas change is represented by about 300 mm. change in the flotation pressure so that the errors in its readings would not be of consequence. This amounts to 1 mm. water in the reading of a single pressure, and gives rise to a possibility of an error of 2 mm. when the difference between two pressures is measured, and this would increase the error by a further 10% when the pressure change was 20 mm. water corresponding to a gas change of approximately 0.12 μl. On the other hand, it can be pointed out that these are maximum estimates which could in all probability be reduced on the basis of further experimental analysis. Further, the technique described is designed to measure the change of oxygen uptake resulting from the addition of reagents without altering the fluid contents of the diver, so that such a change can be measured without introducing the errors due to a calculation of the relationship between the change of flotation pressure and the gaseous change.

If the material used does not undergo gross movement, as is the case in Daphnia, the limits imposed by the rate of diffusion of oxygen become important. In the case of non-motile material the present investigations show that, with divers of the type described, there is an upper limit at the rate of 2 μl. of oxygen per hour. Although this would be greater in the case of uniform cell suspensions or with a chemical reaction proceeding uniformly throughout the fluid to the bulb, nevertheless, a specific investigation of the limits of diffusion would be advisable if the experimental conditions were altered.

SUMMARY
1. The Cartesian diver microrespirometer has been adapted to the measurement of the oxygen consumption of Daphnia. The modification involves an increase in size of the diver to a total capacity of over 100 μl., and absorption of carbon dioxide by sodium hydroxide in a central cup incorporated in the structure of the diver.
The measurement of the oxygen consumption of Daphnia

2. The variation in the rate of oxygen consumption of normal Daphnia has been measured.

3. Sources of error in the modified technique have been investigated and the possibility of its wider use discussed.

The writer is indebted to Prof. R. J. V. Pulvertaft for facilities provided and for many helpful suggestions.

REFERENCES