

CHANGES OF APPARENT IONIC MOBILITIES IN PROTOPLASM

II. THE ACTION OF GUAIACOL AS AFFECTED BY pH

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Under the influence of guaiacol the p.d. of *Valonia* becomes more positive within 30 seconds¹ and the effect of the guaiacol ion appears to be much the same as that of the undissociated molecule. But after longer exposure (5 minutes or more) the cell seems to become sensitized to the guaiacol ion whose effect then appears to be much greater than that of the undissociated molecule.

Fig. 1 shows the p.d. across the protoplasm of a cell (impaled on a capillary¹) in sea water at pH 8.2. At the start it was slightly positive.² On transferring to sea water at pH 6.4 there was practically no change in p.d.¹ On adding 0.01 M guaiacol (called for convenience HG) the p.d. became about 75 mv. positive (the pH was kept³ at 6.4). When the pH was raised³ to 9.6 there was little change in p.d. although the concentration of the guaiacol ion (called for convenience G⁻) was thereby raised⁴ from about 0.000007 M to about 0.0043 M.

In Fig. 1 we see that at pH 9.6 there is a tendency for the curve to rise. This is sometimes absent but in some cases it is much more pronounced than in Fig. 1. (After a longer exposure to HG we see the opposite effect since a rise in pH then causes the curve to fall as in Fig. 2.)

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

² "Positive" means that the positive current tends to flow from the vacuole across the protoplasm to the sea water. The cells are usually slightly negative in the sea water but a slight positivity, such as we see here, seems to be without significance.

³ This is done by adding NaOH. The technique and material were the same as in the previous paper.¹

⁴ See page 689.

