THE CARTESIAN DIVER BALANCE

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I. PRINCIPLE OF METHOD

The principle of the Cartesian diver has been adapted (Zeuthen 1947, 1948a) for the determination of underwater or reduced weights ($RW$) of small samples of living cells or of single cells about the volume of single large amoeba (0.01–0.1 μ). Later, single smaller amoebae (0.001 μ) have been accurately weighed.

Whereas the Cartesian diver gasometers are constant volume, changing pressure systems, the balance is a changing volume, changing pressure device. When the system is loaded the air bubble is expanded so much that the excess gas volume will carry the load. The balance (Fig. 1) is a minute flask with a long narrow tail which serves as a brake for the diffusion of gases, but permits pressure equilibration between the interior of the flask and the surroundings. On to the flask is attached a plastic cup.
which permits the loading of the diver on the outside with known standards and unknown samples. The diver is floated by means of a small, round air bubble, the size of which can be finely adjusted by the operation of a double-branched water manometer (cf. Holter's article in this volume) which is connected to the air space of the flotation vessel. The general set-up is shown diagrammatically in Fig. 2. The manometer \((P, m)\) is equal to the one shown in Holter's Fig. 1 (this volume). The compensation bottle \((c)\) is a 51. air volume which is closed from the atmosphere and is open to the manometer \((c)\) and the flotation vessel \((F, 3\text{ ml medium})\) is submerged in a finely regulated \((0.002^\circ\text{C})\) bath (cf. Holter's Fig. 2). All other parts are in air. A horizontal cathetometer (cf. Holter's Fig. 2), or better a horizontally mounted dissection microscope with a scale in the one ocular is used for the optical control.
The equilibrium pressure \((h, \text{mm } H_2O)\) of the unloaded diver—floating at a defined level—is read on the water manometer. The ground joint on top of the flotation vessel is opened and a sample is dropped on to the diver balance. The equilibrium pressure (e.p.) is read again. The numerical change in e.p. may be \(\Delta p_s\) and \(\Delta p_u\) mm H2O for the standard and for the unknown. From these two readings the \(RW\) of the unknown is calculated as described on p. 71.

II. MAKING THE DIVER BALANCE

In making the glass part of the instrument, proceed in one of the ways indicated in Fig. 3. Use thin-walled 0.3-0.5 mm Pyrex capillary. In procedure I a loop of an electrically heated wire is the heating source. The temperature can be varied by means of a Variac. In procedure II heating is in an electrically heated spiral coil (e.g. from an electric bulb, glass removed). In procedure III the diver balance is blown in a microflame. Different individuals have different preferences with regard to methods I-III. The author recommends I and III. For making the cup, proceed as shown in Fig. 4 (a)-(f). Use a 5% polystyrene solution in benzene, or commercially available polystyrene cement for gluing children’s play-toys. It is essential that the atmosphere is dry where the cup is to be made. It may be necessary to mount an incandescent lamp next to the diver which is being processed. The polystyrene solution is taken into a vertically mounted braking pipette. The glass part to which the polystyrene cup should be added is held vertically, using an artery clamp or a clothes-pin which rests on a stand (Linderstrøm-Lang and Holter, 1940). This stand can be moved up and down in a finely controlled manner. If this is not available a microscope stand may serve the purpose. The jaws of the clothes-pin, or of the clamp, should be protected with rubber. After the
bubble is blown as indicated, the solvent is given time to evaporate, so that the bubble hardens. In later stages of the drying process, the blowing pressure may be held mechanically. The clamp which holds the diver is gently removed after stage \((e)\). The finished diver is separated as indicated in \((f)\), by the cut of a razor or of a razor blade.

\[\text{Fig. 4. Techniques of blowing the polystyrene cup. (Zeuthen, 1948a.)}\]

III. SELECTION OF DIVER FOR USE

It pays to produce diver balances in series. Many can be made in the course of a day, but some must be discarded for one reason or another. The diver always floats cup up, but the shape of the cup should meet specific requirements of the experiment. The tail should be shaped to form an effective barrier for diffusion. How to select divers with proper tails has not been described before. Below, it is presented in some detail.

Krogh\'s diffusion coefficient (1919) for O\(_2\) in water (ml diffusing at 20°C in 1 min across a 1 ml cube, with a pressure head of 1 atmosphere) is 0·000034. This corresponds to 0·204 \(\mu\)l (N.T.P.) per hour through a cube of 1 \(\mu\)l, at a pressure of 1 atmosphere. This value equals the "standard rate of passage", \(\rho\)\(_i\) (cf. Holter\'s article in this volume), except that the latter is defined at \(\bar{f}\). The amount of air diffusing at a pressure head of 1 atmosphere is the sum of O\(_2\) and of N\(_2\) travelling, and it is equal to:

\[
\frac{0.204 \times 21}{100} + \frac{0.204 \times 79 \times \sqrt{32} \times 0.0155}{100 \times \sqrt{28} \times 0.031} = 0.129 \mu\text{l/hr (N.T.P.)}
\]

The figures introduced in the calculation are the percentage composition of the atmosphere for O\(_2\) (21%) and N\(_2\) (79%), the molecular weight of
these two gases (32 and 28) and their absorption coefficients in water at 20°C (0.031 and 0.0155).

The diver’s tail (length = \( l \) mm) is accepted as an effective brake for diffusion if the diver loses air (\( \Delta V \ \mu l \)) by this route at a maximum rate of 0.01% per hour of the floating diver’s air volume \( V_D \) (radius \( R \)). The equilibrium pressure would for this reason change 1 mm H\( \text{O} \) per hour. When the diver is not loaded we can assume that steady states prevail. The medium is saturated with air at the pressure of the manometer. The pressure head with which air diffuses from the diver is \( h_M + \sigma \). \( h_M \) is the height of the column of water (mm) above diver, and \( \sigma \) is the height of rise (mm) of the flotation medium (water) in a capillary with radius \( R \) mm. When necessary, the diver may be floated near the surface of the flotation medium. Therefore, \( h_M \) may be disregarded so that, in the present context, \( \sigma \) is the pressure head to be considered. \( \sigma \) and \( R \) are related (water at 20°C) according to the expression: \( \sigma = 14.8/R \). Below, \( \pi r^2 \) equals the area of an average inner cross-section of the tail (radius \( r \)). Thus, for a diver which meets the requirements mentioned the following must hold true:

\[
\Delta V = \frac{4/3\pi R^3 \times 10^{-4}}{lR \times 10300} = \frac{0.129 \times \pi r^2 \times 14.8}{lR \times 10300}
\]  

(1)

We shall limit our considerations to divers in which the length of the tail (\( l \)) is 10 mm; equation (1) then reduces to: \( r/R^2 = 2.7 \). Divers with tails which leak only the accepted minimum of air may now be selected using Table I. (In practice, deviations from \( l = 10 \) mm are, of course, perfectly

