

A THEORY OF INJURY AND RECOVERY.

II. EXPERIMENTS WITH MIXTURES.

By W. J. V. OSTERHOUT.

(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)

(Received for publication, January 15, 1921.)

The electrical conductivity of *Laminaria* changes when the plant is transferred from sea water to 0.52 M NaCl, or to 0.278 M CaCl₂, and is subsequently replaced in sea water. It has been found that these changes can be predicted with considerable accuracy by means of certain equations. According to the theory of the writer,¹ the same equations should enable us to predict the changes produced by transferring tissue from sea water to mixtures of NaCl and CaCl₂ and then replacing the plant in sea water.

In order to test this theory experiments were made with a variety of mixtures. The solutions employed are given in Table I. The electrical resistance of the tissue in these solutions is shown in Fig. 1.

The experimental data are in good agreement with the values calculated by means of the formula:

$$\text{Resistance} = 2,700 \left(\frac{K_A}{K_M - K_A} \right) \left(e^{-K_A T} - e^{-K_M T} \right) + 90 e^{-K_M T} + 10$$

This is evident from Fig. 1, which shows the curves calculated by means of this formula and also the observed values.²

¹ Osterhout, W. J. V. *J. Gen. Physiol.*, 1920-21, iii, 145. For corrections see the slips inserted in this and in the preceding number of the *Journal*.

² The points shown in Fig. 1 (except those for the solutions containing 1.41 and 2.44 per cent CaCl₂) are taken from Fig. 1 of a previous paper (cited below), but the curves here given are not identical with those shown in that figure since the curves of the present paper represent the calculated values (using the velocity constants given in Table II), while those in Fig. 1 of the former paper are merely smoothed curves drawn through the observed points.

The results described in the former paper as obtained with solutions containing

TABLE I.
Composition of Mixtures.

0.52 M NaCl	0.278 M CaCl ₂	Molecular proportions in the mixture.	
		NaCl	CaCl ₂
cc.	cc.	per cent	per cent
973	27	98.59	1.41
955	45	97.56	2.44
914	86	95.24	4.76
751	249	85.00	15.00
496	504	65.00	35.00
247	753	38.00	62.00

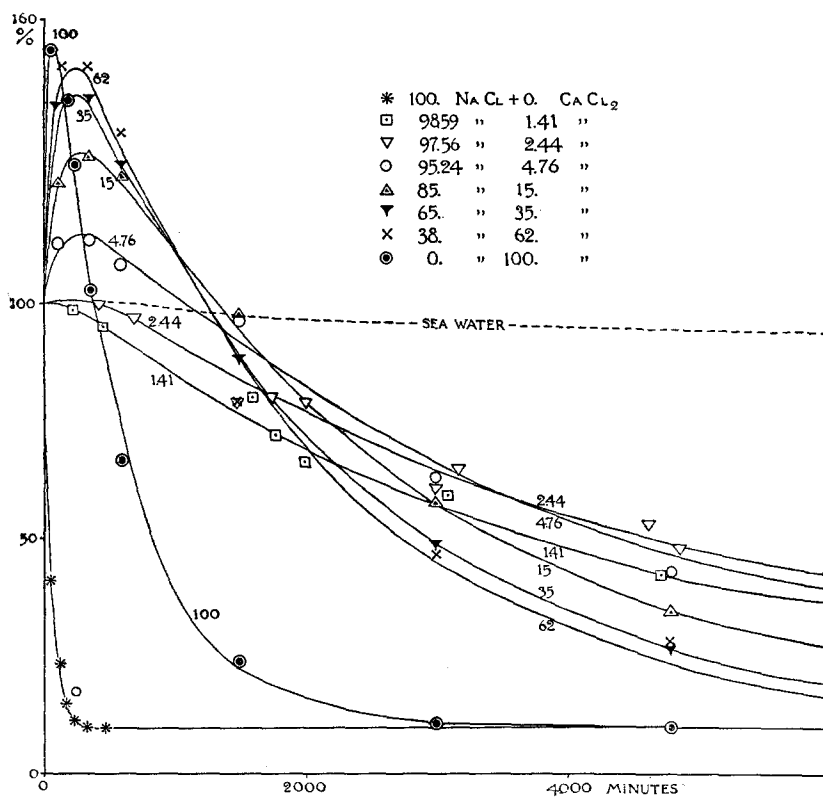
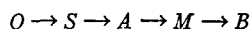


FIG. 1. Curves showing the electrical resistance of *Laminaria agardhii* in 0.52 M NaCl, in 0.278 M CaCl₂, and in mixtures of these (the figures attached to the curves show the molecular per cent of CaCl₂ in the solution). The curves show the calculated values (from constants obtained by trial, which are given in Table II); the points show the observed values (some are omitted in order to avoid undue crowding); each represents the average of six or more experiments. Probable error of the mean less than 10 per cent of the mean.

