THE DEATH WAVE IN NITELLA.

I. APPLICATIONS OF LIKE SOLUTIONS.

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Experiments with chloroform have led us to predict¹ that the current of injury will be positive when the cell is in contact with concentrated solutions (such as 0.1 M KCl) and negative with dilute solutions (such as 0.001 M KCl).

The experiments on cutting here described justify this prediction. They also reveal a new feature: the rapid spread of the effects of injury,¹ a study of which may assist our understanding of the propagation of stimuli. From the point where the cell is cut a wave of some sort, which we may for convenience call the death wave, passes along the cell, setting up at each point it touches a death process which has greater speed and intensity the nearer it is to the cut.

The experiments were performed on Nitella flexilis, arranged as shown in Figs. 1, 4, or 6: the changes in potential difference being recorded photographically by means of a string galvanometer. The technique has been fully described in a previous paper. The average temperature was about 23°C, but as the experiments were very brief there was little variation in any one experiment.

The results remained practically the same when the pH value of the solutions varied from 4 to 9.

Let us first consider the experiments in which sap² or 0.05 M KCl was applied at A and B (Fig. 1) after which the cell was cut at Z by means of a sharp knife insulated by rubber from contact with the hand (a clean cut was made by cutting downward against the paraffin

¹ This has been referred to in certain previous publications, cf. (a) Osterhout, W. J. V., J. Gen. Physiol., 1927–28, xi, 83; (b) Osterhout, W. J. V., and Harris, E. S., J. Gen. Physiol., 1927–28, xi, 673.
² 0.05 M KCl gives the same results as sap for such experiments as are here described.
on which the cell rested). The cut caused $A$ to become more positive after which the potential difference approached zero (Fig. 2).

Our interpretation of this phenomenon may be presented by describing how it arose. Our first experiments of this sort aroused the suspicion that all the changes produced at $A$ are followed by similar ones at $C$, and that the observed curve merely represents the resultant of the

$^a$The microscopic appearance of $C$ changed in much the same way as that of $A$ and so did its response to the tests for normality described in a former paper (Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, xi, 417). The hypothetical curves of $A$ and $B$ imagined at that time were almost the same as those we use at present.
opposing potential differences at A and C. To illustrate this we made
(Fig. 3a) a tracing of the curve in Fig. 2 and drew (Fig. 3b) hypoth-
etical A and C curves to make it evident that the difference between
them would give the curve in Fig. 2. But there was no way of testing

![Fig. 3a. Tracing of the curve in Fig. 2 with certain ordinates drawn for com-
parison with Fig. 3 b.](image)

![Fig. 3 b. Theoretical interpretation of Fig. 3 a. Fig. 3 a shows only the
observed potential difference but Fig. 3 b shows that this is equal to the dif-
ference between the hypothetical curve A (unbroken line) and the hypo-
thetical curve C (broken line): each ordinate is equal to the one directly above it in Fig. 3 a. A and C are the (hypothetical) “true” curves of A and C. At the start A is more nega-
tive than C which accords with the fact that the curve in Fig. 3 a is negative at the
start (this curve shows the potential difference of A with respect to C). When
the value of A falls to that of C the curve in Fig 3 a becomes zero; when A be-
comes positive to C the curve in Fig. 3 a becomes positive; the two curves then
approach each other and the curve in Fig. 3 b approaches zero.

![Fig. 4. Like Fig. 1 except for the addition of B.](image)

this conception until it was observed that chloroform may kill C
without immediately affecting A and B. It was found that when C is killed its electromotive force falls to zero and remains constant while the cut is being made at Z and
the resulting disturbances are recorded. This is illustrated by Fig. 5 in which the record starts after the disturbances produced by chloro-

![Fig. 5. Photographic record of potential differences, the experiment being arranged as in Fig. 4 with 0.05 M KCl at A, B, and C. The record begins after C has been killed by 0.05 M KCl saturated with chloroform so that the A and B curves have their “true” values (and hence will be called the “true” curves of A and B since the death of C reduces its electromotive force approximately to zero). At the start these curves are negative (A more so than B) but on cutting at Z they approach zero. The vertical marks represent 5 second intervals. Selected as typical from 30 experiments.]

