

CHANGES OF APPARENT IONIC MOBILITIES IN PROTOPLASM

IV. INFLUENCE OF GUAIACOL ON THE EFFECTS OF SODIUM AND POTASSIUM IN NITELLA

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Guaiacol not only changes the behavior of *Valonia*¹ and of *Halicystis*,² but, as this paper demonstrates, is likewise potent in *Nitella*. The results show differences as well as similarities in the three species and a comparison is instructive.

In normal cells of *Nitella* the effect of K^+ on the p.d. is so much greater than that of Na^+ that normal cells are able to distinguish between K^+ and Na^+ in somewhat the fashion of a potassium electrode.

This ability is greatly lessened by guaiacol (hereafter called HG for convenience) because it increases the effect of Na^+ on p.d. but not that of K^+ . As a result Na^+ acts more like K^+ .

This ability is likewise lessened by leaching in distilled water which removes an organic substance (or group of substances) called for convenience³ R_p . In this case the effect of K^+ is lessened but not that⁴ of Na^+ .

In both cases the effects of K^+ and Na^+ are made to approach each other and their action on p.d. becomes more nearly identical.

The effect of HG shown in Fig. 1 is fairly typical except that recovery is somewhat quicker than usual.⁵

¹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, **20**, 13.

² Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 707.

³ Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 105. Hill, S. E., and Osterhout, W. J. V., 1937-38, **21**, 541.

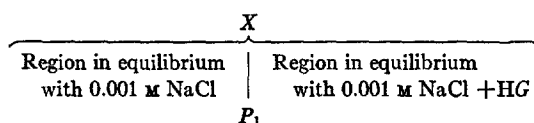
⁴ Osterhout, W. J. V., and Hill, S. E., *Proc. Nat. Acad. Sc.*, 1938, **24**, 427.

⁵ The experiments were performed on *Nitella flexilis*, Ag., using the technique employed in former papers (cf. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541). Temperature 20 to 25°C. The cells were freed from neighbor-

At the start the P.D. is positive⁶ to the extent of 100 mv. When 0.01 M NaCl + 0.025 M HG is applied the P.D. begins to fall off so that the curve rises, suggesting that in the presence of HG the behavior of Na⁺ becomes more like that of K⁺ which has a strongly negativating effect.⁷

This might be due to an increase in u_{Na} , the mobility of Na⁺ in the outer protoplasmic surface, X (see Fig. 2), or to an increase in its partition coefficient S_{Na} (S_{Na} = concentration of Na⁺ in X ÷ concentration of Na⁺ in external solution).

Presumably we should then have the following situation in X



We might assume that the falling off in P.D. caused by guaiacol is due to the inwardly directed (negative) diffusion potential⁸ at P_1 caused by the action of guaiacol in raising the value of u_{Na} or S_{Na} . Such alterations do not seem improbable since HG changes mobilities in *Valonia*¹ and in *Halicystis*:² in *Nitella* partition coefficients can be changed by various means, e.g. by distilled water,⁴ by action currents,⁹ and by calcium.¹⁰

ing cells and kept for 30 days or more at $15 \pm 1^\circ\text{C}$. in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, **17**, 87). These cells belonged to Lot B (cf. Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312; cf. also Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1938-39, **22**, 139). They were kept at room temperature for about 2 hours before the experiment was performed.

There was no sign of injury in these experiments; the concentration of HG was in all cases 0.015 to 0.025 M (which gave similar effects); the use of 0.03 M HG was avoided as it may be toxic in longer experiments.

It may be added that the cells are quite variable and in order to avoid confusion the presentation has adhered in general to the more typical behavior.

⁶ *I.e.*, the positive current tends to flow across the protoplasm from the sap to the external solution.

⁷ Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541.

⁸ Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715.

⁹ Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541.

¹⁰ Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1938-39, **22**, 139.

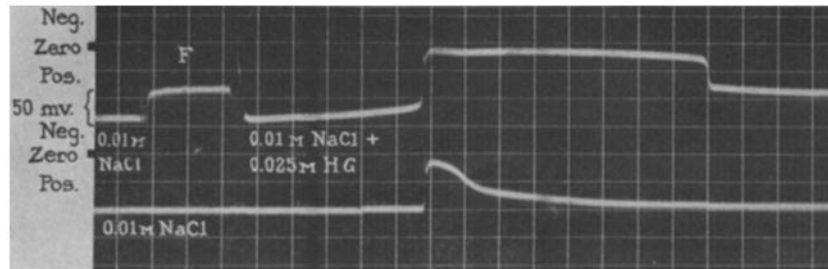


FIG. 1. The effect of HG as shown by a photographic record of changes at *C* and *D* (Fig. 2): at the start the spot *F* was in contact with 0.01 M KCl.

