THE PERMEABILITY OF TOLYPELLOPSIS CELLS FOR HEAVY WATER AND METHYL ALCOHOL

BY

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Introduction.

Among the substances most often used in studies of cellular permeability water occupies a very special position. Although the monovalent alcohols and especially the methyl alcohol, considered as methylated water, form a connecting link between the water and organic anelectrolytes, only very few of the permeability series so far published comprise the water or the monovalent alcohols along with the other anelectrolytes.

On the other hand there is no lack of special studies concerned with the water or alcohol permeability of many different cell types, but their results are on the whole not very accurate and cannot be directly compared with those of the systematic permeability studies.

This is due, mainly, to two causes.

1. It is usually necessary to study the permeation of water as a mass movement or filtration process, brought about by differences in osmotic or hydrostatic pressure, while the permeation of other substances is studied as a diffusion process. The results are therefore expressed in totally different units of measure\(^1\). It is true that the osmotic pressure can be described as a water concentration difference and thus the permeation rate for water expressed in the same way and by the same units as employed for other compounds; but even so it is possible to doubt whether the result would be the same if the permeation of water could take place as diffusion pure and simple, like that of other substances.

2. The second cause which compromises the comparison of water and alcohol with the rest of the anelectrolytes is the extremely high permeation rate of the firstnamed substances which makes sufficiently accurate determinations extremely difficult to obtain. In plant cells we have as a further difficulty the effect of the cellulose wall which reduces the permeability to an extent which it is usually impossible to determine.

The first difficulty can be overcome by means of heavy water, which opens up the possibility of working with a solution of water in water and allows a comparison with, say, alcohol in water as quite similar diffusion processes.

\(^1\) Comp. BACHMANN (1939) and the discussions by FREY-WYSSLING and v. RECHENBERG-ERNST (1943) which latter are not, however, quite to the point.
There is no doubt a definite difference between the rates of permeation of ordinary and heavy water (Brooks 1935, Parpart 1935, Brooks 1937), but the permeation mechanism should be practically the same for both.

In an earlier study (Wartiovaara 1942) the writer was able largely to overcome the technical difficulties due to high permeation rates by measuring the rates at which the compound dealt with would come out from large cells of Characeae into small volumes of water. The quantity obtained in each experimental period is thus directly determined by analysis. Using a somewhat altered setup the permeation of methyl alcohol and heavy water could be measured easily and with sufficient accuracy in periods down to 10 seconds.

The experiments to be described were carried out on the internodial cells of Tolypellopsis stelligera, an alga belonging to the Characeae, partly because these cells are well suited for the purpose, but also because they are nearly related to the Chara ceratophylla, on which Collander and Bärlund made their extensive permeability studies, and can be taken to show very similar permeabilities (Wartiovaara 1942 p. 75).

Methods.

The plants used were collected at the southern shores of Finland and sent to Copenhagen packed in moist paper. They were kept there for 7 weeks in artificial brackish water of $1^{0}/_{60}$ salinity at $12^\circ$ C and exposed only to a weakened daylight. The growth during this period was very slight. The plants which were collected about the end of their natural vegetation period were evidently in a state of rest, very favourable from the point of view of the uniformity of the material for physiological purposes.

Single internodial cells were prepared a few hours before each experiment. Their diameter varied from 0.6 to 0.8 mm with a length of about 70 mm. They were regular cylinders with a volume of about 30 $\mu$l. Each experiment was made on one single cell.

The essential part of the apparatus shown in fig. 1 is a glass tube (d) about 4 mm in diameter (fig. 2), expanded at the top and provided with a side tube, while the lower end is narrowed down to capillary dimensions. The cell to be studied is placed in this tube and pressed lightly against one side by means of two 0.5 mm glass rods sealed together at both ends (fig. 2, right). In consequence of this arrangement air bubbles can glide freely upwards and break without producing spray, and when the tube is emptied the water will flow off quickly and almost completely.

The tube is first filled with a solution of the substance to be studied, which is kept in contact with the cell for several minutes and well mixed by air.
bubbles sucked through from below. Thereupon this solution is allowed to drain off, a water reservoir (b) is connected to the top of (d), a piece of rubber tubing (i) to the side tube and the whole is arranged in a waterbath (c). This is filled with water of the desired temperature which is kept mixed by air. The opening of the reservoir (b) is so narrow that the water cannot flow out except under pressure. Pressure can be applied through (g, k, l) and causes an air bubble to enter (b). By the same pressure the solution is emptied out from (d). When next suction is applied (h, k, l) a definite volume of water is let out from (b) and covers the cell and at the same time air begins to bubble through from (e). The volume of water, let out every time suction replaces pressure, is regulated by the rubber membrane (a) which is adjusted by a screw.

The connection of the apparatus with either the suction or the pressure was brought about instantaneously by a clip (i). Suction and pressure were regulated to about the same difference from the atmosphere of about 20—50 cm water. The capillary (k) regulates the flow of air and slows down the rise in pressure sufficiently to allow the solution to drain off from the walls of the tube (d) and the cell. The dimensions of the separate parts were tried out until a volume of water of 100—200 μl could be exchanged in one second.

