A NEW METHOD OF STUDYING PERMEABILITY

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(WITH TWO FIGURES)

The writer[1] has shown the desirability of a study of permeability by some method which should be entirely independent of other methods, and yield data the interpretation of which need not depend upon any unverifiable assumptions. A method is here presented which seems to fulfil these requirements. It has proved to be exceedingly reliable; and the experiments point clearly to the errors previously made in the interpretation of the data secured by many methods, and to the validity of the conclusions based on the evidence of certain others.

Method

The method depends upon diffusion of salts or other substances through a diaphragm of living tissue. For this purpose fronds of one of the common kelps of the New England coast, *Laminaria Agardhii* (formerly identified as *L. saccharina*), proved to be extremely satisfactory material because of absence of air spaces in the tissue, ease of manipulation, resistance to adverse conditions, and especially because it was possible to secure thin sheets of tissue in which there were no wounded surfaces in contact with the solutions.

The method of experimentation was as follows. Sections of glass tubing of 18 mm. internal diameter were cut; one end of each piece was flared and the end ground flat. The resulting "cells" were either 2.5 cm. or 4 cm. in length, and were combined in pairs, each consisting of one long and one short cell (fig. 1, *A*, *B*). The unground end of the longer cell was closed by a rubber tube and pinchcock (fig. 1, *C*, *D*). Disks were cut from the fronds of *Laminaria* of such a size as nearly to cover the ground ends of the tubes.

[1] Brooks, S. C., Methods of studying the permeability of protoplasm salts. Bot. Gaz. 64:230–249. 1917.

In the experiments with living material the surface of these disks was quickly dried with filter paper, the disks (fig. 1, *E*) placed between the ground ends of a pair of cells, and the joint made tight with a stiff cement consisting of a mixture of vaseline and beeswax (fig. 1, *F*). Thus there were formed two cells separated by a diaphragm of *Laminaria* tissue. The cell supplied with the rubber tube and pinchcock (hereafter called the "lower cell") was then filled with solution and the pinchcock closed, care being taken that no air bubbles were included in the cell. The apparatus was then inverted and the upper cell filled with solution, covered to check evaporation (fig. 1, *G*), and set in a suitable support. During these operations each disk was in contact with the air less than two minutes, which was not sufficient to cause any appreciable drying-out of the tissue.

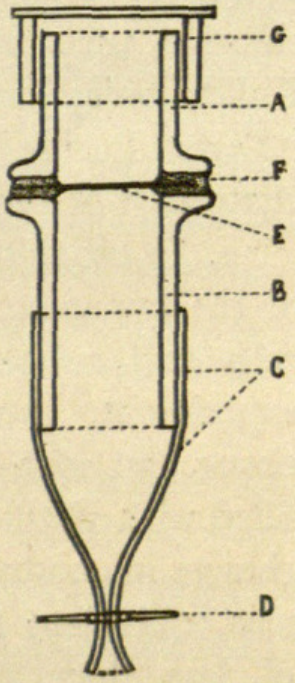

FIG. 1

In order to obtain dead tissue for experiments on the permeability of the intercellular substance, living disks were exposed, after cutting, to an atmosphere saturated with chloroform vapor at room temperature for 16–24 hours. They were next exposed to the air about one hour to allow the complete evaporation of any chloroform which remained in the tissue, and then placed in a large volume of sea water for about 24 hours to allow the establishment of equilibrium between the electrolytes of the sea water and those in the dead cells. At the end of this time the surface of the disks of tissue was dried with filter paper, and the apparatus set up as in the experiments with living material. Tissue which had died a natural death gave results in every way similar to those given by tissue killed in this manner.

The permeability of the tissue was shown by the rate of passage of salts through the diaphragm as shown by diminution of the difference of concentration between the solutions in the upper and lower cells. It is possible to measure rapidly, and with extreme accuracy, slight changes in the concentration of the solutions in either cell by determining the change in electrical conductivity. This method was therefore employed.

