

A THEORY OF INJURY AND RECOVERY.

I. EXPERIMENTS WITH PURE SALTS.

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Previous researches¹ indicate that the process of death conforms to the laws of chemical dynamics and that further investigation of this subject may throw light on the fundamental mechanism of normal life processes. The present series of papers continues these studies and deals with the recovery of cells after exposure to toxic solutions.

When the marine alga *Laminaria* is transferred from sea water to a solution of sodium chloride (of the same conductivity as the sea water) its electrical resistance falls steadily until it reaches a stationary condition, which indicates death. If the tissue is replaced in sea water before this point is reached we observe that the resistance rises; this may be called recovery. In earlier stages of the death process recovery may be complete (*i.e.* the normal resistance may be regained) but this is not the case in the later stages. This is evident from Fig. 1, which shows the death curve in a solution of NaCl and recovery curves after various periods of exposure to the solution.²

If in place of sodium chloride we employ calcium chloride and various mixtures of these salts, varying the times of exposure, and sometimes transferring the plant from one of these solutions to another, instead of replacing it in sea water, a very complicated set of curves is obtained. It is of interest to find that these may be predicted

¹Osterhout, W. J. V., *Proc. Am. Phil. Soc.*, 1916, lv, 533; *J. Gen. Physiol.*, 1920-21, iii, 15.

²*Cf.* Osterhout, W. J. V., *J. Gen. Physiol.*, 1920-21, iii, 15. The recovery curves rise to definite levels at which they may remain for days under favorable conditions. Often, however, there is a gradual decline which may be more rapid than that of the control.

with considerable accuracy by assuming that the electrical resistance is proportional to the amount of a substance in the cell which increases or decreases according to the relative proportion of salts in the external solution.

We assume that this substance (M) is formed and decomposed by a series of reactions³ of the type⁴

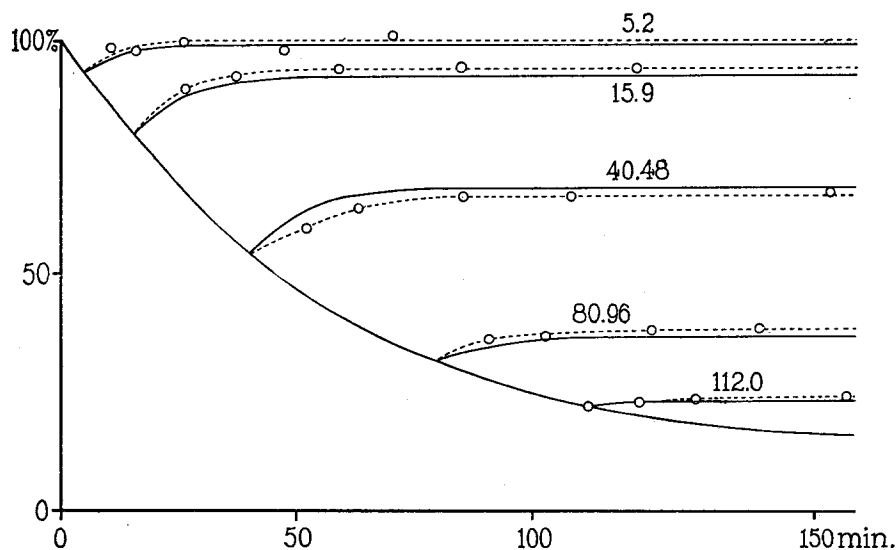
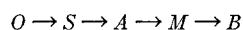


FIG. 1. Curves showing the fall of electrical resistance of *Laminaria agardhii* in 0.52 M NaCl (descending curve) and recovery in sea water (ascending curves). The figure attached to each recovery curve denotes the time of exposure (in minutes) of the solution of NaCl.

In the recovery curves the experimental results are shown by dotted lines, the calculated results by the unbroken lines (the curves are extended beyond the last observed point here shown because of later observations which are not shown in the figure).

The observed points represent the average of eight or more experiments; probable error of the mean less than 10 per cent of the mean.

³ These are regarded as irreversible or practically so.