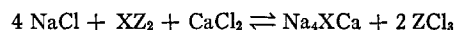
This formula is based upon the assumption that the electrical resistance is proportional to a substance, M , which is formed and decomposed by the reactions³



We assume that when the tissue is transferred from sea water to NaCl, or to CaCl₂, or to a mixture of these two solutions, the reactions $O \rightarrow S \rightarrow A$ cease, while the reactions $A \rightarrow M \rightarrow B$ continue. By assuming various values of K_A (the velocity constant of the reaction $A \rightarrow M$) and of K_M (the velocity constant of the reaction $M \rightarrow B$), and employing these in the formula, we obtain curves which closely approximate those which we find by experiment. The values of the velocity constants which are thus obtained are given in Table II.

It is evident from Table II that as the per cent of CaCl₂ in the mixtures increases (beginning at 1.41 per cent CaCl₂) the value of K_M first falls and then rises, its minimum value occurring in 97.56 NaCl + 2.44 CaCl₂ (which is the mixture in which the tissue lives the longest). It seems reasonable to assume that in each mixture a substance is formed which reduces the value of K_M . We may assume that the decrease of K_M is directly proportional to the amount of this substance, which may be assumed to occur in maximum amount in 97.56 NaCl + 2.44 CaCl₂.

The simplest assumption which we can make is that NaCl and CaCl₂ combine with some constituent of the protoplasm, as XZ_2 , to form a compound.⁴ If we suppose that the compound is Na₄XCa, formed by the reversible reaction



we can calculate the amount of Na₄XCa which is formed in each mixture of NaCl and CaCl₂.

2 per cent CaCl₂ are omitted, as it was subsequently found that this solution contained more than 2 per cent CaCl₂.

For the data concerned and for the derivation of the formula see Osterhout, W. J. V., *Proc. Am. Phil. Soc.*, 1916, lv, 533. In the present paper the formula previously used is multiplied by 305 and 10 is added.

³ These are regarded as monomolecular and irreversible or practically so.

⁴ It is assumed that XZ_2 , Na₄XCa, and ZCl₂ are in solution. Since the per cent of XZ_2 which is transformed to Na₄XCa is negligible, the concentration of XZ_2 may be regarded as constant.

TABLE II.
Velocity Constants at 15°C.

Reaction.	Velocity constant.	Value of the velocity constant in*									
		NaCl	NaCl 98.59 CaCl ₂ 1.41	NaCl 97.56 CaCl ₂ 2.44	NaCl 95.24 CaCl ₂ 4.76	NaCl 85.0 CaCl ₂ 15.0	NaCl 65.0 CaCl ₂ 35.0	NaCl 38.0 CaCl ₂ 62.0	CaCl ₂		
<i>N</i> → <i>O</i>	<i>K_N</i>	0.03	0.00046	0.0004	0.00061	0.00091	0.0013	0.00134	0.005033		
<i>O</i> → <i>P</i>	<i>K_O</i>	0.0297	0.0004554	0.000396	0.0006039	0.0009009	0.001287	0.0013266	0.0049827		
<i>R</i> → <i>S</i>	<i>K_R</i>	0.04998	0.0005623	0.00056	0.001055	0.001606	0.0026	0.003509	0.012532		
<i>S</i> → <i>T</i>	<i>K_S</i>	0.02856	0.0008048	0.000874	0.00151	0.0017446	0.00254	0.00319	0.010848		
<i>A</i> → <i>M</i>	<i>K_A</i>	0.018	0.000222	0.000187	0.000245	0.000364	0.000481	0.00053	0.0018		
<i>M</i> → <i>B</i>	<i>K_M</i>	0.540	0.00666	0.00546	0.00590	0.00730	0.00859	0.009	0.0295		

* The figures at the heads of columns refer to molecular proportions in the mixture.

For reasons given in a former paper,⁵ we assume that this reaction occurs at the surface of the cell and that CaCl_2 accumulates in the surface to a greater degree than NaCl . The increase in concentration of CaCl_2 in the surface is supposed to be ten times as great as the corresponding increase of NaCl , so that the proportions in the surface are those given in Table III. For example, when the proportions in the solution are $97.56 \text{ NaCl} + 2.44 \text{ CaCl}_2$, the proportion of NaCl to CaCl_2 in the surface is as 97.56 to 24.40, which is equivalent⁶ to $80 \text{ NaCl} + 20 \text{ CaCl}_2$.

TABLE III.
Amount of Na_4XCa .