Because the heavy water is hygroscopic the air used for mixing had to be dry, and at the same time the lower end of (d) had to be easily accessible in order to collect the solution samples. The dry air was let in through (e). Even if this was perhaps unnecessary on account of the low concentration of the solutions and the brief experimental periods, the tube was useful as a protection for the capillary. When the solution had to be changed a micro

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1 This detail was not fully satisfactory as the volume variations were too large. Some kind of pump would no doubt be better.
test tube was introduced so that the capillary reached well into it and for one second pressure was applied instead of the normal suction.

Before any determinations of the diffusion from the cell were made the water was changed several times during a period corresponding more or less to the elimination of one half of the available quantity of the substance to be determined, in order to have a regular concentration gradient. Then followed the changes in accurately measured periods of the same length, and the solutions emptied out were collected for analysis. The sampling tubes were closed with paraffin stoppers.

The solution used for the experiments was distilled water with 30 mg/l CaCl₂ and neutralized to pH 7 with 13.7 mg/l NaHCO₃. This solution corresponds fairly well to the natural environment of the plants and at the pH of 7 is not too sensitive to the CO₂ of the atmosphere. All experiments were made at 20° C.

The D₂O had a concentration between 30 and 50 %. The same quantity of 0.3 ml could be used to saturate cells several times before becoming too dilute.

From the point of view of the accuracy of the results the highest possible concentration is to be preferred. Even concentrations of about 100 % do not apparently damage the cells, but when brought from this into ordinary water the cells would often burst. This never happened with 50 % D₂O. The plasma rotation continued undisturbed throughout, and the cells would keep alive at least for several days afterwards.

The methyl alcohol was used as 5 %, that is about 1.6 molar, solution. It had no visible effect on the cells during or after the experiments. The narcotic effects of methyl alcohol on plant cells is known to be slight and the osmotic effect small only.

The volumes of the water samples were determined by weighing and no account was taken of the very slight differences in specific gravity.

The concentration of DHO in the samples was at first determined in the usual way, after purification by repeated distillation, by determination of the specific gravity of the distillate according to the method of LINDESTROM-LANG (1938), in which drops of the unknown solution are placed in a vertical tube containing mixtures of bromo-benzol and gasoline, showing a regularly increasing specific gravity from top to bottom, and their position accurately compared with drops of known specific gravity; but it was found that the concentrations of other constituents remained so constant that a direct determination of the specific gravity of the samples by the same method could give results of sufficient accuracy.¹

¹ The determinations were kindly carried out by dr. USsING and MRS. SCHAUFUSS.
The determinations of methyl alcohol were carried out as follows:

A 0.01 molar solution of potassium bichromate in conc. sulphuric acid was added to each sample by a syringe pipette (Krogh 1935). After cooling the mixture was washed into a larger vessel with about 20 volumes of water and the surplus bichromate titrated iodometrically by means of thiosulphate.

The permeation constant was calculated from the equation (Collander & Bärlund 1933 p. 26)

\[
P = \frac{v}{t \cdot q} \ln \frac{C}{C-c}
\]

in which \( P \) is the permeation constant, \( t \) the time, \( v \) the cell volume, \( q \) the cell surface, \( C \) the concentration of the diffusing substance at the beginning of each period and \( c \) the decrease in concentration during the period \( t \).

In practice the following equation was employed

\[
P = 3.45 \frac{d}{t} \log k
\]

in which \( d \) is the cell diameter in mm, \( t \) the time in minutes and \( k \) the relation between quantities of the substance diffusing out in consecutive periods. In this equation \( P \) is obtained in cm/hour, although \( t \) and \( d \) are measured in minutes and mm. The equation is obtained from (1) by changing into ordinary logarithms, substituting \( \frac{d}{q} \) for \( \frac{v}{q} \) (relation between cylinder volume and surface) and combining all constants into one. The substitution of \( k \) for \( \frac{C}{C-c} \) can be made, because the rate of diffusion at any moment is directly proportional to the inside concentration at that moment.

This latter relation is of special interest, because in my experiments the quantities diffusing out of the cell in consecutive periods are determined instead of the concentration in the cell sap. When the periods are of the same length we get a series of \( k \)-values (in principle identical) from which to calculate the permeation constant according to equation (2).

The experimental arrangement adopted makes it possible to avoid one source of error which is very common in experiments on rapid diffusion. When namely the diffusion resistance of the plasma membrane is of the same order of magnitude as that of the other constituents of the cell — including the sap — it is necessary to allow for the time which is required at first to establish a definite concentration gradient. With my arrangement it is unnecessary to begin measuring the rate of permeation just after changing from the saturating solution to water. One can wait until the concentration gradient is
stabilized. Without this arrangement it would scarcely be possible to measure the rate of methyl alcohol permeation in freshly killed cells, which show a half-value period only 3 times longer than that of a corresponding cylinder of pure water.

**Sources of systematic error and corrections.**

The very rapid permeation of the substances dealt with will cause certain rather large errors in the results as directly obtained, for which quantitative allowance must be made.

The analyses are mainly liable to positive errors. Negative errors, due e.g. to evaporation, will, on account of the low concentrations, be insignificant, but accidental, even if small, impurities will have a definite positive effect. Larger errors of this kind will show up as distinct aberrations from the curves representing the experiments.

