POSITIVE AND NEGATIVE CURRENTS OF INJURY IN RELATION TO PROTOPLASMIC STRUCTURE.*

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Much may be learned about the nature of protoplasm by studying its transformations during the process of death. Such alterations have been observed by a method which has marked advantages since it permits us to follow very rapid changes which are often of primary importance. It may be added that certain complications inherent in tissues have been avoided by using single multinucleate cells.

The outcome, which supports the idea that protoplasm is made up of layers differing considerably in their properties, appears to necessitate a change in the traditional view that the current of injury is negative¹ for the observations show that it can be rendered positive or negative according to the will of the experimenter.

The experiments described in this paper were performed on single multinucleate cells of *Nitella flexilis*² according to the methods given in a previous paper³ (which contains a description of the technique and of the apparatus). A flowing contact was used in most cases. Especial care was taken to employ only normal cells, and to avoid injury (except in applying toxic agents). The current of injury was produced by chloroform which has previously been found useful for experi-

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¹ A discussion of the literature will be deferred to a later paper dealing with cell groups and tissues. It may be mentioned that a few experiments on the effect of chloroform on *Nitella* were performed by Haacke (Haacke, O., *Flora*, 1892, lxxv, 455). Hörmann (Hörmann, G., *Studien über die Protoplasmaströmung bei den Characeen*, Jena, 1898) states that stimulation of one end of a *Nitella* cell produces a wave of negativity.

² Kindly identified by Dr. J. S. Karling.

³ Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, xi, 391.

ments on injury.⁴ The experiments were carried out at room temperature, ranging from 20° to 25°C. but the variation in any one experiment was less than 1°C.

The procedure may be illustrated by an experiment with 0.05 M KCl⁵ in contact with the cell at *A* and *C* (as shown in Fig. 1). Fig.

FIG. 1. Diagram to show the arrangement of the experiments. In most cases flowing contacts are employed at *A* and *C*. In some cases the ends of the cell dipped into two cups hollowed out of a block of paraffin: in experiments with sap cotton soaked in sap is applied. When necessary (with more concentrated solutions) cotton soaked in distilled water is applied for a short stretch between *A* and *C* but a space is left on each side of the cotton to prevent short circuiting.

2 shows at the start a small potential difference⁶ between *A* and *C*.⁷ We now substitute for 0.05 M KCl at *C* a solution of 0.05 M KCl saturated with chloroform. This injures the protoplasm at *C* and produces characteristic changes in the curve but it subsequently reaches a steady state which is assumed to mean that the protoplasm

⁴ Osterhout, W. J. V., *J. Gen. Physiol.*, 1922-23, v, 709. Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927-28, xi, 391. Chloroform is soluble to the extent of about 0.4 per cent by volume (about 0.6 per cent by weight) and hence does not noticeably affect the osmotic pressure or concentration of electrolytes.

⁵ This gives practically the same result as sap or artificial sap. It would appear that this result must be due primarily to the effect of the cations since the halide content of sap is about the same as that of 0.1 M KCl, but the K content of sap is much less.

⁶ To avoid misunderstanding we may state that we use the term electromotive force to designate the force present at an electrode surface: if we tap off at two points in the circuit we obtain a potential difference which depends on the resistances involved.

⁷ The recorded values would probably be a trifle higher if we could eliminate short-circuiting effects in the cell (Osterhout, W. J. V., *J. Gen. Physiol.*, 1927-28, xi, 83). Diffusion potentials are small enough to be neglected (in view of the concentration of KCl present).

at *C* is dead.⁸ For reasons given in a previous paper³ we now regard the potential difference as due entirely to the living protoplasm at *A*. We now apply 0.05 M KCl saturated with chloroform to *A* and obtain a curve similar to that already observed except that the signs are reversed. This is to be expected since the curve always records the state of *A* with reference to *C* and any changes occurring at *C* will have their signs reversed: hence to determine the effect of chloroform on the protoplasm we must take the latter part of the curve (when the chloroform is applied to *A*) where the signs have their true value (or we may take the first part of the curve and reverse the signs).

We see that when *A* is injured by applying chloroform dissolved in 0.05 M KCl *A* becomes more positive⁹ with reference to *C*. Since the

⁸ The use of a flowing contact³ largely eliminates disturbances due to the coming out of sap immediately after the death of *C*. If the flowing contact were not employed we should not have the same salt solutions in the cell wall at *A* and *C* and in consequence there might be a potential difference (sap coming out at *C* earlier than at *A* would tend to make the cell wall at *C* more negative so that on the record *A* would appear more positive). The flowing contact promptly washes the sap out of the cell wall and thus removes this source of disturbance. As an example of this we may consider the following experiment: Live cells were placed in pure chloroform (not in an aqueous solution of chloroform) which quickly kills them without allowing salts to escape from the cells. One end of the cell was then cut and the protoplasm and sap squeezed out for a distance of an inch so that the lumen was filled with air and could not supply salts to the cell wall any more than the living protoplasm would. We will call this end *A* and the other *C*. Both *A* and *C* were alike in having the cell wall imbibed with sap, due to the death of the protoplasm (which is not quite the same thing as applying sap from the outside, as will be discussed in a later paper), but they differ in that *C* has a reservoir of sap in the lumen of the cell which *A* has not. On leading off from *A* to *C*, with 0.05 M KCl applied to each, there was practically no potential difference but when 0.001 M KCl (flowing junction) was applied to *A*, it quickly became positive showing that the salts were being rapidly washed out. When the value became constant, 0.001 M KCl (flowing junction) was applied at *C* and the potential difference rapidly fell to zero showing that the salts were washed out of the cell wall despite the reservoir of sap in the lumen of the cell.