⁴ It is assumed that O is present in relatively large amount so that it may be regarded as practically constant despite the fact that it very slowly decomposes to furnish S , A , M , and B .

and that in sea water these processes are in equilibrium (so that the amount of M remains constant) but that when the tissue is transferred to a solution of sodium chloride M is decomposed faster than it is formed and hence the resistance falls. On replacing the tissue in sea water, M is formed more rapidly than it is decomposed and in consequence the resistance rises.

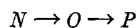
Let us assume that when the tissue is transferred from sea water to the solution of NaCl the reactions $O \rightarrow S \rightarrow A$ cease and that the velocity constant K_A of the reaction $A \rightarrow M$ increases from 0.0036 to 0.0180 while the velocity constant K_M of the reaction $M \rightarrow B$ increases from 0.1080 to 0.540. We may then calculate the resistance in the solution of NaCl after any length of exposure by means of the formula⁵

$$\text{Resistance} = 2,700 \left(\frac{K_A}{K_M - K_A} \right) \left(e^{-K_A T} - e^{-K_M T} \right) + 90 e^{-K_M T} + 10 \quad (1)$$

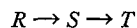
in which T is the time of exposure in minutes, and e is the basis of natural logarithms. 10 is added in the formula because the base line is taken as 10 (not as 0) for the reason that the resistance sinks to 10 (as shown in Fig. 1) when the tissue dies.

We assume that when the tissue is replaced in sea water the reactions $O \rightarrow S \rightarrow A$ recommence and that the values of K_A and K_M become 0.0036 and 0.1080 respectively, while the other velocity constants likewise acquire the values which they normally have in sea water. Under these conditions M will be formed faster than it is decomposed and the resistance will rise.

The fact that the rise does not reach as high a level after a long exposure as after a short one indicates that during the exposure O gradually diminishes; we assume that this takes place by the reactions



We likewise assume that during exposure to the solution of NaCl the amount of S changes by means of the reactions



and that on transferring to sea water S is rapidly converted into A .

⁵ For an explanation of the formula see Osterhout, W. J. V., *Proc. Am. Phil. Soc.*, 1916, lv, 533. The constants 8.853 and 0.2951 are here multiplied by 305, becoming 2,700 and 90 respectively.

In order to calculate the rate of recovery we find by trial the most satisfactory values of the velocity constants. The values thus found are given in Table I.

TABLE I.
Velocity Constants.

Reaction.	Velocity constant.	Value at 15°C. in	
		NaCl	CaCl ₂
$N \rightarrow O$	K_N	0.03	0.0045
$O \rightarrow P$	K_O	0.0297	0.004455
$R \rightarrow S$	K_R	0.04998	0.0145
$S \rightarrow T$	K_S	0.02856	0.007
$A \rightarrow M$	K_A	0.018	0.0018
$M \rightarrow B$	K_M	0.540	0.0295

As an example of the method of calculation we may take the case of tissue exposed for 15 minutes to a solution of 0.52 M NaCl at 17°C. The net⁶ resistance in sea water at the start was 960 ohms; in the course of 15 minutes in the solution of NaCl it fell to 775 ohms, which is 80.69 per cent of the original resistance.⁷ The fall of resistance is a little more rapid than in the "standard curve" obtained in a previous investigation.⁸ If we assume that this is due to the difference in temperature (these measurements were made at 17°C. while those on which the standard curve is based were obtained at 15°C.) we may introduce a correction by multiplying the abscissa by the factor⁹ 1.06, which makes it 15.9 minutes, and causes it to agree with the standard curve. All the abscissæ are multiplied by the same factor.¹⁰ The

⁶ The net resistance is that of the tissue itself, obtained by subtracting the resistance of the apparatus from the total resistance.

⁷ For convenience all results are expressed as per cent of the original resistance.

⁸ Osterhout, W. J. V., *J. Biol. Chem.*, 1917, xxxi, 585.

⁹ This agrees closely with the temperature coefficient as determined in a previous investigation. Cf. Osterhout, W. J. V., *Biochem. Z.*, 1914, lxxvii, 272.

¹⁰ This procedure may displace the points on the curve so that where several curves are averaged it may be necessary to employ interpolation in order to average points on the same ordinate. In many cases curves were obtained by averaging the ordinates of death curves and recovery curves before multiplying by the factor.

effect of this is to make the process appear to proceed at 15 instead of at 17°C. If the difference between the two curves is due wholly to difference in temperature this introduces no error, and if the difference is due in part to other factors, the error, if any, is less than the usual experimental error.