The fact that a certain fraction of the solution in the experimental vessel is not emptied out and takes up some of the substance diffusing out diminishes the concentration gradient and causes a negative error. This error is nearly constant and can be eliminated by calculation. When the water is changed at constant intervals a definite relation is established after a few changes between the surplus left over from one change and the deficit of the next. Since the quantities diffusing out decrease regularly the surplus is always larger than the deficit and in the same proportion throughout.

The external concentration which reduces the permeation has a minimum, just when the water has been changed, and a maximum just before the next change. When the external concentration is kept low compared with the internal it can be assumed that the error of the permeation constant, as calculated directly from the analyses, is proportional to the (relative) external concentration. Just after a change of water we have approximately:

\[
\frac{\text{External conc.}}{\text{Internal conc.}} = \frac{v}{p} \frac{k - 1}{V 1-pk}
\]

and just before the next change

\[
\frac{\text{External conc.}}{\text{Internal conc.}} = \frac{v}{V} \frac{k - 1}{1-pk}
\]

in which \(v\) is the cell volume, \(V\) the external water volume, of which \(pV\) is left over, and \(k\) the relation between two consecutive quantities diffused out\(^1\). The volumes both of the cell and the external solution are easily deter-

\(^1\) If each analysis corresponds to two samples \(Vk\) is to be substituted for \(k\) in the equations (3) and (4).
minded by weighing. The residual water is determined by filling in a solution which does not penetrate the cell. When such a solution is let out and the apparatus then washed, an analysis of the washing fluid will give the residue. The highly colloidal pigment T 1824 which can easily be determined colorimetrically was employed for the purpose.

The average \( \frac{\text{External conc.}}{\text{Internal conc.}} \) which opposes the diffusion is calculated from the figures \( v=30 \mu l, V=150 \mu l, p=0.2 \). For \( k=1.5 \) we find for (3) the value 0.03 and for (4) 0.114 with the average 0.085 or about 9 \%

The result is highly dependent on the value of \( k \) and becomes 20 \% for \( k=2 \) and 36 \% for \( k=2.5 \).

The equations (3) and (4) are arrived at as follows:

The quantity diffusing out from the cell is reduced by \( \frac{1}{k} \) for each period and, since it is proportional to the inside concentration, the quantity left inside is also reduced by \( \frac{1}{k} \) during each period.

Taking the inside quantity at the beginning of the first period as unity we have at the beginning of the second period \( \frac{1}{k} \) left, and the difference \( 1 - \frac{1}{k} = \frac{k-1}{k} \) has diffused out. At the beginning of the \( n^{th} \) period we have inside the cell \( \frac{1}{k^{n-1}} \) and at the end \( \frac{1}{k^n} \) while \( \frac{k-1}{k^n} \) has diffused out.

The quantities diffusing out are reduced at each change of water to \( \frac{pV}{V} \), and at the beginning of the \( n^{th} \) period after \( n-1 \) changes of water we have left from the first portion \( \frac{k-1}{k} \) only \( p^{n-1} \frac{k-1}{k} \) and from the following increasing fractions up to and including \( p \frac{k-1}{k^{n-1}} \) or in all

\[
p^{n-1} \frac{k-1}{k} + p^{n-2} \frac{k-1}{k^2} + \ldots + p^2 \frac{k-1}{k^{n-2}} + p \frac{k-1}{k^{n-1}} = \left( k-1 \right) \left( p^{n-1} \frac{k-1}{k} + p^{n-2} \frac{k-1}{k^2} + \ldots + p^2 \frac{k-1}{k^{n-2}} + p \frac{k-1}{k^{n-1}} \right)
\]

and the relation

\[
\frac{\text{External conc.}}{\text{Internal conc.}} = \frac{v}{V} \left( k-1 \right) \left( p^{n-1} k^{n-2} + \ldots + p^2 k + p \right) = \frac{v}{V} \left( k-1 \right) \left( \sum_{1}^{n} p \left( pk \right)^{n-2} \right) = \frac{v}{V} \left( k-1 \right) \left( \sum_{1}^{\infty} p \left( pk \right)^{n-2} \right) \text{ when we put } n=\infty.
\]

Similarly we find for the end of the \( n^{th} \) period
\[
\frac{\text{External conc.}}{\text{Internal conc.}} = \frac{V}{V} (k - 1) (p^{n-1} k^{n-1} + p^{n-2} k^{n-2} + \ldots + p^2 k^2 + pk + 1)
\]

\[
= \frac{V}{V} (k - 1) \left[ \sum_{i=1}^{n} (pk)^{n-1} \right] = \frac{V}{V} \frac{k - 1}{1 - pk}
\]

The error introduced by taking \( n = \infty \) is insignificant, because the series converges rapidly towards 0 when \( p \) is sufficiently small.

The analytical results when corrected will therefore give a true picture of the diffusion, except for the irregular variations due to variations in the quantity of water left over from one period to the next. These are accidental and will check out in the mean.

The time during which the cell is dry at each change of solution causes an apparent lowering of the permeation rate. This error is very difficult to estimate, but one cannot go far wrong by judging it to make up about \( \frac{1}{2} \) the total time for each change. In the experiments on dead cells, in which the water was changed every 10 seconds, the period should therefore be reduced by 5%. In experiments on living cells the correction is smaller, because the periods were longer.

Some methyl alcohol is evaporated into the air sucked through. In a control experiment with a known alcohol solution the error on this account amounted to a few %. In the actual experiments it is largely eliminated by the calculation of the permeation constant according to equation (2), because only the alcohol diffused out into the water is exposed to evaporation and the relative loss must be approximately constant.