Molecular proportions.				Amount of Na_4XCa	Increase in Na_4XCa
In the solution.		In the surface.			
NaCl	CaCl ₂	NaCl	CaCl ₂		
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
100.0	0.	100.0	0	0	0
98.59	1.41	87.5	12.5	0.000902	0.000855
97.56	2.44	80.0	20.0	0.000936	0.000889
95.24	4.76	66.67	33.33	0.000870	0.000823
85.0	15.0	36.27	63.73	0.000488	0.000441
65.0	35.0	15.66	84.34	0.000177	0.000125
38.0	62.0	5.78	94.22	0.000047	0
0	100.0	0	100.0	0	0

We calculate the amount of Na_4XCa by the usual formula:

$$K = \frac{(C_{\text{Na}_4\text{XCa}}) (C_{\text{ZCl}_2})^2}{(C_{\text{NaCl}})^4 (C_{\text{CaCl}_2}) (C_{\text{XZ}_2})}$$

but since $2C_{\text{Na}_4\text{XCa}} = C_{\text{ZCl}_2}$ we may write

$$K = \frac{(C_{\text{Na}_4\text{XCa}}) (2 C_{\text{Na}_4\text{XCa}})^2}{(C_{\text{NaCl}})^4 (C_{\text{CaCl}_2}) (C_{\text{XZ}_2})}$$

⁵ Osterhout, W. J., V., *Proc. Am. Phil. Soc.*, 1916, lv, 533.

⁶ As explained in a former paper,⁵ it is assumed that the reaction takes place in a surface which is saturated with respect to NaCl and CaCl_2 , so that while one of these may be displaced by the other (in case their relative proportions in the solution are altered) the total concentration does not change; for convenience this concentration is taken as 100 and the sum of $\text{NaCl} + \text{CaCl}_2$ is therefore always equal to 100.