The advantages of this procedure are that we can employ for our calculations the constants already obtained for the standard curve and also compare the theoretical curves which start from the same points. This procedure has therefore been followed throughout and the corrected results (*i.e.* the figures multiplied by a suitable factor) are employed in the following description.

When the tissue was replaced in sea water the resistance began to rise. At the end of 10 minutes it had risen from 80.69 to ~~92.57~~ 89.10 per cent.¹¹ Since, however, the abscissæ of the death curve have been multiplied by 1.06 the same thing must be done for the recovery curve and in place of 10 minutes we must put 10.6 minutes. Proceeding in this manner we obtain the recovery curve which is labeled 15.9 in Fig. 1.

In order to calculate the course of the recovery curve we must consider the reactions which determine the amount of electrical resistance. When the tissue is placed in the solution of NaCl the reactions which occur are: (1) $A \rightarrow M \rightarrow B$; (2) $R \rightarrow S \rightarrow T$; and (3) $N \rightarrow O \rightarrow P$. Let us first consider the reactions $A \rightarrow M \rightarrow B$. The value of A in sea water is taken as 2,700 and that of M as 90. As explained in a former paper¹ the value of A will diminish during exposure to NaCl according to the formula

$$A e^{-K_A T} \quad . \quad (2)$$

Since $K_A = 0.018$ (see Table I) the value of A after 15.9 minutes in sea water is

$$2,700 e^{-(0.018) 15.9} = 2,027.96$$

The value of M at the end of 15.9 minutes is the observed resistance 80.69 less 10 (since the base line of the curve is not 0 but 10).

¹¹ In earlier experiments it was found that complete recovery was possible after the resistance had fallen to about 80 per cent. This was not the case in the present series; the difference may be due to differences in material or in technique. Cf. Osterhout, W. J. V., *Bot. Gaz.*, 1915, lix, 242.

On replacing the tissue in sea water, therefore, we start with $M = 70.69$ and $A = 2,027.96$, but this value of A is at once augmented by the conversion of S into A . In order to find the amount of this augmentation we must know the value of S .

During exposure to NaCl the reaction $R \rightarrow S \rightarrow T$ occurs. The value of S may be easily calculated by employing formula (1) and substituting the appropriate constants. We thus obtain

$$S = R \left(\frac{K_R}{K_S - K_R} \right) \left(e^{-K_R T} - e^{-K_S T} \right) + S_0 e^{-K_S T} \quad (3)$$

in which S_0 denotes the value of S at the start of the reaction. The value of R in sea water is taken as 1,041.77 and that of S as 2.7. In the solution of NaCl the values of K_R (the velocity constant of the reaction $R \rightarrow S$) and K_S (the velocity constant of the reaction $S \rightarrow T$) are taken as 0.04998 and 0.02856 respectively (see Table I). Hence the value¹² of S at the end of 15.9 minutes is 447.26. When the tissue is replaced in sea water S is rapidly converted into A so that the total value of the latter becomes $447.26 + 2,027.96 = 2,475.22$.

On replacing the tissue in sea water $A = 2,475.22$ and $M = 70.69$. The resistance after any given time T in sea water is obtained by modifying formula (1) which becomes

$$\text{Resistance} = 2,475.22 \left(\frac{K_A}{K_M - K_A} \right) \left(e^{-K_A T} - e^{-K_M T} \right) + 70.69 \left(e^{-K_M T} \right)^{+10} \quad (4)$$

The velocity constants K_A and K_M have the normal values in sea water, 0.0036 and 0.1080 respectively. Hence the resistance at the end of 10.6 minutes is 87.44.

We must likewise remember that on replacing the tissue in sea water the reactions $O \rightarrow S \rightarrow A$ recommence and produce a certain amount of A ; this breaks down to form M , which in turn decomposes. The resulting amount of M may be easily calculated. It will be recalled that in sea water all processes are so adjusted that the amount of M remains constant; it is evident that if the reactions $O \rightarrow S \rightarrow A$ were suddenly to stop, allowing $A \rightarrow M \rightarrow B$ to continue, the amount of M would diminish. At the start the total resistance is 100. If O

¹² In general the greater the rise in recovery the greater the value of S , while the greater the fall the less the value of S .

should stop producing this would diminish and we may call the loss of resistance L . Now if O were producing normally it would just replace this loss, so as to keep the resistance constant at 100: hence the amount produced from O in any given time will be equal to the loss L which would occur in that time if O were to stop producing.