Because the vapour pressure is reduced more rapidly than the concentration the quantities analysed will be more slowly diminished than those actually diffusing out, but the error on the permeation constant is so small as to be completely overshadowed by the accidental variations.

The sources of error now discussed cause the permeation constant to be reduced by 10 to 12% and are easily allowed for. Two further factors viz. the diffusion resistance of the cell wall and the equilibrium concentration of the substance investigated are decidedly more important and have to be taken quantititively into account before it is possible to obtain any information of value concerning the permeability of the protoplasm membranes.

If we assume that the abolition of the semipermeability of the plasma membranes is the only change in permeability brought about by the death of the cell the diffusion resistance of the membranes can be calculated by subtracting the resistance of the freshly killed from that of the living cell. Since the diffusion resistance is inversely proportional to the permeability we have
in which \( P \) is the permeability of the plasma membranes. \( P_1 \) that of the living and \( P_d \) that of the dead cell (WARTIOVAARA 1942).

The above assumption is probably not completely correct. The diffusion resistance of the plasma between the two membranes is probably altered by the death of the cell, but it seems probable that the influence of this thin and very watery layer is extremely small whether dead or alive and probably not much higher than that of a corresponding layer of water.

When the cell dies the turgor is abolished and the volume shrinks about 5%, corresponding to a reduction in diameter of about 2.5% which is taken into account in the permeability calculations. It is possible, however, that the permeability of the cell wall is somewhat reduced by the shrinkage.

Determinations of the permeability of dead cells were made on the same cells which had served for experiments in the living state. The cell was left in the tube (d) and killed by placing this in hot water (60–70\(^\circ\)). Thereupon all substances able to diffuse out through the cellulose wall were removed by several hours treatment with running water (fig. 3). The determinations of permeability were made as with living cells.

The »non-solvent space« of Tolypellopsis cells is evidently very small. With ethyleneglycol for instance osmotic equilibrium is reached only when the concentration inside the cells is equal to that outside. DHO will no doubt behave in the same way, and it was deemed unnecessary to specially determine the equilibrium concentration. Methyl alcohol on the other hand belongs to that group of substances which are at equilibrium definitely less concentrated in the cell sap than outside, at least in the case of Chara (COLLANDER & BÄRLUND 1933). It appeared necessary, therefore, to find out whether the cells of Tolypellopsis would show the same peculiarity, because that would mean that the cell would be more rapidly saturated (and again emptied) or, in other words, that the permeation is more rapid than corresponding to the real permeability. It was to be expected, further, that no such anomaly exists in the cells when killed, so that the \( P \) value, calculated according to (5) would be even more dependent on the equilibrium content of methyl alcohol than the \( P_1 \) value.

On account of the volatility of the methyl alcohol the cell sap could not
Fig. 4. Arrangement for determining the equilibrium concentration. Above the capillary tube, containing the cells, is shown an air bubble which, sliding back and forth, causes a flow of solution through the capillary tube.

be analysed directly and the following procedure was adopted. Cells were weighed and enclosed in a thinwalled capillary with two openings (fig. 4) and rocked for about a quarter of an hour in a test tube containing a solution of methyl alcohol. The capillary was then rapidly emptied out, wiped off on the outside, transferred to a second, weighed test tube with a known amount of water and rocked until the alcohol was again eliminated. Analyses of the water then gave the total alcohol in the cells and adhering to them in the capillary. This latter amount could be determined from the weight of the second test tube after the introduction of the capillary, the weight of which + the cells alone was known. After subtraction of the adhering amount of alcohol the equilibrium concentration in the cells could be made out.

In similar determinations on killed cells the shrinkage had to be specially determined by weighing without removing them from the capillary. As the cells could not be wiped dry the capillary was filled with a solution of dialysed starch, emptied out and weighed and the quantity of starch left over washed out and determined by analysis. The determination showed a volume diminution by killing of 5%, which agrees perfectly with the decrease in weight observed when living cells are allowed to transpire in air until the turgor is lost. The killed cells would collapse when brought into a 1 molar alcohol solution. They were therefore first treated with a solution containing some NaCl and thereupon with several changes of pure alcohol solution. Shrinking at first they would thus regain their normal volume.

In the killed cells a distribution coefficient of $0.95 - 0.97$ was obtained showing that no gross errors were involved in the procedure. The fact that the value is lower than unity can be ascribed mainly to the non solvent parts of the cell wall and protoplasm which are included in the weight. On the living cells the mean of 4 determinations was $0.86$ ($0.840, 0.855, 0.850, 0.844$).

When the correction factor for dead cells is put at 1.0, which we need not hesitate to do, the corresponding factor for living cells must be raised to 0.89.

Results.

The results obtained with heavy water are summarized in tables 1 and 2 and in the curve fig. 5. In the tables both the analytical determinations and the permeation constants calculated from equation (2) without any cor-
Table 1.
Results of experiments with heavy water. Living cells. $t$ time in minutes, $d$ diameter of cells in mm., $a$ quantities eliminated in arbitrary units, $k$ relation between successive quantities.

