THE CONCENTRATION EFFECT WITH VALONIA: POTENTIAL DIFFERENCES WITH CONCENTRATED AND DILUTED SEA WATER

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The concentration effect promises to be of considerable importance as a means of revealing properties of protoplasm otherwise unsuspected. In view of this, a series of studies has been started, one of which has recently appeared.[1] The present paper deals with the P.D. between the interior of cells of *Valonia macrophysa* and concentrated or diluted sea water applied externally.[2]

In an earlier paper,[3] it was pointed out that in measurements of P.D. across the protoplasm in *Valonia*, the presence of parallel circuits through the cell wall may cause serious difficulties. These make it undesirable to apply two different solutions simultaneously at more or less widely separated spots on the same cell.[4] In the present experiments we have established connection[3] with the interior of the cell through a fine glass capillary filled with artificial sap, on which the cell is impaled, while contact with the outside is made through a continuous stream of solution which flows over the entire surface of the cell. The protoplasm thus comes into the circuit only once, and the P.D. across it is measured directly. On standing, the protoplasm becomes sealed to the glass capillary, preventing a possible short circuit between the external solution and the vacuolar sap through a

[1] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 761.

[2] The experiments reported in this paper were carried out at Bermuda. The apparatus and experimental technique have been described in earlier communications.[3] The Compton quadrant electrometer used in these measurements, however, was an improved model made by the Cambridge Instrument Company. This was adjusted to give deflections of 3.5 to 5.0 mm. per mv. with the scale 4 feet from the mirror.

[3] Damon, E. B., *J. Gen. Physiol.*, 1929–30, **13**, 207. See also Osterhout, W. J. V., Damon, E. B., and Jacques, A. G., *J. Gen. Physiol.*, 1927–28, **11**, 193.

[4] With *Nitella*, however, and probably also with other fresh water plants, the resistance of the cell wall is so high that such parallel circuits may be shown to be of only negligible importance.

film of solution between the protoplasm and the glass. We believe that the values measured when there is a good seal actually represent the E.M.F. acting across the protoplasm of the impaled cell (leaving open the question of how much the cell may be altered by impalement[5]).

We apply a series of different solutions to the same cell, and compare the P.D.'s to which they give rise. By combining the values observed in actual measurements on chains of the type:

sap (inside) | protoplasm | solution *A*

and

sap (inside) | protoplasm | solution *B*

we may calculate the E.M.F. of the chain:

solution *A* | protoplasm | sap | protoplasm | solution *B*

for the ideal case (*i.e.*, in which parallel circuits through the cell wall play no part).

The following convention which we have adopted for reporting the signs of the P.D.'s may assist in avoiding confusion as to which type of chain is meant. For all measurements between an external solution and the vacuolar sap, made with the use of a capillary, the sign of the interior of the cell is reported, thus: "inside positive" or "inside negative." Such expressions as "sea water positive" or "solution at *A* positive" are reserved for actual or fictitious chains in which we lead off from two different points on the outside of the cell. In either case, the solution or spot reported as positive is the one from which positive current tends to flow through the measuring instrument in the external circuit.

In case the P.D.'s fluctuate so as to indicate that the system is changing continuously during the measurement or the solution first applied produces in the cell alterations which affect its behavior in later measurements,[6] we may compare the values with a given solution, measured on one series of cells with those of another solution measured on a different series; in this case, however, variations among individual cells must be considered.

The concentration effect with sea water presents an especially favorable case for comparing measurements with a series of solutions on the

[5] *Cf.* Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13**, 361.

[6] Experiments with artificial sap[3] illustrate these difficulties.

same cell, for the observed changes in P.D. are regular and reproducible within certain limits. If the solution is too dilute, or its application too long continued, secondary changes occur in the cell which result in the change of the sign of the P.D. These changes are reversible up to a certain point, beyond which they become irreversible, involving permanent injury. These secondary changes are delayed by making the dilute solutions isotonic with ordinary sea water by the addition of glycerol.[7] Further studies of these secondary changes are being carried on.

Changes in the protoplasm produced by the solutions applied to the cells are always a source of difficulty. An advantage of the capillary method is that it makes these changes less liable to be overlooked or misinterpreted.[8]

The sign of the concentration effect with sea water, *i.e.*, the sign of the E.M.F. of the (imaginary) chain:

sea water, concn. 1 | *Valonia* | sea water, concn. 2

is made evident by the changes in P.D. across the protoplasm observed when sea water is replaced by concentrated or diluted sea water. We find that, until secondary changes begin to appear, the more

[7] In practice, the sea water is diluted with a solution of glycerol, 8.7 per cent by weight, in distilled water. The freezing point of this solution is approximately the same as that of Bermuda sea water. We have used sugar solutions, also, but prefer glycerol since it is less liable to be contaminated by molds.