<table>
<thead>
<tr>
<th>( V_D ) (( \mu l ))</th>
<th>( R ) (mm)</th>
<th>( \sigma ) (mm)</th>
<th>( r ) (( \mu ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 4 \times 10^{-6} )</td>
<td>0.01</td>
<td>1480</td>
<td>0.26</td>
</tr>
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<td>0.02</td>
<td>740</td>
<td>1.00</td>
</tr>
<tr>
<td>( 5 \times 10^{-4} )</td>
<td>0.05</td>
<td>296</td>
<td>6.70</td>
</tr>
<tr>
<td>( 4 \times 10^{-3} )</td>
<td>0.10</td>
<td>148</td>
<td>26.00</td>
</tr>
<tr>
<td>( 3 \times 10^{-2} )</td>
<td>0.20</td>
<td>74</td>
<td>103.00 (50)\textsuperscript{a}</td>
</tr>
<tr>
<td>( 5 \times 10^{-1} )</td>
<td>0.50</td>
<td>30</td>
<td>660.00 (100)\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\( R \) = radius of air bubble.

\( r \) = radius of tail.

\textsuperscript{a} Suggested values, cf. p. 66.

acceptable. It should only be remembered that—other factors being equal—leakage is inversely proportional to the length of the tail. When \textit{in operation} the equilibrium pressure sometimes changes considerably
faster than discussed for the diver which is not in use. The reasons will be discussed. Every time the diver is loaded the inside pressure is temporarily reduced by a value $\Delta p$. The saturation pressure in the medium remains practically constant. The gradient for air diffusing immediately becomes $\sigma + \Delta p$. $\sigma$ is positive and $\Delta p$ negative. Thus, upon loading the diffusion gradient becomes smaller or changes direction. In work with larger divers the numerical value of $\Delta p$ may exceed that of $\sigma$ several times. So for the loaded diver to be reasonably tight for gases, $r$ must be smaller than calculated in Table I. How much depends on the size of the diver, on the type of experiment to be performed, and also on mechanical considerations relating to the process of making diver balances. The author's suggestions are given in parentheses in Table I.

Another factor deserves consideration at this place. The air in the diver is mostly in the bubble, but some is dissolved in the water in the diver. The amount of dissolved air varies with the pressure. It constitutes a small but not constant fraction of the finite amount of air in the system. Some air is shifted back and forth between water and bubble when the diver is operated as a balance. How much depends on the water/air ratio in the diver, and how fast, depends on the diffusion distances ($b$) in the water in the diver. Equilibrium conditions are sufficiently approached within a few minutes after loading and re-balancing of the diver if the water/air ratio is low (0.5-3), and if $b$ is less than 0.25 mm. For larger divers $b$ cannot be kept very small. However, in this case it is easy to reduce the water/air ratio in the diver, and thus to minimize the fraction of air which is dissolved. In either case the diver should be shaped like a sphere with a long narrow tail (Fig. 1); not like a half-sphere containing the air bubble and a piece of very wide tail followed by a long narrow tail (Hagens, 1958). In the former type of divers the solubility factor is similarly involved in the weighings of the unknown and of the standard, and it therefore usually cancels out. This need not be the case in Hagens' diver balance because steady states may not adjust themselves for hours. The reader who wants to go deeper into these questions should consult Linderstrøm-Lang (1943).

Further selection of diver balances must be based on actual tests for stability of the equilibrium pressure. Many divers leak more air than can be accounted for as described. Air sometimes diffuses right through the glass (Løvtrup, 1950a) in amounts which far exceed the negligible diffusion to be calculated from known properties of glasses. Submicroscopic pores may be present, perhaps formed by boiling of the melted glass. This trouble is the more frequent the smaller and more thin-walled is the diver. Sometimes it is therefore advisable to make the diver's glass part thick-walled. The glass part with the air bubble will now not float by
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itself, much less carry the weight of a cup of polystyrene which is more dense (about 1.05) than water. If the cup is made of polyethylene (density 0.92) as suggested by Hagens (1958), such a heavy diver may still be made buoyant. However, it is a disadvantage that this small diver tends to become sluggish because of the large volume of plastic which must now be applied. Also, it is inconvenient that polyethylene is opaque. Polystyrene is translucent. (In Zeuthen’s earliest diver balances a non-compressible flotation body—air in glass—was inserted between the compressible air space and the cup (Zeuthen, 1948a)). The cup was then made of glass like the rest of the balance. The size \( V_D \) of the diver must fit into the range of the reduced weights to be measured. The air bubble (in \( \mu l \)) should be around 20–100 times the \( RW \) (in mg) of the samples. The pressure changes upon loading will then be of the convenient order of 500 to 100 mm H\(_2\)O.

IV. MAKING THE DIVER BALANCE FLOAT

The newly made diver balance is hydrophobic for a few hours after it has come in contact with water. At the end of this time it is pipetted into the flotation vessel. If it is not pushed against the glass walls of the vessel, the diver stands rough treatment. Air in the cup and other outside bubbles may be removed using braking pipettes, or, brutally, by the use of small glass rods. Time will do the trick too, and particularly so if initially the flotation medium is slightly under-saturated with gases. This latter precaution also reduces the danger of new air bubbles developing when the diver is in actual use.

At this stage the diver is filled completely with air and should drift upwards quickly. The manometer should now be closed off. Subsequent suction (through \( a, b \), Fig. 2) removes separate small volumes of air as bubbles through the capillary tail. Every time a few bubbles have been removed, the diver is tested for buoyancy at atmospheric pressure. Suction is discontinued when the diver floats at atmospheric pressure + 20 cm H\(_2\)O. Suction is definitely not intended to bring the manometer fluid into the flotation vessel. Therefore, before sucking remember to close the manometer off by using stopcock III, Fig. 2. Air bubbles tend to form on the diver at reduced pressure. Also, when the pressure is reduced the diver can easily get stuck in the surface film. The measures already mentioned may then be taken. In addition, tapping with a finger nail, and the introduction of short periods of increased pressure may help to shake loose, or dissolve outside bubbles; or to loosen the diver from the surface of the medium.

The diver is now ready for use. When, after some time, too much air
has leaked out, the diver is removed from the flotation vessel using a pipette. If necessary, it is cleaned by pipetting into concentrated sulphuric acid for a short exposure, or into a hexametaphosphate cleaning solution. After washing in water and removal of outside water with filter paper, the diver is freed of inside fluid by suction with a pump. A tiny piece of filter paper in contact with the tip of the tail absorbs outcoming fluid. The diver is re-balanced and used over again.