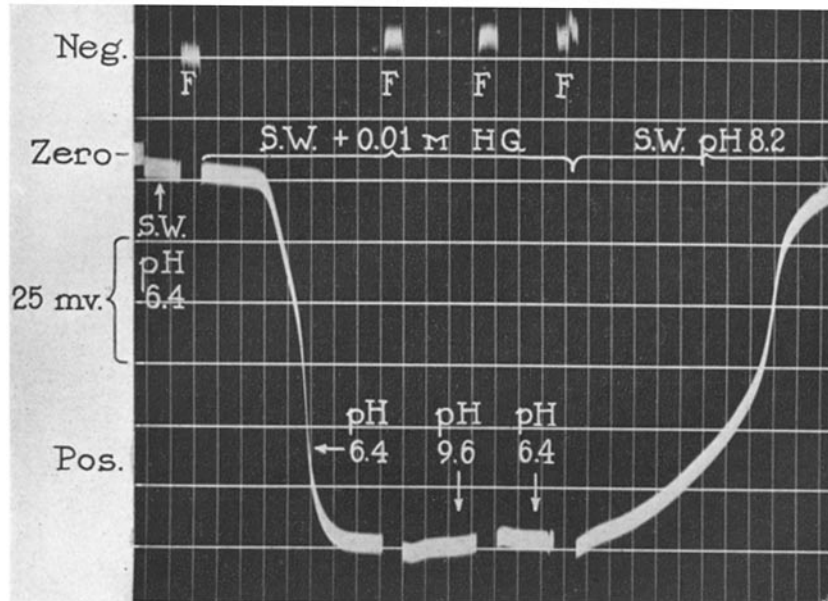


FIG. 1

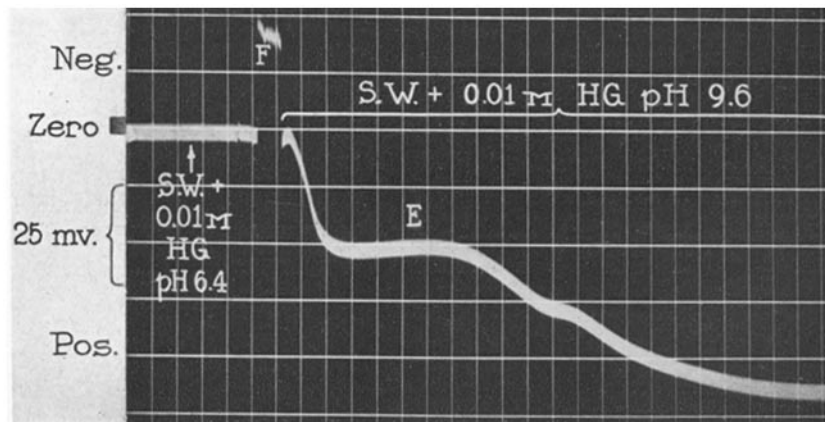


FIG. 2

FIG. 1. Photographic record showing effect of replacing sea water at pH 6.4 by sea water + 0.01 M HG at pH 6.4 (where the concentration of the guaiacol ion is negligible). In sea water the cell was slightly positive. When the cell was lifted out of the sea water the curve jumped up to *F*, the free grid of the vacuum

Fig. 1 shows that when the cell is replaced in sea water (at pH 8.2) it recovers its original P.D. This also happens when the pH of the sea water is 6.4. If the cell is not replaced in sea water the curve slowly rises and the P.D. may reach the original value:⁵ for convenience we speak of this as "recovery" but without implying that the cell returns to its original state (this has been discussed in a previous paper¹).

Let us now see what happens after a longer exposure to HG (such observations were first made in 1931 by L. R. Blinks, who kindly tested the effect of guaiacol at the request of the writer). Fig. 2 shows a typical result. The cell, which had a P.D. at the start of 3 mv. negative, was placed in HG 0.01 M at pH 6.4 for 300 seconds during which time the curve fell to 55 mv. positive and then rose nearly to zero where it remained fairly steady. This is the condition shown in the figure at the start. The pH was then raised to 9.6 and the curve began to fall after approximately 2 seconds, which is hardly long enough to be regarded as a latent period.

tube amplifier; when it was placed in sea water + 0.01 M HG it jumped back again and after a latent period¹ of about 14 seconds began to descend. After it reached its lowest level the pH was raised to 9.6 which produced very little change. Changing back to pH 6.4 left the P.D. practically unaltered.

Time marks 5 seconds apart. Temperature 22°C.

The positive drop in this case was larger than the average reported in the previous paper.¹ The amount was quite variable in this lot of cells.

FIG. 2. Photographic record showing the effect of a rise in pH (and hence in the concentration of the guaiacol ion) on a cell of *Valonia* which had been previously exposed to sea water + 0.01 M HG for 300 seconds during which time the P.D. became about 50 mv. more positive than at the start and then returned to the original value.

At the start of the curve shown in the figure the cell was in sea water at pH 6.4 containing 0.01 M guaiacol. When it was lifted out the curve jumped to the free grid (*F*) of the vacuum tube amplifier. When it was again placed in the same solution with its pH raised to 9.6 the curve began to descend after about 2 seconds.

The fall in the first part of the curve (about 30 mv.) may be attributed to the effect of the guaiacol ion on the outer protoplasmic surface; the remainder (after the letter E, amounting to 34 mv.) may be due to other causes.

Time marks 5 seconds apart. Temperature 20°C.

⁵ This happens in sea water + 0.01 M HG at pH 6.4 or 9.6. The "recovery" in this case is much slower than in sea water and is much more apt to be incomplete.