<table>
<thead>
<tr>
<th>$t$</th>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Cell 3</th>
<th>Cell 4</th>
<th>Cell 5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d = 0.80$</td>
<td>$d = 0.70$</td>
<td>$d = 0.71$</td>
<td>$d = 0.66$</td>
<td>$d = 0.70$</td>
</tr>
<tr>
<td>0—1</td>
<td>$a$</td>
<td>66.1</td>
<td>2.32</td>
<td>80.6</td>
<td>2.29</td>
</tr>
<tr>
<td>1—2</td>
<td>28.5</td>
<td>2.41</td>
<td>26.9</td>
<td>2.21</td>
<td>30.4</td>
</tr>
<tr>
<td>2—3</td>
<td>11.8</td>
<td>2.95</td>
<td>12.2</td>
<td>2.54</td>
<td>13.5</td>
</tr>
<tr>
<td>3—4</td>
<td>4.0</td>
<td>4.8</td>
<td>3.7</td>
<td>4.7</td>
<td>8.1</td>
</tr>
<tr>
<td>Mean</td>
<td>1.13</td>
<td>0.99</td>
<td>0.89</td>
<td>0.89</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Table 2.
Results of experiments with heavy water. Killed cells.

<table>
<thead>
<tr>
<th>$t$</th>
<th>Cell 1</th>
<th>Cell 2</th>
<th>Cell 3</th>
<th>Cell 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d = 0.80$</td>
<td>$d = 0.70$</td>
<td>$d = 0.71$</td>
<td>$d = 0.66$</td>
</tr>
<tr>
<td>0°—0°20</td>
<td>29.3</td>
<td>1.86</td>
<td>41.5</td>
<td>1.66</td>
</tr>
<tr>
<td>0°20—0°40</td>
<td>15.7</td>
<td>2.15</td>
<td>25.0</td>
<td>3.43</td>
</tr>
<tr>
<td>0°40—1°00</td>
<td>7.3</td>
<td>1.16</td>
<td>7.3</td>
<td>2.08</td>
</tr>
<tr>
<td>1°00—1°20</td>
<td>6.3</td>
<td>3.5</td>
<td>6.3</td>
<td>5.3</td>
</tr>
<tr>
<td>Mean</td>
<td>2.01</td>
<td>2.39</td>
<td>2.49</td>
<td>2.32</td>
</tr>
<tr>
<td>P</td>
<td>2.56</td>
<td>2.74</td>
<td>2.91</td>
<td>2.49</td>
</tr>
</tbody>
</table>

The initial quantity of heavy water for each cell, put at 100 in the diagram, was calculated as the sum of the geometric series formed by the analytical results. In order to make the results obtained on cells of different dia-
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Fig. 5. Elimination of heavy water from living (lower curve) and dead cells. Abscissa, time in minutes, calculated for a cell diameter of 0.7 mm. Ordinate, eliminated quantities in percent of total quantity.

meter directly comparable I have used different time units on the abscissa, since the time \( t \) and the diameter \( d \) are according to equation (2) in inverse proportion. That is why only the points corresponding to cells of 0.7 mm diameter are placed above the whole minute points, although the changes of solution in all cases took place at whole minute (or 20 sec.) intervals.

In view of the difficulties due to the high permeation rate of the heavy water the values obtained on different cells show a satisfactory agreement, and it is possible to utilize them for a calculation of the plasma membrane permeability.

Theoretically this should perhaps be calculated for each cell separately, but since the dispersion of the results is due evidently more to experimental and analytical errors than to real differences in permeability of the cells it seems better to utilize the mean in which the accidental errors have been to a certain extent smoothed out.

The \( P \) value obtained on living cells is somewhat reduced by the incomplete change of solution and by the brief dry periods at each such change. According to the discussion of the sources of error the first of these factors can be put at \(-9\%\) and the second at \(-1.7\%\).

The \( P \) value should therefore be corrected by multiplication with \( \frac{100}{100-9} \times \frac{100}{100-1.7} = 1.12 \), and we have \( P_1 \) (corr.) = 1.12 \( P_1 = 1.04 \text{ cm/h} \).

The \( P \) values for the dead cells in table 2 were calculated without regard to the diminution in volume at death. This causes a positive error of 2.5 \%. The negative errors due to the changes of solution can be put at 9 \% and 5 \% and we get the final correction.
In both cases the corrections are relatively small and their possible errors cannot have any serious influence.

From the corrected values the permeability of the living protoplasmic membrane for heavy water at 20°C is calculated according to (5) as 1.6 cm/h.

This value belongs probably to the molecules DHO which are greatly in excess in dilute solutions.

When cells filled with D₂O are apt to burst when put into ordinary water this is no doubt due to the slower permeation of heavy water (comp. Brooks 1937). The difference must be pretty small, however, since only concentrations above 50% (or about 25 moles) D₂O begin to be dangerous in this respect.

In the methyl alcohol experiments the dispersion, as seen from tables 3 and 4, appears larger than for the DHO, but this is due, partly at least, to the distribution of the quantity eliminated on a larger number of analyses. This reduces the difference between consecutive analyses (k becomes smaller) and the accidental variations become more conspicuous, but the increase in dispersion is counteracted by the larger number of analyses so that the calculated mean P values amounting to 0.85 cm/h for living and 1.59 cm/h for dead cells are of about the same reliability. The curves in fig. 6 show that the exosmosis as a whole is regular and the variations in single points accidental and due to errors, analytical and others.

