STIMULATION BY COLD IN NITELLA

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Umrath[1] has noted that action currents are produced in *Nitella* by local application of cold.[2]

In order to see just what happens the following procedure was adopted.[3] Cells which had been kept for 7 to 12 days in Solution A[4] were tested, using the arrangement shown in Fig. 1. All responded normally to electrical stimulation (about 120 mv. D.C. applied by means of saturated calomel electrodes).

The cell whose record appears in Fig. 2 was placed on a paraffin block at room temperature (22°C.) with Solution A at *C* and *D* (the cell wall was imbibed with Solution A). Fresh Solution A at 22°C. was then applied at *C*, causing no change.[5] Solution A at 1°C. was then applied at *C* which became more negative[6] to the extent of 13 mv., without setting up a characteristic action current: this happened in about 6 per cent of the cells (the alteration ranged from 10 to 20 mv.).

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

[2] This had also been observed in this laboratory by W. J. V. Osterhout and E. S. Harris and photographically recorded in 1927 (personal communication).

[3] The technique, unless otherwise stated, is as in previous papers (Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1930–31, **14**, 473; 1933–34, **17**, 87). The experiments were carried out at room temperature 22 ± 1°C. The material was *Nitella flexilis*, Ag.

[4] This is a nutrient solution: for its composition see Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87.

[5] The solutions were poured rapidly and continuously on a strip of filter paper which passed under the cell at *C*, precautions being taken to avoid mechanical or electrostatic shock. In some cases there are irregular small changes (2 to 3 mv.) as the result of this procedure.

[6] That this change is not due to streaming potential is shown by the fact that it does not occur if the solution is at room temperature.

As the normal potential across the protoplasmic surface is outwardly directed (positive) the fact that *C* becomes more negative means that there is a partial loss of P.D.

As explained elsewhere the measured P.D. depends on a number of E.M.F.'s[7] and of resistances.[8] The temperature coefficients need not

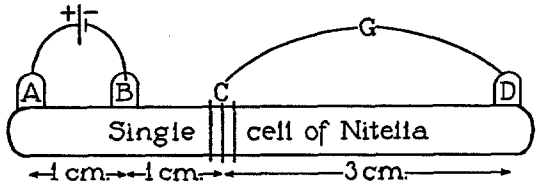

FIG. 1. Arrangement for testing cells (placed on a block of paraffin) with solutions at various temperatures. The electrical stimulation was applied through saturated calomel electrodes at *A* and *B*. *C* was a flowing contact for changing solutions during recording. The recording instrument (*G*) was a Cambridge string galvanometer with thermionic amplifier (making the measurement essentially electrostatic).

be the same for all of these. Hence (even in the absence of secondary effects and of stimulation) we do not know just how much lowering of P.D. to expect with a given fall of temperature,[9] but it seems safe to

[7] The lowering of temperature would, of course, lower the E.M.F. and raise the resistance. Lowering the temperature from 22°C. to about 3°C. increased the average resistance of 10 *Nitella* cells 44.2 per cent. This is the combined ohmic and polarization resistances at one spot, 1 cm. long, in contact with Solution A. The other contact was chloroformed. The measurement of resistance was made according to the method of Blinks (Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13,** 495).

According to Bernstein (Bernstein, J., Elektrobiologie, Braunschweig, F. Vieweg und Sohn, 1912, 91) the P.D. in muscle is directly proportional to the absolute temperature. Bernstein (Bernstein, J., *Arch. ges. Physiol.*, 1910, **131,** 589) also states that the demarcation potential of muscle changes when the temperature is altered at the intact surface, but not when the change is made at the cut end. Verzár (Verzár, F., *Arch. ges. Physiol.*, 1912, **143,** 252) repeating this experiment, found an effect also at the cut end.

[8] For a discussion of E.M.F.'s and resistances in *Nitella* see Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1927–28, **11,** 673.

[9] Raising the temperature above that of the room does not produce much change in P.D. The reason for this is not clear but it may depend on the fact that as the E.M.F. increases the resistance falls.

say that it would be of the order of magnitude observed in these experiments.

When the flow of cold solution at *C* ceased the original P.D. was slowly regained.[7] The whole procedure shown in Fig. 2 is fairly typical for those cells which are not stimulated.