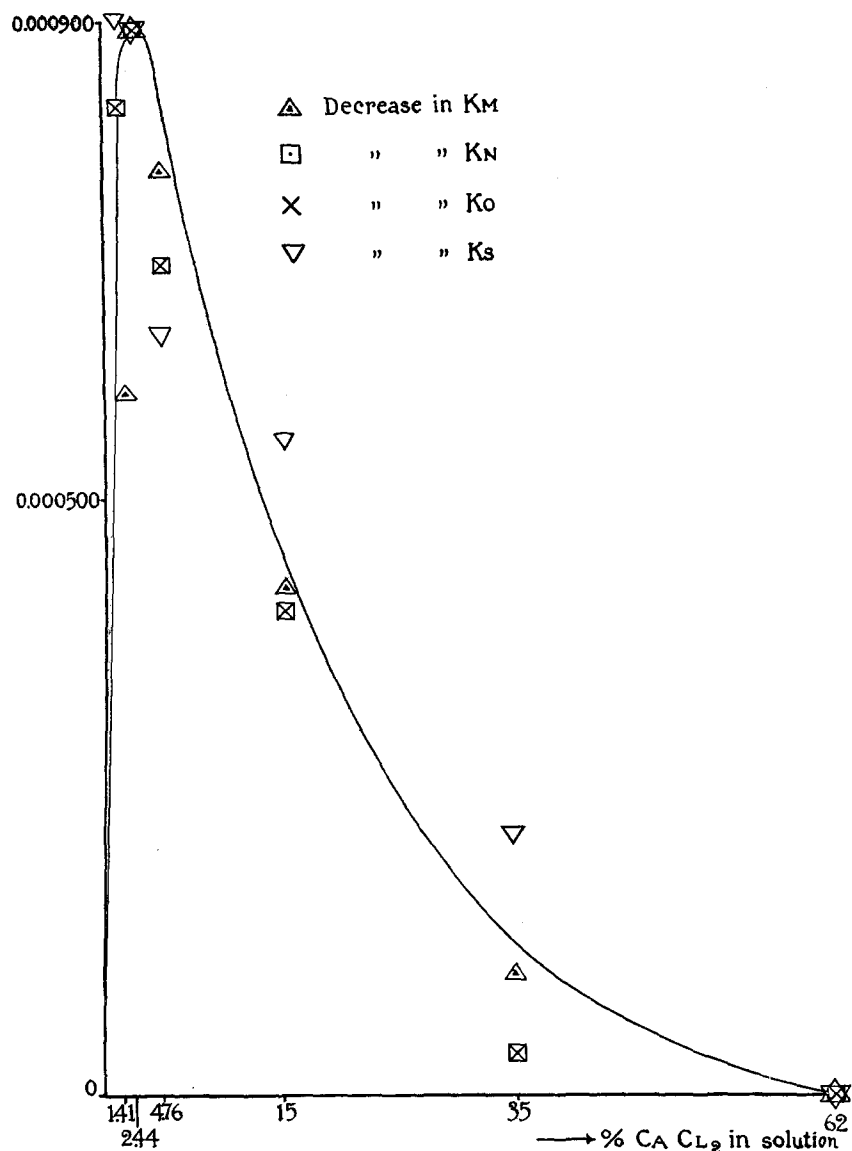


FIG. 2. Curve showing the increase of a hypothetical salt compound Na_4XCa (see Table III); and the corresponding decrease of the velocity constants K_N , K_O , K_S , and K_M (these constants are given in Table II). The figures on the abscissa give the molecular per cent of $CaCl_2$ in the mixture. The mixture containing 62.0 per cent $CaCl_2$ is taken as the standard of comparison: proceeding from this to the mixtures containing less $CaCl_2$ we find that Na_4XCa increases and the velocity constants decrease as shown by the ordinates. In order to facilitate comparison the values of K_N have been multiplied by 0.989; of K_O by 0.991; of K_S by 0.384; and of K_M by 0.251.

Putting $K = 4(10^{-17})$ and $C_{XZ_2} = 0.1$ we get⁴

$$10^{-17} = \frac{(C_{Na_4XC_a})^2}{(80)^4 (20) (0.1)}$$

whence $C_{Na_4XC_a} = 0.000936$.

Proceeding in the same manner with the other mixtures we get the values given in Table III. Starting with the lowest value (that in 62.0 per cent $CaCl_2$) we observe that there is an increase as the per cent of $CaCl_2$ decreases until 2.44 per cent is reached (the amount of this increase is shown in Column 6 of the table). Conversely we find (Table II) that the velocity constants are higher in 62.0 per cent $CaCl_2$ than in any other mixture and that they decrease as the per cent of $CaCl_2$ decreases to 2.44 per cent. Thus in the case of K_M the value in 62.0 per cent $CaCl_2$ is 0.009, in 2.44 per cent $CaCl_2$ it is less by 0.00354, while in 15.0 per cent it is less by 0.0017, and in 35.0 per cent by 0.00041; if we multiply these numbers by the constant factor 0.251 they agree very closely with the figures for the increase in Na_4XC_a . These values are plotted in Fig. 2, which shows that the decrease in K_M is directly proportional to the increase in the amount of Na_4XC_a . Hence we assume that Na_4XC_a acts as a negative catalyzer or inhibitor of the reaction $M \rightarrow B$.

An inspection of Table II shows that the value of K_A fluctuates with that of K_M except that as $CaCl_2$ increases the value of K_A rises more rapidly than that of K_M . This is also obvious from Fig. 1, which shows that the greater the per cent of $CaCl_2$ in the mixture the greater the maximum attained. Since this maximum increases as the value of $K_A \div K_M$ increases, it is evident that the value of $K_A \div K_M$ must rise as the per cent of $CaCl_2$ becomes greater. The value of $K_A \div K_M$ in the solution containing 1.41 per cent of $CaCl_2$ is 0.03333 while in the solution containing 62.0 per cent $CaCl_2$ it is 0.05889, an increase of 0.02556. If we calculate this increase for the other mixtures and plot the values so obtained against the per cent of $CaCl_2$ in the surface, we obtain a straight line as shown in Fig. 3. This indicates that $CaCl_2$ catalyzes the reaction $A \rightarrow M$; for if this were not the case the value of K_A and K_M would rise and fall in such a way that the value of $K_A \div K_M$ would remain constant.

It is evident from Figs. 2 and 3 that the values of K_A and K_M are determined by the amount of Na_4XC_a and by the per cent of $CaCl_2$

in the mixture, and that when these values are experimentally determined for any two mixtures they can be calculated for any other mixture. When this is done we can calculate the course of the death curve in that mixture.

Having thus accounted for the death curves, we may turn our attention to the process of recovery. We find that, when tissue is removed from a mixture of NaCl and CaCl₂ and replaced in sea water, the resistance at once rises or falls and after a time becomes stationary. This rise or fall of resistance may be called recovery.