When tissue is exposed to a solution of NaCl, O diminishes according to the scheme $N \rightarrow O \rightarrow P$. Assuming that at the start $N = 89.1$ and $O = 90$ we find¹³ that the value of O after any given time (T) of exposure to a solution of NaCl may be obtained by changing the constants in formula (1) thus:

$$O = 89.1 \left(\frac{K_N}{K_O - K_N} \right) \left(e^{-K_N T} - e^{-K_O T} \right) - 90 e^{-K_O T} \quad (5)$$

in which K_N (the velocity constant of the reaction $N \rightarrow O$) and K_O (the velocity constant of the reaction $O \rightarrow P$) have the values 0.03 and 0.0297 respectively (see Table I).

We find by this formula that at the end of an exposure of 15.9 minutes the value of O is 92.57; hence it can produce only $(92.57 - 10) \div (100 - 10) = 0.917$ as much of M in any given time as it could produce if it were intact.¹⁴ The amount it could produce, if intact, during recovery in sea water is easily found by subtracting from 100 the resistance obtained by means of formula (1), when $K_A = 0.0036$ and $K_M = 0.1080$ (these are the normal values in sea water). Using these values we find that at the end of 10.6 minutes the amount of resistance, as given by formula (1), would be 98.55. Hence the loss during that time would be $100 - 98.55 = 1.45$, which is the amount

¹³ This value of O is assumed merely for convenience in calculation without reference to other assumed values. Its real value must be much greater than that of A but it is not necessary to assign any definite real value to it, since the only point of interest is to determine what per cent of O remains after any given time of exposure to sea water. It is assumed that in sea water any change in the amount of O is so small as to be negligible. This might be due to the fact that O is present in large amount and decomposes slowly or to the fact that it is formed as rapidly as it decomposes (by the reactions $N \rightarrow O \rightarrow P$).

¹⁴ In other words, if S , T , and A were completely removed, O could raise the level of M to $100 - 10 = 90$ in the course of time. But if, for example, half of O is lost the remainder can raise the level of M to only one-half its former value; *i.e.*, to $45 + 10 = 55$.

O could produce in 10.6 minutes if intact: but as O has diminished to 0.917 times its original value it can produce in 10.6 minutes only $(1.45)(0.917) = 1.33$.

By adding this value to that obtained by formula (4) we find the resistance after 10.6 minutes in sea water to be $87.44 + 1.33 = 88.77$.

In the same manner we may find the resistance at any given time after replacement in sea water. A series of values so obtained is given in Table II. It will be seen that they are in good agreement with the experimental values. The calculated and observed values are also plotted in Fig. 1, in which the abscissæ represent the time in the solution of NaCl plus the time of recovery in sea water (in the case just discussed this would amount to $15.9 + 10.6 = 26.5$ minutes).

TABLE II.

Recovery in Sea Water after Exposure of 15.9 Minutes to 0.52 M NaCl.

Total time = time in sea water + 15.9.	Time in sea water.	Electrical resistance.	
		Observed.	Calculated.
<i>min.</i>	<i>min.</i>	<i>per cent</i>	<i>per cent</i>
26.5	10.6	89.10	88.77
37.1	21.2	93.21	91.34
58.3	42.4	93.99	92.43
84.8	68.9	94.48	92.52
121.9	106.0	94.20	92.55
164.3	148.4	93.81	92.55
545.9	530.0	94.00	92.57
863.9	848.0	93.87	92.57

Proceeding in this manner with different times of exposure we obtain the series of recovery curves shown in Fig. 1. The number attached to each curve denotes the time of exposure to the solution of NaCl. The observed results are plotted as dotted lines, the calculated values as unbroken lines.

It will be seen that the agreement is satisfactory throughout. In general the greater the number of experiments which were averaged to obtain the result the nearer it approached to the calculated curve.