Table 3.

Results of experiments with methyl alcohol. Living cells.

<table>
<thead>
<tr>
<th>t</th>
<th>Cell 6</th>
<th>d = 0.66</th>
<th>Cell 7</th>
<th>d = 0.61</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>k</td>
<td>a</td>
<td>k</td>
</tr>
<tr>
<td>0⁰⁰—0³⁰</td>
<td>1.044</td>
<td>1.699</td>
<td>3.575</td>
<td>3.640</td>
</tr>
<tr>
<td>0³⁰—1⁰⁰</td>
<td>0.827</td>
<td>1.192</td>
<td>2.015</td>
<td>2.472</td>
</tr>
<tr>
<td>1⁰⁰—1³⁰</td>
<td>0.626</td>
<td>0.688</td>
<td>1.222</td>
<td>1.658</td>
</tr>
<tr>
<td>1³⁰—2⁰⁰</td>
<td>0.344</td>
<td>0.456</td>
<td>0.689</td>
<td>0.967</td>
</tr>
<tr>
<td>2⁰⁰—2³⁰</td>
<td>0.286</td>
<td>0.259</td>
<td>0.439</td>
<td>0.525</td>
</tr>
<tr>
<td>2³⁰—3⁰⁰</td>
<td>0.162</td>
<td>0.207</td>
<td>0.287</td>
<td>0.320</td>
</tr>
<tr>
<td>3³⁰—3³⁰</td>
<td>0.126</td>
<td>0.111</td>
<td>0.200</td>
<td>0.202</td>
</tr>
<tr>
<td>Mean</td>
<td>1.44</td>
<td>1.58</td>
<td>1.62</td>
<td>1.63</td>
</tr>
<tr>
<td>P</td>
<td>0.72</td>
<td>0.90</td>
<td>0.88</td>
<td>0.89</td>
</tr>
</tbody>
</table>
Table 4.

Results of experiments with methyl alcohol. Killed cells.

<table>
<thead>
<tr>
<th></th>
<th>Cell 6</th>
<th>d = 0.66</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>t</td>
<td>a</td>
<td>k</td>
<td>a</td>
<td>k</td>
</tr>
<tr>
<td>0°-0 15</td>
<td>3.018</td>
<td>1.56</td>
<td>0.921</td>
<td>1.55</td>
</tr>
<tr>
<td>0 15-0 30</td>
<td>1.950</td>
<td>1.17</td>
<td>0.590</td>
<td>1.74</td>
</tr>
<tr>
<td>0 30-0 45</td>
<td>1.660</td>
<td>1.66</td>
<td>0.339</td>
<td>1.37</td>
</tr>
<tr>
<td>0 45-1 00</td>
<td>1.002</td>
<td>1.38</td>
<td>0.248</td>
<td>1.68</td>
</tr>
<tr>
<td>1 00-1 15</td>
<td>0.725</td>
<td>1.83</td>
<td>0.158</td>
<td>1.13</td>
</tr>
<tr>
<td>1 15-1 30</td>
<td>0.396</td>
<td>1.14</td>
<td>0.140</td>
<td>1.40</td>
</tr>
<tr>
<td>1 30-1 45</td>
<td>0.349</td>
<td>0.100</td>
<td>0.231</td>
<td>0.181</td>
</tr>
</tbody>
</table>

Mean | — | 1.46 | — | 1.47 | — | 1.54 | — | 1.62 |

P | 1.49 | 1.52 | 1.58 | 1.76 |

The average curves (not drawn in fig. 6) cut the horizontal corresponding the elimination of $1/2$, $3/4$ and $7/8$ at points corresponding to the same lengths of time (the half value period) which is for living cells about 0.8 min. and for those killed about 0.4 min., which would give P values of 0.8 and 1.6 respectively.

It is worthy of special notice that no difference in permeability between

![Fig. 6. Elimination of methyl alcohol from living (lower curve) and dead cells. Abscissa, time in minutes, calculated for a cell diameter of 0.6 mm. Ordinate, eliminated quantities in per cent of total quantity.](image-url)
single cells can be observed and that the half value period remains unaltered between concentrations from 0.8 mol$^1$ down to 0.08 mol.

The negative errors on the P values for the living cells are the same as in the heavy water experiments, but in addition there is a positive error of 11 %, because the equilibrium concentration of methyl alcohol within the cells is lower than outside. The final correction is

$$P_1 \text{(corr)} = 0.89 \times 1.12 \cdot P_1 = 0.85 \text{ cm/h}.$$  

The dead cells require the same correction as with heavy water and there is at equilibrium the same concentration inside and outside. We have

$$P_d \text{(corr)} = 1.10 \cdot P_d = 1.75 \text{ cm/h}.$$  

Using the corrected figures in equation (5) we find as permeability of the protoplasmic membranes for methyl alcohol at 20° C 1.7 cm/h.

**Discussion.**

The present paper is a preliminary attempt to obtain absolute values for the plasma membrane permeability of cells inclosed within a cell wall to substances diffusing very rapidly. It shows that one is not limited to the use of naked protoplasts for experiments with such substances, even when fairly reliable results are aimed at.

