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# REVERSIBLE LOSS OF THE POTASSIUM EFFECT IN DISTILLED WATER

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Not only does distilled water take away the irritability[1] of *Nitella*[2] but it also changes its behavior toward potassium. In normal cells potassium is strongly negative to sodium: this will be called for convenience the potassium effect. After sufficient exposure to distilled water this effect disappears but it can be restored by returning the cells to their normal environment or to nutrient solutions. This change in the protoplasm seems to be chiefly confined to its outer surface.

These facts may be illustrated by citing a few typical experiments.[3]

A group of cells was divided, alternate cells being placed in distilled water and in a nutrient solution called Solution *A*.[1] 3 days later the cells were taken out of Solution *A* and placed on paraffin blocks,[4] being surrounded by moist air except at the contacts *C*, *D*, *E*, etc. (Fig. 1). At first Solution *A* was placed at all contacts. Then Solution *A* was replaced at *C* by 0.01 M NaCl which made little change in potential. Substitution of 0.01 M KCl for 0.01 M NaCl caused the potential at *C* to become 86 mv. more negative, a normal potassium effect. In the other group of cells, which had been kept in distilled water 3 days, distilled water at *C* was replaced by 0.01 M NaCl and then by 0.01 M KCl. The change in potential was much less, K becoming only 20 mv. negative to Na.

In a similar experiment with a different lot of cells 0.01 M KCl was 64 mv. negative to 0.01 M NaCl on the control cells in Solution *A*, but

[1] Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933–34, **17**, 87.

[2] This is *Nitella flexilis* Ag., the species used in previous experiments in this laboratory.

[3] The experiments were performed at 21–23°C.

[4] For technique see footnote 1.

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the cells leached in distilled water for 4 days made no discrimination between K and Na.

It would appear therefore that distilled water can leach out of the cell something which is responsible for the potassium effect.

It is of interest to know the potential across the protoplasm in cells which do not show the potassium effect. A group of cells was leached 6 days in distilled water. Then 0.01 M NaCl was placed on contacts *C* and *F*, Fig. 1. The solution at *F* was then changed to 0.01 M KCl, which made no great difference in potential, *F* becoming 12 mv. more negative. The 0.01 M KCl was then replaced by 0.01 M KCl saturated with chloroform, which reduced the potential at *F* approximately to zero: *C*, in contact with 0.01 M NaCl, was then 110 mv. positive to *E*. Since *C* and *F* were previously at nearly the same

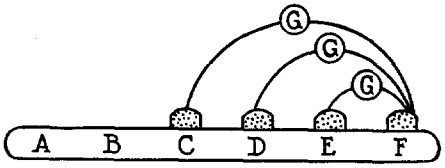


FIG. 1. Arrangement of *Nitella* cells for rapid testing of potassium effects. Contact with the cell is made by means of wads of cotton dipped in the solutions. *G* denotes a string galvanometer in series with a vacuum tube.

potential it is evident that *F* when in contact with 0.01 M KCl (without chloroform) had an outwardly directed P.D. of 98 mv. which is about what would be expected in a cell taken from Solution *A* and placed in contact with 0.01 M NaCl. In other words the P.D. across the protoplasm had not been lessened by the treatment with distilled water.

In a previous paper[1] it was reported that local areas of the cell could be anesthetized by distilled water. It is of interest to determine whether the potassium effect shows a similar behavior.

Cells of *Nitella* which had been kept for several days in Solution *A* were placed in paraffin cups[4] separated by paraffin partitions (Fig. 2). Solution *A* was applied at *A*, *B*, *C*, *D*, *E*, and *F*. We found only a small P.D. between *F* and the other cups. We then substituted 0.01 M NaCl for tap water at *C* and *D*, which made little change at either

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spot. We then applied 0.01 M KCl at *D*. This produced a great change: before applying KCl *D* was 4.0 mv. positive to *F* but afterward it was 64 mv. negative to *F*, a normal potassium effect.