![Fig. 6. Like Fig. 4 but with an additional connection between A and B.]

form are over: A and B are then negative and have their “true” values (i.e. the values observed when the electromotive force of C

4 These will not be discussed here since they have been described in a previous paper, where it is shown that the injury at C does not immediately spread to A and B.
is zero). After cutting they approach zero, the process at A being a little in advance of that at B (this is not evident in Fig. 5 but is shown by other experiments).

Fig. 7a.

Fig. 7b.

Figs. 7a and b. Photographic records of potential differences, the experiment being arranged as in Fig. 6 with 0.05 M KCl at A, B, and C. The records begin after B has been killed (by 0.05 M KCl saturated with chloroform) so that the curves of A and C show their "true" values (both are negative at the start, A being more so than B, but on cutting at Z they approach zero); these are recorded on one instrument while the curve showing the potential difference between A and C is simultaneously recorded on another as shown in Fig. 7b (the slight vertical movements in this curve are due to the alternations of the rotary switch in the other instrument); the value of each point of the curve is equal to the difference between the "true" A and "true" C values. The vertical marks represent 5 second intervals. Selected as typical from 20 experiments.

5 The curves for A and B are recorded simultaneously by means of the rotating switch previously described (Osterhout, W. J. V., and Harris E. S., J. Gen. Physiol., 1927-28, xi, 417).

6 This permits us to study the speed of propagation of the stimulus, which will be discussed in a later paper.
It would seem that leading off from \( A \) to \( B \) must be similar to leading off from \( A \) to \( C \) and that the \( A \) to \( C \) curve must resemble the \( A \) to \( B \) curve and must be approximately equal to the difference between the "true" curves of \( A \) and \( C \) (and hence of \( A \) and \( B \) in Fig. 5) which would give a curve similar to that of Fig. 2. That this is really so became evident as soon as it was possible, by making simultaneous records on two instruments and using the arrangement shown in Fig. 6, to get the \( A \) to \( C \) curve together with the "true" curves of \( A \) and \( C \). Inspection of Figs. 7a and b shows that the difference between the true curves of \( A \) and \( C \) gives the observed \( A \) to \( C \) curve, i.e., if we measure the vertical distance between \( A \) and \( C \) we obtain the vertical distance above or below zero of the \( A \) to \( C \) curve.

In this case we notice that the death wave which starts at \( Z \) must pass over the freshly killed protoplasm at \( B \) before it can affect \( C \), but it can evidently do this, as shown by the behavior of \( C \).

To ascertain whether the killing of \( C \) modifies the \( A \) and \( B \) curves we

\footnote{We should not expect to get the exact difference since, as shown in a former paper, we do not necessarily measure exactly the same fraction of the true \( E \cdot M \cdot \tau \) in both cases. But this would not noticeably affect the form of the curves and might make very little quantitative difference between them.}
have performed the experiment without killing C, the result being shown in Fig. 8. In this case the curves are negative at the start (usually they are near zero under these circumstances) but on cutting

![Diagram](image)

**Fig. 9.** Hypothetical diagram to illustrate the condition of the protoplasm in contact with 0.05 M KCl. The direction in which the positive current tends to flow is shown by the direction of the arrows, the relative magnitude of the electromotive force being indicated by their length. The potential difference across the protoplasm is by convention regarded as negative since the X arrow is longer.

**Fig. 10 a.** Tracing of the "true" A curve in Fig. 5 with certain ordinates drawn for comparison with Fig. 10 b.

**Fig. 10 b.** Theoretical interpretation of Fig. 10 a. Fig. 10 a shows only the observed potential difference, but Fig. 10 b indicates that this is equal to the difference between the (hypothetical) value of x (broken line) with negative sign (as shown by the scale of ordinates at the left) and the (hypothetical) value of y (unbroken line) with positive sign (as shown by the scale of ordinates on the right): each ordinate is equal to the one directly above it in Fig. 10 a.

Since the potential difference across the protoplasm in contact with 0.05 M KCl is negative the x ordinate is made longer at the start (since x is negative by convention). When x falls and becomes equal to y the curve in Fig. 10 a falls to zero; as x continues to fall and becomes less than y the curve in Fig. 10 a becomes positive; when the two curves approach each other the curve in Fig. 10 a approaches zero.

