NEGATIVE VARIATIONS IN NITELLA PRODUCED BY CHLOROFORM AND BY POTASSIUM CHLORIDE

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Experiments by Mr. Harris in 1922 (in collaboration with the senior author) showed that chemical stimulation could set up negative variations in *Nitella*. Owing to the theoretical interest of this result an extensive series of experiments has been carried on since that time. The present paper deals with the effects of chloroform and KCl.

The technique, unless otherwise stated, is that described in previous papers.[1] The experiments were performed on *Nitella flexilis* at a temperature of 19° to 20°C. It was found advisable to keep each cell in a separate dish after cutting (to avoid the effects of sap coming from injured cells) and to let the cells stand for some hours or days after cutting. In placing the cells in the apparatus they were handled with extreme care to prevent as far as possible any mechanical disturbance.

In order to avoid stimulation by static discharge glass-tipped forceps were used, the operator standing on a grounded piece of copper. To avoid stimulation by mechanical shock the cotton saturated with solution was placed very gently on the cell or flowing contacts were used (that these precautions were effective is shown by the fact that applying cotton soaked in 0.001 M KCl did not start a variation).

In applying an E.M.F. between A and C the P.D. at C was measured by killing A with chloroform (this brings its P.D. aproximately to zero). In some cases the chloroform was applied before impressing the E.M.F. and in other cases afterward (in these latter cases a whole series of E.M.F.'s was applied in turn to be sure of getting the right value).

To prevent unintentional stimulation the time signal and the stimulus were completely insulated from each other and the stimulating circuit was closed by a platinum wire dipping into a mercury cup (to avoid an unintentional break in the circuit).

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[1] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11**, 673; 1928–29, **12**, 167.

According to the local circuit theory of stimulation[2] we should expect an application of chloroform to a cell imbibed with tap water or with 0.001 M KCl (*e.g.*, to such a point as *A*, Fig. 1) to start a negative variation for the following reason. As previous studies[3] have shown

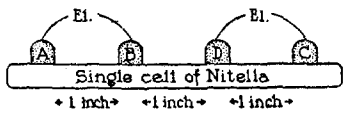

FIG. 1. Diagram to show arrangement of experiments

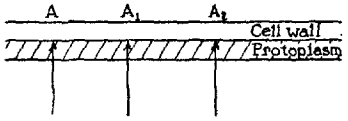

FIG. 2

FIG. 2. Hypothetical diagram of P.D.'s in the protoplasm when the cell wall is imbibed with tap water. The arrows show the direction in which the positive current tends to flow and their length indicates the relative magnitude.

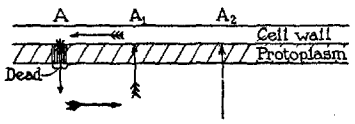

FIG. 3

FIG. 3. As in Fig. 2 but with the protoplasm at *A* shaded to indicate that it has been killed by chloroform. The feathered arrows denote flow of current, the plain arrow represents P.D. (it being assumed, as a convenient fiction, that there is no flow as yet at A_2).

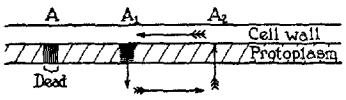

FIG. 4

FIG. 4. As in Fig. 3 but with a flow between A_2 and A_1 (it being assumed, as a convenient fiction, that there is no flow at *A*). The protoplasm at *A* is shaded vertically to indicate that it has been killed by chloroform and that at A_1 shaded horizontally to indicate that it has temporarily lost its E.M.F.

that the P.D. across the protoplasm may be represented as in Fig. 2 and that when 0.001 M KCl saturated with chloroform is applied at *A* the

[2] *Cf.* Lillie, R. S., Protoplasmic action and nervous action, University of Chicago Press, Chicago, 1923. Davis, H., The condition of the nerve impulse, *Physiol. Rev.*, 1926, **6**, 547.

[3] *Cf.* Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11**, 673.