The flowing contact did not produce complications except possibly in very dilute solutions and if disturbances occurred in experiments of any sort (as shown by the fact that the curve did not fall to zero when the cell was killed) the experiments were rejected.

⁹ In all cases we obtain essentially the same result when we apply a toxic agent at *A* whether we have previously killed *C* or not. The only difference is that if *C*

protoplasm at *C* is dead we may regard its electromotive force as approximately zero³ so that all the potential differences observed are

FIG. 2. Photographic record of potential differences, the experiment being arranged as in Fig. 1 with 0.05 M KCl at *A* and *C*. When 0.05 M KCl saturated with chloroform is applied at *C* the curve (which records the potential difference of *A* with reference to *C*) shows that *A* appears to become more negative but since it is only *C* which changes, the curve really shows that *C* is becoming more positive. The potential difference between *A* and *C* soon reaches a fixed value which is practically all due to the living protoplasm at *A*. A solution of 0.05 M KCl saturated with chloroform is then applied at *A*, giving a death curve which resembles that already observed but with opposite signs since the signs now have their true value (*C* being dead remains constant). The curve shows that under these conditions injury causes the protoplasm to become more positive after which the potential difference approaches zero.

The vertical lines represent 5 second intervals. Selected as typical from over 100 experiments.

remains alive it adds a certain constant (positive or negative) value (due to the presence of the living protoplasm at *C*): in consequence the curve of *A* does not approach zero at the end but reaches a steady state at a definite positive or negative value which represents the potential difference across the protoplasm at *C*.

due to *A* which, as shown in a previous paper,³ can apparently remain normal for some time after the death of *C*.

It will be noticed that there is no trace of the traditional negative current of injury. The observed change in potential difference caused by the toxic agent is wholly in the positive direction.

It may simplify matters if before describing other experiments we present an hypothesis which brings the observed phenomena under a single point of view and which may thus facilitate the subsequent treatment.

According to this hypothesis the protoplasm has an outer (*X*) and an inner (*Y*) surface layer, both of which are probably non-aqueous,

FIG. 3 a.

FIG. 3 b.

FIG. 3. 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. Fig. 3 a represents a possible conception of the potential differences; Fig. 3 b presents the same thing in simplified form. The potential difference across the protoplasm is said to be negative since the negative arrow at *X* is longer and the positive current tends to flow as indicated in Fig. 4 b.

with an aqueous layer, *W*, between them. On this basis we may diagram the potential differences in the protoplasm in the manner shown in Fig. 3 a and 3 b, the arrows representing the direction in which the positive current tends to flow and their length indicating the relative magnitude of the potential difference.¹⁰ The underlying theory of these effects has been discussed in a previous paper¹¹ and

¹⁰ The arrows are hypothetical illustrations and their lengths can be varied accordingly.

¹¹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1927-28, xi, 83.

the conclusion reached that in general the cations have a greater tendency to enter X and Y than the anions. Hence we may suppose that at the surfaces a , b , c and d (Fig. 3 *a*) the potential differences may be represented by the arrows if they are due to phase boundary potentials. Since we are merely concerned with the resultant of these

FIG. 4 *a*. FIG. 4 *b*.

FIG. 4 *a*. Diagram of a circuit through the protoplasm at a single spot where an imaginary capillary has been inserted so as to lead off from the outside of the protoplasm to the inside. In this case the potential difference across the protoplasm is said to be positive because the electrometer shows X to be positive to Y .

FIG. 4 *b*. As in Fig. 4 *a* but in this case the potential difference across the protoplasm is said to be negative because the electrometer shows X to be negative to Y .

effects it is more convenient to give the diagram the simpler form shown in Fig. 3 *b* in which only two arrows¹² are employed: this would also be appropriate if diffusion potentials¹³ play a part.¹⁴

In the diagram in Fig. 3 *b* the arrow at X is longer than that at Y indicating that the positive current tends to flow as in Fig. 4 *b*. Hence

¹² The actual length of the arrows is not significant: it is merely intended to show that the X arrow is longer than the Y arrow.

¹³ Such diffusion potentials might well be due to organic substances produced by the cell (*e.g.*, in the layer W). It is commonly said that diffusion potentials cannot produce potential differences of the magnitude found in living cells. This would be true of aqueous solutions but in non-aqueous media or in such structures as the collodion membranes studied by Michaelis and his coworkers higher values may be possible.

¹⁴ Whether we suppose that the potential difference results chiefly from diffusion potentials due to substances produced in W or to phase boundary potentials (following the scheme in Fig. 3 *a*) it seems highly probable that the arrows in Fig. 3 *b* should point in opposite directions.

if we arrange an experiment as in Fig. 1 with 0.05 M KCl at *A* and *C* and lead off from *A* to *C* after *C* has been killed by chloroform (thus reducing its potential difference approximately to zero) we should expect the positive current to flow from *C* to *A* so that *A* would appear negative. This is actually the case, as is evident from Fig. 2.