As a rule the sudden application of cold Solution A stimulates the cell and a negative variation results. The shape of the action curve

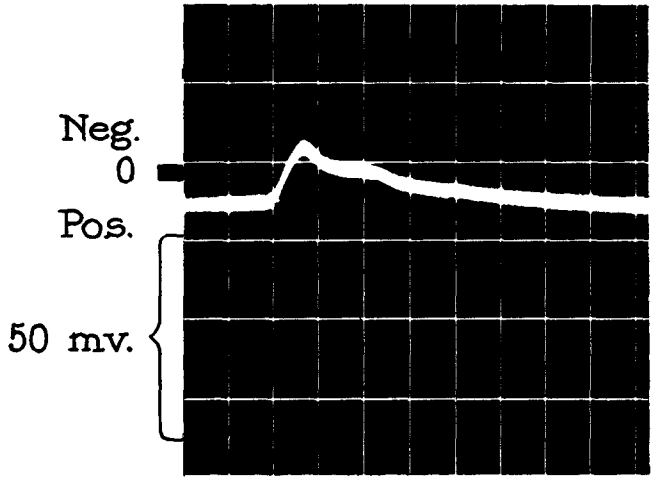

FIG. 2. Photographic record of partial loss of P.D. in *Nitella* caused by application of Solution A at 1°C. at *C* by flowing contact (arranged as in Fig. 1). No characteristic action current was produced. The cell had been kept in Solution A for 6 days. The vertical lines represent 5 second intervals. Room temperature, 22°C.

at the cooled spot differs from that at a normal spot in one or more of the following particulars. (1) The ratio of the height of the first peak to the second is reduced. (2) The total change of P.D. is greater or less at the cooled spot. (3) The recovery of potential is slower at the cooled spot.[10]

Fig. 3 (the experiment being arranged as in Fig. 4) shows the reduction of the first peak and decrease in total change of potential. In

[10] Gasser (Gasser, H. S., *Am. J. Physiol.*, 1931, **97**, 254) states that cooling frog nerve causes a prolongation of the spike accompanied by a considerable falling off in height, and this effect increases as the temperature is lowered.

this case, *C*, *D*, and *E* were in contact with 0.001 M KCl (at 23°C.). The negative variation at *D* (middle curve) which was started by the application (at *D*) of 0.001 M KCl at 1°C. spread along the cell in

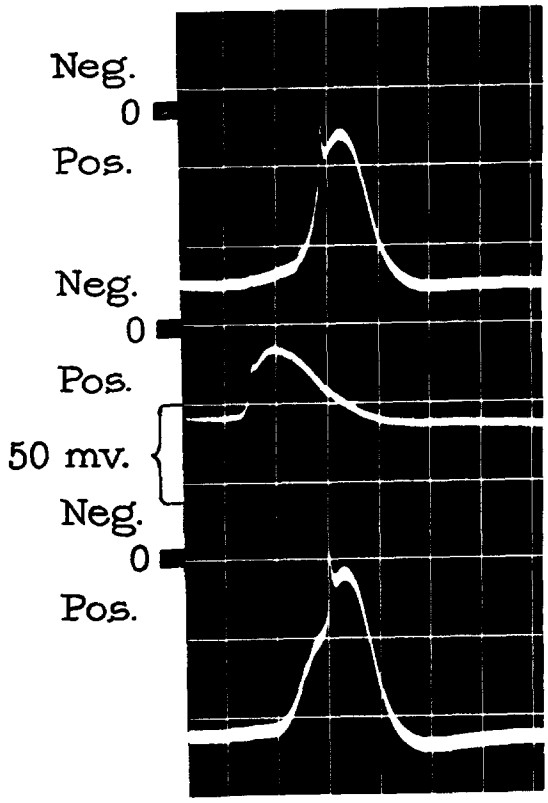

FIG. 3. Photographic record of negative variations in *Nitella* caused by applying 0.001 M KCl at 1°C. at *D* (Fig. 4). Before the record started *F* was killed with chloroform to give monophasic responses. The top curve shows changes at *C*, the middle curve at *D*, and the bottom curve at *E*. All contacts were 0.001 M KCl. The cell had been kept for 4 days before use in Solution A. The vertical lines represent 5 second intervals. Room temperature, 23°C.

both directions, and its behavior at *C* (top curve) and at *E* (bottom curve) appears to be a normal one. Fig. 5 shows a similar result (with the same arrangement) produced on another cell. Here the

first peak is lacking at the cooled spot (middle curve). In both these records *F* had been killed by chloroform at the start to give monophasic responses.