P.D. at that point rapidly approaches zero[3] we suppose that a current, due to the E.M.F. at A_1, flows between A_1 and A as shown in Fig. 3,

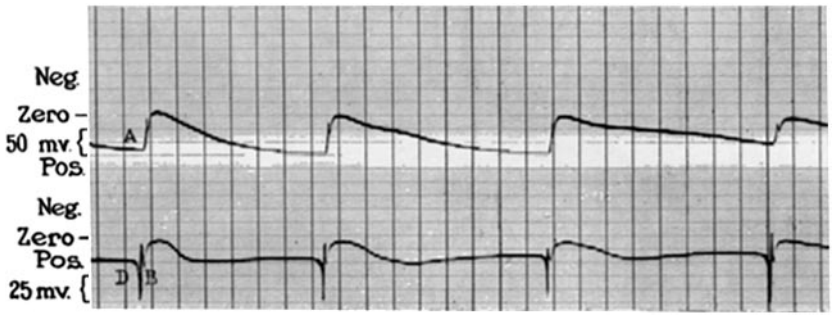

FIG. 5. Photographic record of successive variations produced by application of 0.001 M KCl saturated with chloroform at C (Fig. 5 *a*) with 0.001 M KCl at A, B and D. The two strings of the double string Einthoven galvanometer were connected as shown in Fig. 5 *a*.

A variation starting at C strikes D, making the downward movement labelled D in the record (this is really a negative change at D but appears positive because we record only the P.D. of B with reference to D). When the variation reaches B it makes the upward movement labelled B in the record. The P.D. then approaches zero. The successive variations starting from C produce similar effects.

When the variation reaches A it causes the upward movement labelled A in the record (*i.e.*, a negative variation at A): the variation is monophasic because the P.D. at C remains constant (approximately zero), the protoplasm having been killed by chloroform.

The vertical lines mark 5-second intervals.

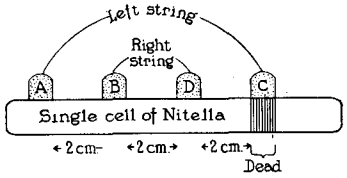

FIG. 5 *a*. Diagram to show arrangement of the experiment recorded in Fig. 5. Protoplasm shaded at C to show that it has been killed by chloroform. The left string gives the upper record in Fig. 5.

which quickly reduces the E.M.F. at A_1 approximately to zero (that it really goes almost or quite to zero is evident when we lead off from A_1

to a spot whose P.D. is zero).[4] We suppose that a flow then starts between A_1 and A_2 (Fig. 4) and thus the variation travels along the cell.[5] The P.D. at A_1 and A_2 soon returns to normal and the whole process can commence anew. Thus successive disturbances can be produced as shown in Fig. 5 (in some cases a dozen or more).

The interval between successive disturbances depends upon the refractory period which follows each stimulation. It has been found by Blinks[6] that when the E.M.F. approaches zero, as the result of the outward flow of positive current, the resistance (as measured by direct current) falls almost or quite to that of a dead spot. There is therefore a period during which cations are moved outward by the current: as

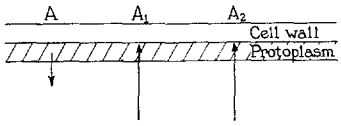

FIG. 6. As in Fig. 2 but with 0.05 M KCl at A in place of chloroform.

a result they may increase in concentration just outside the outer protoplasmic surface. This must decrease the E.M.F. across the protoplasm until such cations are removed by outward diffusion[7] or by reabsorption into the cell aided by the inward current which follows the outward current. This may explain the refractory period after each stimulus and the increasing fatigue following repeated stimuli, but it is possible that chemical or structural changes, such as those suggested by Osterhout and Harris,[3] may also play a part.

We should expect similar results if 0.05 M KCl (without chloroform) were applied at A since this would usually give to the arrow at A the direction shown in Fig. 6[3] which should produce a greater flow of cur-

[4] This will be dealt with in other papers: it does not go all the way to zero in all cases since the all or none law does not always strictly apply.

[5] The variation travels in both directions from A but for convenience only one side is here represented.

[6] Blinks, L. R., Harris, E. S., and Osterhout, W. J. V., *Proc. Soc. Exp. Biol. and Med.*, 1928–29, **26**, 836. Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13**, 495.

[7] This diffusion away from the outer surface can readily occur since, relatively speaking, the actual quantity of cations coming out is exceedingly small. The external solution as a whole therefore remains practically constant as shown by the potentials after successive recoveries.

rent than the application of chloroform. This expectation seems to be realized since it is a simple matter to produce a succession of disturbances by 0.05 M KCl (Fig. 7).