At the start the p.d. at *C* (upper string) was 100 mv. positive in 0.01 M NaCl. When the solution was withdrawn from *C* the curve jumped to *F*, the free grid potential of the amplifier. When 0.01 M NaCl + 0.025 M HG was applied at *C* the curve dropped back and then rose slowly: this slow rise was followed by an action current with delayed recovery; the recovery curve descends suddenly, giving a "square topped" action current.

The action current was propagated to *D* (lower string): here the action current has a normal appearance; *D* was in contact with 0.01 M NaCl.

Heavy time marks 5 seconds apart. Temperature 26°C.

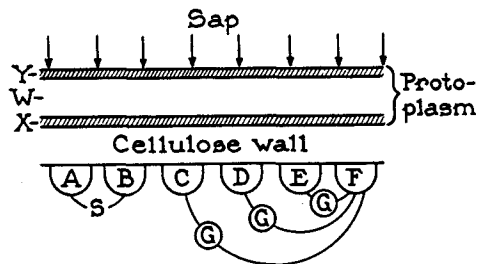


FIG. 2. Diagram to show the arrangement of leads and the supposed structure of the protoplasm which is assumed to consist of an aqueous layer *W*, an outer non-aqueous layer *X*, and an inner non-aqueous layer *Y*.

The arrows show the outwardly directed (positive) p.d. whose seat is supposed to be chiefly at *Y* when the cell is in pond water: hence the p.d. at *X* is regarded as negligible and is not shown. But under some conditions the p.d. at *X* may become important.

Each lead is connected to a separate amplifier and to one string of the 3-string Einthoven galvanometer.

As is evident from previous papers,^{7, 11} we can tell whether u_{Na} is altered if we measure the concentration effect. An increase of u_{Na} would increase the concentration effect, but an increase of S_{Na} would not do so. The concentration effect was measured, as shown in Fig. 3. Before applying HG the substitution of 0.01 M for 0.001 M NaCl (and *vice versa*) gives 23.2 ± 0.9 mv. (22 observations). From this we calculate, by methods described in a previous paper,⁸ the value of u_{Na} as 2.33. A similar measurement, made after the application of HG and the resulting action current, is illustrated in Fig. 3. The

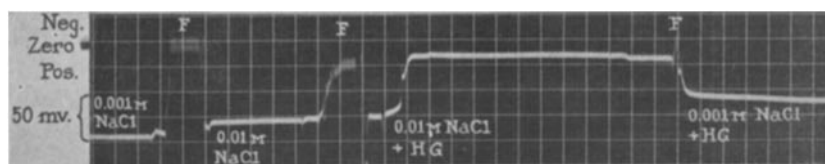


FIG. 3. Action of HG on the concentration effect of NaCl as shown by a photographic record of changes in p.d. at C (Fig. 2) which at the start was in contact with 0.001 M NaCl (*F* was in contact with 0.01 M KCl).

When 0.01 M NaCl was applied the curve rose 18 mv. When 0.01 M NaCl + 0.025 M HG was applied the curve rose slowly and an action current occurred. When 0.001 M NaCl + 0.025 M HG was applied the curve fell 48 mv.

Heavy time marks 5 seconds apart. Temperature 22°C.

Regarding *F* see Fig. 1.

average of 39 observations¹² is 44.0 ± 0.7 mv. From this we get $u_{\text{Na}} = 7.30$, a value considerably higher than before HG was applied. The change from $u_{\text{Na}} = 2.33$ to $u_{\text{Na}} = 7.30$ makes the p.d. more negative. The amount can be calculated as follows.

Since in the presence of HG the behavior of Na^+ is somewhat like that of K^+ we may for purposes of calculation regard it as playing the rôle of K^+ . Hence replacing 0.01 M NaCl with $u_{\text{Na}} = 2.33$ by 0.01 M NaCl with $u_{\text{Na}} = 7.30$ might be regarded as equivalent to replacing 0.01 M NaCl by 0.01 M KCl. If in the latter

¹¹ Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312.