The solution used in the lower cells was either sea water or a pure salt solution of the same conductivity; while the upper cells contained a solution of half the concentration of that in the corresponding lower cell. Solutions of equal conductivity were used in order to facilitate comparison with the work of OSTERHOUT.[2]

Sources of error

In order to obtain accurate data, the following precautions were taken:

1. The solutions were made up with distilled water, which had a specific conductivity of about 2×10^{-6} ohms. The sodium chloride used was Baker's "analyzed"; the calcium chloride, Kahlbaum's; and the lanthanum nitrate, Eimer and Amend's "Tested Purity." For this work an error of 1 per cent in the concentration of the solutions was considered allowable.

2. In order to prevent dissolving of electrolytes from any part of the apparatus, the cells were made of Durox glass, and both cells and rubber thoroughly steamed immediately before each experiment. An apparatus of this type, set up with no *Laminaria* tissue, but with a thick layer of the vaseline-beeswax cement, and filled with distilled water, gave off only traces of electrolytes. The change in conductivity of the water in such a cell during 48 hours was equivalent to an increase of concentration of sodium chloride of less than 1×10^{-7} M. Dissolving of electrolytes from the apparatus has therefore no significance in the experiments.

3. It was necessary to eliminate the influence of temperature. As it was impracticable to conduct the experiments at constant temperature, the cells were placed outdoors, the temperature varying from $-3°$ to $+9°$ C. This amount of fluctuation produced no appreciable change in the rate at which salts passed through the tissue, and the low temperatures were exceedingly favorable to the maintenance of normal permeability.[3]

[2] Unpublished data of OSTERHOUT show that differences of osmotic pressure of the magnitude of those produced by the use of solutions of equal conductivity have little effect on the permeability of *Laminaria* during the length of time occupied by these experiments.

[3] *Laminaria* lives much longer when the temperature is low. While it may be kept alive under laboratory conditions several weeks at 0° C., it perishes rapidly at 24° C.

4. The *Laminaria* thallus is made up of masses of protoplasm (the cells) imbedded in a gelatinous intercellular substance. From this intercellular substance, in which the salts are present in the same concentration as in sea water, the salts will diffuse out into a surrounding medium, and will alter its conductivity if it be other than that of sea water. From the protoplasm, also, a similar diffusion may take place, which for convenience may be designated as "exosmosis."

That there is actual passage of salts through the tissue was shown by the fact that the conductivity of the more dilute solution always increased, while that of the more concentrated solution decreased to a corresponding degree. There was no appreciable change in the volume of either solution even during experiments whose duration was greater than 24 hours. The relative amounts of increase in the upper cell and of decrease in the lower cell, as found in the experiments, were in fair agreement with those calculated. If a given amount of salt passes from one salt solution to an equal volume of another solution having half the concentration of the first, the percentage of increase in the concentration of the latter will be double the percentage of decrease in the former. If there be a difference in volume between the two solutions, the change caused by the addition or removal of a given amount of salt will be inversely proportional to the volume. Thus, in one experiment the increase in concentration in the upper cell was 1 per cent per hour, while the decrease in the lower cell was 0.26 per cent per hour. Since the volume in the upper cell was 5.4 cc. as compared with 12.5 cc. in the lower, and the concentrations were as 1:2, the expected ratio between the changes in the two cells would be $\frac{12.5 \times 2}{5.4} = 4.6$; while the observed ratio was $\frac{1.0}{0.26} = 3.8$.

The agreement was reasonably satisfactory, and it therefore could be assumed that changes in the concentration of the solutions in the upper cells would be nearly proportional to the amount of salt passing through the diaphragm. Two modifications of the method, however, were sufficient to eliminate entirely the errors due to both diffusion and exosmosis. The error due to diffusion of salts from the intercellular substance was eliminated by filling

the cells, when first set up, with half-strength sea water in the upper cell and sea water in the lower. Thirty minutes was ample for the establishment of a steady diffusion gradient through the tissue between the two solutions. The upper solution was then replaced by fresh half-strength sea water, after which regular readings were taken. In order to eliminate the error due to exosmosis from the protoplasm, such as might be occasioned by toxic salts, 3 controls out of each set of 11 to 13 simultaneous experiments had the more dilute solution in both cells. At the end of the experiment the average conductance of the solution in the upper cells of the controls was taken as a standard of measurement, the average conductance of all the other upper solutions being divided by this figure in order to obtain the percentage which expresses their gain as compared with the control. The figures which were obtained in this manner measure the amount of salt which has passed through the tissue, while the errors due to exosmosis from the protoplasm as well as those due to diffusion from the intercellular substance are eliminated.