Although little is known regarding electrical conditions in the protoplasm it may be of interest to consider certain possibilities which would exist if the cell conformed to the diagram in Fig. 5. Here we confine ourselves to the assumption that there is but one seat of electromotive force in *X* and one in *Y* (but they may exist at both surfaces of any

FIG. 5. Hypothetical diagram of electrical conditions in the protoplasm. For the sake of simplicity but one source of electromotive force is assigned to *X* and one to *Y* (those in the cell wall and *W* are regarded as negligible). The resistance in *Y* is regarded as having vertical components (R_{AY} and R_{CY}) and a horizontal component (R_Y): this also applies to *W* and *X*.

layer and possibly in the interior, due to diffusion potential) and we have arbitrarily located this at the outer surface in each case. On this basis the total electromotive force may be regarded as $E_{AY} - E_{AX} - E_{CY} + E_{CX}$.

The resistance in each layer may be regarded as consisting of a vertical component (such as R_{AY}) and a horizontal component (such as R_Y). The resistances R_X and R_Y may be relatively high: R_W and R_S are probably less. As to the resistance R_0 it may be said that the measurements of Dr. Blinks show that when we cut out a portion of a

cell about an inch in length, remove the protoplasm and sap by squeezing, rinse in tap water, squeeze the contents out and dry the surface lightly with filter paper, the resistance is in the neighborhood of a megohm. That of a similar length of intact cell is about half as much which might indicate that under these conditions the resistance of the living protoplasm and sap taken together is not far from that of the cell wall.¹⁵

But, according to Dr. Blinks, it seems possible that the resistance due to the living protoplasm and sap taken together (which we may call R_P) may be largely due to polarization: hence if measured with currents as small as those present in our ordinary experiments R_P might be quite small in comparison with R_O . In that case we might write

$$\text{observed P.D.} = \text{E. M. F. in circuit} \left(\frac{R_O}{R_O + R_P} \right)$$

and the value of $\frac{R_O}{R_O + R_P}$ would be not far from 1 so that the measurements described in this paper would not be far from the true values of the electromotive forces in the circuit. That this is actually the case is indicated by the measurements obtained when a capillary is inserted in the manner employed for *Valonia*.¹¹

Let us now consider what would happen if we had a simpler system from which the layers X and W were omitted. We should then be measuring by means of the electrometer the potential difference across the ends of R_O and we should have

$$\text{observed P.D.} = E_{AY} - E_{CY} \left(\frac{R_O}{R_O + R_{AY} + R_{CY} + R_{YS}} \right)$$

in which R_{YS} is the combined resistance of R_Y and R_S (and is equal to $(R_Y)(R_S) \div R_Y + R_S$).

The application of chloroform to C would presumably do away

¹⁵ If we call the combined resistance of the living protoplasm and the sap R_P we might employ the usual equation for resistances in parallel and regard the effective resistance of the living cell as equal to $(R_P)(R_O) \div R_P + R_O$. If we have $R_P = R_O = 1$ megohm the effective resistance will be equal to 0.5 megohm.

with E_{CY} and reduce R_{CY} to negligible proportions so that the remaining potential difference would be

$$\text{observed P.D.} = E_{AY} \left(\frac{R_O}{R_O + R_{AY} + R_{YS}} \right).$$

When we speak of the potential difference across the protoplasm at A^{16} (after C has been killed) it seems by no means impossible that we must understand it as conforming to a scheme which is similar to this but one which involves all the electromotive forces and resistances in the cell (including any possible eddy currents, as discussed in a previous paper¹¹).

Future investigation must decide to what extent the conditions in the protoplasm are represented by such a scheme as that shown in Fig. 5. It is, of course, evident that the cell wall, X , W and Y are not actually insulated from each other as they are in the diagram nor are the electromotive forces confined to A and C .

We may suppose that if we have a simple system such as is represented by the diagram in Fig. 5 the electromotive force after the killing of C is

$$\text{E.M.F.} = E_{AY} - E_{AX}$$

of which we measure by means of the electrometer only a certain fraction so that we may write

$$\text{observed P.D.} = n(E_{AY} - E_{AX}) = n(E_{AY}) - n(E_{AX}).$$

If we put $y = n(E_{AY})$ and $x = n(E_{AX})$ we may write

$$\text{observed P.D.} = y - x.$$

¹⁶ The observed P.D. does not show much change when we shorten the distance between A and C (both being intact) so that we may regard the observed potential difference as practically that of the area directly under A . This fact indicates that R_O cannot be small in comparison with $R_{AY} + R_{CY}$ for if it were the shortening of the distance between A and C would greatly diminish the observed potential difference since the value of $R_{AY} + R_{CY}$ would remain unchanged.

It is evident that we cannot arrive at the true value of the electromotive force at A (after killing C) unless we know the value of the fraction found by dividing R_O by the other resistances properly combined (measurements with an inserted capillary indicate that this value is not far from 1).

If we could destroy X without altering W or Y it is evident that both E_{AX} and R_{AX} would disappear and that this would not only change the electromotive force but would also alter the value of n . In view of this and possibly other complications (such as the presence

FIG. 6 a.

FIG. 6 b.

FIG. 6 a. Tracing of the latter part of the curve shown in Fig. 2 (after C has been killed by chloroform so that the entire electromotive force is due to the living protoplasm at A). The curve shows that at the start the value of A is negative (-15 millivolts). After the application of chloroform at A it becomes more positive since the potential difference diminishes and approaches zero. Certain ordinates are shaded for comparison with Fig. 6 b.