[8] For example, we have tried measurements on intact (unimpaled) *Valonia* cells, in which we led off from one end with sea water, and from the opposite end with sea water diluted with 8.7 per cent glycerol solution, using flowing contacts. When recently collected cells were employed, we found that the more concentrated solution was positive, but (probably as a result of short circuits through the cell wall) the P.D.'s were smaller than in measurements by the capillary method. The observed P.D.'s could be somewhat increased by wetting the greater part of the surface of the cell with dilute sea water, instead of ordinary sea water. In either case, secondary changes, reversing the sign of the P.D., occurred much sooner than in measurements by the capillary method. When we used apparently healthy cells which had been standing for some time in sea water in the laboratory, the observed P.D.'s were still smaller, and secondary changes set in more promptly. As a result, in many cases, only the P.D. after the reversal of sign was observed. Such experiments alone would have led to reporting the wrong sign for the concentration effect.

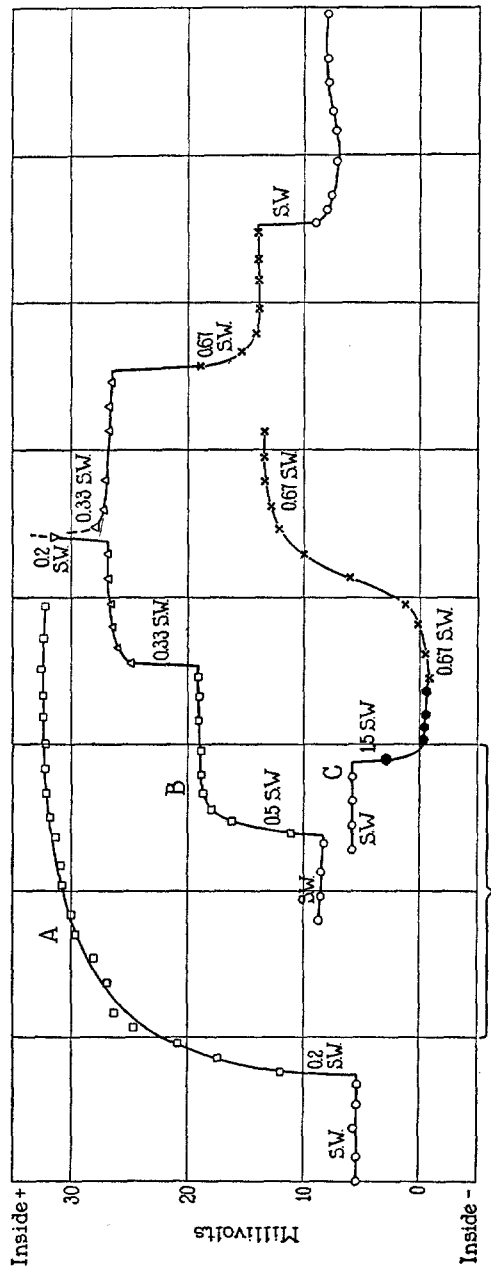

FIG. 1. P.D.-time curves, showing the concentration effect in *Valonia* with concentrated and diluted sea water. The ordinates represent the P.D. between the interior of the cell and the external solution indicated. Curve *B* represents measurements on cell No. 2, Curves *A* and *C* on cell No. 3 of Table I.

concentrated solution is positive (*i.e.*, positive current tends to flow in the external circuit from the more concentrated to the more dilute solution).

The relation between concentration and P.D. is best studied in the range between 1.5 sea water to 0.2 sea water. These concentrations probably represent the limits within which values can be obtained

TABLE I

Effect of Concentration of Sea Water on P.D. *in Valonia**

	Concentration of sea water†					
	1.5	1.0	0.67	0.5	0.33	0.2
	mv.	mv.	mv.	mv.	mv.	mv.
1	−6.6	(0.0)‡	5.3	11.8	18.9	25.2
2		(0.0)	6.0	10.6	18.5	23.8
3	−6.2	(0.0)	7.7	11.3	19.6	27.0
4	−6.0	(0.0)	6.7	11.6	18.1	25.8
5	−5.8§	(0.0)	6.3	11.8	19.5	24.3
6		(0.0)	6.6	11.0		
Average.....	−6.1	(0.0)	6.4	11.4	18.9	25.2

* These measurements were made at Bermuda between Nov. 29 and Dec. 13, 1928. The temperature of the laboratory at this time varied between 17° and 22°C., avg. 20°C. The cells used in these measurements were collected Nov. 19, 1928.

