POSITIVE VARIATIONS IN NITELLA

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Under suitable conditions we find positive and negative variations in the same cell of *Nitella*. As this is of some theoretical interest a brief account of the experiments is here presented.

By way of definition we may say that when stimulation produces a temporary loss of P.D. at a spot which is positive[1] in its resting state the electrical variation is called negative (all the variations described in the literature are of this kind). It follows that when stimulation produces a temporary loss of P.D. at a spot which is negative in the resting state (so that the spot becomes less negative) the electrical variation may be designated as positive.

In some earlier experiments[2] cells were stimulated mechanically by cutting. The cut created a compression wave which traveled along the cell, causing a loss of P.D. at every point. A spot which was in contact with 0.001 M KCl, and therefore positive, became more negative. A spot in contact with 0.1 M KCl, and therefore negative, became more positive. For example, two spots, *A* and *C*, were made negative by applying 0.1 M KCl. The cell was then cut close to *A*. This caused the P.D. to fall to zero[3] more rapidly at *A* than at *C*.[4] In consequence *A* became temporarily positive to *C*.[5]

[1] The P.D. is called positive when the positive current tends to flow from the sap across the protoplasm to the external solution.

Historically the term "negative" was applied to the electrical variation not because it becomes more negative (when the potential is defined in the conventional way) but because a loss is involved. By usage, however, a negative variation has come to mean a change by which a spot becomes more negative and we shall so consider it.

[2] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12,** 167, 355. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14,** 385, 473.

[3] *Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12,** 167.

[4] The process begins almost simultaneously at both places but proceeds more rapidly at *A* because it is nearer to the cut.

[5] Cases have also been described (*cf.* Osterhout, W. J. V., and Harris, E. S., *J.*

This positive variation is not completely analogous to a negative variation since for one thing it is not reversible. But we may stimulate reversibly by pinching or bending the cell instead of cutting it. We thus obtain positive variations which are reversible.

This may be illustrated by some typical experiments. The record in Fig. 1 was made by arranging two cells[6] as in Fig. 2 with a nutrient

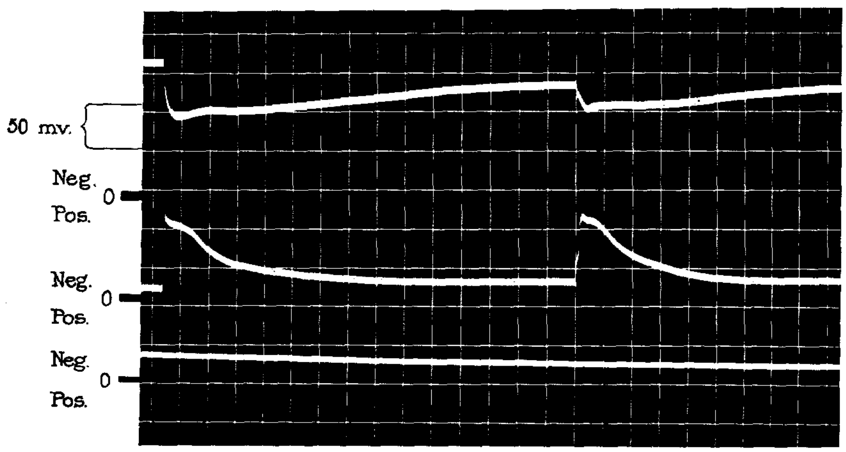

FIG. 1. Photographic record of experiment arranged as in Fig. 2. Room temperature 21°C. The vertical marks are 5 seconds apart. The electrical variations followed a pinch near contact *A*. The top curve records changes at *A*, the middle curve changes at *B*, and the bottom curve shows that little change occurred in the circuit connecting *C* and *D*. Upward deflection of the string represents relative increase in negativity of *A*, *B*, or *D*. Any change of potential at *C* would result in simultaneous movements in the same direction of all three curves, and any change at the junction of the two cells would result in simultaneous movements in the same direction of the two upper curves. (No such change occurs in this case.) *A* was in contact with 0.1 M KCl + 0.01 M $CaCl_2$, and *B*, *C*, and *D* were in contact with Solution A,[7] in which the cell had been kept for 10 days.

Gen. Physiol., 1927–28, **11**, 695 (Fig. 18)) in which chloroform was applied to a spot in contact with 0.1 M KCl, as the result of which the spot became less negative, then more negative, and then went to zero. This might be interpreted as a positive variation superimposed on a death curve.

[6] The plant is *Nitella flexilis*, Ag. The technique, unless otherwise stated, is that described in previous papers (Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14**, 473; 1933–34, **17**, 87).