In Fig. 3 the action current carried the P.D. at the normal contacts to zero or nearly so, while in Fig. 5 the action current reduced the P.D. by only about two-thirds of its value. This is not unusual in *Nitella*, an action current frequently failing to discharge all the polarization of the membrane.

Fig. 6 (with the same arrangement, but with Solution A on contacts *C*, *D*, and *E*, and with 2 cm. spacing between contacts) shows a

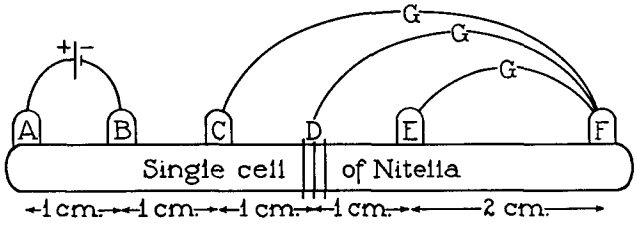

FIG. 4. Arrangement for testing cells (placed on a block of paraffin) with solutions at various temperatures. *D* is a flowing contact for changing solutions during recording. *F* may or may not be killed. All contacts are made through saturated calomel electrodes. *GGG* represent Cambridge string galvanometers with thermionic amplifiers (the measurement is essentially electrostatic). The recording instrument was a Cambridge Type A string galvanometer in which the single string had been replaced by three tungsten wires, each 5μ in diameter. Careful calibration shows the deflections to be proportional to the applied voltage within the limits of the recording paper here employed.

relative increase in the second action curve at the cooled spot. In the first action current at *D* (middle curve), before *D* was cooled, the change of P.D. was approximately the same as at *C* and *E*, but after it was cooled (second action current) the second peak of the curve was higher by about 40 mv. This in itself is not unusual, as such variations have often been observed[11] at ordinary temperatures. In this case (and many other cases like it) the only difference in conditions at the three recorded contacts was the cooling at contact *D*. In Fig. 7 the variation caused by the application at *D* (upper curve) of

[11] The fact that the action curve goes above zero may be due to some positive P.D. at the common contact *E*.

Solution A at 1° was practically the same as at contact *E* (lower curve) at 22°C. The reason for the occasional increase in value of the second peak will be discussed in a later communication.

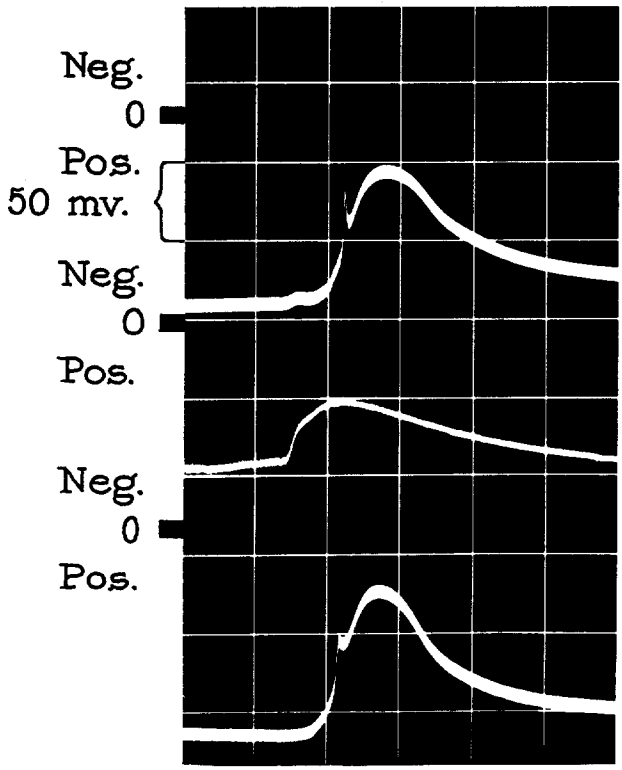

FIG. 5. Photographic record of negative variations caused by the application of 0.001 M KCl at 1°C. at *D* (Fig. 4). The top curve shows changes at *C*, the middle curve at *D*, and the bottom curve at *E*. All contacts were 0.001 M KCl: *F* was killed with chloroform before the experiment started. The cell was kept for 2 days in Solution A. The vertical lines represent 5 second intervals. Room temperature, 21°C.