† Expressed in terms of ordinary sea water. *I.e.*, 0.2 means a fivefold dilution; 1.5 means sea water concentrated by evaporation to two-thirds its original volume. All dilutions were made with 8.7 per cent glycerol solution.

‡ The observed P.D.'s with sea water varied between 5.1 and 8.5 mv., average 6.2 mv., inside of the cell positive.

§ Secondary changes began before the P.D. became constant. 5.8 mv., representing the maximum observed change, is probably not the full value.

without too great errors from secondary changes beginning before the full value of the P.D. has been reached. Even within these limits it is not advisable to expose a cell continuously to dilute or concentrated sea water for very long periods, but to return it to sea water between short series of measurements. In order to postpone secondary changes until after the full value has been attained, the dilute solutions must be made isotonic with sea water by the addition of glycerol (or some other

suitable non-electrolyte). Under these conditions, the P.D.'s come to definite values at which they remain reasonably constant (see the P.D.-

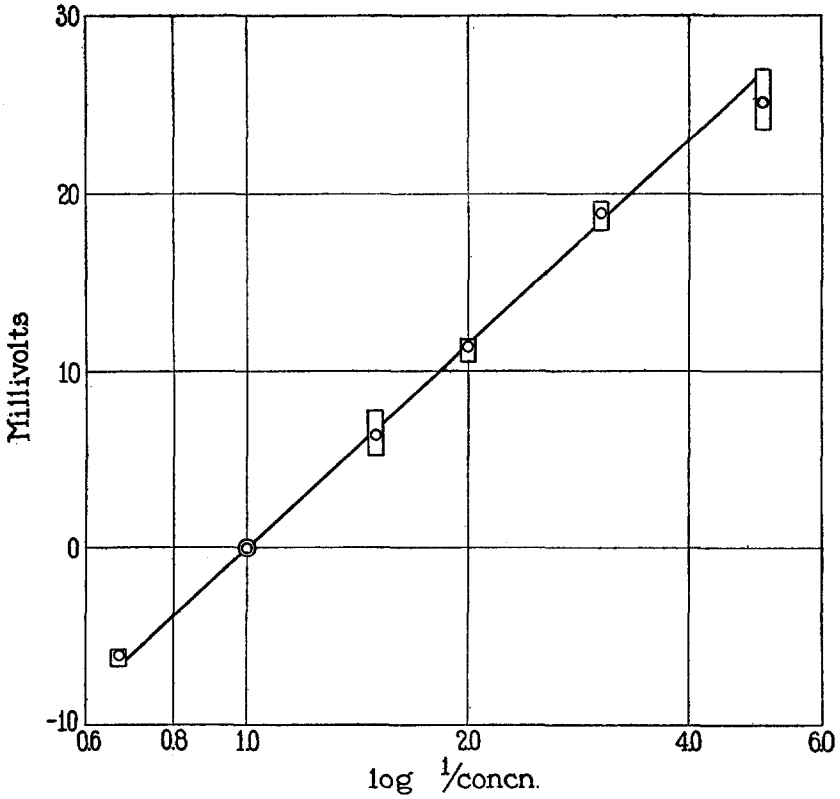

FIG. 2. Graph showing the relation between the concentration of the sea water applied externally and the P.D. across the protoplasm in *Valonia*. (See Table I.) P.D.'s plotted as ordinates are differences between the value measured with the concentration in question and the value observed with sea water. The height of the rectangles shows the variation in the observed values: averages are shown by circles. The slope of the straight line is $\frac{2}{3}\frac{RT}{F}$, where $T = 273 + 20°$, the average temperature.

time curves, Fig. 1). The constant values observed with intermediate concentrations may be approached from either side, *i.e.*, it makes no difference whether the solution in question follows a more concentrated

or a more dilute solution. The values observed with different cells are in reasonably good agreement.

These points are illustrated by the P.D.-time curves in Fig. 1, which represent measurements on two different cells,[9] and by Table I, in which are collected the P.D.'s observed with these cells and with four others. The values reported in the table are differences between the P.D. observed with the solution in question and the constant value previously or subsequently observed with sea water. In other words, the table gives the E.M.F. calculated for the chain:

sea water | *Valonia* | concentrated or diluted sea water

The sign reported is that of ordinary sea water: it will be noted that the more concentrated solution is in every case positive.