V. THE STANDARDS

For standards 5–10 μ thick strips of gold have been proposed, cut on the microtome from the edge of a 0.09 mm gold foil (Levi and Zeuthen, 1946). Also, palladium (Løvtrup, 1950a) or platinum (Løvtrup, 1953a) filaments have been used. We mostly prefer (Zeuthen, 1948a) small polystyrene beads of known density (about 1.05), accurately determined to the fourth decimal place as the density of the KCl solution in which the standard neither rises nor falls. The weight of the polystyrene standard \( g_{st} \) in air (0.1–0.5 mg) is determined with an accuracy of one to two per cent. It exceeds the underwater weight about twenty times. However, the reduced weight of the standard differs with the medium and is:

\[
RW_{st} = g_{st} \left(1 - \frac{\phi_{st}}{\phi_{st}}\right)
\]  

(2)

Using diver balances of graded sizes, and a series of graded weights, it becomes possible to standardize the smallest weights by comparison with very large standards. The principle (Zeuthen, 1948a) is demonstrated in Table II. The first standards were made from gold, and their \( RW \) was determined by weighing (Levi and Zeuthen, 1946) in Linderstrom-Lang’s density gradient (Linderstrom-Lang and Lanz, 1938). The three gold standards had reduced weights: 23.12 μg (B), 15.87 μg (C) and 7.00 μg (D), as determined February 24, 1944. C and D were diver-weighed. Comparison was made with B (gradient-weighed). On January 23–29, 1946, two years later, weights C and D were diver-weighed (diver: 0.15 μl) using weight B as the standard. The results check closely (0.3 and 0.6%) with the earlier diver-weighings. At the same time five polystyrene beads (I–V) were diver-weighed with B as the standard. The polystyrenes were reweighed February 2–7, however now with the diver-weighed D as the standard, and using a smaller (0.1 μl) diver balance. The results of this second series of weighings are within the spread (±1%) of the first series of results. Finally, using a 0.04 μl diver, on March 9, 1946, polystyrene IV was used as a standard for repeated weighings of two amoebae (Chaos chaos). Instead, an even smaller polystyrene bead might have been standardized, and so on.
Standards and unknowns are placed on and removed from the balance with a braking pipette which is held in a vertical position just above the diver balance, with the tip submerged. To protect fragile objects from breakage, the tip of the pipette should be fire-polished.

If suitable standards are not available, or the balance is to be used only for relative measurements, the volume \( V_D \) of the air bubble may be calculated from a microscopic measurement in the floating diver of the diameter of the bubble (Prescott and Mazia, 1954; Geilenkirchen and Zeuthen, 1958). With approximation, \( V_D \) thus measured may be inserted for \( K \) into equation (5). Lense actions in the glass and in the plastic may introduce errors in the diameter measurement. This microscopic calibration of the diver balance has never been properly checked. It has been suggested (Prescott, 1955b) that the errors are within \( \pm 10\% \).
VI. THE ACTUAL WEIGHING

Figure 5 shows the record of an experiment in which two *Chaos chaos* amoebae and then a standard are weighed on the diver balance. The equilibrium pressure of the empty diver is measured repeatedly in the course of the first minutes. After 12 min the side-tube I (Fig. 2) is opened; the ground joint on the top of the flotation vessel is taken apart to give access to the diver and amoeba 1 is dropped on the diver balance. The ground joint of the flotation vessel is re-assembled, and the right meniscus of the manometer is put in a position which should deviate only 5–10 cm from the position before the diver was loaded. This is to ascertain that the bottle pressure is not significantly influenced by the position of the meniscus in the right branch of the manometer. Side-tube I is now closed, the diver is balanced using the pressure device of the manometer and a series of readings of the equilibrium pressure are...
obtained. After 28 min the amoeba is removed. The equilibrium pressure of the empty diver is read again. The diver is loaded with the second amoeba and the pressure is read. For each time the diver is loaded and unloaded there is a jump in equilibrium pressure as indicated in the figure. For calculation of the reduced weight of an object we accept the average of the two opposite changes in equilibrium pressure of the diver, caused by loading, and resulting from unloading, of the diver. The two values seldom deviate more than 2–3 mm H₂O. It is advised that the diver is chosen so that Δp equals or exceeds 100 mm H₂O.

An object with volume \( V_c \) and density \( \phi_c \), in a medium of density \( \phi_M \), has a submerged or “reduced” weight \( (RW) \):

\[
RW_C = V_C(\phi_C - \phi_M)
\]  

(3)

Let \( \Delta p_\text{st} \) and \( \Delta p_x \) be the numerical changes in equilibrium pressure (mm H₂O) of the diver resulting from the loading with the standard and with the unknown. If \( RW_{\text{st}} \) is the reduced weight of the standard (mg), then insertion in the formula (Holter, Linderstrøm-Lang and Zeuthen, 1956—slightly changed from Lovtrup 1950a; this equation is more handy but does not differ significantly from the one first used by Zeuthen, 1948a):

\[
K = \frac{P - \Delta p_\text{st}}{\Delta p_\text{st}} \cdot RW_{\text{st}}
\]  

(4)

gives the diver balance constant, \( K \). For \( \phi_M = 1.00 \) the constant equals the reduced weight (mg) of the diver. Use the expression:

\[
RW_x = K \cdot \frac{\Delta p_x}{P - \Delta p_x}
\]  

(5)

to obtain the reduced weight \( (RW_x) \) of the unknown. The value \( P \) (mm H₂O; 1 atm. ~ 10300 mm H₂O) is the flotation pressure of the empty diver (cf. Holter’s account, this volume pp. 113–114). In the formula changes (due to expansion and compression of the air bubble) of capillary forces residing in the surface of diver’s air bubble are neglected. It is essential that prior to each series of weighings the experimenter equilibrates the pressure in the compensation bottle with the atmosphere, that he again closes the bottle off from the atmosphere, and that he reads the barometric pressure.

VII. SENSITIVITY AND PRECISION OF METHOD

The jump in equilibrium pressure caused by loading the balance is defined with an absolute error of 2–3 mm H₂O (p. 71), perhaps slightly better for larger divers. The diver’s absolute \( RW \) sensitivity \( (S) \) is therefore
obtained if, say, 3 mm is inserted for $\Delta \rho$ in equation (5); $P$ is close to 10,000 mm H$_2$O. In water, $K$ equals $V_D$. Thus:

$$S(mg) = V_D(\mu l) \times 3 \times 10^{-4}$$

It is seen that $S$ varies with $V_D$. The smallest diver balance used thus far (Prescott and Mazia, 1954; Prescott, 1955b, 1956) weighed with an error ($S$) of $\pm 2 \times 10^{-7}$ mg RW. $V_D$ must have been close to $10^{-3} \mu l$. For the smallest diver ($4 \times 10^{-2} \mu l$) described in the original publication (Zeuthen, 1948a), $S$ was $\pm 10^{-5}$ mg RW (Table II).