[7] For the composition of this solution see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87. Its effect on the P.D. is approximately that of 0.001 M KCl.

solution (Solution A[7]) at *B*, *C*, and *D*. *A* was in contact with 0.1 M KCl (containing 0.01 M $CaCl_2$ for purposes of physiological balance) which made the P.D. negative.[8] The P.D. at *C* was positive and *A* was 148 mv. negative to *C* as seen in the top curve which shows the P.D. of *A* with reference to *C*.

When the cell was stimulated mechanically by pinching near *A* there was a loss of P.D. at *A* and *B* (just as when the stimulus was pro-

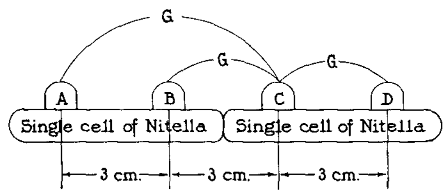

FIG. 2. Arrangement used in making the record shown in Fig. 1 on two naturally united cells from the same filament of *Nitella*. Contacts *B*, *C*, and *D* are Solution A, and contact *A* is 0.1 M KCl + 0.01 M $CaCl_2$. The cells were placed on a paraffin block in a moist chamber and the contacts made through calomel electrodes. *GGG* represent string galvanometers with thermionic amplifiers (all three strings in the same magnetic field). The method of measurement is essentially electrostatic.

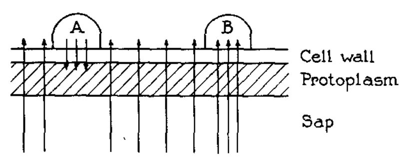

FIG. 3. Hypothetical diagram of the P.D. across the protoplasm of the cell whose record is shown in Fig. 1. *A* is in contact with 0.1 M KCl and *B* with Solution A. The arrows show the direction in which the positive current tends to flow and their length indicates very roughly the magnitude of the P.D.

duced by cutting as already described). But the loss was temporary, and the process was reversible. Although *A* and *B* were 3 cm. apart the start of the response was practically simultaneous at both places as would be expected since the compression wave produced by pinching travels along the cell at a high rate of speed.[2]

[8] This has practically the same effect on the P.D. as 0.1 M KCl. For the latter see Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11,** 673; 1929–30, **13,** 47.

The temporary loss of P.D. at A produced a positive variation (as shown in the top curve) because A was negative: but at B it produced a negative mechanical variation[9] (as exhibited in the middle curve, which shows the P.D. of B with reference to C) because B was positive.

A second pinch near A produced a similar result.

The bottom curve (showing the P.D. of C with reference to D) exhibits no change except a slow drift. This means that the mechanical stimulation did not pass[10] to the cell at the right. Hence it is evident that all the changes recorded in the top and middle curves (except the slow drift just mentioned) occurred at A and B.

If the slow drift seen in the bottom curve occurred at C[11] it means that a correction should be applied to the P.D. of A as seen in the top curve. When this is done it is seen that the recovery after the second positive variation is practically complete, while that after the first positive variation is nearly complete.

We may picture the situation in the cell as in Fig. 3. When the cell is pinched at or near A, a compression wave travels along the cell.[2,3,12] On reaching A it causes a loss of P.D. (partial or complete). Perhaps the simplest assumption is that this is due to a mechanical rupture of the non-aqueous protoplasmic surface layers. Previous experiments indicate that the more violent the mechanical disturbance

[9] Such variations are called mechanical to distinguish them from ordinary negative variations not produced by mechanical stimulation. Some points of difference have been discussed in a previous paper (Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14,** 473). Others appear on comparing the curves given here (and in the previous paper) with the figures showing the ordinary propagated negative variation (as shown in previous papers), but as these figures do not show the great variety of forms which occur in the two kinds of variation caution must be used in drawing conclusions in this respect.

[10] Inasmuch as the circuit from A to C passes through the end of the left-hand cell a disturbance at that spot might be registered. An ordinary negative (non-mechanical) variation does not as a rule affect the bottom curve when the cells are arranged as in Fig. 2. *Cf.* Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1929–30, **13,** 547.

[11] If the slow drift occurred at D it would show in the bottom curve but changes at D would not show in the top or middle curve since in these cases D was not in the circuit.

[12] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14,** 473.

the greater the loss[13] of P.D. (as the compression wave travels along the cell its effects diminish) and the greater the danger of irreversible injury. Repeated pinching is apt to cause injury. Fig. 4 shows that after the first four pinches recovery was complete but not after the fifth and sixth.