We shall first try to make a comparison between the power of permeation of heavy water in <st>stalk</st> cells of *Tolyphellopsis* with that of ordinary water as measured by Palva (1939) on <le>leaf</le> cells of the same species. It is to be noted that Palva used an osmotic method and determined the changes in specific gravity brought about by loss of water. From his data he calculated a permeability for water of 1.08 $\mu$. Atm$^{-1}$. min$^{-1}$ at 20—21° C. This could, of course, be recalculated into cm/h, but it is safer to work up from the experiments themselves.

According to the curves given by Palva (p. 268) 50 % of the water lost in an experiment is eliminated on an average in 1.8 minutes and 75 % in 3.2 min. The loss of water can therefore be considered as an exponential function of the force acting, with a half value period of 1.7 min.

The cell diameters are mentioned as being between 0.5 and 0.8 mm, and the average can be taken as 0.7 mm without committing any serious error. When these values ($d = 0.7$, $t = 1.7$, $k = 2$) are introduced into equation (3) we find the permeation constant 0.45 cm/hour.

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$^1$ The original 1.6 molar concentration was reduced to 0.8 during the introductory period of washing.
The rate of water elimination was therefore in PALVAS experiments certainly not more than 40% of my value for heavy water. It might be assumed that the cells studied by PALVA were less permeable than mine, but it is much more probable that the difference is due to the necessary omission of mixing the solutions used in PALVAS experiments, a source of error which is mentioned by the author, but not considered quantitatively or allowed for.

It appears doubtful whether a valid comparison can be made between an osmotic flow of water and the diffusion of single water molecules, and this doubt applies to all determinations made by means of osmotic methods, even when technically perfect, and it should perhaps be pointed out that generally they are not technically perfect, especially when the object contains several layers of cells.

In the present paper the permeation of DHO is studied as a diffusion process pure and simple, so that the permeation constants both for DHO and for methyl alcohol can be directly compared with those for more slowly permeating substances. They can be included in the permeability series on Tolypellopsis (WARTIOVAARA 1942) as shown in table 5.

Table 5.
The permeability series for Tolypellopsis at 20°, cm/h. (From WARTIOVAARA 1942, except DHO and methyl alcohol).

<table>
<thead>
<tr>
<th></th>
<th>Living cells</th>
<th>Protoplastic membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHO</td>
<td>1.0</td>
<td>1.6</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>0.85</td>
<td>1.7</td>
</tr>
<tr>
<td>Urethylan</td>
<td>0.42</td>
<td>0.75</td>
</tr>
<tr>
<td>Trimethyl citrate</td>
<td>0.044</td>
<td>0.050</td>
</tr>
<tr>
<td>2, 3-Butylene glycol</td>
<td>0.016</td>
<td>—</td>
</tr>
<tr>
<td>Ethyleneglycol</td>
<td>0.010</td>
<td>—</td>
</tr>
<tr>
<td>Urea</td>
<td>0.00084</td>
<td>—</td>
</tr>
<tr>
<td>Hexamethylentetramine</td>
<td>0.00055</td>
<td>—</td>
</tr>
<tr>
<td>Tetraethyleneglycol</td>
<td>0.00051</td>
<td>—</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.000081</td>
<td>—</td>
</tr>
</tbody>
</table>

This series, based on direct determinations of permeating quantities of the single substances, covers P values over a range of $10^5$, from that of glycerol to those of heavy water and methyl alcohol. Since such a series is not available for any other plant it seems justified to attempt a numerical evaluation of the results.

1 HöFLER published in 1934 a permeability series on Majanthemum, also including water, but the errors inherent in the plasmolytic method, especially
As shown by Collander and Bärlund (1933) in the case of Chara the permeation rates of most anelectrolytes is almost directly proportional to their solubility in lipoids. Only those compounds the molecular size of which is below a certain limit (MRD ≤ 15) show a definitely higher rate than corresponding to their lipoid-solubility. That Tolypellopsis will show the same relations is to be expected, if only because of the close relationship to Chara.

Table 6.
The permeability series for Tolypellopsis, arranged according to increasing molecular volume of the substances included. P the permeability constant, k the distribution ratio for ether/water (after Collander and Bärlund 1933 and Wartiovaara 1942). MRD the molecular refraction.

<table>
<thead>
<tr>
<th>Substance</th>
<th>P</th>
<th>k</th>
<th>P/k</th>
<th>MRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHO</td>
<td>1.6</td>
<td>0.018</td>
<td>89</td>
<td>3.7</td>
</tr>
<tr>
<td>Methyl alcohol</td>
<td>1.7</td>
<td>0.27</td>
<td>5.1</td>
<td>8.3</td>
</tr>
<tr>
<td>Urea</td>
<td>0.00084</td>
<td>0.00047</td>
<td>1.8</td>
<td>13.7</td>
</tr>
<tr>
<td>Ethyleneglycol</td>
<td>0.010</td>
<td>0.0048</td>
<td>2.1</td>
<td>14.4</td>
</tr>
<tr>
<td>Urethylan</td>
<td>0.75</td>
<td>0.48</td>
<td>5.4</td>
<td>16.5</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.000081</td>
<td>0.00074</td>
<td>0.11</td>
<td>20.6</td>
</tr>
<tr>
<td>Buthyleneglycol</td>
<td>0.016</td>
<td>0.029</td>
<td>0.55</td>
<td>23.7</td>
</tr>
<tr>
<td>Hexamethylenetetramine</td>
<td>0.00055</td>
<td>0.00026</td>
<td>2.1</td>
<td>38.8</td>
</tr>
<tr>
<td>Tetraethyleneglycol</td>
<td>0.00051</td>
<td>0.0024</td>
<td>0.21</td>
<td>47.1</td>
</tr>
<tr>
<td>Trimethyl citrate</td>
<td>0.050</td>
<td>0.43</td>
<td>0.12</td>
<td>50.3</td>
</tr>
</tbody>
</table>