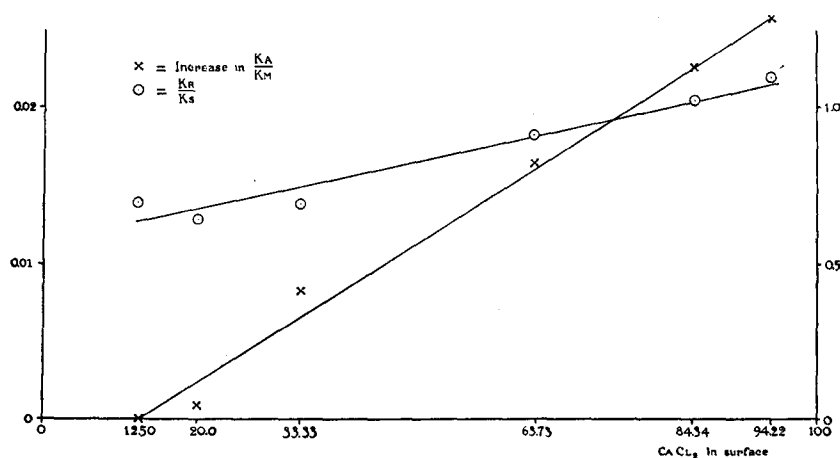


FIG. 3. Graph showing the increase of $K_A + K_M$ and the value of $K_R + K_S$ as the molecular per cent of CaCl₂ increases. The figure shows that CaCl₂ acts as a catalyzer of the reaction $A \rightarrow M$ (which has the velocity constant K_A) and also of the reaction $R \rightarrow S$ (which has the velocity constant K_R). The figures on the ordinate at the right show the values of $K_R + K_S$; those on the ordinate at the left show the increase in the value of $K_A + K_M$ over the value found in the mixture containing 1.41 per cent CaCl₂. The abscissæ denote molecular per cent of CaCl₂ in the surface (not in the solution).

In order to account for the facts we suppose that when we replace the tissue in sea water the reactions $O \rightarrow S \rightarrow A \rightarrow M \rightarrow B$ proceed at the rates which are normal for sea water. The manner in which the rate of recovery is calculated has been explained in detail in a previous paper.¹ It is assumed that during the exposure to any of the mixtures the following reactions occur: (1) $N \rightarrow O \rightarrow P$; (2) $R \rightarrow S \rightarrow T$; (3) $A \rightarrow M \rightarrow B$. By assuming values of the velocity

constants of these reactions we can approximate the observed results. The velocity constants thus found are given in Table II. An inspection of the table shows that all these velocity constants behave like K_A and K_M in that as the per cent of CaCl_2 in the mixture increases (beginning with 1.41 per cent CaCl_2) the value of the velocity constant first falls and then rises, and that this value in every case reaches its minimum in the mixture containing 97.56 NaCl + 2.44 CaCl_2 . It would therefore appear that the reactions $N \rightarrow O \rightarrow P$ and $R \rightarrow S \rightarrow T$ are inhibited by Na_4XCa in the same manner as the reactions $A \rightarrow M \rightarrow B$. This is borne out by an inspection of Fig. 2. in which the decrease⁷ of the velocity constants is plotted, together with the increase of Na_4XCa .

We have seen that the value of $K_A \div K_M$ increases as the per cent of CaCl_2 increases and we interpreted this to mean that the reaction $A \rightarrow M$ is catalyzed by CaCl_2 . In the same manner we infer that the reaction $R \rightarrow S$ is catalyzed by CaCl_2 , since we find that the value of $K_R \div K_S$ increases with increasing percentage of CaCl_2 , as shown in Fig. 3. It is not certain that the curve does not reach a minimum in the mixture of 97.56 NaCl + 2.44 CaCl_2 but for practical purposes we may, for the present, regard it as a straight line.⁸

⁷ By the decrease in the velocity constant is meant the decrease which we observe as we pass from the solution containing the highest per cent of calcium (38.0 per cent NaCl + 62.0 per cent CaCl_2) to mixtures containing smaller per cents of calcium. Thus the decrease of $K_M = 0.009 - K_M$; the decrease of $K_N = 0.00134 - K_N$; the decrease of $K_O = 0.0013266 - K_O$; and the decrease of $K_S = 0.00319 - K_S$. In the same manner we find that the increase in the amount of $\text{Na}_4\text{XCa} = \text{amount of Na}_4\text{XCa} - 0.000047$.

The decrease of the amount of K_A and K_R is not shown in the figure because it depends not only on Na_4XCa but also on the per cent of CaCl_2 .

The fact that even in the presence of the maximum amount of Na_4XCa these velocity constants are greater than in sea water is of course to be attributed to the other substances present in sea water.

⁸ Since in pure NaCl or CaCl_2 the salt compound Na_4XCa is not formed, we should expect that in these solutions all the reactions would be more rapid than in the mixtures. That this expectation is fully realized is evident from Table II.

The velocity constants are somewhat higher in NaCl than in CaCl_2 ; this is not explained by the assumptions already made but it does not seem desirable at present to make additional assumptions for this purpose. We might expect the values of $K_A \div K_M$ and $K_R \div K_S$ to reach a maximum in CaCl_2 . This is actually the case. It might perhaps be expected that these values would fall to a minimum in NaCl. This is the case with $K_A \div K_M$ but not for $K_R \div K_S$.