FIG. 6 b. Theoretical interpretation of Fig. 6 a. The ordinates as drawn represent the observed potential difference which is equal to that of Fig. 6 a. Fig. 6 a shows only the observed potential difference but Fig. 6 b indicates that this is equal to the difference between x with negative sign (as shown by the scale of ordinates at the left) and the value of y with positive sign (as shown by the scale of ordinates on the right). Each ordinate in Fig. 6 b has the same length as the one directly above it in Fig. 6 a, and the

distance between x and y is equal to the distance from zero of the curve in Fig. 6 a. Since x and y are purely hypothetical curves they may be drawn in a variety of ways but the most natural assumption seems to be that they have a simple and regular form and they have been drawn in conformity with this idea.

Since we do not know the absolute value of the ordinates in Fig. 6 b but only the value of the difference between x and y (*i.e.*, the value of the ordinates as drawn) 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 if the x and y curves come together the observed potential difference is zero but this zero has no relation to the absolute zero of Fig. 6 b.

of more sources of electromotive force, eddy currents,¹¹ etc.) we shall employ the equation $P.D. = y - x$ merely for convenience in discussion and only in a qualitative sense, meaning that in a general way the observed potential difference depends on a factor due to X and on one due to Y .

Let us now consider the mechanism of the death process. Since chloroform penetrates with extreme rapidity (often killing in a few seconds) and since the protoplasm is only a few microns thick it seems probable that Y will be affected almost as soon as X . If X and Y are in contact with the same solution (sap) or with solutions which have been found¹⁷ to act alike (*e.g.* if X is in contact with 0.05 M KCl and Y in contact with sap) we may expect both to change at the same rate if both are equally sensitive. This is sometimes observed and we might in that case interpret the result in the manner shown in Fig. 6 *b*, since it seems reasonable to suppose that when chloroform acts on X and Y the values of x and y may fall off in regular fashion, as is observed in the death curves of tissues¹⁸ and of single cells (as shown by the unpublished experiments of Dr. Blinks). This assumption is by no means necessary but we may adopt it as the most natural and as a matter of fact it suffices to explain all the observed curves. If, for example, the application of chloroform + 0.05 M KCl causes x and y to fall off in some such fashion as that shown in Fig. 6 *b* it is evident that if we plot the difference between them ($y - x$)¹⁹ in the usual way we shall get the curve shown in Fig. 6 *a* which is a tracing of the latter part of the curve in Fig. 2.

We do not know the absolute value of the ordinates in Fig. 6 *b* but only the difference between them. Hence the scales of ordinates are interrupted toward the base by dotted lines to signify that below this point the ordinates extend for an indefinite distance. It should be noted that when the x and y curves come together, the observed potential difference (as in Figs. 2 and 6 *a*) is zero, but this zero has no relation to the absolute zero of Fig. 6 *b*.

The nature of the change produced by chloroform is uncertain. It

¹⁷ This is found by applying sap at A and 0.05 M KCl at C when it is observed that both act alike in that the potential difference between A and C is zero. We obtain the same death curve with sap saturated with chloroform as with 0.05 M KCl saturated with chloroform.

¹⁸ Cf. Osterhout, W. J. V., Injury, recovery, and death, in relation to conductivity and permeability, Philadelphia, 1922; *J. Gen. Physiol.*, 1922-23, v, 709. Cf. Klopp, J. W., *J. Gen. Physiol.*, 1924-25, vii, 39.

¹⁹ Since the value of A is negative the ordinates of x (which is negative by convention) are made greater than those of y (which is positive by convention).

may cause a loss both of electromotive force and of resistance.²⁰ It is quite possible that the first effect of chloroform may be to produce in *X* and *Y* very minute openings or small electrical leaks (some of which may be almost instantly repaired). This might have a certain

FIG. 7 a.

FIG. 7 b.

FIG. 7 a. Like Fig. 6 a but showing a different result. This is a tracing of the latter part of the curve in Fig. 8 (after the counter movement).

FIG. 7 b. Theoretical interpretation of Fig. 7 a. The ordinates as drawn show the observed potential difference (which is equal to that of Fig. 7 a). Fig. 7 a shows only the observed potential difference but Fig. 7 b indicates that this is equal to the difference between the value of *x* (with negative sign) and that of *y* (with positive sign). Each ordinate has the same length as the one directly above it in Fig. 7 a. During the period marked *q* the value of *x* remains stationary while that of *y* falls off corresponding to the fact that the curve in Fig. 7 a becomes more negative. During the period marked *s* the two curves in Fig. 7 b approach each other as the curve in Fig. 7 a approaches zero; if they should meet the potential difference would be zero (but this zero has no relation to the absolute zero of Fig. 7 b). Cf. Fig. 6 b.

similarity to the effects of mechanical injury. The fact that *X* and *Y* are changed so readily by chloroform and other lipoid solvents might suggest that they are lipoid: they are also very susceptible to

²⁰ In the case of *Laminaria* chloroform, ether, and alcohol cause a temporary increase of resistance (Osterhout, W. J. V., Injury, recovery, and death, Philadelphia, 1922) but this is not true of all organisms and may not be the case with *Nitella*. The increase of resistance is followed by a decrease which continues until death ensues: during this period recovery (partial or complete) is possible in the case of alcohol and to a very much smaller extent in the case of ether and chloroform.

the action of acid, alkali, concentrated salt solutions, and mechanical injury, but this might not be inconsistent with the assumption that they are lipoid in nature. We must also consider the possibility that they may act like proteins, agar, starch paste or kaolin.²¹

FIG. 8. Like Fig. 2 but showing a different result. After the counter movement following the application of chloroform at *A* (when the signs have their true value) we see that *A* becomes more negative, then more positive: the potential difference then sinks almost to zero. The vertical lines represent 5 second intervals. Selected as typical from over 100 experiments.