¹² This does not include certain low values obtained directly after the spike on passing from 0.001 M to 0.01 M NaCl; see Fig. 4. It includes values obtained on passing from 0.001 M to 0.01 M NaCl and *vice versa* taken later on in the course of the action current, see p. 422.

case the concentration of Cl^- in X remains constant we have according to Henderson's equation (at 20°C .)

$$\text{Change of P.D.} = 58 \log \frac{u_{\text{K}} + v_{\text{Cl}}}{u_{\text{Na}} + v_{\text{Cl}}}$$

Substituting the numerical values¹³ of u_{Na} we have

$$\text{Change of P.D.} = 58 \log \frac{7.30 + 1}{2.33 + 1} = 23 \text{ mv.}$$

Evidently the change from $u_{\text{Na}} = 2.33$ to $u_{\text{Na}} = 7.30$ can make the P.D. 23 mv. more negative. As this is not enough to account in all cases for the total upward movement of the curve during its slowly rising phase it seems probable that S_{Na} has also increased. This is shown to be the case by measurements of the potassium effect (*i.e.* the change in P.D. observed on substituting KCl for NaCl) which will be discussed later (p. 426).

We might therefore attribute the upward movement of the curve in its slowly rising phase in Fig. 1 to the increase¹⁴ in u_{Na} and S_{Na} . But we find that in some cases curves somewhat resembling the upper curve in Fig. 1 are obtained on applying 0.02 m HG in tap water. The cause of this is not clear: it might be regarded as an effect of HG on Y (Fig. 2) but it would then be necessary to assume a very rapid penetration of HG in such cases.

When the P.D. has fallen off sufficiently¹⁵ in the slowly rising phase of the curve an action current¹⁶ occurs. This is probably due, as explained in a previous paper,⁹ to a discharge from a neighboring region and an exit of substances from the sap in that region. If these substances diffuse along W (Fig. 2) to the spot in contact with guaiacol they may cause an action current at that spot.¹⁷

¹³ As in previous papers we put $v_{\text{Cl}} = 1$, *cf.* footnote 8.

¹⁴ This increase in u_{Na} and S_{Na} also occurs when the cell is in contact with 0.001 m or 0.01 m KCl when the curve does not rise (Fig. 5).

¹⁵ When 0.001 m NaCl + HG does not depress the P.D. sufficiently to cause an action current subsequent application of 0.01 m NaCl + HG will do so. Or electrical stimulation may be applied.

¹⁶ This is frequently propagated along the cell (Fig. 1).

¹⁷ As would be expected, an action current can be produced at any time during the slowly rising phase of the curve (Fig. 1) by electrical stimulation. This resembles the action current which occurs without such electrical stimulation.

The delay in recovery may be due to the action of HG upon Y (Fig. 2). The long delay in recovery and the sudden descent of the curve in recovery, giving "square topped" action curves, recalls the situation in cells treated with guanidine.¹⁸

We suppose¹⁹ that the action current involves a loss of p.d. at Y (Fig. 2). If Y recovers its normal p.d. we expect in the present case only partial recovery since in the presence of HG NaCl acts upon X (Fig. 2) somewhat like KCl (because either u_{Na} or S_{Na} or both have increased) and thus produces an inwardly directed (negative) p.d. which lessens the net total outwardly directed (positive) p.d. Hence, in Fig. 1, the level of the curve after recovery is higher than before HG was applied. It would be still higher but for the fact that after recovery u_{Na} and S_{Na} fall off;²⁰ the cause of this is not clear.

It may be added that during the action curve seen in Fig. 1 the stimulated region no longer appears to act as a condenser when tested in a Wheatstone bridge as described by Blinks.²¹ This applies also to the action curve in 0.001 M KCl seen in Fig. 6. In both these cases we may suppose that Y (Fig. 2) has lost its p.d. and a great part of its resistance.

