SALT BRIDGES AND NEGATIVE VARIATIONS

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If a cell of *Nitella* imbibed with tap water be stimulated at such a point as *A* (Fig. 1) a negative variation usually passes to *B*, *D*, and *C*. If sufficient chloroform be applied at *D* the negative variation fails[1] to appear at *C* and we therefore speak of the chloroformed spot as a block.

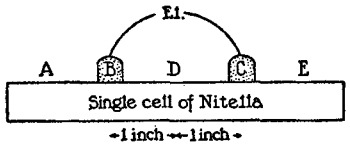

FIG. 1

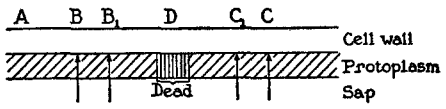

FIG. 2

FIG. 1. Diagram to show arrangement of experiments.

FIG. 2. Hypothetical diagram showing electrical conditions in the protoplasm. The arrows indicate E.M.F. (the direction shows how the positive current tends to flow). The protoplasm at *D* is shaded vertically to indicate that it has been killed by chloroform; it is assumed that it has been previously treated as in Fig. 4 to prevent negative variations from originating at *D*.

According to the local circuit theory[2] we should expect a variation starting at *A* to bring about a variation at *C* if we put a salt bridge around the chloroformed spot. For if the cell is imbibed with tap water (or 0.001 M KCl) we may diagram the protoplasm as in Fig. 2 and assume that a negative variation traveling from *A* to *B* makes an outward flow of current[3] at *B* causing[2c] the E.M.F. at *B* to approach zero and a flow

[1] It is necessary to arrange the experiments so that no negative variation originates at *D*. See footnote 2 *c* and Fig. 4.

[2] *Cf.* (*a*) Lillie, R. S., Protoplasmic action and nervous action, University of Chicago Press, Chicago, 1923; (*b*) Davis, H., *Physiol. Reviews*, 1926, **6**, 547; (*c*) Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1929–30, **13**, 391.

[3] By this is meant the sort of flow indicated by the arrow at B_1 in Fig. 3.

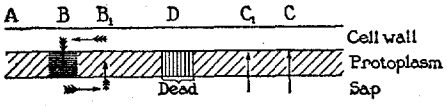

FIG. 3

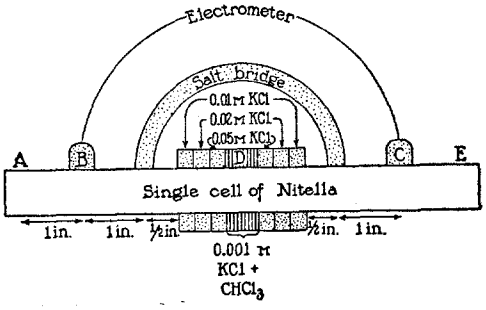

FIG. 4

FIG. 3. As in Fig. 2 but showing a flow of current between B and B_1 (flow is indicated by feathered arrows, E.M.F. by plain arrows). The protoplasm at B is shaded horizontally to indicate that it has temporarily lost its potential (that at D is shaded vertically to indicate that it is dead).

FIG. 4. Diagram to show arrangement of experiments. The protoplasm at D has been killed by 0.001 M KCl saturated with chloroform and various concentrations of KCl are placed on each side to prevent negative variations[2c] from originating at D (and passing along the cell in either direction).

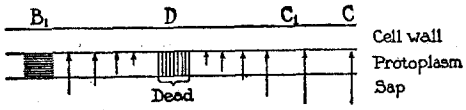

FIG. 5

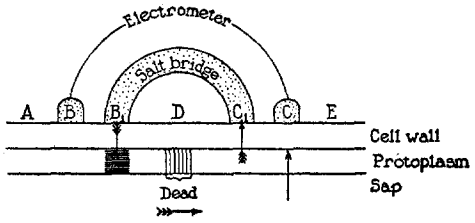

FIG. 6

FIG. 5. As in Fig. 4 but without a salt bridge: the protoplasm at B_1 has lost its potential but the P.D. gradient is not steep enough to cause sufficient outward flow at C_1 to start a variation. The arrows indicate E.M.F.