In *Chara*[12] we observe at the cooled spot a curve which shows less total change in P.D., or slower recovery, or both, than at a spot at room temperature. This is shown in Figs. 8, 9, and 10.[13] Here

[12] This is *Chara coronata*, Ziz.

[13] The arrangement, solutions, and distances were as in Fig. 4.

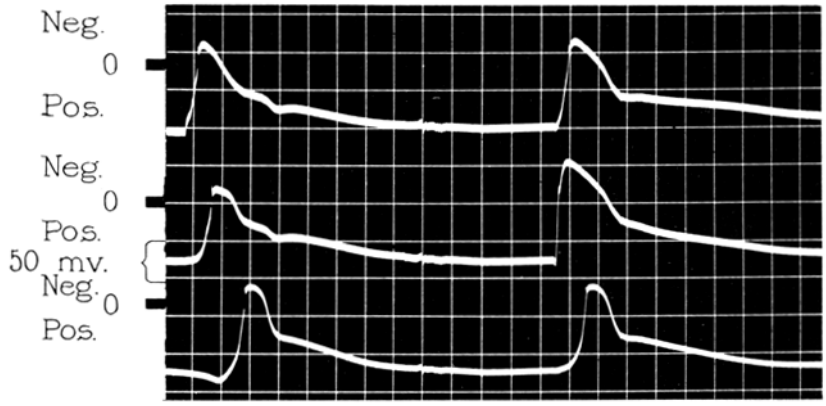

FIG. 6. Photographic record of negative variations in *Nitella*. The first variation was caused by electrical stimulation with 120 mv. D.C. at *B* (Fig. 4). The variation passed along the cell and was recorded at *C* (top curve), *D* (middle curve), and *E* (bottom curve). The application at *D* of Solution A at 22°C. (shown by the slight disturbance on the curves 45 seconds after the record started) was without effect. The second variation was caused by the application at *D* (67 seconds after the record started) of Solution A at 1°C. It passed along the cell in both directions and was recorded at *C* (top curve) and *E* (bottom curve). *F* was brought nearly to zero by application of 0.01 M KCl at *F* before the record started. All contacts were Solution A in which the cell had been kept for 17 days. The vertical lines represent 5 second intervals. Room temperature, 21°C.

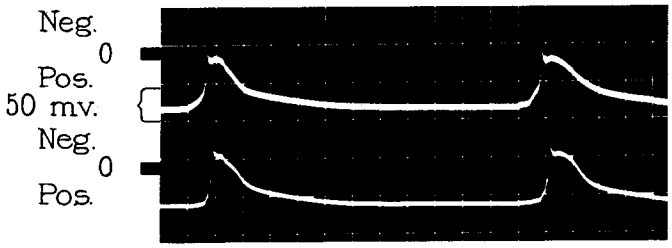

FIG. 7. Photographic record of negative variations in *Nitella*. The first reaction was caused by electrical stimulation at *B* (Fig. 4 with *C* omitted). The variation passed along the cell and was recorded at *D* (upper curve) and *E* (lower curve). The second variation, recorded in the upper curve, was caused by the application at *D* of Solution A at 1°C. The negative variation passed along the cell and was recorded at *E* (lower curve). The P.D. at *F* was brought nearly to zero by the application of 0.01 M KCl before the record started. All contacts were Solution A, in which the cell had been kept for 5 days. The vertical lines represent 5 second intervals. Room temperature, 22°C.

0.001 M KCl at 1°C. was applied at *D* (middle curve), causing a negative variation which spread in both directions (the action curve with a single peak is characteristic of *Chara*). It will be observed in Figs. 8 and 9 that the polarization of the cell was only partly dis-