Let us now consider the behavior of tissues transferred from a solution of 0.278 M CaCl_2 (which has the conductivity of sea water) to sea water. In such a solution the resistance rises and then falls. If

tissue is allowed to remain in the solution for a short time and is then replaced in sea water the resistance falls rapidly, as shown in Fig. 2. This fall of resistance may be regarded as analogous to the rise of resistance which occurs in the experiments with NaCl and the term recovery may be used in both cases. It is evident from the figure that, as the exposure to the solution of CaCl_2 lengthens, the level

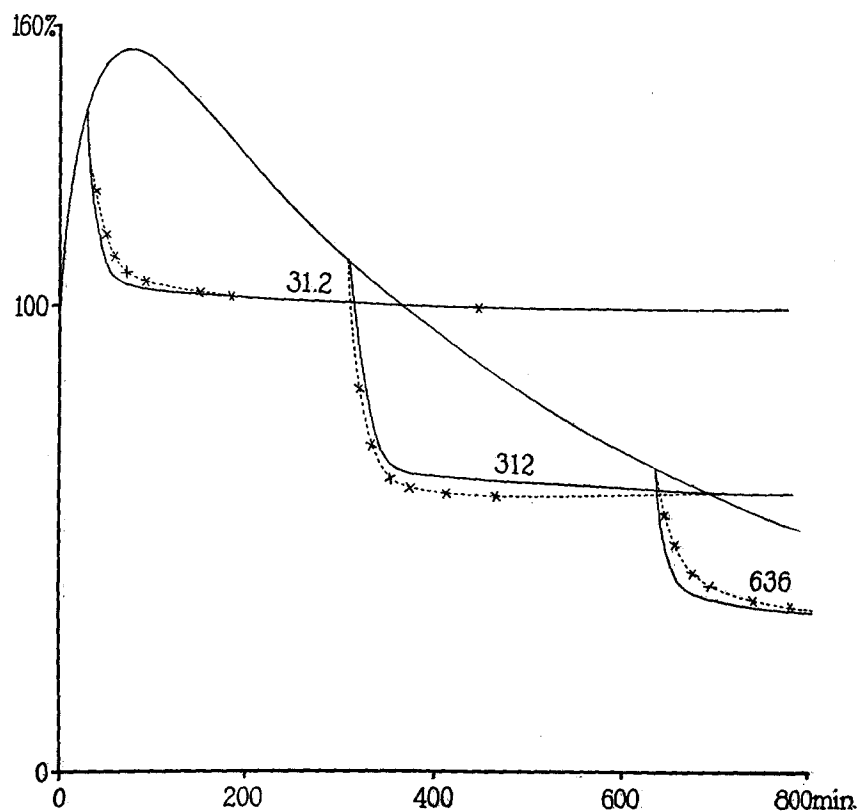


FIG. 2. Curves showing the rise and fall of electrical resistance in *Laminaria agardhii* in 0.278 M CaCl_2 (single curve which rises and falls) and recovery in sea water (descending curves). The figure attached to each recovery curve denotes the time of exposure (in minutes) to the solution of CaCl_2 .

In the recovery curves the experimental results are shown by the dotted lines, the calculated results by the unbroken lines.

The observed points represent the average of eight or more experiments. Probable error of the mean less than 10 per cent of the mean.

which is reached as the result of recovery gets lower. This is precisely what happens in the experiments with NaCl. It would therefore appear as though the same mechanism of recovery were involved. If this is so the same method of calculation should enable us to predict recovery in both cases. This is found to be true. Using the same formulas which have already been employed in the experiments with NaCl we are able to predict the course of the curves obtained in experiments with CaCl₂. This is rather striking in view of the fact that the two sets of curves differ so fundamentally in appearance.

In calculating the curves for CaCl₂ the constants given in Table I are employed. The results are shown as unbroken lines in Fig. 2 (the dotted lines show the experimental results). It is evident that the agreement is very satisfactory.

Some assistance in picturing the reactions which occur during exposure is afforded by Fig. 3, which shows the curve of O_1 in NaCl (unbroken line) and in CaCl₂ (dotted line). These curves are plotted from the calculated values; the observed values are shown as points; it will be observed that they lie fairly close to the calculated curve. The figure also shows the calculated values of S : in this case no observed values are given because such values cannot be very precisely determined. This is owing to the fact that the value of S affects only the speed of recovery (not the final level attained) and as the speed is variable the only satisfactory procedure is to assume such values of K_R and K_S as cause the closest approximation to the observed speed of recovery. When these values have been found the value of S can readily be calculated. The results of these calculations are plotted in Fig. 3.