The relation between K_N and K_O is taken as constant in the proportion of 100 to 99.

It is evident that when the constants have been empirically determined for two mixtures the constants for any other mixture can be calculated at once, since all of them depend in a definite manner on Na_4XCa (K_A and K_R also depend on the per cent of CaCl_2). The agreement between the constants thus obtained by calculation and those found by trial is fairly close, as is evident from Figs. 2 and 3.⁹

It has been shown in a previous paper that the height to which the recovery curve rises depends on the value of O : the value of O

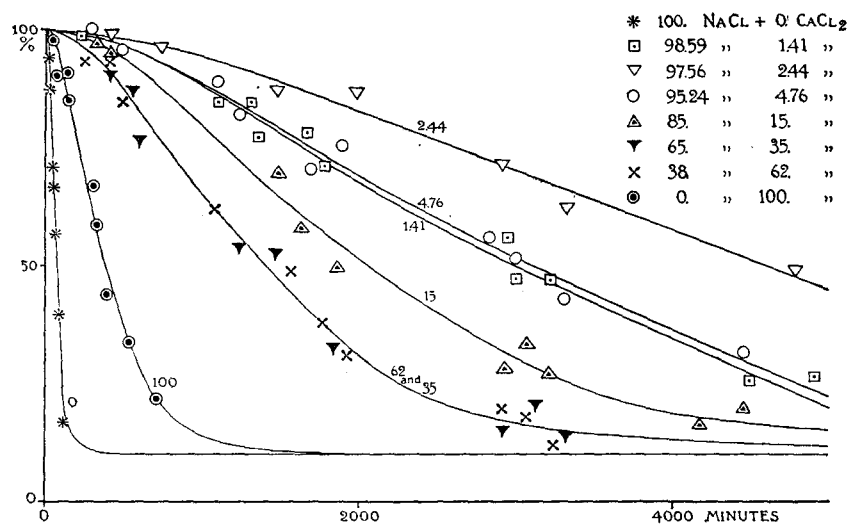


FIG. 4. Curves showing the value of $O + 10$ in 0.52 M NaCl, in 0.278 M CaCl_2 , and in mixtures of these (the figures attached to the curves show the molecular per cent of CaCl_2 in the solution). The ordinates give the relative values of $O + 10$, the value in sea water being arbitrarily taken as 100 per cent. These values are obtained by exposing tissue to toxic solutions and then finding the level to which the resistance rises or falls after the tissue is replaced in sea water: they are therefore a measure of permanent injury. The abscissæ give the length of exposure to the toxic solution. The curves show the calculated values (using the velocity constants given in Table II). The points show the observed values; each represents the average of six or more experiments. Probable error of the mean less than 10 per cent of the mean.

⁹ The constants obtained by calculation would fall exactly on the graphs in these figures while those found by trial are indicated by the points given.

+ 10 is shown for all the solutions¹⁰ in Fig. 4, which shows the agreement between observation and calculation in respect to the final level reached by the recovery curve, but not in respect to speed of recovery, which depends more on the value of S than on that of O . The rate of recovery seems to be about the same in the mixtures as in the pure salts. In general it is found that the rise or fall is nine-tenths completed in about an hour.

Fig. 5 shows the calculated values of S ; observed values are not given because they cannot be very precisely determined. This is