5. We must eliminate the error due to variations in the thickness and maturity of the disks of tissue from different fronds, and also that due to variations in the area of tissue through which salt can pass (such as might be introduced by unavoidable smearing of the cement over the surface of the disks).[4] In order to eliminate all of these errors, controls were established in the following manner. After a preliminary half-hour with half sea water in the upper and sea water in the lower cell, the upper solution was replaced with 5.4 cc. of fresh half sea water, and the rate of change of conductivity determined at the end of 2 hours. Both upper and lower solutions were now replaced with solutions of the salt to be investigated (the fresh solutions having the same conductivity as those which they replaced), and the rate of change of conductivity determined after a further period of 2 hours. By dividing the

[4] A single experiment was conducte d to determine the influence of frond thickness. The results were entirely negative. This is in accord with the results secured by ABEL (ABEL, J. J., ROWNTREE, L. G., and LURNER, B. B., On the removal of diffusible substances from the circulating blood of living animals by dialysis. Jour. Pharm. and Exp. Ther. 5:275. 1914.), who found that diffusion of electrolytes through a collodion membrane was independent of the thickness of the membrane.

figure obtained for the salt in question by that for the control period of the same disks of tissue, we obtain a figure (given in the ratio column of table II) from which all errors due to individual variations of the disks of tissue are eliminated.

6. The method for the determination of the conductance of the solutions was as follows. The solution was poured from the cell into a U tube of such dimensions as to give a conductance of the order of magnitude most accurately determinable, namely, about 1500–2000 ohms$\times 10^{-7}$. The U tube was nearly immersed in a constantly stirred water bath whose temperature, determined to 0°.05 C., varied less than 0°.8 C. in any one set of readings. A temperature correction of 2 per cent per degree Centigrade was applied to the actual readings to reduce them to the average temperature of the set, and the results calculated from the corrected readings thus obtained. A slide wire bridge, a standard 1000-ohm bifilar resistance (supplied with current from the secondary of a Nernst string inductorium at about 300–500 oscillations per second), and a telephone as the zero instrument were used in the customary manner to measure the resistance between bright platinum electrodes immersed in the solution at the opposite ends of the U tube. The distance between the electrodes was fixed. The readings had an error less than ± 0.1 per cent. The check experiments in half-strength sea water usually gave an agreement of corrected readings within ± 0.05 per cent. It will be seen that this degree of accuracy was ample for the purpose.

Results

It is desirable first to find out how fast the various salts pass through the intercellular substance, and whether there is any selective permeability due to any source other than the protoplasm. The data presented in table I show that the cell walls intercellular substance of *Laminaria* are permeable to the salts used, and that the passage through the walls is nearly independent of the nature of the diffusing salt. In dead material the change of concentration is so rapid that owing to the decrease in the concentration gradient the rate of passage of salts through the tissue decreased, as is shown by the lower rate for the longer periods in both sea water and sodium

chloride. It is necessary, therefore, to draw our conclusions from the results of periods of equal length only. The relative permeability to different salts will then be represented by the following figures: calcium chloride 2.2, sea water 2.2, lanthanum nitrate 2.1, sodium chloride 2.1.

TABLE I

PERMEABILITY OF DEAD TISSUE OF *Laminaria*

Solution in upper cell	Solution in lower cell	Duration in hours	Change of conductivity, percentage per hour
Half sea water...	Sea water.....	5.5	1.8
$CaCl_2$, 0.14 M...	$CaCl_2$, 0.28 M..	4.5	2.2
Half sea water...	Sea water.....	12	1.3
" " " ...	" "	5.5	2.2
NaCl, 0.26 M...	NaCl, 0.52 M ..	5	2.0
Half sea water...	Sea water.....	12	1.2
La_2Cl_6, 0.05 M..	La_2Cl_6, 0.10 M..	4.5	2.1
NaCl, 0.26 M...	NaCl, 0.52 M ..	4.5	2.1

It appears probable that the slightly lower rate of diffusion of sodium chloride may have been due to a slight irreversible decrease in the permeability of the intercellular substance caused by the lanthanum nitrate, by which the tissue had been bathed immediately previous to the experiment with sodium chloride. This would be in accord with unpublished data secured by OSTERHOUT by determination of the conductivity of the tissue.