The fact that alcohol produces effects similar to those of chloroform and that they are readily reversible up to a certain point suggests the possibility that the loss of potential difference may be due to an

²¹ Cf. Höber, R., *Z. physik. Chem.*, 1924, cx, 142. Matsuo, T., *Arch. ges. Physiol.*, 1923, cc, 132. Mond, R., *Arch. ges. Physiol.*, 1924, cciii, 247. Fujita, A., *Biochem. Z.*, 1925, clxii, 245. Deutsch, W., *Arch. ges. Physiol.*, 1925, ccix, 675. Beutner, R., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 462.

increase in the conductivity of X and Y rather than to the production of leaks (there is no reason to suppose that the latter process would be irreversible from the start).

It is also possible that the falling off of potential difference may be due to a loss of electromotive force (independent of or combined with a change in resistance). If the electromotive force is due to unequal mobilities of the ions or unequal partition coefficients it is quite possible that any substance which goes into solution in X and Y could induce alterations in this respect (reversible or irreversible).

In the case of Fig. 2 we may suppose that X and Y change simultaneously but this need not be a general rule. If Y should go a little before X we might get curves similar to those in Fig. 7 *b*, corresponding to the curve shown in Fig. 7 *a*, which is a tracing of the latter part of the photographic record in Fig. 8. As a matter of fact such curves are much more common than the type shown in Fig. 2.

In Fig. 8 and some of the other figures the first large movement after the application of chloroform is preceded by a small movement in the opposite direction. This will be called the counter movement. It might be explained as due to the fact that the chloroform strikes X first so that we might expect the change in X to begin a little before that of Y . Since the "counter movement" is not a constant feature and may possibly be explained in other ways it will be omitted from the theoretical diagrams in this paper.

The fact that Y is apt to change more rapidly than X might be due to differences in the layers themselves or to the solutions in contact with them. The latter seems improbable since we find the same thing when we replace 0.05 *M* KCl by sap or artificial sap. We may regard all of these solutions as equally "effective" in this respect (we shall use the word "effective" in this special sense throughout the subsequent discussion, the most "effective" solution being the one which most assists the action of the chloroform in changing the potential difference). On the other hand, we know from previous experiments³ that the two layers are not identical in their properties and we may therefore assume that Y is more sensitive than X to the action of chloroform.

Let us now consider an experiment with a more dilute solution,

FIG. 9. Photographic record of potential differences; the experiment is arranged as in Fig. 1: at the start 0.001 M KCl is applied at *A* and *C*. After the counter movement which follows the application of 0.001 M KCl saturated with chloroform at *A* we see that *A* becomes more negative, then more positive and then approaches zero (*C* being dead remains constant).

The first part of the curve (resulting from the application of 0.001 M KCl saturated with chloroform to *C*) is similar but with signs reversed because the changes are occurring at *C*: after the death of *C* the curve reaches a fixed value which is practically all due to the living protoplasm at *A*. Chloroform is then applied at *A* giving the death curve already described.

The vertical lines represent 5 second intervals. Selected as typical from over 100 experiments.

Calibration marks are shown at four places. Small irregular fluctuations are due to movements of the operator, mechanical disturbances or sound waves: they are more noticeable with dilute solutions which introduce greater resistance in series with the living cell.

namely 0.001 M KCl: the result is shown in Fig. 9. The curve shows that after *C* is dead *A* is positive:²² hence we diagram the protoplasm

²² This is in harmony with the results of unpublished experiments (made by us in 1922) which show that dilute solutions are positive to more concentrated solutions, as seems to be usually the case with uninjured living cells.

as in Fig. 10 with the arrow at Y^{23} longer than that at X . After the counter movement the first effect of chloroform is to make A more negative;⁹ hence we infer that Y changes more rapidly than X . This might be expected on the ground that it is in contact with the more concentrated solution and it appears that in general, even in the absence of chloroform, concentrated solutions render the protoplasm unstable and more easily injured. We therefore regard sap as a more effective solution than 0.001 M KCl.

In order to account for this result we may refer to the diagram in Fig. 11 *b*. We draw hypothetical curves for x and y (just as in Figs. 6 *a* and *b* and 7 *a* and *b*) in such a manner that the values of $y - x$ when plotted as in Fig. 11 *a* give us the latter part of the curve in Fig. 9 (of which the curve in Fig. 11 *a* is a tracing).

FIG. 10.

Hypothetical diagram to illustrate the condition of the protoplasm in contact with 0.001 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 said to be positive since the positive current tends to flow as in Fig. 4 *a* (since the positive arrow at Y is longer).

The variations which are observed are such as the hypothesis might lead us to expect. For example if the process in X were a little slower we should get the situation shown in Fig. 12 *b*, which would give the observed curve shown in Fig. 12 *a*. We should also expect that the y curve might sometimes drop for a time below the x curve in the latter part of its course making the potential difference temporarily negative and such cases are actually observed.