An interesting result is seen in Fig. 4 where 0.001 M NaCl is replaced by 0.001 M NaCl + HG. After a slow rise in the curve an action current occurs and 0.001 M NaCl + HG is then replaced by 0.01 M NaCl + HG and the curve quickly rises 32 mv. On the basis of what has already been said regarding Fig. 3 we should expect a rise of about 44 mv. When the solution is changed back again to 0.001 M NaCl + HG the curve does not go back to the former level but drops much lower: the total drop is 44 mv. which is about what would be expected according to the discussion of Fig. 3 (p. 420).²²

¹⁸ Osterhout, W. J. V., and Hill, S. E., Some ways to control bioelectrical behavior, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1936, **4**, 43. See also, Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, **22**, 91.

¹⁹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 215.

²⁰ This is shown by measurements of the concentration effect of NaCl and of the potassium effect (on the assumption that S_K and u_K remain constant as stated on p. 424).

²¹ Blinks, L. R., *J. Gen. Physiol.*, 1929-30, **13**, 495; 1936-37, **20**, 229. See also Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, 1938-39, **22**, 37.

²² This behavior of 0.01 M NaCl is not seen when it is applied soon after the spike

This can be accounted for if we assume²³ that when 0.01 M NaCl + HG is applied just after the spike of the action current some of it penetrates X (Fig. 2). This seems probable because the experiments of Blinks²¹ and of Cole and Curtis²¹ show that just after the spike of the action current the protoplasm becomes very permeable. Evidently the NaCl which penetrates sets up an outwardly directed (positive) P.D. against X opposing the inwardly directed P.D. due to the 0.01 M NaCl outside and in consequence we find less than the expected effect. After this, however, each change of concentration in either direction produces its full effect²⁴ (*i.e.* the increased concentration effect of 45 to 50 mv.) and the only effect of the NaCl which has penetrated to the region just inside X is permanently to lower the level of the curve.²⁵

In striking contrast to all that has been said about Na^+ is the behavior of K^+ , as seen²⁶ in Fig. 5. Here HG has practically no effect.²⁷ When the cell is stimulated electrically we get an action curve (Fig. 6). The delay in recovery may be due to the action of HG upon Y (Fig. 2). In the absence of HG electrical stimulation in 0.001 M KCl produces a normal action curve with recovery in about 20 seconds.²⁸

of an action current produced by electrical stimulation when the cell is in contact with 0.001 M KCl + 0.02 M HG. Apparently the protoplasmic surface is then in a different state (see p. 425).

²³ If this assumption is not made we must suppose that the low concentration effect just after the spike of the action current means a value for u_{Na} about equal to the normal (*i.e.* about 2.33). The slow rise in the curve preceding the spike in the action current would then be attributed to a rise in S_{Na} rather than in u_{Na} . The increase in the concentration effect observed at the second application of 0.01 M NaCl after the spike might be attributed to a sudden rise in u_{Na} due to substances moving from the sap to X (*cf.* footnote 9).

²⁴ Such values are included in the average of 44 mv. given on p. 420.

²⁵ In some cases the curve after the spike of the action current drifts slowly downward when the cell is in contact with 0.001 M NaCl or 0.01 M NaCl: this may indicate penetration.

²⁶ A similar result is obtained in 0.01 M KCl.

²⁷ In a few cases the curve rises somewhat when HG is applied and an occasional cell is found in which K^+ acts very much like Na^+ . In some cases the curve falls somewhat under the influence of HG and rises somewhat above its original level when HG is removed.

²⁸ Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 215.

If u_K or S_K were changed by HG the level of the curve in Fig. 5 would change. Since there is little change we may conclude that u_K and S_K remain almost constant. This is confirmed for u_K by the following measurements.

