THE PRODUCTION AND INHIBITION OF ACTION CURRENTS BY ALCOHOL

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This paper describes an investigation growing out of preliminary experiments begun in 1922 by Mr. E. S. Harris and the senior author.[1] It deals with lowering of P.D. by virtue of which alcohol is able to start and stop action currents in *Nitella*.

It has already been stated that chloroform and KCl can stimulate by lowering the P.D. at a given spot until the inflow of current from neighboring regions is sufficient to start a negative variation.

Ethyl alcohol can act in the same manner. An interesting example of this appears in Fig. 1.[2] The alcohol was applied near the center of the cell, as shown in Fig. 2, at the spot marked *B*. At the start the P.D. of *B* with reference to *D*, as shown by curve *B*, was about 100 millivolts positive, owing to the fact that *D* had previously been killed by chloroform.[1,3] When alcohol (1.5 M) was applied the P.D. gradually fell toward zero (as shown by the rise of the curve) and when it reached 80 millivolts an action current started as shown by the sudden jump of the curve. The excitation spread in both directions, as shown by the movements of curves *A* and *C* about a second later. At *A* and *C* recovery was normal but at *B* it was very slow. At the point marked 2 on the record the cell was stimulated electrically at *X*. A normal

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

[2] This record was chosen for reproduction from many which were similar. The statements made about it apply qualitatively to all the others, and are in good agreement quantitatively.

[3] The experiments were performed on *Nitella flexilis* with the technique previously described (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). The method of measurement is essentially electrostatic. The temperature was very close to 20°C.

response is seen at A but only a very slight one at B: since the impulse does not pass on to C it is evident that the alcohol at B is acting as a block.

At the point marked 3 the shutter of the camera was closed and the motion of the photographic paper was stopped for 1 minute during

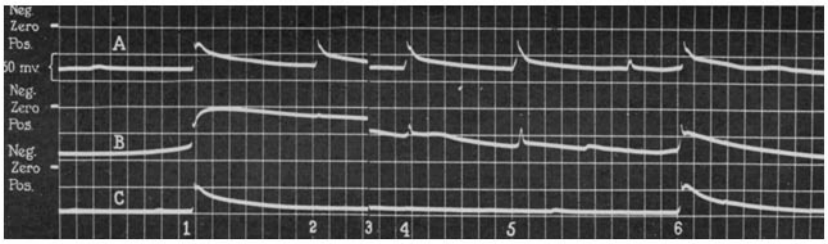

FIG. 1. Photographic record (employing a galvanometer with three strings) of an experiment arranged as in Fig. 2: 0.001 M KCl at all points except at B where 0.001 M KCl containing 1.5 M ethyl alcohol is applied. Curves A, B, and C show the P.D. of A, B, and C with reference to D (which has been killed by chloroform before starting the experiment). The intervals between time marks represent 5 seconds.

The effect of alcohol on starting and stopping action currents is seen in Curve B (for description see page 611).

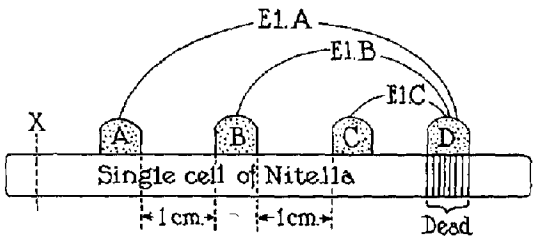

FIG. 2. Diagram to show the arrangement of an experiment: D is killed by the application of chloroform before the start of the experiment.

The cell is supported on a paraffin block, and is surrounded with air except where the contacts are applied.

which the alcohol was washed away by a stream of 0.001 M KCl. Cotton soaked in 0.001 M KCl was then replaced exactly on B: the record was set in motion, the camera was opened and at 4 the cell was stimulated electrically at X, producing a normal response at A, an

incomplete response at B, and none at C. Another stimulus at 5 had much the same result save that the response at B was greater. But the next stimulus (starting at D) at the spot marked 6 produced a much greater response: it will be noted that recovery has now made good progress and we see that the blocking effect of the alcohol has disappeared and that A now responds normally.