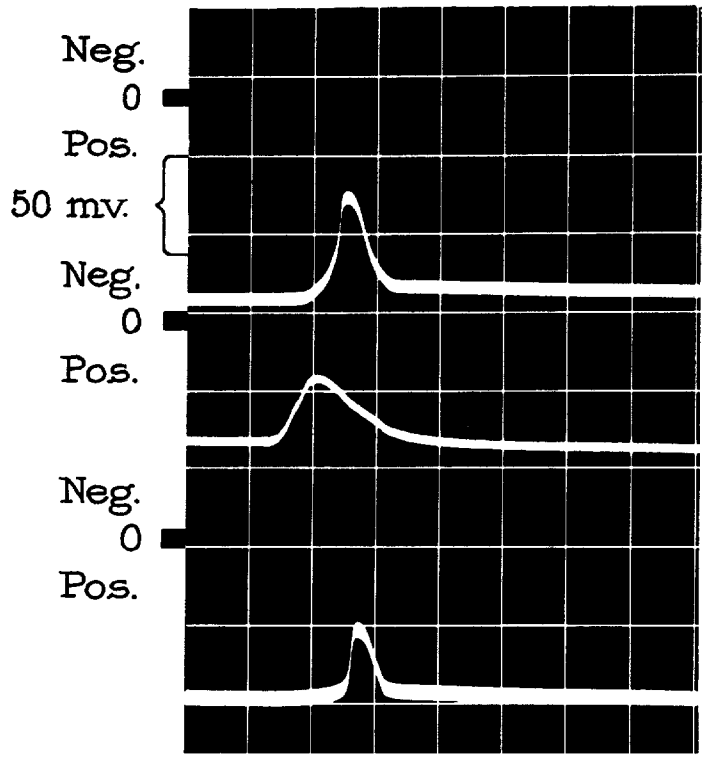

FIG. 8. Photographic record of negative variations in *Chara* caused by the application of 0.001 M KCl at 1°C. at *D* (Fig. 4). The top curve shows the changes in potential at *C*, the middle curve at *D*, and the bottom curve at *E*. All contacts were 0.001 M KCl: *F* was killed before the experiment started. The cell was kept for 5 days before use in Solution A. The vertical lines represent 5 second intervals. Room temperature, 22°C.

charged (*i.e.* the action curve does not go to zero) in these two experiments. It is interesting that the phenomenon of stimulation of single cells by cold solutions could be observed in two genera of plants.

It may be noted that solutions at 14°C. rarely stimulate *Nitella*: those at 10°C. stimulate in about half the cases, those at 8°C. stimulate in about 80 per cent of the cases, and those at 1°C. in about 94 per cent.

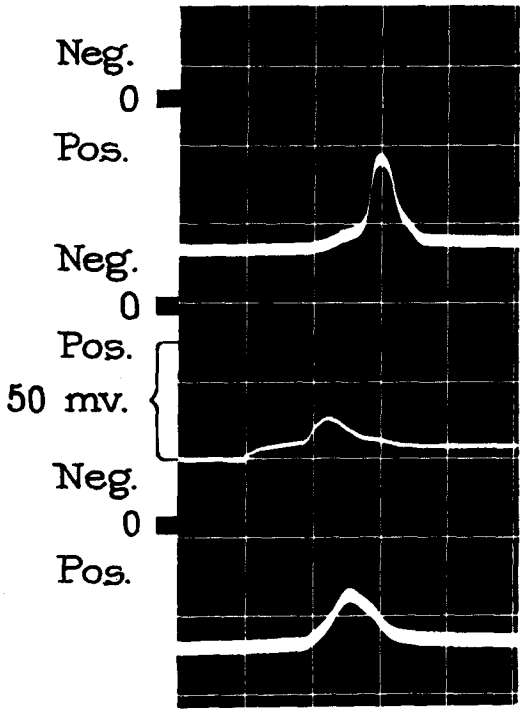

FIG. 9. Photographic record of negative variations in *Chara* caused by the application of 0.001 M KCl at 1°C. at *D* (Fig. 4). The top curve shows the changes in potential at *C*, the middle curve at *D*, and the bottom curve at *E*. All contacts were 0.001 M KCl: *F* was killed before the experiment started. The cell was kept for 5 days before use in Solution A. The vertical lines represent 5 second intervals. Room temperature, 22°C.