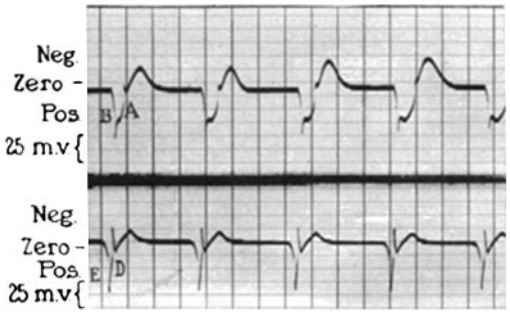

FIG. 7. Photographic record showing successive variations due to application of 0.05 M KCl at *C* (Fig. 7 *a*), with 0.001 M KCl at *A*, *B*, *D*, and *E*. The two strings of the double string Einthoven galvanometer were connected as shown in Fig. 7 *a*.

A variation starting at *C* strikes *E* making the downward movement labelled *E* in the record (this is really a negative change at *E* but appears positive because we record only the P.D. of *D* with reference to *E*). When the variation reaches *D* it makes the upward movement labelled *D* in the record. The P.D. then approaches zero. The successive variations starting from *C* produce similar effects.

When the variation reaches *B* it causes the downward movement labelled *B* in the record (this is really a negative change at *B* but appears positive because we record only the P.D. of *A* with reference to *B*). When the variation reaches *A* it makes the upward movement labelled *A* in the record.

The vertical lines mark 5-second intervals.

The fact that the refractory period is shorter than in Fig. 5 apparently has no relation to the stimulus in this case.

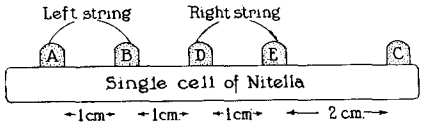

FIG. 7 *a*. Diagram to show arrangement of the experiment recorded in Fig. 7. The left string gives the upper record in Fig. 7.

With lower concentrations the results are different: for example, 0.01 M and 0.02 M KCl usually produce only one wave. This is to be

expected if we consider that a wave can start only when a sufficient P.D. gradient exists: thus in Fig. 8 the P.D. gradient G_1 might be sufficient to start a variation because the flow of current through A_3 could break down its P.D. whereas this might not happen with the P.D. gradient G when the flow through A_1 might be insufficient.

These P.D. gradients will depend on the diffusion gradients: this is illustrated in Fig. 9 which shows the effect of the diffusion gradient

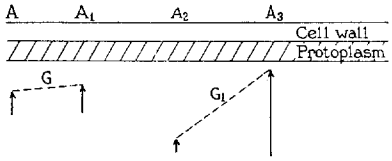

FIG. 8. Hypothetical diagram illustrating a gentle gradient of P.D. (G) and a steep one (G_1).

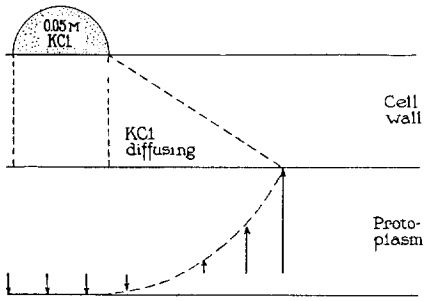

FIG. 9. Hypothetical diagram showing the effect of 0.05 M KCl (diffusing along the cell wall) upon the P.D. gradient in the protoplasm. The concentration of KCl in the cell wall is denoted by the height of the broken line in the cell wall.

on the P.D. gradient. It is evident that the higher the concentration[8] of applied KCl the steeper will be the diffusion gradient (with equal times of diffusion). The longer the time elapsing since the application the less steep will be the diffusion gradient and the smaller the chance of starting a negative variation. Hence it is clear that as we lower the concentration we shorten the time during which the necessary steepness of gradient will persist and finally a concentration will be reached where

[8] For example, excellent results are obtained with 0.1 M KCl.

only one disturbance will occur: at still lower concentrations none will start.

In the same way chloroform (or any substance produced by its action) diffusing along the cell might lower the P.D. gradient which may explain why we often get only a few negative variations (sometimes only one or none at all).