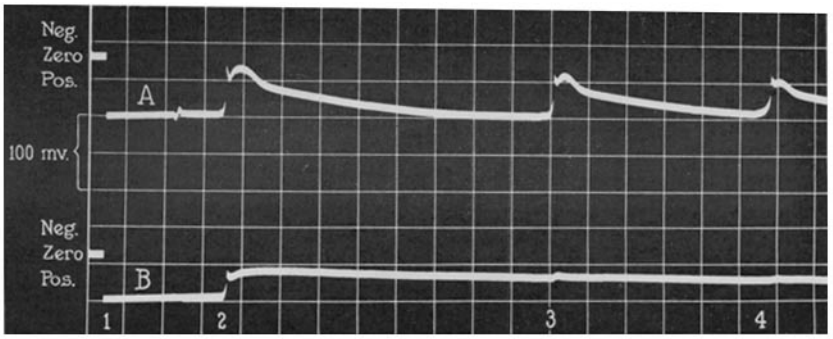

FIG. 3. Photographic record of an experiment arranged as in Fig. 2 with C omitted. At A and D 0.001 M KCl; at B 0.001 M KCl containing molar ethyl alcohol: Curves A and B show the P.D. of A and B with reference to D (which has been killed by chloroform before the start of the experiment). The intervals between time marks represent 5 seconds.

Curve B shows that in this case alcohol had no apparent effect until an action current arrived from another part of the cell (for description see below).

In many cases there was a larger degree of recovery at B after passage of an action current, and the cell could again be stimulated within 30 seconds. In such cases the recovery was less with each successive response, because of the loss of P.D., but the curve rose to practically the same level each time.

It sometimes happens that the application of alcohol produces no apparent effect until the cell is stimulated. This is illustrated by Fig. 3, the experiment being arranged as in Fig. 2 with C omitted. At the start B was 65 millivolts positive to D and this was not altered by the application of molar alcohol at the point marked 1. At the point marked 2 the cell was stimulated electrically at X and a normal response occurred at A. A response is also seen at B (where alcohol is applied) but in this case there is practically no recovery. The subsequent stimuli at 3 and 4 produced only slight responses at B.

As is to be expected, the effect of alcohol varies with the concentration. With 0.5 M alcohol there was no lowering of P.D. and irritability was normal. With molar alcohol, the P.D. slowly fell, the loss not exceeding 20 per cent in 5 minutes when there were no action currents. An action current arriving from another part of the cell sent the P.D. approximately to zero, and recovery of P.D. was slow and incomplete. After several action currents, recovery was absent and propagated variations were no longer transmitted, but a response at the spot in contact with alcohol could still be secured by cutting another part of the cell.[4] On removal of alcohol, and washing, the P.D. returned completely in about 5 minutes, irritability, responses, and transmission becoming normal.

The application of 1.5 M alcohol produced results like those shown in Fig. 1, the loss of P.D. taking place during from 5 to 100 seconds after the application of alcohol. During the loss of P.D. stimulation usually occurred after which the P.D. approached zero. There was little or no recovery and electrical stimulation at such a point as X (Fig. 1) produced no response at B but cutting at X produced a death wave at B. When the alcohol was removed complete irritability returned in from 3 to 15 minutes.

Alcohol at a concentration of 2 M stimulated in a few seconds: the P.D. fell to and remained at zero, irritability and transmission were abolished but a response at B (Fig. 2) could be secured by cutting at X. As in the other cases the action of alcohol was hastened by an action current arriving from another part of the cell. On removing the alcohol no recovery was observed during 10 minutes, but after 20 minutes the potential had risen to approximately the original value and irritability had returned.