Within the limits of diver sizes used thus far ($0.001 \mu l$ to $350 \mu l$ (Løvtrup, 1953a)), the precision (percentage accuracy) of the instrument is roughly represented by the function: $2-3$ (mm H$_2$O): $\Delta \rho$ (mm H$_2$O) $\times 100$. Thus, for $\Delta \rho = 100$ mm H$_2$O, the precision is $2-3\%$; for $\Delta \rho = 1000$ mm H$_2$O, the precision is closer to $0.2-0.3\%$. This is reflected in the standard deviation and in the percentage error of the weighings shown in the two lower columns of Table II: (a) in the diver weighings of gold standards C and D, $\Delta \rho$ was close to 1000 and 500 mm H$_2$O; (b) in the determinations of the reduced weights of polystyrenes I–V, $\Delta \rho$ was about 200–400 mm H$_2$O; (c) in the weighings of the amoebae 1 and 2, $\Delta \rho$ was around 100 mm H$_2$O.

Divers with $V_D = 0.1 \mu l$ and larger are handy instruments, easy to make and to work with. A bit of practical experience and a glance at Table I will convince most investigators that, concerning size, a $10^{-3} \mu l$ diver approaches a lower practical limit. Some newer technical developments concerning the use of ultra-small divers will soon be described by Brzin and Zeuthen (1961) and by Løvlie and Zeuthen (1961). Løvlie and the writer are now exploring what can be gained by increasing the sensitivity of the manometer, and by pipetting in such a way that no mechanical disturbances are produced.

VIII. WHAT IS MEASURED BY THE RW?

The reduced weight of a cell ($RW_C$) is the sum of the reduced weights of its constituents. Let $Pr$, $NA$, $Cb$, $F$, $PL$ and $S$ stand for proteins, nucleic acids, carbohydrates, fats, phospholipids and salts, respectively. Then, if we consider only the major constituents of the cell:

$$RW_C = RW_{Pr} + RW_{NA} + RW_{Cb} + RW_{F} + RW_{PL} + RW_{S} + RW_{\Pi D} \quad (6)$$

The relation between reduced ($RW$) and absolute weights ($g$) are given by equation (2), p. 68, in the section dealing with the standards. It is seen that the $RW$ equals the absolute weight times a factor which depends on the densities of the substance and of the suspension medium.
We shall operate with the following densities (cf. Holter and Zeuthen, 1948): \( \phi_{Pr} = 1.35; \phi_{N_A} = 1.63 \) (sodium salt of DNA (Astbury, 1945)); \( \phi_{Cp} = 1.55; \phi_{F} = 0.925; \phi_{PL} = 1.03; \phi_{S} = 2.1 \) (Holter and Zeuthen, 1948, and general considerations); \( \phi_{H_2O} = 1.00; \phi_{M} = 1.00 \). For weighings in pure water equation (6) then becomes:

\[
RW_C = g_{Pr} \times 0.26 + g_{N_A} \times 0.39 + g_{Cp} \times 0.35 - g_F \times 0.08 + 
+ g_{PL} \times 0.03 + g_S \times 0.52
\]  

(7)

It is seen that the percentage weight contribution of the substances is somewhat different when we consider the reduced and the absolute weights. This is most apparent when we compare the light substances, viz. the fats and the phospholipids, separately and together, with the heavy substances, viz. the proteins, the nucleic acids and the carbohydrates. The neutral fats \( F \) are lighter than water and contribute negative \( RW \), or positive buoyancy. The phospholipids contribute only slightly to the \( RW \). The buoyancy of 1 mg fat corresponds to the \( RW \) of 2.6 mg phospholipid. In most cells the phospholipids correspond to more than 50% of all lipids (Bloor, 1943). So, in cells that are not excessively rich in lipids, the contribution of the lipids to the \( RW \) may be near nil. Consequently, the \( RW \) of a cell is to be considered a measure of the cell’s lipid-free, dry mass. Table III shows the example of a hypothetical cell (Cell I) which lives in and is weighed in pure water. The lipids do not contribute much to the \( RW \), and the water not at all. Per unit dry weight

| TABLE III |
|-----------------|-----------------|
|                | Cell I          | Cell II         |
|                | \( (\phi_M = 1.00) \) | \( (\phi_M = 1.03) \) |
| Weight         | \( RW \)        | Weight         | \( RW \)        |
| Proteins       | 8.0             | 8.0             | 1.90            |
| Nucleic acids  | 1.0             | 1.0             | 0.37            |
| Carbohydrates  | 2.0             | 2.0             | 0.67            |
| Neutral fats   | 0.5             | 0.5             | 0.057           |
| Phospholipids  | 0.5             | 0.5             | 0.00            |
| Salts          | 0.2             | 0.2             | 0.90            |
| Water          | 87.8            | 87.1            | 2.61            |

|                | dry wt. 12.2    | dry wt. 12.9    | 0.377           |
|                | wet wt. 100.0   | wet wt. 100.0   |                 |

\( ^a \) Cf. text.
the carbohydrates contribute 35%, and the nucleic acids 50%, more \( RW \) than do the proteins. The total \( RW \) is 26·6% of the total dry mass. Had the cell contained only protein and water, the \( RW \) of the cell would have equalled 26% of the dry weight. A second example is represented by Cell II which has the same chemical composition as Cell I, except for the salts. This cell is supposed to be in osmotic equilibrium with serum \((\Phi_M = 1·03)\) in which it is weighed. It carries ions which are heavier than the outside ions (e.g. \( K^+ \) instead of \( Na^+ \)) in amounts which, for convenience, are assumed to contribute to the cell the same \( RW_s \) as in the case of cell I. Because of the higher value of \( \Phi_M \), all substances, including the cell water, get a lower \( RW \) (or a higher buoyancy) in Cell II than in Cell I. The resulting buoyancy of the cell water balances a considerable part of the \( RW \) of the dry matter, and the total \( RW \) of Cell II is therefore reduced to 11·6% of that of Cell I. These considerations are meant to demonstrate that for cells which are weighed in pure water, the cell water does not contribute to the total \( RW \). However, when the weighing is performed in a medium which is made heavier by the addition of non-penetrating, high-molecular substances (in the present case, proteins), the cell water contributes buoyancy, or negative \( RW \). It will be shown later that these observations have been put to use in various types of measurements.