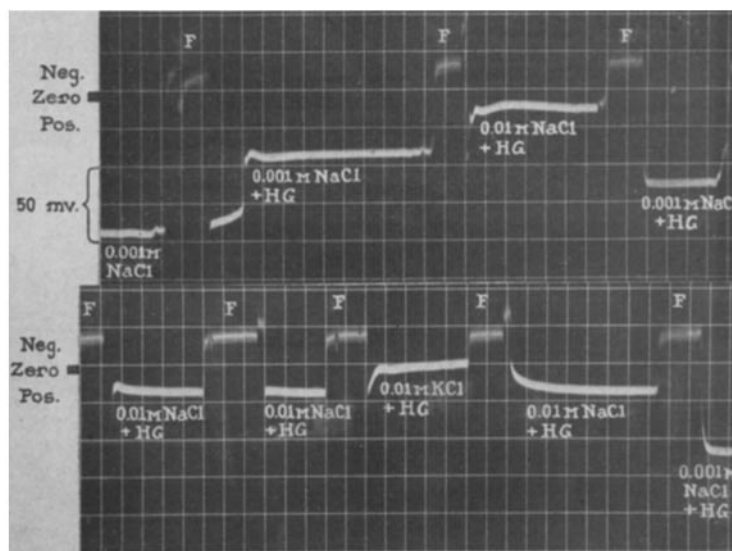


FIG. 4. Action of HG on the concentration effect of NaCl and on the potassium effect as shown by a photographic record of changes of P.D. at D (Fig. 2). The spot F was in contact with 0.01 M KCl.

At the start the P.D. was 94 mv. positive in 0.001 M NaCl. When 0.001 M NaCl + HG was applied the curve rose slowly: this was followed by an action current. When 0.01 M NaCl + HG was applied the curve rose 32 mv. When 0.001 M NaCl + HG was applied the curve fell 50 mv. When 0.01 M NaCl + HG was applied the curve rose 45 mv. (this solution was removed and again applied). When 0.01 M KCl + HG was applied the curve rose 18 mv. and returned to the previous level when 0.01 M NaCl + HG was applied (potassium effect).

The concentration of HG was 0.025 M in all cases. Heavy time marks 5 seconds apart. Temperature 23°C.

Regarding F see Fig. 1.

Before HG is applied the potassium concentration effect (change of 0.001 M to 0.01 M KCl²⁹ and *vice versa*) is 48.5 ± 0.7 (33 observations):

²⁹ Large values due to action currents are rejected. Cf. footnote 9.

during exposure to HG it is 44.2 ± 0.3 (41 observations) before and 45.8 ± 0.6 (44 observations) after electrical stimulation. This indicates that little or no change in u_K is produced by HG. Taking as a general average 46.1 we get⁸ $u_K = 8.76$.

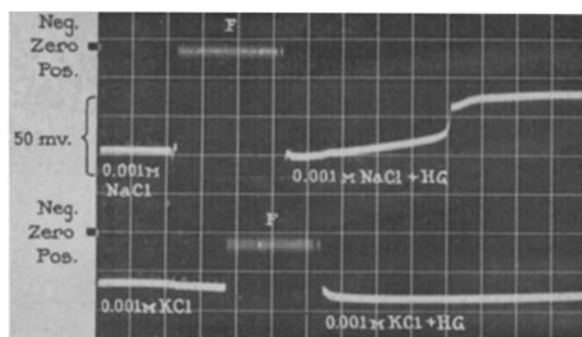


FIG. 5. Contrasting effects of HG in the presence of NaCl and KCl as shown by a photographic record showing behavior of P.D. at *C* and *D* (Fig. 2). At the start *C* was in contact with 0.001 M NaCl, *D* with 0.001 M KCl, and *F* with 0.01 M KCl.

When 0.001 M NaCl at *C* (upper string) was changed to 0.001 M NaCl + 0.02 M HG there was a slow rise of the curve followed by an action current. This did not happen when 0.001 M KCl at *D* (lower string) was replaced by 0.001 M KCl + 0.02 M HG.

Heavy time marks 5 seconds apart. Temperature 23°C.

Regarding *F* see Fig. 1.

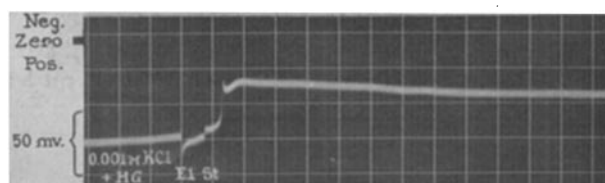


FIG. 6. Effect of electrical stimulation as shown by a photographic record of changes of P.D. at *C* (Fig. 2): *C* was in contact with 0.001 M KCl + 0.02 M HG; *F* was in contact with 0.01 M KCl.

When electrical stimulation was applied at *S* (Fig. 2) an action current occurred at *C* (the preliminary part of the action curve is due to leakage). Recovery was delayed.

Heavy time marks 5 seconds apart. Temperature 23°C.

Regarding *F* see Fig. 1.