The differences which might be expected to arise as an expression of the diffusion coefficients of the salts are evidently of so small an order as to fail to influence appreciably the rate of diffusion through dead tissue. In view of the very imperfect state of our knowledge of diffusion coefficients, it would be unprofitable at the present time to attempt any further explanation of the influence of that factor in our experiments.

It will be seen from the data given in table II that the presence of living protoplasm greatly decreases the permeability of the tissue as a whole. Living protoplasm offers, therefore, a very considerable resistance to the passage of salts. That it is not normally (in sea water) impermeable to salts will appear from the following considerations. The permeability of the protoplasm for conven-

ience may be considered as the amount of salt passing through the tissue, expressed as the percentage of the amount passing through tissue bathed by sea water, as shown in the ratio column of table II. If the protoplasm be assumed to be wholly impermeable to salts of lanthanum, the figure 0.45, expressing the permeability of the tissue as a whole, would in this case represent diffusion through the intercellular substance only. Since this part of the tissue has been shown (cf. table I) to have no appreciable selective permeability, we may assume that not more than 0.45 of the permeability of the tissues to sea water, which is 1.07, is due to passage of salts

TABLE II

PERMEABILITY OF LIVING *Laminaria*

Experiment number	First period				Second period				Ratio
	Upper solution	Lower solution	Duration h. m.	Change of conductivity, percentage per hour	Upper solution	Lower solution	Duration h. m.	Change of conductivity, percentage per hour	Second period / First period
18..	Half sea water	Sea water	2:05	0.73	Half sea water	Sea water	2:00	0.78	1.07
19..	"	"	2:00	0.79	NaCl, 0.26 M	NaCl, 0.52 M	2:00	1.11	1.41
22..	"	"	2:06	0.73	$CaCl_2$, 0.14 M	$CaCl_2$, 0.28 M	2:02	0.51	0.70
17*a*.	"	"	1:35	0.73	$La_2(NO_3)_6$, 0.05 M	$La_2(NO_3)_6$, 0.10 M	1:35	0.33	0.45

through the intercellular substance. There remains $1.07-0.45=0.62$, which represents that part of the salt which passes through the protoplasm. In sea water, therefore, a minimum of $\frac{0.62}{1.07}$, or 58 per cent of the salt, passes through the protoplasm, but the exact significance of this figure is doubtful owing to the arrangement of the protoplasmic masses in the tissue.

In order to show the order of magnitude of the total diffusion through the living tissue, the results may be expressed in terms of the amount of salt in gm. molecules passing through 1 sq. cm. of tissue per hour. Ignoring the exceedingly slight change in molecular conductivity induced by such small changes of concentration,

the conductivity will be proportional to the concentration, and a change of 1 per cent in the conductivity of a 0.26 M solution may be assumed to indicate an increase of 0.0026 M in the concentration. An increase of this size in 5.4 cc. of solution will necessitate the addition of $\frac{5.4}{1000} \times 0.0026$, or 0.0000140 gm. molecules of salt. If we divide the figures obtained in this manner for the various salts, by the area of tissue in sq. cm. through which salts can pass, we obtain the figures given in table III. The figure for sea water was obtained by assuming all of its conductivity to be due to sodium chloride; but since sea water contains about 12 per cent of its electrolyte as salts of bivalent elements, which have a higher molecular conductivity than sodium salts, its actual molecular content is less than that of a sodium chloride solution having the same conductivity, and the figure given in table III is thus slightly too high.