Since we do not know the absolute value of the ordinates but only the value of the difference between them (i.e. the value of the shaded area) the scales of ordinates are interrupted toward the base by a dotted line to signify that below this point the ordinate extends for an indefinite distance. It should be noted that when the x and y curves come together the observed potential difference (such as is given in Fig. 10 a) is zero but this zero has no relation to the absolute zero of the present figure.

they become positive and then approach zero (in the latter part of the record which is not shown here). The difference between the A and
and $B$ curves would evidently give a curve similar to that in Fig. 2. This is to be expected since if we designate the "true" values at $A$, $B$, and $C$ by $a$, $b$, and $c$ we may write as an approximation

\[
\text{Observed } A \text{ to } C \text{ curve} = a - c \\
\text{Observed } B \text{ to } C \text{ curve} = b - c
\]

Taking the difference between these two curves we have

\[
\text{Observed } A \text{ to } B \text{ curve} = (a - c) - (b - c) = a - b
\]

Fig. 11. Photographic record of potential differences (the experiment being arranged as in Fig. 4 with 0.05 M KCl at $A$, $B$, and $C$). The record begins after $C$ has been killed (with 0.05 M KCl saturated with chloroform) so that the curves for $A$ and $B$ show their "true" values and are in consequence negative (their values are so nearly equal that they almost coincide). On cutting at $Z$, $A$ becomes more positive, then more negative, and then approaches zero (resembling an effect often found with 0.1 M KCl): $B$ becomes more negative and then approaches zero (resembling an effect commonly produced by 0.05 M KCl plus chloroform). The vertical lines represent 5 second intervals. Selected as typical from 20 experiments.

In other words we get the observed $A$ to $B$ curve by taking the difference between the "true" curves of $A$ and $B$ or between the observed curves of $A$ to $C$ and $B$ to $C$. 
The experiments indicate that the killing of C does not modify the A and B curves if the cut is made soon enough after the death of C.

Our next step is to try to interpret the “true” curves shown in Fig. 5. These resemble in many cases the “true” curves produced by applying chloroform directly to A and B (instead of producing injury at A and B by cutting at Z). Such curves have been explained on the ground that the protoplasm consists of an outer layer, X, and an inner layer, Y (both of which are probably non-aqueous), with an aqueous layer, W, between them and that the layer in contact with the more concentrated solution is the first to change. On this basis we may diagram the protoplasm as in Fig. 9, the arrows indicating the direction in which the positive current tends to flow and their length the relative magnitude (the direction of the X arrow is called negative by convention and that of the Y arrow positive; and since in this case the X arrow is longer the potential difference across the protoplasm is here said to be negative). If the value of the X arrow is x and that of the Y arrow is y we may assume that the observed potential difference is proportional to y - x. If both these values should fall off simul-
Figs. 13 a and b. Photographic record of potential differences, the experiment being arranged as shown in Fig. 6 with 0.001 $\text{M KCl}$ at $A$, $B$, and $C$. The record starts after $C$ has been killed (by 0.001 $\text{M KCl}$ saturated with chloroform) so that the curves for $A$ and $B$ have their "true" values (both are strongly positive and have about the same value so that they almost coincide; this high value is partly due to the fact that in this material 0.01 $\text{M}$ is positive instead of being negative as usual: they become negative on cutting after which they approach zero); these are recorded on one instrument while the curve showing the potential difference between $A$ and $B$ (marked "A to B") is simultaneously recorded on another; the value of the latter is equal to the difference between the "true A" and "true B" (as illustrated in Figs. 14 a and b). The vertical marks represent 5 second intervals. Selected as typical from 25 experiments.
taneously, as shown in Fig. 10b, we should obtain the curve shown in Fig. 10a which is a tracing of the "true" A curve in Fig. 5.