The effects of 3 M alcohol were much like those of 2 M, except that after the removal of alcohol and washing the cell, there was only par-

[4] Cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14**, 473. The response consisted in a rise of the curve a little above zero and a gradual falling to zero. The stimulus of the cut causes the inner surface to lose its P.D. first, thus causing the curve to go above zero. This may be similar to the effect of cutting when the cell is in contact with 0.01 M KCl, as explained in former papers (Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928–29, **12**, 167) except that in the case of alcohol plus 0.001 M KCl the P.D.'s of each layer may be small.

tial recovery of potential, not to exceed 30 per cent, and no recovery of irritability. Usually the cell died within an hour or two.

The lowering of P.D. has been observed by Jost[5] in *Chara* and by Umrath[6] in *Nitella*.

In chemical stimulation repeated action currents are commonly observed[7] and it has been stated that this appears to depend on a rather sharp boundary of the stimulating agent which results in a rather steep electrical gradient in the neighborhood of the boundary. Unless this can be obtained we should not expect repeated action currents. In the case of alcohol a sharp boundary would not be expected since where alcohol and water meet a rather violent mixing occurs. This applies to a lesser extent when solutions of alcohol in water come in contact with water in the film covering the cell wall. It is therefore not surprising that we do not observe repeated action currents in our experiments.

It is most interesting to find that a non-electrolyte can reversibly alter P.D. to such an extent and it is important to discover how it is done. No doubt structural changes could account for it, *e.g.* by reversible coagulation or the formation of openings (as elsewhere discussed[1]), by changes in surface tension. On the other hand, alteration of the non-aqueous surfaces involving changes in the mobility of ions might bring it about. Alcohol might tend to dissolve out some of the non-aqueous constituents and thus change mobilities: it is probably more polar than the surface layers of the protoplasm and would therefore tend to increase their conductivity and the solubility of electrolytes in them.[8] Such changes might affect the inner and outer surfaces unequally, since these surfaces appear to differ. Further experiments will be undertaken to clear up some of these points.

Similar experiments on the sciatic nerve of the leopard frog (April)

[5] Jost, L., *Sitzungsber. Heidelberger Akad. Wissensch.*, Abt. B, 1927, Abhandl. 13, Nov.

[6] Umrath, K., *Protoplasma*, 1930, **9**, 576.

[7] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1929–30, **13**, 459.

[8] Merely increasing the conductivity without changing relative mobilities would reduce the resistance of the protoplasm without changing the P.D., but the observed value of the P.D. might be slightly raised for reasons given elsewhere (Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1929–30, **12**, 761).

gave negative results. The nerve was exposed near its origin by opening the dorsal body wall in the lumbar region: in this way injury to the nerve could be easily avoided. In some cases the nerve was exposed just above the knee. Concentrations of ethyl alcohol ranging from 1 to 5 molar (in Ringer's solution) were then applied without causing the leg muscles to twitch although subsequent electrical stimulation showed that the nerve was functioning normally.

This result does not seem to be due to the fact that the alcohol does not penetrate to the nerve fibres, for alcohol (3.5 M) applied to the nerve established a complete block in about 4 minutes without causing an action current. It is quite possible that the absence of action currents is due to the absence of a sharp boundary, as already suggested for *Nitella*.

Concentrations up to 5 M applied to the intact skin did not cause the characteristic wiping reaction which was, however, promptly elicited by acetic acid.

On the other hand it must be remembered that one can taste 1.5 M ethyl alcohol without difficulty.

Kemp and Waller[9] state that 5 to 10 per cent alcohol in saline gives a temporary contracture of the frog's sartorius when the muscle is immersed in the solution. This is evidently somewhat different from the negative variation observed in *Nitella*.

SUMMARY

Suitable concentrations of ethyl alcohol (1 to 1.5 M) applied to a spot on a cell of *Nitella* lower the P.D. enough to cause action currents. The alcohol then suppresses action currents arriving from other parts of the cell and acts as a block. After the alcohol is removed the normal P.D. and irritability return.

Similar experiments on the sciatic nerve and skin of the frog produced only a negative result.

[9] Kemp, H. P., and Waller, A. D., *J. Physiol.*, 1908, **37**, xliii (Proc.).