If chloroform or 0.05 M KCl does not produce a sufficiently steep gradient to start a variation we may easily remedy the trouble by

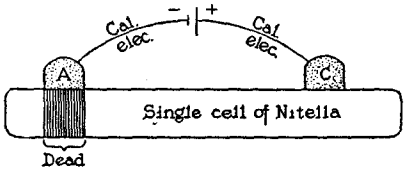

FIG. 10. Diagram to show the method of imposing E.M.F. between A and C. The protoplasm at A is shaded to show that it has been killed by chloroform.

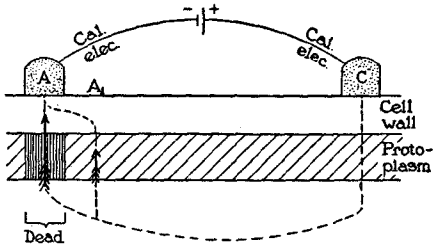

FIG. 11. Diagram to show flow of current when an E.M.F. is imposed between A and C: the flow is greater at A than at A_1. The protoplasm at A is shaded to show that it has been killed by chloroform.

running a wire from the calomel electrode at the spot in question to the calomel electrode at a spot some distance away where the protoplasm is in contact with 0.001 M KCl and hence has a high P.D. This gives a sufficiently high gradient and a variation starts at once at the distant point.

When 0.05 M or 0.01 M KCl has stopped producing variations we can usually start them by moving the wad of cotton to a fresh spot, thus starting a new gradient.

A variation cannot pass a spot to which 0.001 M KCl saturated with

chloroform has been applied nor, as a rule, one which is in contact with 0.01 M KCl or 0.05 M KCl. This is to be expected since all of these agents either reduce the P.D. to a low value or reverse its direction.

We may therefore use these substances to block the passage of a variation: very convenient for this purpose is 0.02 M KCl which very seldom starts more than one; for still greater certainty we may use a piece of cotton saturated with 0.05 M KCl on each side of which we place cotton saturated with 0.02 M KCl.

These results indicate that a variation may usually be started by bringing the P.D. at any point to a low value when the rest of the protoplasm has a sufficiently high P.D. to ensure the necessary outward flow of current, and it might seem possible to bring about this result by means of an applied E.M.F. It may be worth while to consider this briefly in the light of the negative results of our experiments on this point.

If the cell is in the state shown in Fig. 2 (with each arrow representing 100 mv.) an E.M.F. applied between A and C might start a flow such as is seen in Fig. 3 between A and A_1. If we first reduce the E.M.F. at A approximately to zero and apply an E.M.F. of 100 mv. (as in Fig. 10) we merely replace the original P.D. at A by the applied E.M.F. so that there is no more reason for a variation to start than when the cell is in the condition[9] shown in Fig. 2. If we now increase the applied E.M.F. to 200 mv. most of the outward flow will be through the dead protoplasm at A (shaded in Fig. 11) and much less will flow through A_1 (where the resistance is much higher): what flows through A will not start a variation but what flows through A_1 may do so if the flow is sufficient.

A variation coming down the cell and reaching such a point as C in Fig. 10 may not be able to pass since the applied E.M.F. tends to form an inward current (by an inward current is meant one like that shown by the arrow at A in Fig. 3): this, according to unpublished experiments by Dr. Blinks, must reach a relatively high value before it can reduce the E.M.F. of the protoplasm to zero ("anodal stimulation") but this can be done by a relatively small outward current

[9] The chief difference is that A and C are connected by a wire as well as by the cell wall but this cannot start a variation unless sufficient difference of P.D. exists between them.

(*i.e.*, a current like that shown at A_1 in Fig. 3) and this is what happens when a negative variation travels along the cell, *i.e.*, each spot which is traversed by an outward current loses its E.M.F. in turn. If such outward currents are sufficiently reduced by an opposing E.M.F. as at *C* in Fig. 10 the variation cannot pass. This is an "anodal block."

The successive variations in *Nitella* recall those observed in the heart and in certain cases in nerve (since in nerve there is no contraction the analogy may be closer). In all these cases the action currents are similar in form and magnitude but in *Nitella* the process is much slower. These facts suggest the possibility of producing successive variations in muscle and nerve in somewhat the same way as in *Nitella*: experiments are being made to test this suggestion.

SUMMARY

The results of applying chloroform and KCl to *Nitella* indicate that a negative variation may be started whenever it is possible to set up along the protoplasm a gradient of potential difference sufficiently steep to produce the necessary outward flow of current. Successive variations may thus be set up.