Let us now consider the potassium effect; *i.e.*, the change in P.D. produced by substituting KCl for NaCl. This was measured as shown in Fig. 4. Before the application of HG this is 94.4 ± 2.1 mv. (22 observations). As explained in previous papers¹⁰ this may be calculated by means of Henderson's equation if we know the values of u_{Na} and u_{K} . Before the application of HG we have $u_{\text{K}} = 8.76$ and $u_{\text{Na}} = 2.33$ (p. 420) and we find that we must put $S_{\text{K}} \div S_{\text{Na}} = 38$ to get the observed³⁰ P.D. of 94.4 mv. After the application of HG we have $u_{\text{K}} = 8.76$ and $u_{\text{Na}} = 7.30$ (p. 420) and we must then put $S_{\text{K}} \div S_{\text{Na}} = 2.35$ to get the observed value³¹ of 20.9. Since S_{K} is not changed by HG, as is shown by the behavior of the curve in Fig. 5, we may suppose that HG has multiplied the value of S_{Na} by 16.2 which would reduce the ratio from 38 to 2.35. (We have already seen that HG changes u_{Na} from 2.33 to 7.30.)

We may conclude that HG increases u_{Na} and S_{Na} with little or no effect on u_{K} or S_{K} .

This recalls the situation in *Halicystis* where HG appears to increase u_{Na} without affecting u_{K} . In *Valonia*¹ u_{Na} is increased and u_{K} is decreased.³²

The changes produced by HG in the concentration effect and in the potassium effect are to some extent predictable from the electrical behavior of HG. Preliminary experiments in this laboratory, in collaboration with J. W. Murray, show³³ that when the *Nitella* cell is

³⁰ The value for the change from 0.01 M NaCl to 0.01 M KCl and *vice versa* is 94.4 ± 2.1 (22 observations).

³¹ The value for the change from 0.01 M NaCl to 0.01 M KCl and *vice versa* is 20.9 ± 0.7 mv. (32 observations): this is after the action current has been produced as in Fig. 4, in the presence of NaCl: when the action current has been produced as in Fig. 6, in the presence of KCl, the corresponding value is 24.7 ± 1.4 mv. (6 observations).

³² Cf. footnote 1. An exposure of about 5 minutes is necessary to produce this result. But it does not occur in *Nitella* or in *Halicystis* even with an exposure of 10 minutes to 0.02 M HG.

³³ In measuring the concentration effect the HG was first shaken with the more dilute solution and left in contact with it in a U-tube; the more concentrated solution was then placed in contact with the other surface of the HG. The two aqueous solutions were then connected through calomel electrodes to a Compton electrometer. The sign in all cases is that observed in the external circuit.

In measuring the potassium effect the HG was shaken with NaCl and KCl was then placed on the opposite side.

replaced by a liquid layer of HG the concentration effect of NaCl is somewhat smaller than that of KCl, which agrees with *Nitella*. The dilute solution is positive in both cases, as in *Nitella*.

For the potassium effect (0.1 M NaCl vs. 0.1 M KCl) the value is about 14 mv. The KCl is negative to NaCl, as in *Nitella*.

Since the potassium effect is less in HG than in *Nitella* we might expect the addition of HG to the non-aqueous protoplasmic surface layer, X, to lessen the value of the potassium effect, as is indeed the case. And since the concentration effects of KCl and NaCl are nearer together in HG than in *Nitella* we might expect the addition of HG to bring these effects nearer together in *Nitella*, as is the case.

The fact that u_{Na} is increased in *Nitella* and u_K left unaffected is not explained by these considerations. Further investigation is needed.

It is evident that the action of HG cannot be accounted for on the ground that the protoplasmic surface is a pore system since a change in the pores could not increase u_{Na} and leave u_K unaffected as in *Nitella* and *Halicystis* or increase u_{Na} and simultaneously decrease u_K as in *Valonia*.

SUMMARY

In *Nitella*, as in *Halicystis*, guaiacol increases the mobility of Na^+ in the outer protoplasmic surface but leaves the mobility of K^+ unaffected. This differs from the situation in *Valonia* where the mobility of Na^+ is increased and that of K^+ is decreased.

The partition coefficient of Na^+ in the outer protoplasmic surface is increased and that of K^+ left unchanged.

Recovery after the action current is delayed in the presence of guaiacol and the action curves are "square topped."