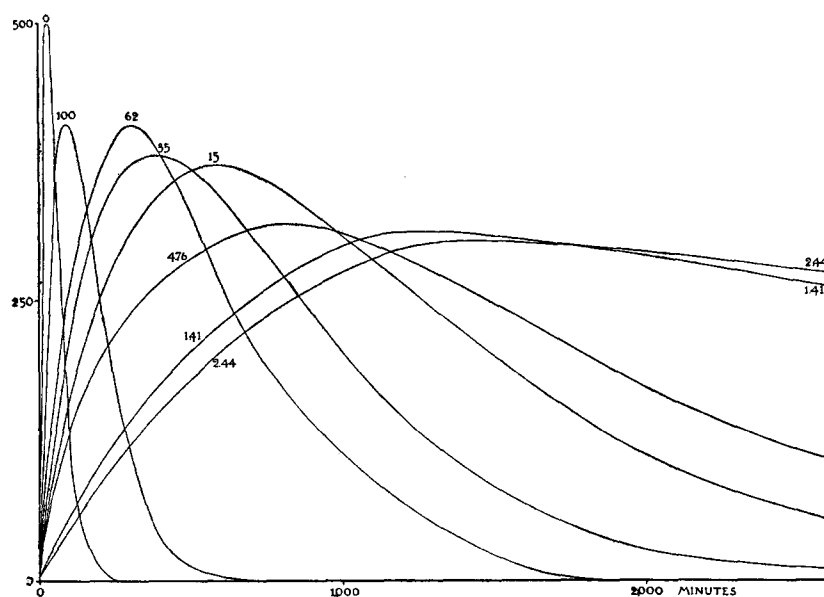


FIG. 5. Curves showing the (calculated) values of S in 0.52 M NaCl, 0.278 M CaCl_2 , and in mixtures of these (the figures attached to the curves show the molecular strength of CaCl_2 in the solution). The curves show the values calculated from constants obtained by trial, which are given in Table II. The abscissæ represent the time of exposure to the toxic solution. The value of S at the start is in all cases 2.7.

¹⁰ The values of $O + 10$ for solutions containing 2.44 and 15.0 per cent CaCl_2 differ slightly from those given in a former paper (Osterhout, W. J. V., *J. Gen. Physiol.*, 1920-21, iii, 15) for the reason that the curves here presented include a larger series of experiments. 10 is added to the value of O because the base line is taken as 10, just as in the case of M .

owing to the fact that S affects only the speed of recovery (not the final level attained) and as the speed is variable the most satisfactory procedure is to assume such values of K_R and K_S in the equation¹¹

$$S = R \left(\frac{K_R}{K_S - K_R} \right) \left(e^{-K_R T} - e^{-K_S T} \right) + S_0 e^{-K_S T}$$

as cause the closest approximation to the observed speed of recovery. The values of S thus obtained for each solution are shown in the figure. In general the speed of recovery, as calculated from these values of S , is in satisfactory agreement with the observations.

By means of the equations given in the previous paper, and of the velocity constants in Table II of this paper, we are able to calculate the recovery curves for any solution after any length of exposure.

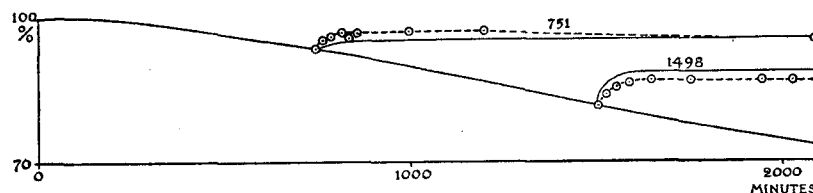


FIG. 6. Curves showing the electrical resistance (descending curve) of *Laminaria agardhii* in a mixture containing 97.56 mols of NaCl to 2.44 mols of CaCl₂ and recovery in sea water (ascending curves). The figure attached to each recovery curve denotes the time of exposure (in minutes) to the toxic solution. In the recovery curves the experimental results are shown by the broken lines, the calculated results by the unbroken lines. The observed points represent the average of six or more experiments. Probable error of the mean less than 10 per cent of the mean.

Lack of space prevents a tabulation of the observed and calculated values, but it is possible to exhibit graphically the data for three mixtures and for this purpose one in which recovery consists in a rise of resistance (Fig. 6), one in which it shows a moderate fall (Fig. 7), and one showing a very decided fall (Fig. 8) are presented. In general the agreement between observation and calculation is satisfactory for all the solutions employed in the investigation.

It might be thought that the number of constants is sufficient to make it possible to fit any sort of experimental curve and that the

¹¹ Cf. equation (3) of the preceding paper (Osterhout, W. J. V., *J. Gen. Physiol.*, 1920-21, iii, 145).

consequent agreement between observed and calculated results is less significant than would otherwise be the case. But, as a matter of fact, the constants are so related to each other and to the salt compound, Na_4XCa that the whole set of curves fits into a consistent scheme, so that when the constants are determined for any two mixtures the theoretical curves for all the other mixtures are thereby fixed. Under these circumstances the close agreement in the six different mixtures (ranging from 1.41 to 62.0 per cent CaCl_2) seems to be significant.

There seems to be no doubt that the behavior of the tissue is such as to indicate an underlying mechanism which is the same in all cases.¹² We have assumed that this mechanism consists in the production and decomposition of a substance, M , the amount of which, in the mix-

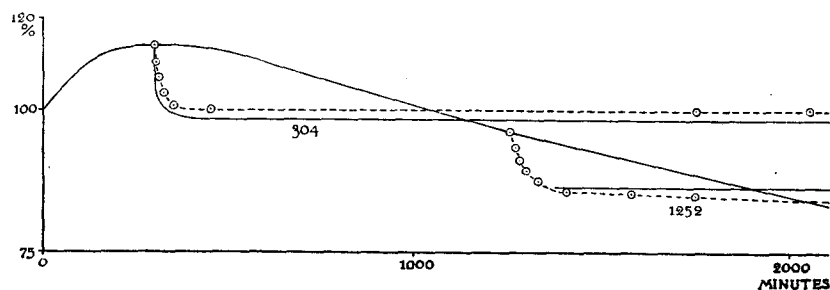


FIG. 7. Curves showing the electrical resistance (curve which ascends and descends) of *Laminaria agardhii* in a mixture containing 95.24 mols of NaCl to 4.76 mols of CaCl_2 , and recovery in sea water (descending curves). The figure attached to each recovery curve denotes the time of exposure (in minutes) to the toxic solution.