Let us now consider an intermediate concentration (0.01 M KCl): this is illustrated by Fig. 13 which shows that after C is dead A has a value⁹ which approximates zero, hence in Fig. 14 we make the arrows equal. As Y is in contact with the more "effective" solution we expect it to change more rapidly and we picture the process (after the counter movement) as shown in Fig. 15 *b* from which we plot $y - x$, obtaining

²³ This presumably remains unaffected by the external solution, at least in short experiments.

the curve in Fig. 15 *a* which is a tracing of the latter part of the curve in Fig. 13 (the crossing of the curves at the right in Fig. 15 *b* is apparently a frequent phenomenon at all concentrations and results in the observed curve changing sign for a while in the latter part of its course).

FIG. 11 *a*. FIG. 11 *b*.

FIG. 11 *a*. Tracing of the latter part of the curve shown in Fig. 9 (after the counter movement). Certain ordinates are drawn for comparison with Fig. 11 *b*.

FIG. 11 *b*. Theoretical interpretation of Fig. 11 *a*. The ordinates as drawn represent the observed potential difference (which is equal to that of Fig. 11 *a*). Fig. 11 *a* shows only the observed potential difference but Fig. 11 *b* shows that this is equal to the difference between the value of x (with negative sign) and that of y (with positive sign). Each ordinate has the same length as the one directly above it in Fig. 11 *a*. The curve begins at the end of the counter movement when the value of A is 65 millivolts. During the period marked q the value of x remains stationary while that of y falls off as the curve in Fig. 11 *a* becomes more negative. During the period marked r the value of x falls off as the curve in Fig. 11 *a* becomes more positive. During the period marked s the curves approach each other, as the curve in Fig. 11 *a* approaches zero; after they cross the curve in Fig. 11 *a* becomes negative.

The behavior in 0.01 *M* KCl raises an interesting question. In the case of 0.001 *M* KCl it might be suggested that the layer which changes most rapidly is the one with the greatest potential difference across it. But in the case of 0.01 *M* KCl where the potential differences across X and Y are equal it would appear that the determining cause is the "effectiveness" of the solution rather than magnitude of the potential difference.

It will be noted that in Fig. 2 (and in Fig. 9 after the counter movement) the first result of the application of chloroform to *A* is a loss of potential difference. But with 0.01 M KCl even when the value of *A* before injury is negative we find (disregarding the counter movement) an increase on applying chloroform. For example in the latter part

FIG. 12 a.

FIG. 12 b.

FIG. 12 a. Like Fig. 11 a but showing a different result.

Fig. 12 b. Theoretical interpretation of Fig. 12 a. The ordinates as drawn represent the observed potential difference which is equal to that of Fig. 12 a. Fig. 12 a shows only the observed potential difference but Fig. 12 b indicates that this is equal to the difference between the value of *x* (which has a negative sign) and that of *y* (with positive sign). Each ordinate has the same length as the one directly above it in Fig. 12 a.

During the period marked *q* the value of *x* remains constant while that of *y* falls as the curve in Fig. 12 a becomes more negative. During the period marked *s* the value of *x* falls off and the two curves approach each other as the curve in Fig. 12 a approaches zero.

of the curve in Fig. 16 (when *C* is dead but *A* is still normal) *A* is negative²⁴ and when chloroform is applied to *A* it becomes more negative and then commences to fall toward zero. It would seem

²⁴ The fact that the potential difference across the protoplasm in contact with 0.01 M KCl is sometimes negative (Fig. 16) and sometimes zero (Fig. 13) may be due to variations in sap. It might also be due to variations in *X* and *Y*. It has been shown in a former paper³ that these are not alike, and this accords with the diagram in Fig. 14 for if *X* and *Y* were alike we could not make the arrows in this diagram of equal length since sap is approximately equivalent to 0.05 M KCl.

that this would afford a crucial test of the ability of our hypothesis to explain all the facts. As a matter of fact it is precisely what the

FIG. 13. Photographic record showing potential differences. The experiment starts with 0.01 M KCl at *A* and *C*: 0.01 M KCl saturated with chloroform is applied to *C* and then to *A*. Just before applying chloroform to *A* it is slightly negative. When chloroform is applied we see that after the counter movement *A* becomes more negative, then more positive, and finally approaches zero (*C* being dead remains constant).

The first part of the curve (resulting from the application of chloroform to *C*) is similar but with signs reversed because the changes occur at *C*. After the death of *C* the curve reaches a fixed value which is practically all due to the living protoplasm at *A*. Chloroform is then applied to *A*, giving the death curve already described.

The vertical marks represent 20 second intervals. Selected as typical from over 100 experiments.

hypothesis would lead us to expect, since it demands that in all cases where *Y* changes more rapidly the first large jump (after the counter

movement) will be in the negative direction even though (as here and in Fig. 8) the value across the protoplasm is already negative.

To interpret Fig. 16 we diagram the protoplasm as in Fig. 3 *b* and

FIG. 14. Hypothetical diagram of the condition of the protoplasm in contact with 0.01 M KCl. The potential difference across the protoplasm is zero since the two arrows are equal.

FIG. 14.

FIG. 15 *a*. Tracing of the latter part of the curve in Fig. 13 (after the counter movement) with certain ordinates drawn for comparison with Fig. 15 *b*).

FIG. 15 *b*.

FIG. 15 *b*. Theoretical interpretation of Fig. 15 *a*. The ordinates as drawn indicate that the observed potential difference is equal to the difference between x (with negative sign) and y (with positive sign) and corresponds to the observed potential difference in Fig. 15 *a* (each ordinate as drawn has the same length as the one directly above it in Fig. 15 *a*). During the period marked q the value of x remains stationary while that of y falls off as the curve in Fig. 15 *a* becomes more negative; during the period marked r the value of x falls off as the curve in Fig. 15 *a* becomes more positive; during the period marked s the two curves approach each other as the curve in Fig. 15 *a* approaches zero (this zero has no relation to the absolute zero of Fig. 15 *b*).

we picture the process (after the counter movement) as shown in Fig. 17 *a* and *b*: Y changes more rapidly since it is in contact with the more effective solution.