We then applied distilled water at *C* and *D* for 2 days. On substituting 0.01 M NaCl for distilled water at *C* and at *D* very little change was observed. We then substituted 0.01 M KCl for 0.01 M NaCl at *C* and *D*. This substitution which formerly produced a great change now had very little effect, 0.01 M KCl becoming only 14 mv. negative to 0.01 M NaCl. Hence it would appear that something had been leached out of *C* and *D* so that they no longer behaved normally toward KCl. In other words the normal potassium effect had disappeared as the result of the exposure to distilled water.

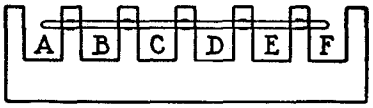


FIG. 2. Diagram of a series of paraffin cups *A*, *B*, *C*, etc., with a single cell of *Nitella* passing through all of them (in each partition the *Nitella* cell is sealed in with vaseline). We lead off from one cup to another through a string galvanometer in series with a vacuum tube.

In order to ascertain to what extent this effect of distilled water is reversible the experiment was repeated. After the leaching of *C* and *D* by distilled water 0.01 M NaCl was applied. As before this produced little change nor did the substitution of KCl for NaCl. The KCl was then replaced by Solution *A*. The P.D. between *C* and *F* was then 11 mv., *C* being positive: that between *D* and *F* was 7 mv.

After 24 hours Solution *A* was replaced by 0.01 M NaCl which made little change. Then 0.01 M KCl was substituted for NaCl. This made a great change, *C* becoming 64 mv. and *D* 55 mv. more negative. It would therefore seem that the normal state of the protoplasm had been restored, as the potassium effect before leaching was 68 mv. By applying Solution *A* saturated with chloroform at *F* we found that the P.D. across the protoplasm at *C* was 3 mv. and at *D* 4 mv. which is about the usual value for a normal spot in contact with 0.01 M KCl.

It therefore appears that Solution *A* can restore the P.D. across the protoplasm to normal after it has been leached by distilled water.

All this could be easily explained by saying that the cell constantly produces certain substances, which may collectively be called *R*, which move into the protoplasmic surfaces and ensure its normal behavior. When *R* is leached out by distilled water faster than it is produced the behavior becomes abnormal. But this does not happen in the presence of tap or pond water or of Solution *A*, presumably because in these cases *R* is produced faster than it is leached away. It is quite possible that calcium is an important factor in this situation and that it tends to prevent the rapid leaching of *R*. This has been discussed in a previous paper.[1]

The leaching effect does not appear to affect the inner protoplasmic surface greatly nor to lower the concentration of potassium in the sap because the P.D. across the protoplasm does not fall off. This P.D. appears to be due for the most part to the action of the potassium salts in the vacuole on the inner protoplasmic surface, as explained in previous papers.[5]

It may be added that the loss of the potassium effect appears to precede the loss of irritability. This will be discussed in later papers. It was observed that the treatment did not stop the protoplasmic motion.

It is interesting to note that cells collected in June (a season of active growth[6]) cannot as a rule be stimulated electrically[1] and that the potassium effect is greatly reduced or altogether lacking. Placing them in Solution *A* does not alter this situation. Hence we conclude that it does not arise from a change in the pond water but rather from a change in the cells which probably are not producing the normal amount of *R* at this season.

## SUMMARY

Not only does distilled water take away the irritability of *Nitella* but it also changes its behavior toward potassium. In normal cells potassium is strongly negative to sodium but after sufficient exposure to distilled water this effect disappears. It can be restored by returning the cells to their normal environment or to a suitable nutrient solution. This change in the protoplasm seems to be chiefly in its outer surface.

[5] *Cf.* Osterhout, W. J. V., *Biol. Rev.*, 1931, **6**, 369.

[6] The cells used in the experiments may be mature or nearly so and hence need not be actively growing.