FIG. 6. As in Fig. 5 but with a flow of current through a salt bridge as indicated by the feathered arrows (the horizontal flow in the protoplasm and cell wall is relatively small and is therefore not indicated). Plain arrows indicate E.M.F. and feathered arrows indicate current.

to start between B and B_1 as shown in Fig. 3. This in turn will cause the E.M.F. at B_1 to approach zero and in this way the variation will travel along the cell.[4]

[4] *Cf.* Blinks, L. R., Harris, E. S., and Osterhout, W. J. V., *Proc. Soc. Exp. Biol. and Med.*, 1928–29, **26**, 836.

The situation when a block is placed in the center (as in Fig. 4 but without the salt bridge) probably resembles that shown in Fig. 5. The P.D. gradient is not steep enough to permit sufficient outward flow at C_1 to reduce its E.M.F. to zero and start a negative variation.

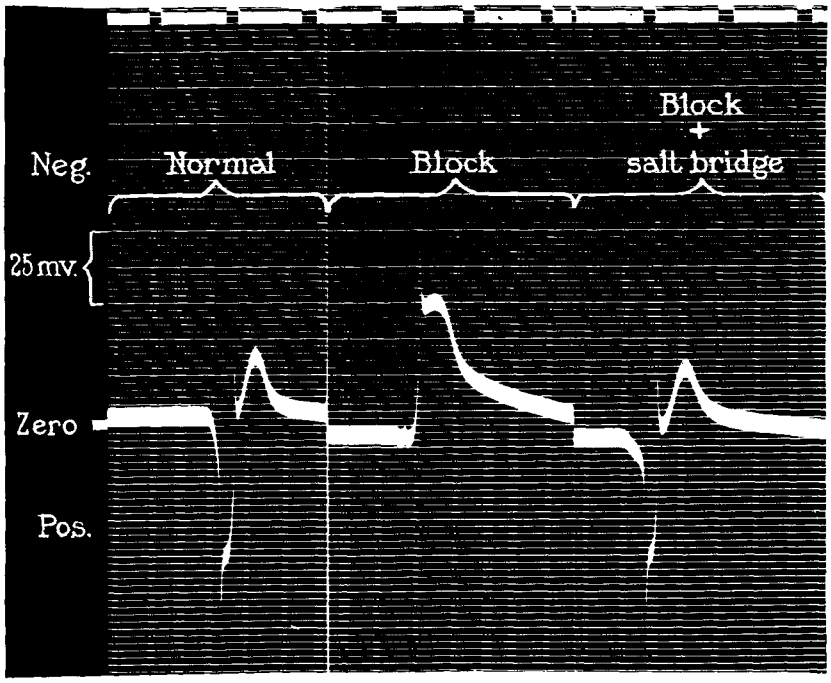

FIG. 7. Photographic record showing the P.D. of B in relation to C.

The part marked "normal" shows a diphasic action current, the experiment being arranged as in Fig. 1 (with 0.001 M KCl at B and C); the cell is stimulated at E (by applying 0.05 M KCl^{2c}) and the negative variation reaching C makes B appear positive because we are recording the P.D. of B with reference to C; afterward it reaches B making it appear negative.

The part marked "block" shows the effect of stimulation at A when the experiment is arranged as in Fig. 4 but without a salt bridge. A negative variation starting at A reaches B but since it cannot pass D the action current is monophasic.

The part marked "block plus salt bridge" shows the action current produced when the experiment is arranged as in Fig. 4 (with salt bridge). The action current produced by stimulation at E is diphasic like the one labelled "normal" (at the start of the record) for reasons given in the text.

The time marks represent 5-second intervals.

But if we put a salt bridge[5] between B_1 and C_1 and the E.M.F. at B_1 falls to zero (as the result of a negative variation) a flow can take place as shown in Fig. 6, which will reduce the E.M.F. at C_1 to zero, starting a negative variation.[6] We thus obtain the record shown in Fig. 7.

The experiment referred to in Fig. 7 was arranged as in Fig. 4, putting a piece of cotton soaked in 0.001 M KCl saturated with chloroform in the middle of the cell