Our hypothesis states that the layer in contact with the more concentrated solution will be the first to change and since 0.05 M KCl acts much like sap we might expect that when it is applied to the outside of the

![Fig. 14 a. Tracing of the curve in Fig. 13 a with vertical and horizontal scales made identical with those in Fig. 14 b. Certain ordinates are drawn for comparison with Fig. 14 b.](image)

![Fig. 14 b. Interpretation of Fig. 14 a. Fig. 14 a shows only the observed potential difference but Fig. 14 b shows that this is equal to the difference between the "true" curves of A and B (traced from Fig. 13 b); each ordinate is equal to the one directly above it in Fig. 14 a. At the start A is positive to B corresponding to the fact that the A to B curve of Fig. 14 a is positive (this curve shows the potential difference of A with reference to B): when A becomes negative to B the curve in Fig. 14 a becomes negative: A then becomes positive to B and then as A and B approach each other the curve in Fig. 14 a approaches zero.

cell X and Y would behave as if in contact with similar solutions and upon cutting would change at about the same rate, or that sometimes one and sometimes the other would go faster. This is actually the case: when there is a difference it is usually Y which goes first, just as in the case of chloroform, so that the potential difference across the protoplasm first becomes more negative and then approaches zero (curve B, Fig. 11). Occasionally it would seem that X goes first,
giving curves like the $A$ curve shown in Fig. 22a (see curve $A$, Fig. 11).$^8$

Let us now consider the results obtained with a solution less concentrated than sap, e.g. 0.001 M KCl. We find (Fig. 12) that the cut makes $A$ negative, then positive, after which the potential difference approaches zero (Fig. 12). When the experiment is arranged as in Fig. 6 we observe (Figs. 13a and b) after killing $C$ that $A$ and $B$ are positive (showing their "true" values).$^{10}$ On cutting both become negative, after which they approach zero. It is evident that if we should lead off from $A$ to $B$ we should obtain approximately the difference between the "true" curves, giving a curve of the type seen in Fig. 12. This is the case, as seen in Fig. 13a and shown more clearly by Figs. 14a and b.

$^8$ The same cell may show both types of curves (as in Fig. 11) but in many cases but one is observed. The resulting $A$ to $B$ curves may resemble those shown in Figs. 2, 7b or 22b. The variations may be due to differences in the sap or in the protoplasm.
Figs. 16 a and b. Photographic record of potential differences, the experiment being arranged as in Fig. 6 with 0.001 M KCl in contact with A, B, and C. The record starts after B has been killed (by 0.001 M KCl saturated with chloroform) so that the curves for A and C have their “true” values: before cutting both have about the same positive value (this is very high corresponding to the fact that with this material 0.01 M KCl is positive instead of being negative as usual) and coincide but after cutting they become negative and then approach zero; these are recorded on one instrument while the curve showing the potential difference between A and C (marked “A to C”) is simultaneously recorded on another: the value of the A to C curve is equal to the difference between the “true A” and “true C” (cf. Figs. 14 a and b). The vertical lines represent 5 second intervals. Selected as typical from 20 experiments.
If we should perform the experiment without killing C it would still be true (as previously explained) that the difference between the curves of A and B should give the curve observed in leading off from A to B. The result of such an experiment is shown in Fig. 15 and it is evident

that if we pursue the scheme shown in Figs. 14a and b we shall have a similar result. In order to get the "true" C curve we employ the scheme shown in Fig. 6 and obtain the result in Figs. 16a and b (the C curves show considerable variation which will be discussed in a subsequent paper).
The next step is to interpret the "true" curves in 0.001 M KCl, such as the A curve in Fig. 13b. For this purpose we may diagram the protoplasm as in Fig. 17 making the Y arrow longer in order to show that the potential difference across the protoplasm is positive. If cutting caused the value of y to fall off more rapidly than that of x we might get the curve shown in Fig. 18a (which is a tracing of the "true" A curve of Fig. 16b). This would be expected on the basis of our hypothesis which states that in general the changes produced (by chloroform or by cutting) in any protoplasmic layer are more rapid the
higher the concentration of the salt solution in contact with it. As sap is approximately equivalent in these experiments to 0.05 M KCl it is evident that in this case we should expect Y to go before X which is in contact with 0.001 M KCl.