For Tolypellopsis it is now possible, however, to estimate the diffusion resistance of the isolated protoplast even for substances which permeate very rapidly. This makes it possible to estimate the influence of molecular size more accurately than in the case of Chara. If the permeation rate of the substances studied depended solely on their lipoid-solubility — or rather on their distribution ratio between ethylether and water, which has been selected as a measure of lipoid solubility — we should in table 6 find the relation $\frac{P}{k}$ constant, P being the permeation constant and k the distribution ratio ether/water. (This ratio for DHO is given below). This relation is in fact of the same order of magnitude (viz. 0.1 to 0.6)\(^1\) for those compounds which have molecular refractions $\text{MRD} \leq 21$.

For the smaller molecules ($\text{MRD} = 8-17$) the relation $\frac{P}{k}$ is about ten times higher and for the smallest, heavy water ($\text{MRD} \approx 3.7$), a few hun-

\(^1\) Only hexamethylenetetramine behaves abnormally in this as in other respects.
dred times higher. Closer relations between the permeation ability and the physical constants used (somewhat arbitrarily) for comparison could probably only be expected for very nearly related compounds (within homologous series) and at present we cannot do more than point out the relation in order of magnitude between what is called permeation through pores and the molecular volume as expressed by the molecular refraction.

The distribution ratio for DHO as given in table 6 was not directly determined, but arrived at in the following way. When shaken together at 20° ether and ordinary water will separate into a water phase containing about 95 g and an ether phase containing 1.7 g water per 100 ml. A content (not too large) of DHO is assumed to become distributed in the same way, and the distribution ratio will thus be \( \frac{1.7}{95} = 0.018 \). This treatment of the problem is in formal agreement with that adopted for other anelectrolytes when the molecules of DHO are considered as dissolved, but it is by no means clear that the result is of the same significance from a physiological point of view. The distribution ratio for water depends directly on its absolute solubility in ether which is not the case regarding other distribution ratios. On the other hand we have to remember that the determination of distribution ratios represents only a model in which the ether phase represents the lipoids of the plasma membrane. Also in the physiological conditions represented it seems natural to assume that the possibility for water to penetrate the membrane by «solution» in its lipoids is limited by the absolute solubility, while that of other anelectrolytes, present in dilute solutions, is governed by the distribution ratio lipid/water. The distribution ratio as calculated for heavy water appears therefore also physiologically plausible.

As evident from table 6 there is a very large interval between the \( \frac{P}{k} \) value for heavy water and that for the next substance, methyl alcohol. It will probably be difficult to fill up this interval. Such compounds as CO, O\(_2\), N\(_2\) or perhaps even CO\(_2\) and NH\(_3\) are to be considered, but it will be difficult to obtain accurate determinations of plasma permeability with any of them. It is, nevertheless, probable that the «pore permeability» increases regularly with decreasing molecular volume and finally becomes much more important than the «solution permeability». The enormous permeation ability of heavy water as compared with its slight solubility in lipoids is therefore probably no real exception from a general rule. The exceptional position of water is probably apparent only and due to the fact that we have so far no other substance, with similar small lipoid solubility and small molecular volume, for which the permeation rate has been determined with similar accuracy.
Summary.

A method is described for direct determination of the permeation constant of very rapidly permeating substances into single large plant cells. This method is available also for heavy water and allows the determination of its permeation as a diffusion process.

On internodial cells of *Tolypellopsis stelligera* (Characeae), both in the living state and freshly killed, the permeation constants of heavy water and methyl alcohol were determined, and from these determinations the diffusion resistance of the isolated living protoplast calculated.

The following permeation constants were obtained: For DHO on living cells 1.04, killed cells 3.08, isolated protoplasts 1.6, for methyl alcohol on living cells 0.85, killed cells 1.75, isolated protoplasts 1.7. (The permeability for methyl alcohol remained the same between alcohol concentrations of 0.8 to 0.08 molar). The permeation rate of ordinary water is higher than that of DHO, but the difference is probably slight.

The permeability series for the protoplasts of *Tolypellopsis* as now supplemented with the ratio for DHO and methyl alcohol are discussed. The permeation rate for heavy water is several hundred times higher than those for substances of the same relative solubility in ether, but much larger molecular volumes, while methyl alcohol does not differ significantly from other molecules of relatively small size (MRD < 17) which on an average permeate 10 times more rapidly than the larger ones showing a similar solubility in ether.

I wish to thank the chief of the Zoophysiological Laboratory of Copenhagen University professor A. Krogh most heartily for the opportunity to do this piece of work and for his help and support, freely given in many instances. I am very grateful for a grant from the Rask-Ørsted Foundation which made this study financially possible. Some final experiments were carried out in the Botanical Institute of the University of Helsinki, towards which I received a grant from the Finnish Academy of Science. I wish to thank also professor Runar Collander for valuable help and counsel.
Literature.