TABLE III

GRAM MOLS DIFFUSING PER SQ. CM. PER HOUR THROUGH LIVING *Laminaria*

Upper solution	Lower solution	Gm. mols diffusion
Half sea water.....	Sea water.....	0.0000425
NaCl, 0.26 M.....	NaCl, 0.52 M.....	0.0000610
$CaCl_2$, 0.14 M.....	$CaCl_2$, 0.28 M.....	0.0000150
$La_2(NO_3)_6$, 0.05 M..	$La_2(NO_3)_6$, 0.10 M...	0.0000034

The data of tables II and III also show that there is a selective permeability to the salts used. Sodium chloride is allowed to pass through the tissue most rapidly, the salts of sea water next, calcium chloride considerably less rapidly than sea water, and lanthanum nitrate least of all. That the effect is produced in large part by the kations, as was to be expected, is shown by the fact that preliminary experiments with lanthanum chloride (lacking the preliminary comparison period in sea water) showed a permeability comparable with that to lanthanum nitrate. Thus in one experiment with lanthanum chloride the change of conductivity of the upper solution was 0.30 per cent per hour, while that quoted for lanthanum nitrate is 0.33 per cent per hour. Whether

protoplasm is at all permeable to lanthanum salts cannot be decided with the data furnished by these experiments.

It might be supposed that the protoplasm was normally more permeable to sodium chloride than to the other salts of sea water, and that therefore when bathed by pure sodium chloride solution more salt would pass through the diaphragm. On the assumption that the tissue is permeable only to the sodium and potassium chlorides, the molecules of which constitute 88 per cent of the molecules of salt in sea water, the rise in permeability on substitution of sodium chloride solutions for sea water would be only that from 88 to 100. The observed rise is much greater, namely, from 76 to 100, and in addition it must be remembered that the calcium and magnesium salts of sea water are probably able to penetrate the tissue to some extent. Sodium chloride must increase the permeability of the tissue therefore.

By analogy, it might be assumed that the permeability of the protoplasm decreased under the influence of calcium and lanthanum salts. In order to obtain more exact information in respect to this question, a set of experiments was conducted in which the permeability was determined during successive periods of treatment with a given salt. The solutions in both the upper and lower cells were renewed at the beginning of each period. The results are shown in table IV and fig. 2.

From these experiments it will be seen that the increase of permeability due to sodium chloride is progressive, and that it leads in the course of about 4 hours to a permeability of the tissues corresponding to that of dead material. The effect of calcium chloride, on the other hand, is to cause a temporary decrease in permeability, followed by a rise which at the end of about 12 hours leads to a permeability comparable with that for dead material. At the end of this time the material had assumed the green color characteristic of dead material.

The experiment with sea water was conducted under conditions extremely unfavorable to the maintenance of normal permeability, the temperature rising to 14° C. during the third and fourth periods. Partial recovery is shown in the succeeding periods during which the temperature decreased. The last period was begun about 24

hours after the beginning of the experiment, and shows that the tissue, which had only partially recovered its normal permeability,

TABLE IV

PROGRESSIVE CHANGES IN PERMEABILITY OF TISSUE OF LIVING *Laminaria;* EXPRESSED AS RATE OF CHANGE OF CONDUCTIVITY OF SOLUTION IN UPPER CELL, IN PERCENTAGE PER HOUR

UPPER SOLUTION, HALF SEA WATER; LOWER SOLUTION, SEA WATER			UPPER SOLUTION, NaCl, 0.26 M; LOWER SOLUTION, NaCl, 0.52 M			UPPER SOLUTION, $CaCl_2$, 0.14 M; LOWER SOLUTION, $CaCl_2$, 0.28 M		
Period begun at	Duration in min.	Rate of change	Period begun at	Duration in min.	Rate of change	Period begun at	Duration in min.	Rate of change
6:45 A.M..	120	0.67	2:40 P.M....	120	1.11	9:33 A.M....	122	0.51
9:15 A.M..	124	0.78	5:10 P.M	150	2.30	12:05 P.M....	124	0.82
11:45 A.M..	121	1.01	8:15 P.M....	130	2.50	2:45 P.M....	121	0.69
2:20 P.M..	132	1.27	.....	.....	.....	5:25 P.M....	120	1.37
5:00 P.M..	170	0.81	.....	.....	.....	8:10 P.M....	135	2.05
8:20 P.M..	139	0.97	.....	.....	.....	.....	.....	.....
6:05 A.M..	120	0.96	.....	.....	.....	.....	.....	.....