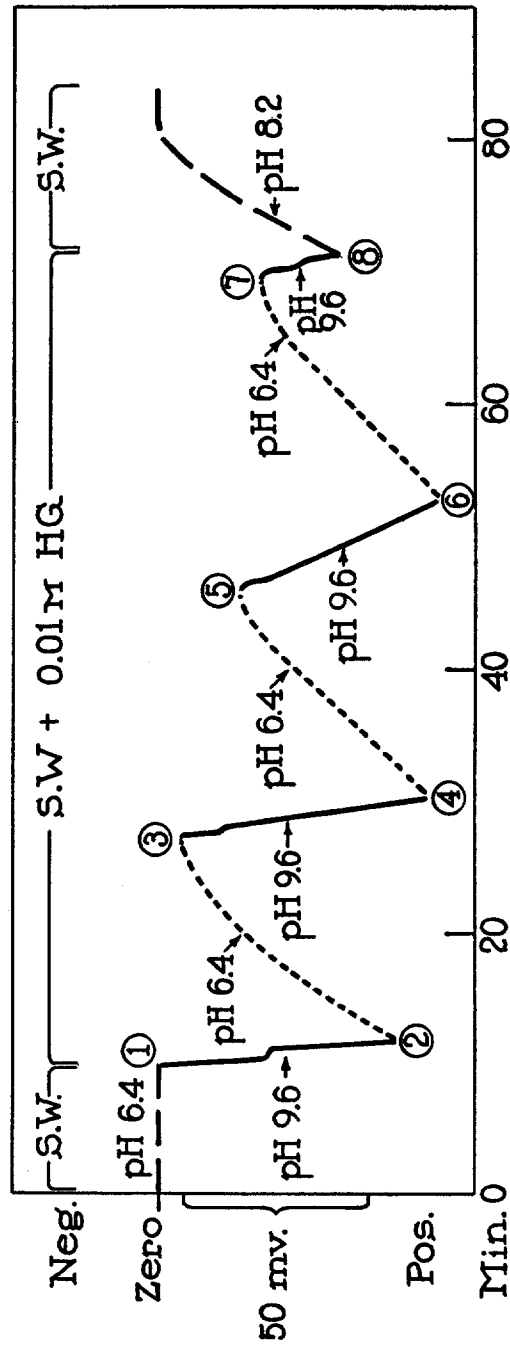


FIG. 3. Reproduces approximately the curve shown in Fig. 2 with a much smaller time scale and shows the subsequent behavior of the cell. At 1 the cell was transferred from sea water containing 0.01 M guaiacol at pH 6.4 to the same solution with the pH raised to 9.6. At 2 the pH was lowered to 6.4 and this alternation continued throughout the curve until at 8 the cell was placed in sea water. Temperature 20°C.

When the pH is lowered to 6.4 the curve rises. By changing the pH several times we obtain the effects shown in Fig. 3.

It may be added that in these experiments⁶ there was as a rule no evidence of permanent injury.¹ The impaled cells lived on for several days after the experiment.

DISCUSSION

The positive drop in Fig. 2 coincides with the increase in the concentration of the guaiacol ion (G^-) from about 0.000007 M to about 0.00429 M due to the change in pH.

The concentration of G^- was calculated as follows. According to Shedlovsky and Uhlig⁷ the pK of HG at 25°C. is 10.1. Subtracting 0.375 for the ionic strength of sea water⁸ gives pK = 9.725. Accordingly⁹ at pH 6.4 the degree of dissociation is 0.07 per cent and if the concentration of HG is 0.01 M the concentration of G^- is 0.000007 M. At pH 9.6 the degree of dissociation is 42.9 per cent and the concentration of G^- is 0.00429 M.

During the first part of its course the form of the curve in Fig. 2 is just what we should expect if G^- had a high mobility and were brought suddenly in contact with the outer protoplasmic surface, *i.e.* there is little or no latent period and the curve is steep at first and gradually flattens out just as when an ion (*e.g.* K^+)¹⁰ which has a high mobility is applied to *Valonia*¹¹ or to *Nitella*.¹²

After becoming horizontal¹³ the curve begins to descend again. It

⁶ If any sign of injury appeared the experiment was rejected.

⁷ Shedlovsky, T., and Uhlig, H. H., *J. Gen. Physiol.*, 1933-34, **17**, 567.

⁸ Jacques, A. G., *J. Gen. Physiol.*, 1935-36, **19**, 397.

⁹ This is obtained by means of the formula $\text{pH} = \text{pK} + \log \frac{a}{1-a}$, where a is the degree of dissociation. Cf. Clark, W. M., *The determination of hydrogen ions*, Baltimore, The Williams and Wilkins Company, 3rd edition, 1928, pp. 15 and 677.