In the recovery curves the experimental results are shown by broken lines, the calculated results by unbroken lines. The observed points represent the average of six or more experiments. Probable error of the mean less than 10 per cent of the mean.

¹² This is shown, for example, by the fact that the rapidity of permanent injury (as observed after replacement in sea water) corresponds throughout with the rate of death, and that the rate of change of M corresponds throughout with the rate of change of O , S , and A . In other words if we change the solution in such a way as to increase (or decrease) the rate of one of the reactions on which the resistance depends we simultaneously increase (or decrease) the rates of all the others in a definite and predictable manner.

tures, depends largely on a compound Na_4XCa formed by the combination of Na and Ca with a constituent X of the protoplasm. It is not necessary to discuss these assumptions more fully at present. But it may be pointed out that two things seem to be fairly well established; (1) a consistent mechanism underlies the entire behavior of the

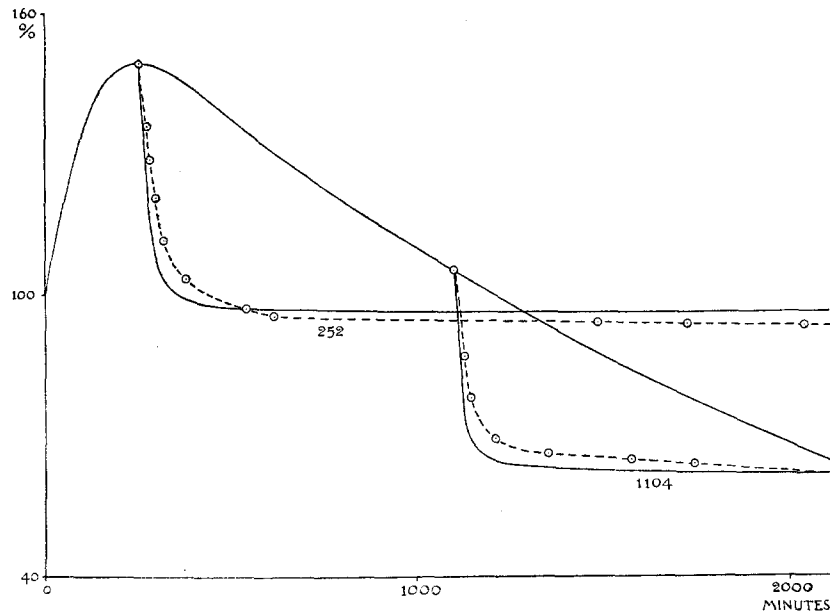


FIG. 8. Curves showing the electrical resistance (curve which ascends and descends) of *Laminaria agardhii* in a mixture containing 38 mols of NaCl to 62 mols of CaCl_2 , and recovery in sea water (descending curves). The figure attached to each recovery curve denotes the time of exposure (in minutes) to the toxic solution. In the recovery curves, the experimental results are shown by the broken lines, the calculated results by the unbroken lines. The observed points represent the average of six or more experiments. Probable error of the mean less than 10 per cent of the mean.

tissue, and (2) its operation can be predicted with a fair degree of accuracy by means of the equations which have been developed. The predictive value of these equations may be regarded as permanently established, since it does not depend on our views regarding the underlying assumptions.

SUMMARY.

1. The equations which serve to predict the injury of tissue in 0.52 M NaCl and in 0.278 M CaCl₂ and its subsequent recovery (when it is replaced in sea water) also enable us to predict the behavior of tissue in mixtures of these solutions, as well as its recovery in sea water after exposure to mixtures.

2. The reactions which are assumed in order to account for the behavior of the tissue proceed as if they were inhibited by a salt compound formed by the union of NaCl and CaCl₂ with some constituent of the protoplasm (certain of these reactions are accelerated by CaCl₂).

3. In this and preceding papers a quantitative theory is developed in order to explain: (a) the toxicity of NaCl and CaCl₂; (b) the antagonism between these substances; (c) the fact that recovery (in sea water) may be partial or complete, depending on the length of exposure to the toxic solution.