It appears possible that the action current starts as the result of a sudden break in the non-aqueous surface layers of the protoplasm.[14] At room temperatures we suppose these layers to be liquid but if they

[14] For a discussion of such layers see Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 1021.

were partly or wholly solidified by a fall of temperature[15] they might possibly be ruptured by the protoplasmic movement which is constantly present in these cells and which continues for 10 seconds to

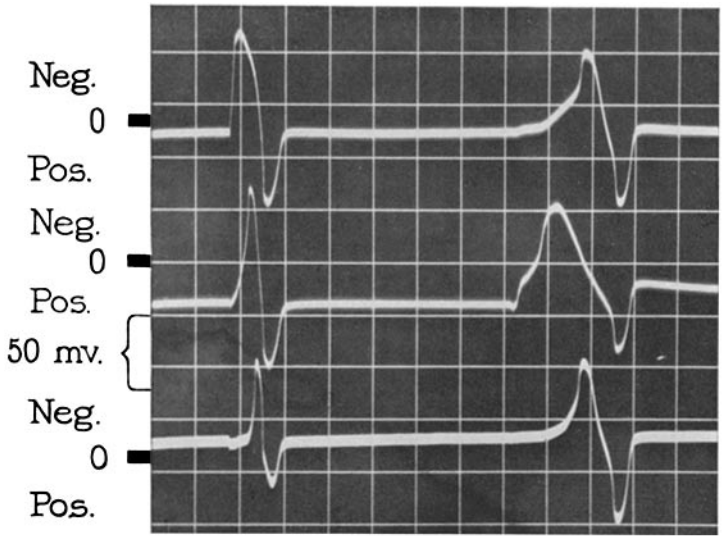

FIG. 10. Photographic record of negative variations in *Chara*. All contacts were tap water, in which the cell was kept for 2 days before use. The first variation was caused by stimulation with 120 mv. D.C. at *B* (Fig. 4). The negative variation passed along the cell, being recorded at *C* (top curve), *D* (middle curve), and *E* (bottom curve). The second negative variation was caused by the application of tap water at 1°C. at *D* (Fig. 4). This negative variation passed along the cell in both directions, being recorded at *C* and *E*.

The simultaneous downward movement of the three curves (diphasic response) is caused by the response at *F*, the common contact.

The vertical lines represent 5 second intervals. Room temperature, 21°C.

several minutes after the start of the action current set up by the cooling. The writer has found that a spot in contact with Solution A at 1°C. is much more sensitive to mechanical stimulation than at

[15] As the temperature is lowered certain oils show sudden and great increases in viscosity (*cf.* Gasser, H. S., *Am. J. Physiol.*, 1931, **97**, 254). If the non-aqueous protoplasmic surface behaved in this way it might easily be ruptured below the critical temperature.

room temperature. The outer layer X would seem to be less susceptible to rupture when chilled, owing to its adherence to the cellulose wall and its distance from the actively streaming protoplasm. When the action curves seen at the chilled spot show a double peak[16] this can be accounted for if we suppose X to remain intact when Y is ruptured.

Slow cooling does not appear to produce action currents. This might be due to the fact that protoplasmic streaming falls off so rapidly that it can no longer rupture the protoplasmic surface at the time when the latter has become solidified. Other changes may occur on cooling which contribute to this result.

Chilling to 10°C. or lower may cause a block so that a negative variation may be unable to pass the chilled spot. This may be due to the fact that the chilled and partly solidified non-aqueous surface layers are incapable of the sudden increase of permeability (due to electrical changes) which is apparently characteristic of the negative variation.[16]

It may be added that the salting out ratio alters with temperature.[17] A change of temperature could therefore cause changes of potential by causing salts to move in or out of the non-aqueous protoplasmic surfaces which are the chief seats of E.M.F. Whether this has any effect in this case is uncertain.

SUMMARY

Sudden local chilling causes action currents to be set up in *Nitella* and in *Chara*, an effect which does not follow gradual local chilling. This may be due to a partial solidification of the non-aqueous protoplasmic surfaces which makes them susceptible to rupture by the protoplasmic streaming. This movement continues usually for several minutes after the chilling, whereas if stimulation occurs at all it occurs immediately on chilling. It is found that a chilled spot is much more sensitive to mechanical stimulation than is a spot at room temperature.

Chilling is accompanied by a rise of resistance, a lowered rate of recovery following stimulation, and usually by a falling off in the magnitude of the action curve.

[16] Osterhout, W. J. V., *J. Gen. Physiol.*, 1934–35, **18**, 215.

[17] Randall, M., and Farley, C. T., *Chem. Rev.*, 1927, **4**, 291.