¹⁰ In the case of K^+ the curve rises instead of falling because of the opposite charge on the ion.

¹¹ Damon, E. B., *J. Gen. Physiol.*, 1932-33, **16**, 375. Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, **20**, 13 (Figs. 8 and 9).

¹² Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715 (Fig. 2).

¹³ In some cases the horizontal part of the curve is very short or in place of the horizontal part of the curve there is a gradual descent as if the second process had begun before the first was over. In a few cases there was no indication of two

seems possible that this second descent is due to an alteration of the protoplasm by G^- somewhat analogous to that produced by undissociated HG .¹

If we adopt this as a working hypothesis for the time being and regard the first descent of the curve as the result of a change in diffusion potential due to the high mobility of G^- we can calculate the apparent mobility of G^- by means of Henderson's equation.

This may be written (for 20°C.)

$$\text{P.D.} = 58 \frac{(U_I - V_I) - (U_{II} - V_{II})}{(U_I + V_I) - (U_{II} + V_{II})} \log \frac{U_I + V_I}{U_{II} + V_{II}}$$

Since the concentration of H^+ is very small it may be neglected and then we have $U_I = U_{II}$ so that the equation becomes

$$\text{P.D.} = 58 \log \frac{U_I + V_I}{U_{II} + V_{II}}$$

where

$$\begin{aligned} U_I = U_{II} &= C_{Na} U_{Na} + C_K U_K \\ &= (0.488)(0.2) + (0.012)(20) \\ &= 0.388 \end{aligned}$$

We also have¹⁴ (putting for convenience $V_{Cl} = 1$) at pH 9.6

$$\begin{aligned} V_I &= C_{Cl} V_{Cl} + C_G V_G \\ &= (0.5)(1) + 0.00429 V_G \end{aligned}$$

and at pH 6.4

$$V_{II} = (0.5)(1) + 0.000007 V_G$$

When a rise of pH from 6.4 to 9.6 causes a change of P.D. of 30 mv. (as in the first descent of the curve in Fig. 2) we have

$$30 = 58 \log \frac{0.388 + 0.5 + 0.00429 V_G}{0.388 + 0.5 + 0.000007 V_G}$$

whence $V_G = 450$.

steps but the curve fell somewhat as in Fig. 1 (perhaps because the exposure was too short).

Occasionally after the first descent of the curve we find instead of a short horizontal stretch a rise (as though recovery had begun) followed by a fall of the curve.

¹⁴ Regarding the numerical values see Damon, E. B., *J. Gen. Physiol.*, 1932-33, 16, 375.

We thus arrive at a very large value (450) for the apparent mobility of G^- (when that of Cl^- is taken as unity) which is the more surprising since it does not appear to exist after a brief exposure to HG. At that time G^- appears to have much the same mobility as Cl^- as shown by the fact that increasing the concentration of G^- does not increase the positive drop¹⁵ (Fig. 1). (It seems rather to have somewhat the opposite tendency, at least in some cases.)

This raises the question whether the effect of pH on the first part of the positive drop in Fig. 2 may be due to something else than a change in the apparent mobility of G^- (as is probably the case with the second part of the drop in the curve). It might be suggested, for example, that injury is responsible but injury would be expected to carry the P.D. toward zero rather than away from it.

If we adopt as a working hypothesis the suggestion that exposure to HG increases the apparent mobility of G^- in the protoplasmic surface we may ask how this comes about. As stated in a former paper¹ such great changes can hardly be due to alterations in partition coefficients or in formation of compounds in the protoplasm. It is more plausible to assume that they are the result of the formation of charged complexes.