IX. APPLICATIONS OF METHOD

A. METABOLISM OF STARVING AMOEBAE

The decreases in \( RW \) resulting from starvation have been followed in seven individual \textit{Chaos chaos} (Holter and Zeuthen, 1948). In the course of 16–33 days, the \( RW \) and the respiratory rate decreases to one-third or one-sixth of the initial value. The amount of oxygen used in respiration is constant at 0·01 \( \mu l \) per hour and per \( \mu g \) \( RW \) (Fig. 6). Apparently, \( RW \) is the expected good measure of the respiring mass. We shall now compare the total oxygen taken up during starvation, and the total \( \Delta RW \) occurring during the same period. We shall then investigate what can thereby be learned about the amounts of the various substrates combusted. We shall accept a value of 1·00 for the density of the amoebae’s mixed lipids. Then, \( \Delta RW \) is due to the combustion of the heavy substrates alone, and if we disregard the part taken by the carbohydrates, of the proteins. It is permitted to neglect the carbohydrates because the argument presented is thereby only weakened. On this basis, \( \Delta RW \) (Table IV, d) may be calculated as \( \Delta \)-protein (Table IV, e) by the multiplication with a factor 3·7 (cf. note in Table IV). A value (“protein-O\(_2\)”, Table IV, g) may be calculated for the amount of O\(_2\) necessary to remove the proteins by complete combustion. Oxygen consumed in
TABLE IV

STARVATION IN THREE AMOEBAE (*Chaos chaos*)
(Table modified from Holter and Zeuthen, 1948)

<table>
<thead>
<tr>
<th>Amoeba No.</th>
<th>3</th>
<th>7</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>a. Initial RW (μg)</td>
<td>1.95</td>
<td>1.56</td>
<td>0.99</td>
</tr>
<tr>
<td>b. Initial vol. (μl)</td>
<td>0.098</td>
<td>0.078</td>
<td>0.05</td>
</tr>
<tr>
<td>c. Final RW (μg)</td>
<td>0.59</td>
<td>0.25</td>
<td>0.36</td>
</tr>
<tr>
<td>d. ΔRW (μg)</td>
<td>1.36</td>
<td>1.31</td>
<td>0.63</td>
</tr>
<tr>
<td>e. Δ protein (μg)</td>
<td>5.03</td>
<td>4.85</td>
<td>2.33</td>
</tr>
<tr>
<td>f. Total O₂ consumed (μl)</td>
<td>7.95</td>
<td>5.58</td>
<td>3.90</td>
</tr>
<tr>
<td>g. Protein-O₂ (μl)</td>
<td>5.13</td>
<td>4.95</td>
<td>2.38</td>
</tr>
<tr>
<td>h. Lipid-O₂ (μl)</td>
<td>2.82</td>
<td>0.63</td>
<td>1.62</td>
</tr>
<tr>
<td>i. Δ lipid (μg)</td>
<td>1.31</td>
<td>0.30</td>
<td>0.76</td>
</tr>
<tr>
<td>k. Δ protein combusted (μg protein)</td>
<td>3.80</td>
<td>16.20</td>
<td>3.10</td>
</tr>
<tr>
<td>l. Minimum initial lipid/cell (i/b) vol. %</td>
<td>1.40</td>
<td>0.39</td>
<td>1.50</td>
</tr>
<tr>
<td>m. Days of starvation</td>
<td>28</td>
<td>33</td>
<td>25</td>
</tr>
</tbody>
</table>

Line e: Calculated from the ratio: 1 μg RW ~ 3·7 μg protein.
Equation (7) indicates a relationship between RWᵣ and gₚₓ × 0·26 (or between 3·8 × RWᵣ and gₚₓ). During starvation the volume of water in which the combusted proteins were dissolved is excreted (Zeuthen, 1948b). Assuming that the protein/salt ratio is regulated to constancy throughout starvation there will be a correction for salts excreted. This changes the factor 0·26 to 0·27 and the factor 3·8 to 3·7.
Line g: 1 μg protein ~ 0·98 μl O₂.
Line i: 1 μg lipid ~ 0·47 μl O₂.

excess of this (Table IV, h) must be “lipid-O₂”. The amount of combusted lipid calculated (Table IV, i) is a minimum measure of the total lipids present in the non-starved cell. The main result of the investigation is that the share of the lipids in the metabolism of the amoeba is considerably higher than can be accounted for by the amount of microscopically visible fat droplets. The latter is at the most 0·5% of the amoeba’s wet weight, (Andresen and Holter, 1944); the former may amount to 1·5% (Table IV, l). Vacuole coalescence (Andresen and Holter, 1944) appears to take place whenever the amount of cytoplasmic masked lipids has seriously diminished. At this time the visible fat globules have not demonstrably decreased in amounts.

Quite often the starvation curve for RW is triphasic suggesting that heavy substrates (carbohydrates or proteins, or both) are combusted early and late in starvation, while light substrates (lipids and fats) are combusted in mid-starvation (Holter and Zeuthen, 1948; Zeuthen, 1948b).
Fig. 6. A. Decrease in reduced weight per day in seven starving amoeba (Chaos chaos) as a function of the starvation days. B. Parallel decrease in respiration. C. Constancy of the amount of respiration per unit RW. (From Holter and Zeuthen, 1948.)
B. Metabolism of Developing Amphibian Embryos

Based on measurements of changes in RW and in the rate of oxygen consumption (Fig. 7), Lovtrup (1953a,b) studied the succession of energy sources in the development of *Amblystoma mexicanum*. Chemical analyses showed that protein reserves were not used during the first 12 days of development. It was therefore considered that either carbohydrates or neutral fats were combusted. Two equations were set up from which the combusted absolute weights of the two could be calculated. (Lovtrup selected a value 0·8 for the density of the combusted lipids which the present author considers too low. In the present context this point is not of high significance. However, equation (8) is given with the constants from equation (7).)

\[
-\Delta RW = 0.35 \times \Delta g_{Ch} - 0.08 \times \Delta g_{F}
\]

\[
\Delta Q_{O_2} = 0.83 \times \Delta g_{Ch} + 2.02 \times \Delta g_{F}
\]

\(\Delta RW\) (μg RW lost (-) or gained (+)) and \(\Delta Q_{O_2}\) (μl O₂ taken up in the course of the same time) are both measured. \(\Delta g_{Ch}\) (μg carbohydrate) and \(\mu g_{F}\) (μg fat) are calculated.

Chemical analysis shows that after 12 days most of the carbohydrate is gone, and that protein now begins to disappear. Two other equations (the first of which is given here with the constants from equation (7)) are now used for calculating the amount of protein (μg) and of fat (μg) used:
The calculated curves \((\phi_F = 0.8)\) for the consumption of carbohydrate, fat and protein are shown in Fig. 8. The chemical determination of carbohydrate disappeared is indicated by the level of the dotted line. It seems that the energy sources are used in the succession: carbohydrate, fat, protein.

![Diagram of energy consumption](image)

**Fig. 8.** Total consumption of energy sources in developing *Amblystoma* embryos in the course of development. (From Lövtrup, 1953a.) (The constants used are Lövtrup’s.)

The use of \(\Delta RW\) values in the study of the metabolism of starving organisms involves many uncertainties: What is the density of the energy-substrates? Are the combustions complete? Are the end-products eliminated? Do the inorganic constituents change significantly? Broad conclusions may be drawn after thorough consideration of the uncertainties involved. The validity of many pooled guesses may be checked by chemical analyses.\(^1\) The \(RW\) data may appear to lend themselves to simple interpretations as those suggested above. It may also become apparent that complicating factors, in themselves perhaps interesting, significantly blur the calculated picture. In *Amblystoma*, the early decreases in \(RW\) could not be fully explained by the extent to which oxygen is consumed. Despite efforts, the increase in \(RW\) from the 12th to the 17th day (Fig. 7) is not easily explained solely by the suggestion that lipids are combusted. The complicating factors remain unknown.

