EFFECTS OF HEXYLRESORCINOL ON NITELLA

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The effects of hexylresorcinol¹ show some resemblance to those of guaiacol,^{2, 3} as might be expected in view of their chemical similarity.

But interesting differences exist. Guaiacol increases the mobility of Na^+ (u_{Na}) and leaves that of K^+ (u_{K}) unchanged, but hexylresorcinol decreases⁴ both mobilities. Hexylresorcinol 0.0003 M brings about as much negative change in P.D. as does 0.03 M guaiacol. The latter is not toxic in brief exposures (up to 5 minutes) but with the same exposure hexylresorcinol shows some toxicity at 0.003 M since some cells recover their normal P.D. when replaced in 0.001 M NaCl but others fail to do so. At 0.0003 M complete recovery may occur in a few minutes but such cells do not always live well afterwards.

Fig. 1 shows the effect of applying⁵ 0.0003 M hexylresorcinol.⁶ The curve

¹ $\text{C}_6\text{H}_5(\text{OH})_2(\text{C}_6\text{H}_{13})$.

² $\text{C}_6\text{H}_4(\text{OH})(\text{OCH}_3)$ 1:2.

³ Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, **22**, 417; 1939-40, **23**, 171.

⁴ It is assumed that the mobility of Cl^- (v_{Cl}) remains unchanged since v_{Cl} is taken as unity. Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715.

⁵ The cells, after being freed from neighboring cells, stood in the laboratory at $15^\circ \pm 1^\circ\text{C}$. in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87) until used. They belonged to Lot B (cf. Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sci.*, 1938, **24**, 315) unless otherwise stated.

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). The temperature varied from 20 to 29°C .

Unless otherwise stated there was no indication of injury.

Two spots, *D* and *E*, were recorded but the record of *E* is omitted in the reproductions given here. Both *D* and *E* were connected through the galvanometer to a spot *G* at the end of the cell. Any change at *G* would be revealed by simultaneous changes in the records of *D* and *E*: no such change occurred in the records here given.

After an exposure of some minutes with 0.0003 M hexylresorcinol at *D* and 0.01 M NaCl at *E* there was sometimes a sudden loss of P.D. at *E*, as though hexylresorcinol had passed from *D* to *E*, a distance of 1 cm. where the cell was surrounded by moist air: since hexylresorcinol is surface-active this may have played a rôle.

⁶ The hexylresorcinol was kindly donated by the firm of Sharp and Dohme of Glenolden, Pa.

records the difference between two spots, *D* (in contact with 0.01 M NaCl) and *G* (in contact with 0.01 M KCl which reduces the P.D. approximately to zero). At the start, *D* had a positive⁷ P.D. of about 65 mv. When the reagent was applied at *D*, the curve, after a latent period of about 32 seconds, rose slowly, indicating a loss of P.D. The latent period and the slow rise of the curve recall the effects of guaiacol on *Nitella*,⁸ *Halicystis*,⁹ and *Valonia*.¹⁰

The average duration of the latent period was about 25 seconds with 0.0003 M hexylresorcinol: it became shorter as the concentration was increased.¹¹ It seems possible that this is due, in part at least, to the time necessary for the reagent to penetrate through the protoplasm to the inner protoplasmic surface, *Y*, which is the chief seat¹² of the P.D.

When the depression of the P.D. reaches a certain point it may call forth an action current,¹³ as seen in Fig. 2. This has also been observed when the P.D. is depressed¹⁴ by KCl and has been explained as due to the discharge from a neighboring region.

A few cells gave curves like that shown in Fig. 3. Here the record shows the difference in P.D. between two spots, *D* and *G*, both in contact with 0.01 M NaCl. On applying hexylresorcinol at *D* the curve fell and then rose, indicating an increase in the positive¹⁵ P.D. followed by a decrease. The promptness of the initial change indicates that the effect is on the outer surface of the protoplasm. The subsequent rise of the curve appears to be of the usual sort, probably involving penetration to the inner protoplasmic surface.

⁷ The P.D. is called positive when positive current tends to flow from the vacuole across the protoplasm to the external solution.

⁸ Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, **22**, 417.

⁹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 707.

¹⁰ Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, **20**, 13. In this case the P.D. becomes more positive.

¹¹ The values varied from 3 to 180 seconds.

¹² Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 215. The upward movement of the curve also occurs when we apply 0.0003 M hexylresorcinol dissolved in distilled water to a spot previously in contact with distilled water. Hence it does not depend on the salts in contact with the external surface.

¹³ This may be propagated along the cell but usually is not. It is more apt to be propagated when the latent period is short.

¹⁴ Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541.

¹⁵ A change in the positive direction is produced by guaiacol in *Valonia*. Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, **20**, 13.

In order to get at the cause of the positive change determinations were made of the concentration effects of NaCl and KCl. With normal cells

FIG. 1. Shows the effect of hexylresorcinol 0.0003 M. At the start the recorded spot (*D*) in contact with 0.01 M NaCl had an outwardly directed (positive) P.D. of about 65 mv. When its contact with the solution was broken the curve jumped to *F*, the free grid of the amplifier. Contact was then made (marked by arrow) with 0.01 M NaCl + 0.0003 M hexylresorcinol and after a latent period of about 32 seconds the curve slowly rose approximately to zero. (The zero is labelled "App. zero" since it is only an approximation.)