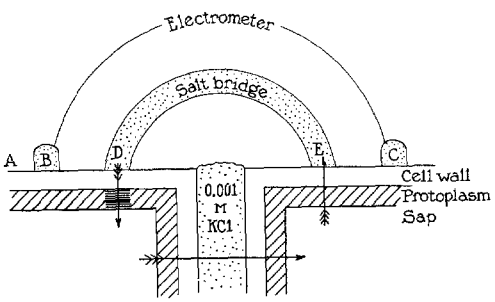

FIG. 8

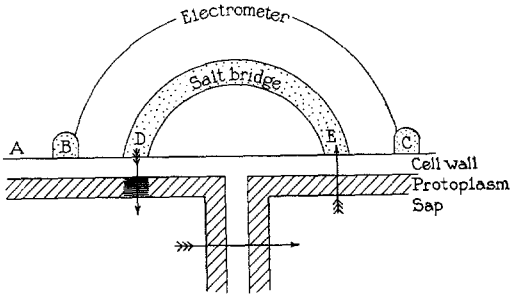

FIG. 9

FIG. 8. Hypothetical diagram of electrical conditions in two cells (from different plants) in contact with cotton soaked with 0.001 M KCl and connected by a salt bridge. The protoplasm at D has lost its potential (as indicated by the horizontal shading) and in consequence there is a flow of current between D and E as indicated by the feathered arrows. (Since the horizontal flow in the horizontal cell walls and the vertical flow in the vertical cell walls is relatively small it is not indicated.)

FIG. 9. Hypothetical diagram of electrical conditions in two cells in their natural union: they are connected by a salt bridge. The protoplasm at D has lost its potential as indicated by the horizontal shading and in consequence there is a flow of current as indicated by the feathered arrows (since the horizontal flow in the horizontal cell walls and the vertical flow in the vertical cell walls is relatively small it is not indicated).

and on each side pieces of cotton soaked in various concentrations of KCl (as shown in the figure) so arranged as to make a gradual gradient (this, as shown in a previous paper, prevents the chloroformed spot from starting negative variations). At B and C 0.001 M KCl was applied and 0.05 M KCl was placed at A or E to start a series of negative variations.

The salt bridge was composed of cotton soaked in 0.001 M KCl in all cases.

All experiments were performed on *Nitella flexilis* at 19° to 20°C. the technique being that described in previous papers unless otherwise stated.

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[5] Two calomel electrodes connected by a wire may serve.

[6] This would, of course, travel in both directions.

In view of this result it does not seem strange that a salt bridge enables a negative variation to set up a similar variation even in a cell taken from another plant and placed beside it at a distance of half an inch or more, as shown[7] in Fig. 8. In this case the circuit passes 4 times through living protoplasm (instead of twice, as in the preceding experiments). Since the resistance of the protoplasm is very high[8] we might expect the flow of current to be greatly reduced but in spite of this a negative variation starting at A and reaching D is promptly followed by one in the other cell when the salt bridge has a sufficiently low resistance (*e.g.*, cotton soaked in 0.001 M KCl).

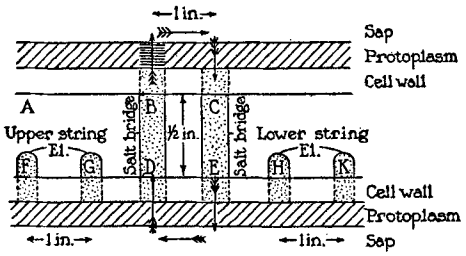

FIG. 10. Hypothetical diagram of electrical conditions in two cells (from different plants) connected by two salt bridges. The protoplasm at B has lost its potential (as indicated by the horizontal shading) and in consequence there is a flow of current as indicated by the feathered arrows. Since the horizontal flow in the protoplasm and in the horizontal cell walls is relatively small it is not indicated.

If an experiment be arranged as in Fig. 9, employing two cells in their natural union, the negative variation starting at A and reaching D can start a variation at E by the aid of a salt bridge but this happens much less frequently when the salt bridge is absent: apparently the cell wall may act as a salt bridge in some cases, as was observed by Mr. Harris and previously reported.[4]

A positive result is also obtained when the experiment is arranged as in Fig. 10. In this case the currents would presumably be as indi-

[7] Experiments arranged as in Figs. 8 and 9 were checked in all cases by using a double string galvanometer and employing two contacts connected with one string on one side of the salt bridge and on the other side two contacts connected to the other string as in Fig. 10.

[8] Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13**, 495.

cated so that in the lower cell there would be an outward current at *D* which could stimulate. This is commonly the case. Preliminary experiments of this sort were carried out by Mr. Harris and have been previously reported.[4]

SUMMARY

A negative variation in *Nitella* is unable to pass a spot killed by chloroform but can set up a negative variation beyond this spot when a salt bridge is put around it. It can likewise set up a negative variation in a cell of another plant if connected to it by two salt bridges.