\(^1\) In theory it should be possible to account for the separate participation of proteins, carbohydrates and lipids in the respiratory metabolism by continuously following \(RW\), \(O_2\)-consumption, and the elimination of the end-products of the protein catabolism.
C. Growth in Single Amoebae

Diver-weighings at frequent intervals of single *Amoeba proteus* (Prescott, 1955b, 1956) have shown that the growth of the cell is most rapid immediately following division. The rate declines steadily through most of the interphase, and virtually ceases during the period of about a few hours before division (Fig. 9). Data for growth as measured by *RW* should be interpreted with the same caution as discussed for degrowth during starvation. It is therefore useful that Prescott checked his *RW* data by independent measurements of cellular protein (using a selected group of cells for one measurement), and of cell volume. In both measurements he obtained curves which closely resembled the *RW* curves. Artificially produced unequal division results in sister cells of different sizes. A large cell grows more slowly but still has a shorter than normal generation time. A small cell grows more rapidly and divides later than normal. In all cases normal cell size is reached at the normal division following the unequal one. A pre-division period of non-growth is always apparent.

D. *RW* as a Reference Measure of Mass

The reduced weight of whole *Chaos chaos* or of parts thereof was used as a measure of mass, to which was referred activities of proteolytic enzymes (Holter and Løvtrup, 1949), of succinodehydrogenase and cytochrome oxidase (Andresen, Engel and Holter, 1951), and of acid phosphatase (Holter and Lowy, 1959). In other studies fragments of the myxomycete *Physarum polycephala* were similarly studied (Holter and Pollock,
Changes in the content of peptidases during amphibian embryogenesis at different temperatures were referred to the RW-changes (Lovtrup, 1953c). Several of the above papers include observations on the weighing technique, and Lovtrup’s contribution (1950a) is devoted to this topic.

E. Determination of Cell Volume and of Cell Density

A cell (Chaos chaos) is weighed, first in water (RW₁), and then (RW₂) in water to which has been added a high-molecular, non-penetrating substance which confers a slightly higher density to the medium. From these two reduced weights, the volume (V_c), density (φ_c) and absolute weight (g_c) of the cell may be calculated using the equations (Zeuthen, 1948a,b):

\[ V_c = \frac{RW_1 - RW_2}{\phi_2 - \phi_1} \]  
\[ \phi_c = \frac{\phi_2 \times RW_1 - \phi_1 \times RW_2}{RW_1 - RW_2} \]  
\[ g_c = \frac{\phi_2 \times RW_1 - \phi_1 \times RW_2}{\phi_2 - \phi_1} \]

φ₁ is the density of the first, φ₂ of the second medium. Zulkowski soluble starch (3·3 g per 100 ml) was used as the high-molecular substance.

If a density gradient is available, the second weighing may be replaced by a direct density measurement of the cell (Lovtrup, 1950b). In the above equations, RW₂ is then zero, and \( \phi_c \) equals \( \phi_2 \). It is now recommended (Holter and Møller, 1958) that Zulkowski starch be replaced by another polymer, Ficoll. This substance is less liable to become infected and does not precipitate in the course of time as starch tends to do, particularly when salts are present.

The “two-weighings” method has been checked against polystyrene standards. Serial volume determinations deviated less than 4%. The mean differed 6% from the mean of results obtained with two other methods (Zeuthen, 1948a). The density gradient method operates with about the same accuracy (Lovtrup, 1950b; Lumsden and Robinson, 1953). Density measurements on Chaos chaos using Zeuthen’s method averaged 1·019. The density gradient yielded results which averaged 1·018 (Lovtrup, 1950b), 1·018 (Holter and Lowy, 1959), and 1·017 (Cowey and Holter, 1961).
When cholinesterase (ChE) splits acetylthiocholine (AThCh), equimolar amounts of acetic acid and of thiocholine are liberated. The acetic acid liberated by the ChE of a single end-plate from mouse gastrocnemius may be measured (Brzin and Zajicek, 1958) with the Cartesian ampulla-diver (Zajicek and Zeuthen, 1956). Thiocholine may be precipitated as Cu-thiocholine (Koelle and Friedenwald, 1949). The rate at which the precipitate forms in and on a single end-plate may be determined by continuous diver-weighing (Brzin and Zeuthen, 1961). We ran the two tests in parallel. The gasometric method was used as the standard with which the gravimetric test was compared. A $5 \times 10^{-4}$ ml balance with a cup made of a plastic lighter than water (Hagens, 1958) was used. In the two tests, the incubation medium was identical except for the buffers (Table V). Table VI compares the molar amounts of acetic acid formed (a) and of Cu-thiocholine precipitated (c, d) by single end-plates from mouse gastrocnemius. In the calculations, a measured value of 2.16 was inserted for the density of the Cu-thiocholine. The density of the medium was 1.012. The reduced weight of 1 mg precipitate then equals 0.58 mg. The results of column c are calculated on the assumption that one copper combines with one thiocholine (Malmgren and Sylvén, 1955). The values of column d are based on the suggestion (Bergner and Bayliss, 1955).
TABLE VI
CHOLINESTERASE ACTIVITY OF SINGLE END-PLATES FROM MOUSE GASTROCNEMIUS MUSCLE

<table>
<thead>
<tr>
<th>Medium</th>
<th>Gasometrically</th>
<th>Gravimetrically</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$m\mu E \times 10^{-2}/h/E.pl.$</td>
<td>$RW, \mu g \times 10^{-3}/h/E.pl.$</td>
</tr>
<tr>
<td></td>
<td>$a$</td>
<td>$b$</td>
</tr>
<tr>
<td>pH</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>6.14</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>9.1</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td>5.36</td>
<td>6.2</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>2.43</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>1.62</td>
</tr>
<tr>
<td></td>
<td>2.7</td>
<td>1.82</td>
</tr>
<tr>
<td>Means</td>
<td>7.28</td>
<td>2.26</td>
</tr>
</tbody>
</table>

$m\mu E$ = millimicroequivalents. 
$E.pl.$ = motor end-plate.

1952) that copper combines with two thiocholines. The averages of columns $a$ and $d$ are statistically different ($P = 0.01$); so are the averages of columns $a$ and $c$ ($P < 0.001$). Thus, whether we use the one or the other equation for the composition of the precipitate, it must be concluded that a considerable part of the thiocholine actually formed fails to come down in or on the end-plate, or in its near surroundings represented by the cup of the diver balance.

It is thus clear that the thiocholine gravimetric method is not quantitative. Other histo- and cytochemical methods may be. Some of them would lend themselves to gravimetric measurement such as here attempted for the Koelle-Friedenwald procedure. Figure 10 shows the diver balance and an end-plate falling on to it. The loaded diver is transferred to the flotation vessel in the narrow pipette shown.