The data recorded in Table I are also shown in the graph, Fig. 2, in which the P.D. is plotted against the logarithm of the reciprocal of the concentration. The variation in the observed values is indicated by the height of the small rectangles; averages are shown by circles. Evidently the P.D. is a logarithmic function of the concentration. The straight line drawn through the observed points corresponds to the empirical equation:

$$E = \frac{2}{3}\frac{RT}{F}\ln\frac{\text{concn.}_1}{\text{concn.}_2} \tag{1}$$

for the average temperature, 273 + 20°. In other words, the P.D. across the protoplasm when the outside of the cell is in contact with concentrated or diluted sea water is given by the expression:

$$\text{P.D.} = \text{P.D.}_{\text{S.W.}} + \frac{2}{3}\frac{RT}{F}\ln\frac{1}{\text{concn.}} \tag{2}$$

where $\text{P.D.}_{\text{S.W.}}$ is the observed P.D. across the protoplasm for the cell in question when the external solution is sea water.

[9] It will be noted that when the external solution is changed the rise (or fall) of the P.D. to its new value is rather slow. This delay represents the time necessary for salts to be leached out of the cell wall (or to diffuse in) so that the concentration there is the same as in the external solution. The maximum effect cannot be observed until the salts in the entire cell wall have been brought to the new concentration.

This result (dilute solution negative) is unlike that hitherto found[10] for living cells,[11] although some non-living structures[12] show a concentration effect with dilute solution negative. This might suggest a difference between *Valonia* and the other organisms hitherto investigated, perhaps analogous to that found by Beutner[13] between acid "oils" (which give dilute solution positive) and basic "oils" (which give dilute solution negative). It is not certain, however, that *Valonia* behaves like Beutner's basic "oils" in giving with all salts a concentration effect in the same direction. We have some evidence that with KCl the dilute solution may be positive. Unfortunately, in studying the concentration effect in *Valonia* with solutions other than sea water, secondary changes are encountered which make the data hard to interpret.

Beutner found that with "oils" in most cases the concentration effect is less at higher concentrations than at low concentrations, a number of "oils" showing with the more dilute solutions the theoretical maximum of 58 mv. for a tenfold dilution. So also, *Nitella*[1] gives about 56 mv. for KCl between 0.01 and 0.001 M, but much less between 0.1 and 0.01 M. This might suggest that with *Valonia* higher values might be expected if we could employ lower concentrations of sea water. There is no hint of this, however, in the P.D.'s which we observe with concentrations down to 0.2 sea water, which proves to be the greatest dilution which we can apply to *Valonia* without inducing too rapid secondary changes.

[10] For the literature, see Osterhout and Harris.[1] With *Nitella*, dilute sea water is positive to more concentrated sea water (unpublished experiments).

[11] The only exception is the result reported by Brooks and Gelfan for *Nitella* (Brooks, S. C., and Gelfan, S., *Protoplasma*, 1928, **5**, 86) on applying sap, which is the opposite of that found for K salts in *Nitella* by Jost (Jost, L., *Sitzungsber. Heidelberger Akad. Wissensch., Abt. B.*, 1927, Abhandl. 13, Nov.) and for sap and for NaCl and for many salts (unpublished experiments), and especially for KCl, by Osterhout and Harris;[1] in these experiments the dilute solution was positive in all cases.

[12] *E.g.*, dead frog skin at low pH values (Amberson, W. R., and Klein, H., *J. Gen. Physiol.*, 1927–28, **11**, 823), the chorion of the egg of the marine fish *Fundulus*, at low pH values (Sumwalt, M., *Biol. Bull.*, 1929, **66**, 193), collodion mixed with rhodamine (Mond, R., and Hoffmann, F., *Arch. ges. Physiol.*, 1928, **220**, 194).

[13] Beutner, R., Die Entstehung elektrischer Ströme in lebenden Geweben. Stuttgart, 1920.

