

CHEMICAL RESTORATION IN NITELLA

IV. EFFECTS OF GUANIDINE

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The irritability¹ of *Nitella* and likewise its ability to distinguish electrically between Na⁺ and K⁺ (potassium effect) may be removed by leaching with distilled water. This apparently dissolves out a group of organic substances called for convenience *R*.²

Since presumably very little *R* is present it is not probable that enough can be obtained for analysis. We may, however, try to learn something about its nature by seeking substitutes which resemble it in being able to restore irritability and the potassium effect.²

Substances of this sort occur in blood.³ To a certain extent ammonia and some of its compounds can take the place of *R*.⁴ Guanidine⁵ has a similar action and some of its effects will be briefly described in this paper.

Cells were kept in distilled water⁶ until they had lost their irritability and the potassium effect.⁷ They were then soaked in guanidine hydrochloride⁸ for various lengths of time and tested for irritability and the

¹ By this is meant the ability to produce propagated action currents.

² Osterhout, W. J. V., *J. Gen. Physiol.*, 1939-40, **23**, 429.

³ Osterhout, W. J. V., *J. Gen. Physiol.*, 1935-36, **19**, 423.

⁴ Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 987.

⁵ Guanidine, NH:C(NH₂)₂, is a strong base.

⁶ The cells, after being freed from neighboring cells, stood in the laboratory at 15° ± 1°C. in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87) for several days. They belonged to Lot B (cf. Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312).

The measurements were made on *Nitella flexilis*, Ag., using the technique described in former papers (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541). Temperature about 20-26°C.

There was no indication of injury in these experiments.

⁷ During the leaching in many cases the potassium effect disappears before the irritability and the treatment with guanidine may restore the irritability earlier than the potassium effect.

⁸ NHC(NH₂)₂·HCl.

potassium effect. In many cases both of these were restored by the treatment with guanidine. In some cases the irritability was restored after 15 seconds in 0.1 M guanidine hydrochloride. In other cases a much longer treatment was needed, especially when the reagent was applied at lower concentrations (0.01 to 0.0001 M). Much apparently depends on the condition of the cells⁹ which appear to be highly variable in this respect: this is also true of the restoration of the potassium effect.¹⁰

During the treatment single peaks persisted for a time but after the potassium effect was restored double peaks made their appearance, as might be expected in view of what has been stated in previous papers.¹¹

We do not know whether guanidine acts like *R* or is a constituent or catalyst of reactions forming *R*. But the rapidity with which guanidine produces its effects in some cases suggests that it does not merely cause *R* to come out of the vacuole, as has been suggested in connection with the restorative action of certain inorganic salts.¹²

It should be noted that the rapid restoration of irritability presumably involves penetration of guanidine to the inner protoplasmic surface abutting on the vacuole. The thickness of the protoplasm is less than 10 microns and may in some places be considerably less, and the penetration of an organic substance, such as guanidine, may be rapid.

The fact that double-peaked action curves make their appearance when the potassium effect has been restored and the outer protoplasmic surface has become sensitive to K^+ supports the suggestion made in previous papers.¹¹ According to this the double peaks depend upon the outward movement of K^+ which sets up a positive P.D. when it reaches the outer protoplasmic surface provided the latter is sensitive to K^+ . When it is not sensitive, as in leached cells, we see only a single peak but when it has been made sensitive to K^+ by treatment with guanidine double-peaked action curves make their appearance.

SUMMARY

Leaching in distilled water may remove irritability and the potassium effect in *Nitella* but both of these may be restored by appropriate treatment with guanidine.

⁹ Some cells did not respond readily to treatment with guanidine.

¹⁰ When the guanidine has not acted sufficiently the potassium effect may be delayed (Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, **22**, 107) or incomplete.

¹¹ Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1939-40, **23**, 743.

¹² Osterhout, W. J. V., and Hill, S. E., *Proc. Nat. Acad. Sc.*, 1939, **25**, 3.