In this figure the ordinates give the values of O_1 ; these must be multiplied by 6.75 to obtain the values of S . In all curves the value of S at the start is 2.7 (the value of S in sea water¹⁵); this appears on the ordinate in the figure as $2.7 \div 6.75 = 0.4$. The curves rise to a maximum and then fall to zero. The curves for O_1 start at 100 and fall to 10 (since the base line is taken as 10, just as in the curve of M).

¹⁵ The normal value of S in sea water is taken as 2.7 which is exceedingly small as compared with the amount of O . The amount of S which is produced from O in each unit of time is relatively large but S is so rapidly transferred into A that its amount in sea water never becomes greater than 2.7.

It will be observed that the rate of recovery is approximately the same in all cases; this applies to the experiments with CaCl_2 as well as with those in NaCl . In general it may be said that it usually requires about 60 minutes for the curve to complete nine-tenths of the total rise or fall which occurs in recovery.

With so large a number of constants it might seem possible to fit any sort of curve and hence the significance of the actual accomplish-

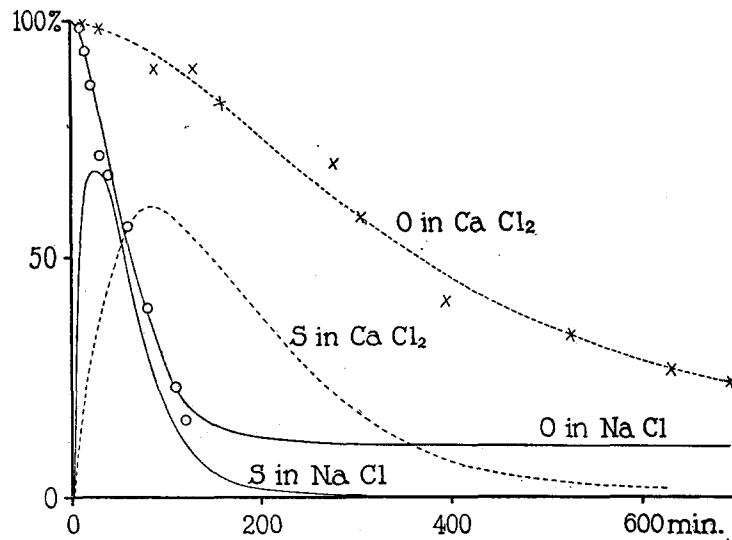


FIG. 3. Curves showing the values of O in NaCl (upper unbroken line) and in CaCl_2 (upper dotted line); also the values of S in NaCl (lower unbroken line) and in CaCl_2 (lower dotted line). The ordinates give the values of O ; these must be multiplied by 6.75 to obtain the values of S .

The observed points represent the average of eight or more experiments; probable error of the mean less than 10 per cent of the mean.

ment might be lessened. This, however, is by no means the case. Moreover, the fixing of one or two constants affects the others in such a way as largely to determine the character of all the curves.

In the foregoing account many details are necessarily omitted, owing to lack of space. These, however, are not essential to the main purpose, which is to show how the process of injury and recovery may be analyzed and subjected to mathematical treatment. Starting

with certain assumptions we have formulated equations by means of which we can predict the behavior of the tissue. If the predictions are fairly accurate it is natural to infer that the assumptions are in accordance with the facts. It is evident from an examination of the figures that the equations enable us to predict with considerable accuracy the behavior of tissues in solutions of NaCl and CaCl₂, as well as the recovery curves after any length of exposure to either of these solutions. But we must not lose sight of the fact that the predictive value of the equations does not depend on the validity of these assumptions and would in no way be impaired if they were to be given up. The equations have a permanent value which is independent of assumptions.

SUMMARY.

1. *Laminaria* exposed for a short period to 0.52 M NaCl loses a part of its electrical resistance but recovers it completely when replaced in sea water. When the period is lengthened recovery is incomplete. If the exposure is sufficiently prolonged no recovery occurs. (After exposure to 0.278 M CaCl₂ the resistance falls when the tissue is replaced in sea water.)

2. Equations are developed which enable us to predict the resistance of the tissue during exposure to NaCl or CaCl₂ as well as the recovery curves after any length of exposure to either of these solutions.