According to Höber the protoplasmic surface of *Valonia* has some areas which are permeable to cations and other areas permeable to anions.[14] The fact that the dilute solution is negative would be interpreted to mean that the areas permeable to anions dominate the situation, and by analogy with similar rhodamine-collodion membranes the concentration effect should fall off rapidly with increasing dilution. It is evident from our results that this is not the case. There are other objections to Höber's theory which need not be mentioned here.[15]

The empirical equation (1) expressing the concentration effect with sea water in *Valonia* obviously resembles the familiar Nernst formula for the P.D. arising from a diffusion gradient:

$$\text{P.D.} = \frac{u - v}{u + v} \frac{RT}{F} \ln \frac{\text{concn.}_1}{\text{concn.}_2}$$

This formula has been derived by Riesenfeld[16] for chains in which aqueous solutions containing the same electrolyte at different concentrations are separated by a solution of the electrolyte in a non-aqueous solvent immiscible with water, assuming that the ionic distribution ratios are equal at the two phase boundaries, and hence that the P.D.'s at the two boundaries are equal and opposite. In this case, u and v represent the mobilities of the cation and anion in the non-aqueous phase. Michaelis[17] has employed this equation for calculating transfer numbers in molecular seive membranes.

If this expression holds also for the sea water concentration effect in *Valonia*, *i.e.*, if the factor, $\frac{2}{3}$, in equation (1) represents the difference between the transfer numbers of anion and cation, we may calculate the transfer numbers in the protoplasm (or its external surface layer) assuming that a single ion pair is responsible for the P.D. Since the

[14] Höber, R., and Höber, J., *Arch. ges. Physiol.*, 1928, **219**, 260. Höber, R., and Hoffmann, F., *ibid.*, 1928, **220**, 558.

[15] *Cf.* Cooper, W. C., Jr., Dorcas, M. J., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1928–29, **12**, 427.

[16] See reference to Riesenfeld and Reinhold, *Z. physik. Chem.*, 1909, **68**, 459, in the chapter by Cremer, M., *Handb. norm. u. path. Physiol.*, 1928, **8**, 1003–4.

[17] Michaelis, L., Molecular sieve membranes, in Molecular physics in relation to biology, *Bulletin National Research Council*, 1929, No. 69, pp. 119–145.

more concentrated solution is positive, and hence the mobility of the anion is greater than that of the cation, we set:

$$\frac{v - u}{v + u} = \frac{2}{3}$$

and find $v = 5u$, *i.e.*, that the mobility of the anion is five times as great as that of the cation. The transfer number of the anion, $\frac{v}{u + v}$, is then 0.83, and that of the cation, $\frac{u}{u + v}$, is 0.17. The anion and cation in question are apparently Cl^- and Na^+.

It can be shown experimentally that changes in P.D. produced by dilution of the external sea water are due principally to changes in concentration of Na^+ and Cl^-, the changes in concentration of other ions in sea water playing a relatively small part. Attempts to measure the concentration effect with solutions of pure NaCl indicate that the more concentrated solution is positive, as with sea water.

Accurate measurements are impracticable, however, since fluctuations in the P.D. show that the system is changing continuously. Evidently the protoplasm is altered by contact with such unbalanced solutions. We can demonstrate the effect of the concentrations of Na^+ and Cl^- in another way, however. We compare the P.D. observed (1) with diluted sea water, in which the concentrations of all salts are reduced by the same factor, and (2) with an artificial sea water in which the concentrations of Na^+ and Cl^- alone are reduced, the other principal constituents remaining approximately the same as in undiluted sea water.

In Fig. 3 are shown graphically the results of such an experiment, comparing the P.D.'s with 0.33 natural and artificial sea water and

the P.D. with what we may call $Na^+/_3$, $Cl^-/_3$ sea water,[18] in which Na^+ and Cl^- have the same concentration as in the 0.33 sea water, while the other principal constituents of sea water, except $SO_4^=$, remain approximately unchanged. (An excess of sulfate is added to replace a part of the chloride, since the decrease in Cl^- is greater than in Na^+.)[18] It will be noted that the effect of changing the concentrations of Na^+ and Cl^- alone is even greater than that of diluting all the salts. The discrepancy is not large, however: after the P.D. with 0.33 sea water has reached its full value, all readings with all three solutions lie within the range 23.8 ± 0.8 mv. This is in good agreement with the value, 23.6 mv., calculated by adding $\frac{2}{3}\frac{RT}{F}$ ln 3 to the 5.0 mv., observed with sea water (temperature, 21°C.).

[18] The following table gives the composition of the $Na^+/_3$, $Cl^-/_3$ sea water, and, for comparison, the formula for artificial sea water on which it is based. This is a simplified form of the formula recommended by McClendon, Gault, and Mulholland, *Publ. No. 251, Carnegie Inst. of Washington* (1917). It will be noted that the $Na^+/_3$, $Cl^-/_3$ sea water contains an excess of sulfate, added to replace a part of the chloride, since the decrease in Cl^- is greater than in Na^+. Glycerol is added to make the solution isotonic with sea water.