G. THE BUOYANCY OF PLANKTON DIATOMS

Observations by Gross on marine plankton diatoms (notably *Ditylum brightwelli*) had indicated that, under suitable physical conditions,
the specific gravity of these cells equals that of sea water. In cultures, the cells remain suspended throughout the water column and only sink to the bottom of the culture vessel when the population density becomes very high, when the light is dim, or when the temperature gets too high or too low. The heavy cells (resting spores) show the appearance indicated in Fig. 11 (b), in contrast to the appearance (a) of the suspended vegetative cells. In the resting spore, the plasma membrane is retracted from the siliceous cell wall, and the protoplast is shrunken to a small spherical body. In the course of a few hours under optimal conditions, the resting spores may germinate and form vegetative cells capable of keeping themselves suspended, or of rising in the sea water.

Fig. 10. A motor end-plate from rat gastrocnemius, with a piece of muscle fibre attached to it, is falling on a 0.001 μl diver-balance. The end-plate is made visible by faint staining with Cu-thiocholine precipitate. It appears white on the picture which is a photographic negative. (From Brzin and Zeuthen, 1961.)
From such observations it was concluded that the cell sap which fills the bulk of the vegetative cell is lighter than sea water. It is expelled when the resting spore is formed. From microscopic measurements, the average volume of the whole resting cell was calculated to be $13.6 \times 10^{-4}$ μl. The average volume of the shrunken resting spore was $1.1 \times 10^{-4}$ μl. The difference, equal to the volume of the expelled sap, was $12.5 \times 10^{-4}$ μl. The $RW$ of a single resting spore (120–394 were weighed together) was $3.12 \times 10^{-6}$ mg. Thus, in the vegetative cell, the buoyancy of $12.5 \times 10^{-4}$ μl sap balanced $3.12 \times 10^{-6}$ mg $RW$. From this it was calculated that the density of the cell sap was lower by 0.0025 than the density of the sea water (Gross and Zeuthen, 1948). It was suggested that the mechanism underlying buoyancy in plankton diatoms is similar to that involved in the flotation of $Halicystis$ cells, and consists in the maintenance of very low concentrations of the relatively heavy divalent ions in the cell sap. Various experiments with respiratory inhibitors demonstrate that this would be the result of a steady expenditure of energy.

H. $RW$-Changes through Cleavage in Developing Sea Urchin Eggs

The reduced weight of small samples (303–460) of simultaneously dividing, naked eggs of $Psammechinus$ $micro$-$tuberculatus$, was followed through cleavage (Geilenkirchen and Zeuthen, 1958). The normally
cleaving eggs lose $RW$ faster than can be accounted for solely by combustions; the same was found for eggs in which cleavage was suppressed by 0·0001 M colchicin while a nuclear rhythm persisted. It was suggested that there is uptake into the cells proper of small volumes of water or of saline lighter than sea water. The swelling may be of the order 0·15–0·6% per division cycle (this cannot be microscopically observed), slightly more

---

**Fig. 12.** *Psammechinus microtuberculatus*. Exp. 14/4'55. Rhythm and overall changes in $RW$ of dividing eggs, and mitotic nuclear rhythm plotted against time. Abscissa: time scale. Right ordinate: $RW$/egg in $\mu g \times 10^{-2}$. Curve IIa: measured overall $RW$ change per egg during the experiment. Curve II: experimental points plotted on curve IIa as a base-line. (From Geilenkirchen and Zeuthen, 1958.)
or less, depending on the gain or loss of dry matter from routes other than combustion.

The loss in $RW$ goes in mitotic steps, in the normal eggs through division cycles 1–5 or longer, in the colchicin eggs through cycles 1–3. The $RW$ of the samples corresponded to values of $\Delta p$ of the order of 1000–2000 mm $H_2O$. The amplitude of the $RW$ variations (Fig. 12) was 0·03–0·1 per cent of this. The $RW$ rhythm can be demonstrated in a reproducible manner only by the use of a sensitive manometer (Zeuthen, 1953). The use of a drifting bath in which temperature oscillations cannot occur (Zeuthen, 1960) was found to be essential. There are many possible explanations of the observed $RW$ rhythm. The $RW$ rhythm might be recalculated into changes of “chemical volume” of a closed system. A 0·03% periodic $RW$ change would then correspond to a volume change of $2 \times 10^{-5}$ ml/g egg.

This value should be compared with Hartmann’s (1934) value ($5 \times 10^{-5}$ ml/g) for frog gastrocnemius muscle tetanised for 2 sec. This comparison makes us prefer a less dramatic interpretation according to which the swelling mentioned before is discontinuous. It is recalled that an egg which is suspended in sea water, acquires a lower submerged weight if it swells by the uptake of $H_2O$ or of water low in salts. For every division cycle the cells proper may swell between 0·15 and 0·6%, however, in such a way that the net inflow of water should be interrupted at every anaphase-telophase. There is evidence of a reduced weight rhythm also in other marine eggs.

I. STUDIES WITH DEUTERIUM OXIDE

The Cartesian diver balance has been proposed as a tool for studying the exchange of water across cellular membranes using $D_2O$ as an indicator (Pigoń and Zeuthen, 1951). The exchange in single Chaos chaos (Levtrup and Pigoń, 1951), single Amoeba proteus (Prescott and Mazia, 1954) and in eggs of the frog and of the zebra-fish (Prescott and Zeuthen, 1953), was studied extensively with this method. The information obtained is about volumes of water exchanging, and about rates at which $D_2O$ crosses the membrane. In the experiment, the cell is transferred from a $H_2O$-environment to the cup of a diver which floats in a balanced salt solution containing low (5–15) percentages of $D_2O$. (The reverse experiment may also be performed.) The increase in $RW$ which results from the replacement of inside cell water with outside $D_2O$ is recorded by the change ($\Delta p$) in equilibrium pressure of the diver-cell system (Fig. 13). When, for each new interval of time, the logarithm of the $\Delta p$ is plotted against the time of the experiment, a straight line is obtained,
showing that all water molecules in the cell have the same chance of exchanging with the environment. Generally, it takes a couple of minutes before reliable measurements can be obtained. This is due partly to disturbances at the time of pipetting, and partly to the fact that a layer of H\textsubscript{2}O adheres to the cell for some time after it has been dropped into the D\textsubscript{2}O-medium. It is therefore useful to allow the cell to drop a few centimetres through the D\textsubscript{2}O-medium before it comes to rest on the diver’s cup. Extrapolation to zero time gives the value of log \Delta p when the cell contains only H\textsubscript{2}O. The time \((t = \frac{1}{2})\) at which \Delta p has dropped to \frac{1}{2} and log \Delta p has decreased by 0.3 is the time when half the cell H\textsubscript{2}O has exchanged with the outside H\textsubscript{2}O–D\textsubscript{2}O. The time \((t = \infty)\) at which the cell has reached a constant RW in the new medium is the time at which all the cell water has exchanged. From the records, \(RW_{t=\infty}, RW_{t=1/2}\) and \(RW_{t=0} = 2(RW_{t=\infty} - RW_{t=1/2})\) can be obtained. The amount of exchanging water in a cell \((V_{H\textsubscript{2}O})\) may be calculated from the expression:

\[
V_{H\textsubscript{2}O} = \frac{RW_{t=\infty} - RW_{t=0}}{\phi_2 - \phi_1}
\]

where the denominator is the difference in density between heavy and light medium. Values for \(V_{H\textsubscript{2}O}\) thus measured in single frog’s eggs check closely (Prescott and Zeuthen, 1953) with determinations derived by subtracting the directly determined weight of the dry matter of a cell from its wet weight (Table VII). Thus, where studied, the exchange curves involve the total cell water.