The fact that the loss of P.D. is gradual may be due to a series of ruptures followed by immediate repair in the fashion of a contractile vacuole discharging itself by a rupture of the surface.[14] The loss of

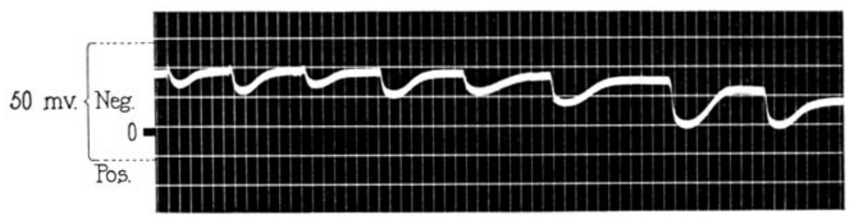

FIG. 4. Photographic record of experiment arranged as in Fig. 5. Room temperature 25°C. The vertical marks are 5 seconds apart. The electrical variations were set up by bending near contact *A*. Contact *B* was killed by chloroform before the record started, and the movement of the string results wholly from change in potential at *A*. Downward deflection of the curve shows that *A* is becoming more positive.

P.D. would then depend on the number of ruptures existing at a given instant and might be gradual (this would also apply to recovery).

Since the outside concentration of K^+ is greater than that inside there may be some penetration of K^+ (due to the rupture or other changes taking place during the positive variation) in which case recovery would not be complete until the penetrating K^+ had diffused away into neighboring regions.

When we consider Fig. 3 we see how the compression wave acts. When it causes a loss of P.D. at *A* this spot becomes more positive but

[13] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 179 (Figs. 16 *a* and 16 *b*); Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14**, 481 (Fig. 11).

[14] The senior author has made artificial models which illustrate this perfectly. In some of these models numerous ruptures occur simultaneously or in quick succession, each being followed by repair.

when it causes a loss of P.D. at *B* this spot becomes more negative (here too the loss may be partial or complete).

It is evident that the procedure is quite different from that in a propagated negative variation of the usual sort.[12] According to the local circuit theory such a variation would not be able to pass *A* because it is propagated by setting up at successive spots on the cell an outgoing electrical current. When it reached *A* it could not do this and in consequence *A* would act as a block. This is confirmed by experiment.

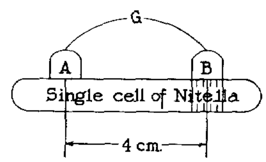

FIG. 5. Arrangement used in making record shown in Fig. 4. Contact *A* is 0.1 M KCl + 0.01 M $CaCl_2$, and contact *B* is Solution A + $CHCl_3$. The cell was kept in Solution A[7] for 10 days before use. The cell was placed on a paraffin block in a moist chamber and contact made through calomel electrodes to *G*, representing a string galvanometer with thermionic amplifier. The method of measurement is essentially electrostatic.

It is likewise evident that a propagated negative variation could not pass a killed spot but that a compression wave might do so. This is also confirmed by experiment.[12]

An interesting experiment is recorded in Fig. 4 which shows a monophasic record of eight positive variations set up by successively bending[15] the cell near *A* (the cell was arranged as in Fig. 5). *B* was killed by means of chloroform so that it could exhibit no changes in potential: *A* was in contact with 0.1 M KCl + 0.01 M $CaCl_2$ and was 24 mv. negative to *B* as shown by the record (since the P.D. at *B* was practically zero the values shown in the record are those at *A*). The bending at *A* was carefully done, and the first four responses, while variable in extent, showed complete recovery.[16] Recovery after the fifth and sixth stimuli was not complete. The seventh and eighth

[15] The number of pinches which the cell can endure without injury depends on the magnitude of the mechanical disturbance and the state of the cell. Sometimes a single pinch causes permanent injury.

[16] It will be observed that the coordinates have been given values which emphasize the responses. The sensitivity of the string, as compared with that in

stimuli, which sent the potential approximately to zero, were also followed by incomplete recovery.

It is evident that the positive variations described in this paper are analogous to the negative mechanical variations described earlier and it therefore seems appropriate to call them positive mechanical variations.

SUMMARY

The reversible electrical variations hitherto described for plants and animals consist in a reversible loss of positive potential at a stimulated spot by which it becomes more negative.

In this paper we describe changes which consist in a reversible loss of negative potential at a stimulated spot whereby it becomes more positive. We suggest that this be called a positive variation.

The stimulation was produced in all cases by pinching or bending the cell. This produced a compression wave which traveled along the cell, producing a negative variation at a spot which was positive and a positive variation at a spot which was negative (due to application of 0.1 M KCl).

The response produced by the compression wave differs in several respects from an ordinary propagated negative variation and may be termed a positive mechanical variation.

Fig. 1, is a little more than four times as great, while the 5 second marks are only one-half as far apart.