	Artificial sea water	$Na^+/_3$, $Cl^-/_3$ sea water
	cc.	*cc.*
Normal solutions of $CaCl_2$	22.0	22.0
$MgCl_2$	50.4	
$MgSO_4$	57.2	107.6
KCl	10.2	10.2
NaCl	483.6	156.5
NaBr	0.8	0.8
$NaHCO_3$	2.5	2.5
Na_2SO_4		2.5
8.7 per cent solution of glycerol		550.0
Distilled water to make	1000.0	1000.0
Total chlorides	566.2	188.7
Total sodium salts	486.9	162.3

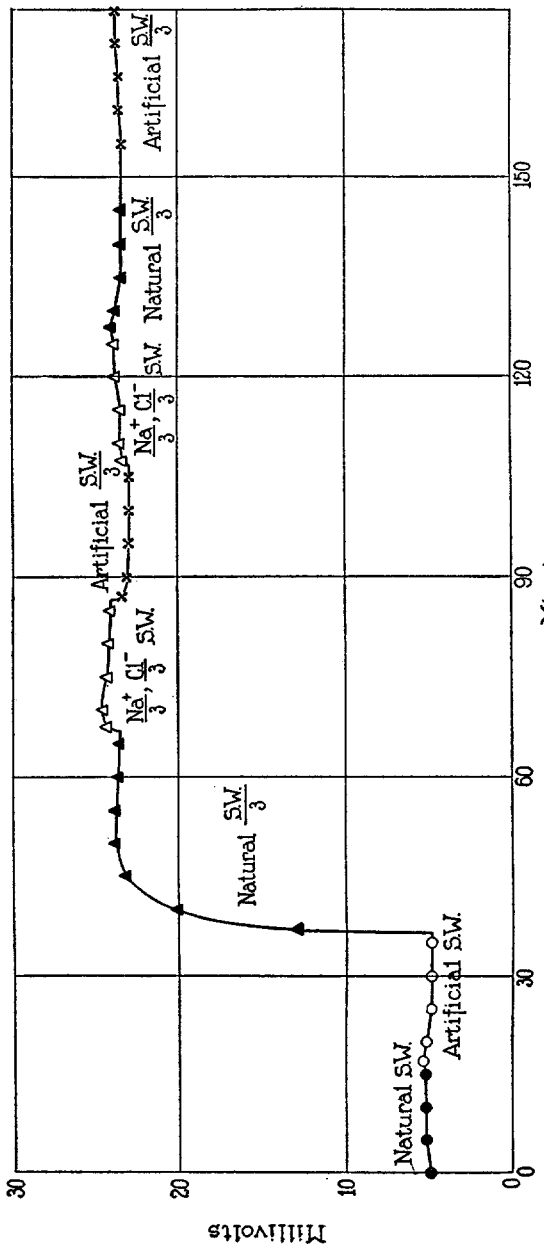

FIG. 3. P.D.-time curve, comparing the P.D.'S with 0.33 (natural and artificial) sea water and that with an artificial solution in which the concentrations of Na^+ and Cl^- are approximately the same as in 0.33 sea water, while the concentrations of the other principal ions of sea water are the same as in ordinary sea water, or somewhat greater ($SO_4^=$).

SUMMARY

The P.D. between the interior of a cell of *Valonia macrophysa* and concentrated or diluted sea water applied externally is given by the empirical equation:

$$\text{P.D.} = \text{P.D.}_{\text{s.w.}} + \frac{2}{3}\frac{RT}{F}\ln\frac{1}{\text{concn.}}$$

where $\text{P.D.}_{\text{s.w.}}$ is the value observed when the external solution is sea water. The sign is that of the interior of the cell. In the chain:

sea water, concn._1 | *Valonia* | sea water, concn._2

therefore, the more concentrated solution is positive in the external circuit to the more dilute solution.

This holds for the concentration range 1.5 sea water to 0.2 sea water, when the dilute solutions are made isotonic with sea water by the addition of a suitable non-electrolyte (such as glycerol). Prolonged exposure to these solutions, or brief exposure to very concentrated or very dilute sea water, or to hypotonic solutions, produces in the cell secondary changes which are made evident by the reversal of the sign of the P.D. These changes are to a certain extent reversible, but if allowed to proceed too far they become irreversible, involving permanent injury to the cell.

The concentration effect with sea water is shown to be practically the concentration effect for NaCl, the part played by the other salts in sea water being relatively small.