Let us now consider the effects of a more concentrated solution, namely 0.1 M KCl, as shown in Fig. 18. After C is dead we find that

A is negative.²² We therefore diagram the protoplasm as in Fig. 3 *b*.¹⁰ Since X is in contact with the more effective solution we expect it to change more rapidly and we therefore picture the process as in Fig. 19 *b* where x and y fall off in such fashion that when the values of $y - x$

FIG. 16. Photographic record showing potential differences. The experiment starts with 0.01 M KCl at A and C : 0.01 M KCl saturated with chloroform is applied to C and then to A . Just before applying chloroform to A its value is negative: when chloroform is applied we see that after the counter movement it becomes more negative and the potential difference then falls to zero and afterward becomes slightly negative.

The vertical lines represent 20 second intervals. Selected as typical from over 100 experiments.

are plotted we obtain the curve shown in Fig. 19 *a*, which is a tracing of the latter part of the curve⁹ in Fig. 18.

Here also we expect variations similar to those found in other concentrations and this expectation is fulfilled.

The hypothesis would also lead us to predict that 0.005 M KCl would produce effects similar to those of 0.001 M KCl. The experiments show that this is so.

The success of the hypothesis in predicting the behavior of the cell under so many conditions indicates that it may be a useful guide in future investigations.

FIG. 17. *a*.

FIG. 17 *a*. Tracing of the latter part of the curve in Fig. 16 (after the counter movement) with certain ordinates drawn for comparison with Fig. 17 *b*.

FIG. 17 *b*.

FIG. 17 *b*. Theoretical interpretation of Fig. 17 *a*. The ordinates as drawn indicate that the observed potential difference in Fig. 17 *a* is equal to the difference between x with negative sign and y with positive sign (each ordinate has the same length as the one directly above it in Fig. 17 *a*). During the period marked q the value of x remains stationary while that of y falls as the curve in Fig. 17 *a* becomes more negative. During the period marked s the two curves approach each other as the curve in Fig. 17 *a* approaches zero (this zero has no relation to the absolute zero of Fig. 17 *b*). After this the curves diverge as the curve in Fig. 17 *a* becomes more negative.

It is evident that the hypothesis would be equally satisfactory if in Figs. 3 *a* and *b*, 10 and 14 we should reverse the direction of the arrows and assume that the layer in contact with the more dilute solution is the one which changes more rapidly. It is difficult to test this experimentally with chloroform since it penetrates so quickly as to reach Y almost as soon as X but if we employ a substance which enters less rapidly, first attacking X and slowly making its way through X and W to attack Y , we may be able to decide the question.

For this purpose 0.16 M formaldehyde in 0.01 M KCl was applied at *A* (with 0.01 M KCl at *C*). The result is shown in Fig. 20; it is evident

FIG. 18. Photographic record showing potential differences. The experiment starts with 0.1 M KCl at *A* and *C*: 0.1 M KCl saturated with chloroform is applied at *C* and then at *A*. Under the influence of chloroform *A* becomes more positive, then more negative, and the potential difference then falls to zero.

The first part of the curve (resulting from the application of chloroform to *C*) is similar but with signs reversed because the changes occur at *C*. After the death of *C* the curve reaches a fixed value which is practically all due to the living protoplasm at *A*. Chloroform is then applied at *A* giving the death curve already described.

The vertical lines represent 5 second intervals. Selected as typical from over 100 experiments.

that the protoplasm becomes more positive. The action is gradual, presumably because the formaldehyde penetrates slowly. This is just the opposite from the effect of chloroform, which produces a

rapid negative change as shown in Fig. 21 where 0.01 M KCl saturated with chloroform was applied at *C* causing its value to become more negative (it appears on the record to become more positive because the curve records the state of *A* with reference to *C*, hence the signs are reversed): 0.16 M formaldehyde was then applied at *A* which slowly became more positive.

FIG. 19 a.

FIG. 19 b.

FIG. 19 a. Tracing of the latter part of the curve in Fig. 18 with certain ordinates drawn for comparison with Fig. 19 b.

FIG. 19 b. Theoretical interpretation of Fig. 19 a. The ordinates as drawn indicate that the observed potential differences in Fig. 19 a are equal to the difference between *x* with negative sign and *y* with positive sign (each ordinate has the same length as the one directly above it in Fig. 19 a). During the period marked *q* the value of *y* remains constant while that of *x* falls off as the curve in Fig. 19 a becomes more positive; during the period marked *r* the value of *y* falls off as the curve in Fig. 19 a becomes more negative; during the period marked *s* the two curves approach each other as the curve in Fig. 19 a approaches zero (this zero has no relation to the zero of Fig. 19 b).