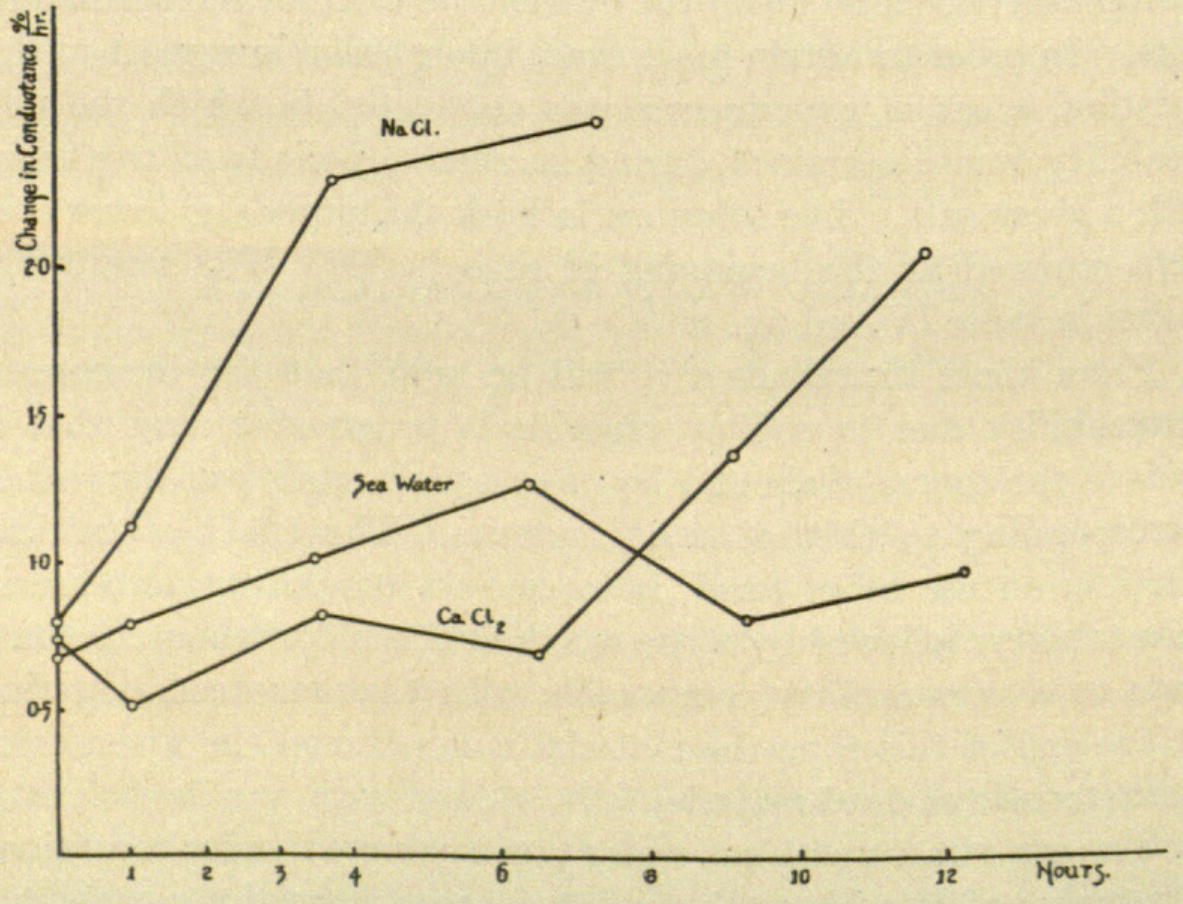

FIG. 2

suffered no further injury during the period of low temperature (0–4° C.) intervening between the fifteenth and twenty-fourth

hours. The disks were still brown and apparently uninjured even after 48 hours in the apparatus. Certain experiments with lanthanum salts indicated that the effect of lanthanum would resemble that of calcium, differing chiefly in that the alterations of permeability would take place more rapidly.

Summary

1. The protoplasm of *Laminaria* is normally permeable to the salts of sea water.

2. Sodium salts cause an increase of permeability which culminates in death.

3. Calcium and lanthanum salts cause a decrease in permeability, followed by an increase which culminates in death.

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