TABLE VII
WATER VOLUMES MEASURED BY TWO METHODS\textsuperscript{a}

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Exp. No.</th>
<th>H\textsubscript{2}O vol., density method ((\mu)l)</th>
<th>H\textsubscript{2}O vol., D\textsubscript{2}O method ((\mu)l)</th>
<th>Difference ((\mu)l)</th>
<th>Per cent by volume, of H\textsubscript{2}O in the cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog</td>
<td>1</td>
<td>1.70</td>
<td>1.68</td>
<td>+0.02</td>
<td>76%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>2</td>
<td>1.47</td>
<td>1.48</td>
<td>−0.01</td>
<td>75%</td>
</tr>
<tr>
<td>Egg</td>
<td>3</td>
<td>1.54</td>
<td>1.50</td>
<td>+0.04</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.59</td>
<td>1.59</td>
<td>0.00</td>
<td>75%</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.62</td>
<td>1.64</td>
<td>−0.02</td>
<td>76%</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1.46</td>
<td>1.48</td>
<td>−0.02</td>
<td>75%</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>1.56</td>
<td>1.56</td>
<td>0.00</td>
<td>75%</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Prescott and Zeuthen, 1953.
The permeability constant $P_d$ (mm/sec) for $D_2O$ is calculated from the expression (Prescott and Zeuthen, 1953; slightly modified from Løvtrup and Pigoń, 1951):

$$\log \Delta RW = -\frac{P_d \times Q}{2 \times 3026} \times t + \log C \times (\phi_{D_2O} - \phi_{H_2O}) \times V_{H_2O}$$  \hspace{1cm} (16)

$\Delta RW$ is the change in $RW$ (mg) taking place in the time $t$ (sec) after loading the diver balance in a $H_2O-D_2O$ mixture. In practice, $\Delta RW$ at the time for half-exchange is inserted. $Q$ equals the surface area of the cell ($mm^2$) divided by the cell volume ($\mu l$) and $(\phi_{D_2O} - \phi_{H_2O})$ is the difference between the densities of pure $D_2O$ and ordinary water. $C$ is the concentration of $D_2O$ in the experimental medium in per cent (which equals the mole fraction). In practice, $C$ and $(\phi_{D_2O} - \phi_{H_2O})$ are combined because $C(\phi_{D_2O} - \phi_{H_2O})$ represents the density difference between the $D_2O$-mixture and a similar medium without $D_2O$.

![Diagram](image.png)

**Fig. 13.** Zebra fish, non-developing, shed egg in frog-Ringer containing 15% $D_2O$. $a, a'$: before shrinkage. $b, b'$: after 3 hr of shrinkage in double concentrated frog-Ringer. Scale to the left: e.p. for exp. $a$; exp. $b$ brought to the same scale using 1 min as the reference point. Scale to the right: log e.p. calculated for successive 2 min periods (curves $a', b'$). (From Prescott and Zeuthen, 1953.)

A different permeability constant, $P_f$, for $H_2O$ using an osmotic difference as the driving force may be calculated from the expression:

$$\Delta V_{H_2O} = P_f \times a \times t(c_i - c_e)$$  \hspace{1cm} (17)
\[ \Delta V_{H_2O} \] is the change (\( \mu l \)) in the water content of the cell after \( t \) sec of exposure to a hypotonic, or a hypertonic medium. \( a \) is the surface of the cell (\( \text{mm}^2 \)) and \((c_i - c_o)\) equals the osmotic concentration difference across the membrane. In Fig. 13, curves \( a, a' \) represent the results obtained for a non-developing zebrafish egg. Curves \( b, b' \) show the repetition of the experiment with the same egg, though after 3 hr of shrinkage in double concentrated Ringer. \( P_d \) and \( P_f \) show independent variation. This may be interpreted to fit in with Ussing’s views (1952) that mass flow of water, measured by the values of \( P_f \), occurs through pores in the cell surface. In *Amoeba proteus*, the value of \( P_d \), measured with \( D_2O \) (Prescott and Mazia, 1954) is identical in whole cells and in half cells which have been without a nucleus for 38 hr. In *Chaos chaos*, \( P_d \) is the same (Lovtrup and Pigoń, 1951) whether it is measured with \( H_2O, D_2O \) or \( H_2O^{18} \). The permeability constant is the same whether the suspension medium is \( H_2O \), Pringsheim solution or Pringsheim solution to which calcium ions (10 mM), sucrose (55 mM), urethane (100 mM) or azide (5 mM) has been added. The active transport across the membrane was calculated to correspond to 2–4\% of the cell volume per hour. It was suggested that the energy expenditure associated with this transport is negligible.

**X. CONCLUSIONS**

Cartesian diver balances which measure reduced weights (\( RW \)) with a sensitivity down to \( 10^{-5} \) mg are easy to make and to work with. The highest published sensitivity (Prescott, 1955b; Brzin and Zeuthen, 1961) is around \( 10^{-7} \) mg. The diver balance works in the range of single large protozoa, or other cells or fragments of cells of this size (e.g. single motor end-plates). It is always supposed that the cell does not, by its own movements, significantly influence the \( RW \). This condition is not fulfilled by flagellated or ciliated cells. No attempt has yet been made to use the diver balance for studying the capacity of cells for self-propulsion or other movement. The \( RW \) of non-mobile cells is a measure of the lipid-free, dry mass. The sensitivity in dry mass determination does not approach that obtained by interference microscopy and by X-ray absorption techniques. However, the diver method for dry mass shares with the microscopic method the essential feature that the cell is studied live, and among the three methods, the diver balance is unique for its high precision (1\%). The diver balance has been applied in studies of cell metabolism. Periodic deviations from a constant or smoothly changing \( RW \) may be demonstrated when the amplitude is as low as 0.03\%. The balance has also been used in cytochemical studies. It has been found particularly useful in the study of cell water.
Divers with air bubbles down to $0.1 \mu l$ are easy to make and to work with. Divers down to a few thousandths of this have been fabricated and used by skilled workers.

REFERENCES