These results indicate that the *X* arrow has the direction shown in the figures. This does not seem to be in harmony with the widely accepted view that the inside of the plasma membrane is negative to the outside²⁵ for in that case the arrow at *X* would have to be reversed. The idea that the inside of the plasma membrane is negative was adopted to account for the negative current of injury but it seems probable that in *Nitella* at least the negative current of injury described in the literature can be accounted for in a different way. The

²⁵ If, as we assume, the inside of *X* is positive we should find on inserting a capillary through *X* so as to lead off from the inside of *X* to the outside that the positive current would flow from the inside through the capillary and the electrometer to the outer surface of *X*.

negative currents of injury described in this paper may be too brief to correspond to the traditional negative current of injury referred to in the literature and it is questionable to what extent they could be observed in cells of ordinary size with the methods usually employed.

FIG. 20. Photographic record showing potential differences. The experiment starts with 0.01 M KCl at *A* and *C*: then 0.16 M formaldehyde in 0.01 M KCl is applied at *A* (with 0.01 M KCl at *C*). Formaldehyde makes *A* more positive but the action is gradual.

The vertical lines represent 5 second intervals. Selected as typical from 30 experiments.

In order to obtain results comparable with those described in the literature it would seem to be necessary to make experiments with portions of a plant of *Nitella* consisting of two or more cells. If we do this we find, on cutting or crushing a cell at one end, that sap, escaping at the opposite end and coming in contact with a neighboring intact cell, causes the injured cell to appear more negative and this

condition may persist for a long time. This will be fully discussed in a subsequent paper.

It will be remarked that in the previous discussion no account is taken of any possible potential differences in W : this does not imply that none exist but only that they are regarded as negligible for our present purpose. It would be possible to set up another hypothesis which would attribute more importance to W . For example, in the

FIG. 21. Photographic record showing potential differences. The experiment starts with 0.01 M KCl at A and C . The application of 0.01 M KCl saturated with chloroform to C causes it to become more negative (the curve becomes positive because the signs are reversed owing to the fact that the change occurs at C but the curve shows the state of A rather than that of C). During this time A is in contact with 0.01 M KCl. Formaldehyde (0.16 M in 0.01 M KCl) applied to A causes it to become more positive. Compare with Fig. 20.

The vertical lines represent 5 second intervals. Selected as typical from 10 experiments.

case of 0.001 M KCl we might suppose that both X and Y are simultaneously destroyed leaving W exposed to 0.001 M KCl on the outside and to sap on the inside: if W reacts in the opposite way²⁶ from

²⁶ Beutner (Beutner, R., *Die Entstehung elektrischer Ströme in lebenden Geweben*, Stuttgart, 1920), experimenting on organic substances immiscible with water, found that basic substances give an effect opposite to that of acid substances with such salts as KCl.

X and Y (e.g., if, in contact with W 0.001 M KCl is negative to sap and to 0.05 M KCl) the value across the protoplasm would change from positive (as in Fig. 4 *a*) to negative (as in Fig. 4 *b*).

But this hypothesis would not account for such cases as those shown in Figs. 9 and 18 where the curve moves first in one direction and then in the other, nor for Fig. 16 where the value of A is negative and becomes more so, nor would it explain why a spot treated with sap + chloroform commonly behaves as shown in Fig. 8, since in that case W would have sap on both sides and unless we assume that W is composed of two unlike layers we should expect that when chloroform is applied the potential difference would at once fall to zero.

Another possible explanation is that the application of chloroform alters the protoplasm in such fashion as to let sap pass out (at least momentarily) and come in contact with the outside of X without altering its power to give a potential difference. If we had 0.001 M KCl at the outside at the start it would be changed to the equivalent of something between 0.001 M and 0.05 M KCl by the coming out of sap. This would lessen the potential difference and might even make it negative. On the other hand 0.1 M KCl which gives a negative value across the protoplasm would be diluted by the coming out of sap and would become less negative but it could never become temporarily positive, as often happens in cutting, because if the 0.1 M KCl were completely changed to sap the value would still be negative (as shown in a previous paper³). In addition this hypothesis fails to explain why a spot treated with sap saturated with chloroform commonly behaves as shown in Fig. 8.

After the completion of this paper we received through the kindness of Professor Jost a reprint of his paper²⁷ which deals with *Chara* and *Valonia*. He makes no mention of experiments with chloroform but his results with ether, ethyl alcohol, amyl alcohol, cutting and crushing agree (except in minor details) with ours which have been repeated many times during the last 5 years. We think it desirable, however, to make it clear that we interpret the lasting negative current of

²⁷ Jost, L., *Sitzungsber. Heidelberger Akad. Wissensch., Abteilung B*, 1927, Abhandlung 13, Nov.

injury, observed when we lead off with dilute solutions from a cut or crushed cell, as due to an effect upon the neighboring intact cell (from which we are in effect leading off at one point with a dilute solution and at another with sap escaping from the injured cell, the circuit passing through the injured cell). If the solution applied to the uninjured cell is as "effective" as sap the current of injury will be of relatively brief duration. But if the solution applied to the uninjured cell is more "effective" than sap the current of injury may last for a long time and will be positive.

In view of the fact that the current of injury is sometimes explained by differences in acidity it may be well to state that varying the pH value from 5 to 8 produces relatively little effect as long as the concentration of cations other than H^+ is not changed.

SUMMARY.

Experiments on single multinucleate cells of *Nitella* show that the current of injury may be made positive or negative. For example, with chloroform in 0.1 M KCl the current of injury is positive but with 0.001 M KCl it is negative.

The changes which occur during the process of death receive a simple explanation upon the basis of the theory of protoplasmic layers. It seems possible that each layer has a death curve of simple and regular form, the more rapid alteration of the outer layer producing a positive current of injury and the more rapid alteration of the inner giving rise to a negative current of injury.