In conclusion it seems desirable to say something about "recovery." Recovery at pH 6.4 in sea water + HG, after a brief exposure, has been attributed¹ to the penetration of undissociated HG producing on the inner protoplasmic surface much the same effect as on the outer but opposite in sign, so that the P.D. returns toward its normal value.¹⁶

After the cell has been sensitized by a long exposure to HG and the concentration of G^- is then suddenly raised the change of P.D. occurs so quickly that we may ascribe the first drop in the curve to a change in diffusion potential at the outer non-aqueous protoplasmic surface. This will tend to disappear as G^- penetrates and lowers the concentration gradient across the non-aqueous layer at the protoplasmic surface. In consequence the curve would tend to rise and produce recovery.

In addition to this phenomenon we have the positive drop following

¹⁵ It was stated in a former paper¹ that the positive drop was 28.0 mv. at pH 6.4, and 26.2 at pH 8.2 (see Table I, p. 16).

¹⁶ Of course, if the pH in the protoplasm is higher than 6.4 there would be a corresponding change in the dissociation of HG.

the horizontal stretch of the curve in Fig. 2. This may perhaps be due to alteration of the non-aqueous surface layer which brings about a change in the diffusion potential set up by organic ions in the aqueous layer of the protoplasm as they diffuse outward through the outer surface. When G^- reaches the inner protoplasmic surface and alters it there may be a corresponding change in the diffusion potential which will be opposite in sign because in this layer the organic ions are diffusing inward.¹ This would tend to produce recovery.

These are merely suggestions which must await further tests before any definite conclusion can be reached. The situation is complicated by other variables. For example we should expect ions to penetrate more slowly than undissociated molecules,¹⁷ but on the other hand CO_2 would pass out rapidly when the pH of the sea water was raised and this would raise the pH of the protoplasm and consequently increase the concentration of G^- in the protoplasm. This might change the P.D. since the apparent mobility of G^- may not be the same in the inner and outer protoplasmic surfaces.¹⁸ Moreover the concentration gradient of G^- across the two protoplasmic surfaces would not be affected in the same way in both cases.

It must be remembered that during the exposure to HG the apparent mobility¹ of K^+ and of Na^+ , as well as that of G^- , appears to change so that a complicated set of variables is in play.

In a former paper¹ it was found that after a brief exposure recovery in sea water + HG was slower at pH 8.2 than at 6.4 but in the present experiments (with longer exposure) it was sometimes slower and sometimes more rapid at 9.6 than at 6.4. After an exposure of 5 minutes it was on the whole much slower at 9.6 and the degree of recovery was highly variable.

It may be added that when the P.D. has become normal after recovery in sea water + 0.01 M HG at pH 6.4 or 9.6 (after long exposure) a change to normal sea water at pH 8.2 (containing no HG) usually has little or no effect on the P.D. This may mean that the HG and G^- come out of the cell in such fashion as to affect both protoplasmic surfaces in much the same manner.

Likewise after a brief exposure to HG recovery at pH 9.6 may bring

¹⁷ Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 967; *Bot. Rev.*, 1936, **2**, 283.

¹⁸ Damon, E. B., *J. Gen. Physiol.*, 1931-32, **15**, 525.

the curve back to normal and it is then found that lowering the pH to 6.4 does not raise it above normal.

SUMMARY

The normal P.D. across the protoplasm of *Valonia macrophysa* is about 10 mv. negative (inwardly directed). On adding 0.01 M guaiacol to the sea water the P.D. becomes positive and then slowly returns approximately to the normal value.

In many cases this behavior is not much affected by raising the pH and so increasing the concentration of the guaiacol ion but in other cases such an increase makes the P.D. somewhat more negative.

But if we wait until the exposure to guaiacol has lasted 5 minutes (and the P.D. has returned to its normal value) before we raise the pH, the result is very different. The cell then behaves as though it had been sensitized to the action of the guaiacol ion which appears to be far more effective than undissociated guaiacol in making the P.D. more positive. This may be due in part to the high apparent mobility of the guaiacol ion and in part to alterations which it produces in the protoplasm (such alterations increase the P.D. across the protoplasm whereas ordinary injury would be expected to lower it and the cells live on after this treatment and show no signs of injury).

This action of the guaiacol ion is in marked contrast to the behavior of other anions whose effect resembles that of Cl^- .