Figs. 20 a and b. Photographic record of potential differences, the experiment being arranged as in Fig. 6 with 0.025 M KCl in contact with A, B, and C. The record starts after B has been killed (with 0.025 M KCl saturated with chloroform) so that the curves for A and C have their “true” values (both are negative at about the same value so that they almost coincide). On cutting at Z they become more negative and then approach zero (the process being more rapid at A than at C); these are recorded on one instrument while the curve showing the potential difference between A and C (marked “A to C”) is simultaneously recorded on another; the value of the A to C curve is equal to the difference between the “true A” and “true C” (cf. Figs. 14 a and b). The vertical lines represent 5 second intervals. Selected as typical from 20 experiments.
When we compare the "true" curves in Fig. 13b with those obtained (as described in a previous paper\textsuperscript{18}) by applying chloroform directly to \(A\) and \(B\) (instead of injuring them indirectly by cutting at \(Z\)) we see a general resemblance but they differ in details.\textsuperscript{9} All that we can say at present is that the forms of these curves seem to depend on the relative rates of change of \(x\) and \(y\) without attempting to explain why cutting in many cases produces effects which are somewhat different from those observed with chloroform. (An example of another type of curve commonly found with chloroform is shown in the \(C\) curve in Fig. 19.\textsuperscript{10})

Let us now consider concentrations (0.01 and 0.025 M KCl) which have a special interest because in some cases they produce a negative potential difference across the protoplasm. Our hypothesis predicts that in spite of this they will act on cutting like 0.001 M KCl which (prior to making the cut) shows a positive potential difference across the protoplasm. The following considerations show why this is so.

\textsuperscript{9} Foot-note \textsuperscript{1b}, Fig. 12 \textit{a}.

\textsuperscript{10} Foot-note \textsuperscript{1b}, Fig. 9.
Since sap acts like $0.05 \text{ m KCl}$, $0.01$ and $0.025 \text{ m KCl}$ are less effective solutions and we therefore expect the layer $Y$ (which is in contact with sap) to go first; this would at first make the protoplasm more negative (just as in the case of $0.001 \text{ m KCl}$). Fig. 20a shows that this is the case.

The interpretation of the “true” curves in Fig. 20a is like that of the curves obtained with $0.001 \text{ m KCl}$ (cf. Figs. 18a and b).

11 The word “effective” is used here in a technical sense. A solution is less effective than sap if it acts like dilute sap as tested by electrical criteria.
There is another case which should be considered, that of a solution more effective than sap (Fig. 21). Since in this case X is in contact with the more effective solution we should expect it to go first. This would make the protoplasm more positive after which the potential difference would approach zero. That this is the case is shown in Fig. 22a. The behavior of the "true" B curve resembles that found with chloroform but in the case of the "true" A curve the first positive movement of the curve goes further and carries it past zero. The "true" curves show a positive drop at the start after which the potential difference approaches zero (in this case so slowly that only a part of

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the curve is given): the process is more rapid and more pronounced at \( A \) than at \( B \).

In this case we diagram the protoplasm as in Fig. 9 (but making the \( x \) arrow longer than in the figure) and interpret the "true" \( A \) curve in Fig. 22a in the manner shown in Figs. 23a and b.

These experiments confirm in a remarkable way the predictions of the hypothesis set forth in a previous paper\(^{15}\) and indicate that it may prove a useful guide.

We see that the injured protoplasm is sometimes positive and sometimes negative to uninjured protoplasm because the electromotive force of the dead protoplasm is nearly zero and its relation to normal protoplasm will depend on what solutions are employed. By making the concentration of KCl 0.01 or thereabouts we can always bring the living protoplasm of *Nitella* to the same potential difference as the dead protoplasm. If we use more concentrated KCl the injured spot will be positive to the uninjured one and with more dilute solutions it will be negative.\(^{12}\)

Certain interesting phenomena connected with the death wave, e.g. the fact that it traverses the cell so rapidly and can pass over a killed spot (though unable to affect adjoining cells) as well as the fact that the death process is more rapid and more pronounced the greater its nearness to the cut, will be discussed in forthcoming papers.

**SUMMARY.**

Experiments on cutting confirm the prediction that the current of injury will be positive when the cell is in contact with concentrated solutions and negative with dilute solutions. They support the idea that the protoplasm is made up of layers differing considerably in their properties, each having a death curve of simple and regular form, the more rapid alteration of the outer layer making the protoplasm more positive and the more rapid alteration of the inner making it more negative.

From the point where the cell is cut a wave of some sort, which we may for convenience call a death wave, passes along the cell, setting up at each point it touches a death process which has the greater speed and intensity the nearer it is to the cut.

\(^{15}\) *i.e.* always using identical solutions at both the injured and uninjured spots: otherwise there may be an effect due to the cell wall (concentration effect).