Two spots, *D* and *E* (1 cm. apart), were connected through the galvanometer to a spot *G* at the end of the cell in contact with 0.01 M KCl (which reduced the P.D. at *G* approximately to zero). Any change at *G* would be shown by simultaneous changes in the records of *D* and *E*. No such change occurred. The record of *E* (not shown here) shows that no changes occurred at *E*.

Vertical marks 15 seconds apart.

The cell was freed from neighboring cells and kept 5 days in Solution A at $15 \pm 1^\circ\text{C}$. The experiment was performed at 21°C .

FIG. 2. Shows an action current induced by hexylresorcinol. At the start the spot recorded (*D*) in contact with 0.015 M NaCl had a positive P.D. of about 95 mv. When its connection with the solution was broken the curve jumped to *F*, the free grid of the amplifier. It was then placed in contact with 0.015 M NaCl + 0.0003 M hexylresorcinol. After a latent period of about 27 seconds the curve rose gradually until an action current occurred.

Two spots, *D* and *E*, were recorded (the record of *E* is not shown): both were connected through the galvanometer to a spot *G* at the end of the cell in contact with 0.01 M KCl (which reduces the P.D. approximately to zero). The records show that there was no change of P.D. at this spot or at *E* during the experiment.

Vertical marks 15 seconds apart.

The cell was freed from neighboring cells and kept for 3 days at $15 \pm 1^\circ\text{C}$. in Solution A. The experiment was performed at 23°C .

the following values were obtained. The average concentration effect¹⁶ of NaCl (0.01 M followed by 0.001 M or *vice versa*) varied from 20 to 41 mv.

¹⁶ The dilute solution is positive in the external circuit.

(depending on which lot of cells was measured). The corresponding values for KCl¹⁷ are 28 to 49 mv.

During exposure to hexylresorcinol 0.0003 M the concentration effect¹⁸ of NaCl falls off and may approach zero.¹⁹ This indicates that the mobility of Na⁺ (u_{Na}) is approaching that of Cl⁻ (v_{Cl}).

When this happens, NaCl has less tendency to lower the p.d. and the result is a downward (positive) movement of the curve, as seen in Fig. 3. But if the change in u_{Na} is delayed this effect may be masked by the tendency of the curve to rise as the result of other changes. This happens in

FIG. 3. Shows an effect of 0.003 M hexylresorcinol. At the start the spot recorded, *D*, in contact with 0.01 M NaCl, had an outwardly directed (positive) p.d. of about 103 mv. When its contact with the solution was broken the curve jumped to *F*, the free grid of the amplifier. It was then placed in contact with 0.01 M NaCl + 0.003 M hexylresorcinol. The curve fell, indicating an increase in p.d. and then rose approximately to zero which is here taken as though 0.01 M KCl were at *G*.

Two spots, *D* and *E*, were connected through the galvanometer to a spot *G* at the end of the cell in contact with 0.01 M NaCl. The record of *E* (not shown here) shows that no change occurred at *E* or *G* during the experiment.

Vertical marks 5 seconds apart.

The cell was freed from neighboring cells and kept for 5 days in Solution A at $15 \pm 1^\circ\text{C}$. The experiment was performed at 29°C .

many cases, as seen in Figs. 1 and 2. The duration of the positive dip therefore varies considerably.

The concentration effect of KCl also falls off during exposure to hexylresorcinol indicating that u_K is approaching v_{Cl} .

Before exposure the average potassium effect, *i.e.* 0.01 M KCl followed by 0.01 M NaCl, amounts to from 53 to 76 mv. (depending on which lot

¹⁷ When 0.001 M KCl is followed by 0.01 M, action currents may occur which make the change in p.d. unduly large. Cf. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541. In all cases the change was made from 0.01 to 0.001 M.

¹⁸ *I.e.*, the effect of substituting 0.001 M for 0.01 M, both solutions containing 0.0003 M hexylresorcinol.

¹⁹ Although the test may be made after the curve has risen approximately to zero the spot is not dead for it recovers its normal p.d. when the hexylresorcinol is removed unless the exposure has been prolonged beyond 5 minutes and even then there is recovery in many cases.

of cells is measured). During exposure to the reagent this also falls off. This indicates that K^+ and Na^+ are becoming more alike in respect to mobility²⁰ or partition coefficient or both.

It may be added that the variability of the cells used in these experiments was unusually great. This was due in part to the fact that they were collected at intervals throughout the year and covered an unusual range of seasonal variations.

A recent paper by Höber and coworkers²¹ states that hexylresorcinol reversibly depresses the resting P.D. of frog muscle and of frog nerve. They ascribe this to a dispersing effect on the colloids of the surface. It is probable that hexylresorcinol produces structural changes in *Nitella* but the nature of these alterations requires further investigation.

SUMMARY

In some ways the effects of hexylresorcinol on *Nitella* resemble those of guaiacol but in others they differ.

Both substances depress the P.D. reversibly and both decrease the potassium effect.

Hexylresorcinol decreases the apparent mobility of Na^+ and of K^+ . Guaiacol increases that of Na^+ but not of K^+ .

The action of hexylresorcinol is more striking than that of guaiacol since 0.0003 M of the former is as effective as 0.03 M of the latter in depressing the P.D.

It is evident that organic substances can change the behavior of inorganic ions in a variety of ways.

²⁰ Regarding this see footnote 3.

²¹ Höber, R., Andersh, M., Höber, J., and Nebel, B., *J. Cell. and Comp. Physiol.*, 1939, **13**, 195.