CHANGES OF APPARENT IONIC MOBILITIES IN PROTOPLASM

III. Some Effects of Guaiacol on Halicystis

By W. J. V. OSTERHOUT

(*From the Laboratories of The Rockefeller Institute for Medical Research*)

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Valonia shows remarkable changes in the apparent mobility of ions as the result of exposure to guaiacol.[1] These changes differ in several respects from those observed in *Halicystis*.

Guaiacol makes the P.D. of *Valonia* more positive and that of *Halicystis* more negative. In *Valonia* the apparent mobility of K^+ is lessened but this does not happen in *Halicystis*. In some respects both plants agree, *e.g.* in both the apparent mobility of Na^+ is increased by guaiacol.

The effects of guaiacol (called H*G* for convenience) in sea water at pH 8.2 might theoretically be due to the undissociated molecule or to the ion (which will be called G^-). In order to eliminate the effects of G^- the pH of the sea water was lowered to 6.4. Since this change of pH affects the P.D. it will be described before taking up the effects of H*G*.

Fig. 1 shows[2] the effect of lowering the pH of sea water from 8.2 to 6.4. After a short latent period[3] the positive[4] P.D. fell[5] from 68 to

[1] Osterhout, W. J. V., *J. Gen. Physiol.*, 1936–37, **20**, 13.

[2] The experiments were performed on *Halicystis Osterhoutii* (Blinks, L. R., and Blinks, A. H., *Bull. Torrey Bot. Club*, 1930–31, **57**, 389), using the technique described in a former paper (see footnote 1). Regarding the amplifier see Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21**, 541. Temperature 21 to 25°C. (The results obtained with this species are not entirely the same with the Pacific Coast *H. ovalis* (Lyngb.) Aresch. (Blinks, L. R., private communication).)

[3] The latent period with change of pH and with H*G* (see footnote 1) varies a good deal and presumably depends to some extent on the state of the cellulose wall. It may in some cases be lengthened by bacterial jelly covering the cells

55 mv. The average of 22 observations gave a change of 16.5 $\pm$ 0.68 mv.

On returning the cell to sea water at pH 8.2 the P.D. became normal and there was no sign of injury on this or on the following days.

When the cell was not returned to sea water at pH 8.2 the P.D. remained practically constant at the lowered value for at least 15 minutes (longer exposures were not made).

Such exposure (up to 15 minutes) did not affect the apparent mobilities of K^+ or Na^+. This was shown by experiments in which the cell after exposure was placed in sea water in which K^+ was increased or in which the sea water was diluted[6] with an equal volume of an isotonic solution of mannite[7] (as described later in experiments with H*G*).

It may be added that raising the pH of the sea water from 8.2 to 9.6 had little or no effect on the P.D.

Blinks[8] states that lowering the pH from 8.2 to 6.0 has no immediate effect on the P.D. The writer has found in a few instances that a change from 8.2 to 6.4 had little or no effect. The cells which show no change in P.D. do not seem to be injured. The high positive P.D. which is normal for *Halicystis* in sea water at pH 8.2 is present in these cells and they respond normally to increases in the concentration of KCl or to dilution of the sea water. Their behavior depends

(this may give them a slippery feeling which was not present in the cells here described). A latent period was observed with KCl and with 0.005 M NH_4Cl at pH 8.2 (but not with 0.3 M NH_4Cl at pH 5.8, 6.4, and 7.2, the P.D. being rendered negative in each case), and with sea water diluted with an isotonic solution of non-electrolyte. In the presence of 0.01 M H*G* there was no latent period with potassium sea water (see Figs. 7 and 8).

[4] The P.D. is called positive when positive current tends to flow from the sap across the protoplasm to the external solution.

[5] The average value is 67 mv. (*cf.* Blinks, L. R., *J. Gen. Physiol.*, 1933–34, **16**, 147).

[6] *Cf.* p. 714.

[7] Regarding this see Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 75.

[8] Blinks, L. R., *J. Gen. Physiol.*, 1929–30, **13**, 229. Blinks states that lowering the pH to 5 makes the P.D. reversibly less positive (Blinks, L. R., *J. Gen. Physiol.*, 1933–34, **17**, 118, Fig. 7). In the cells dealt with in the present paper this treatment carried the P.D. reversibly to zero. See also Blinks, L. R., *J. Gen. Physiol.*, 1934–35, **18**, 409.

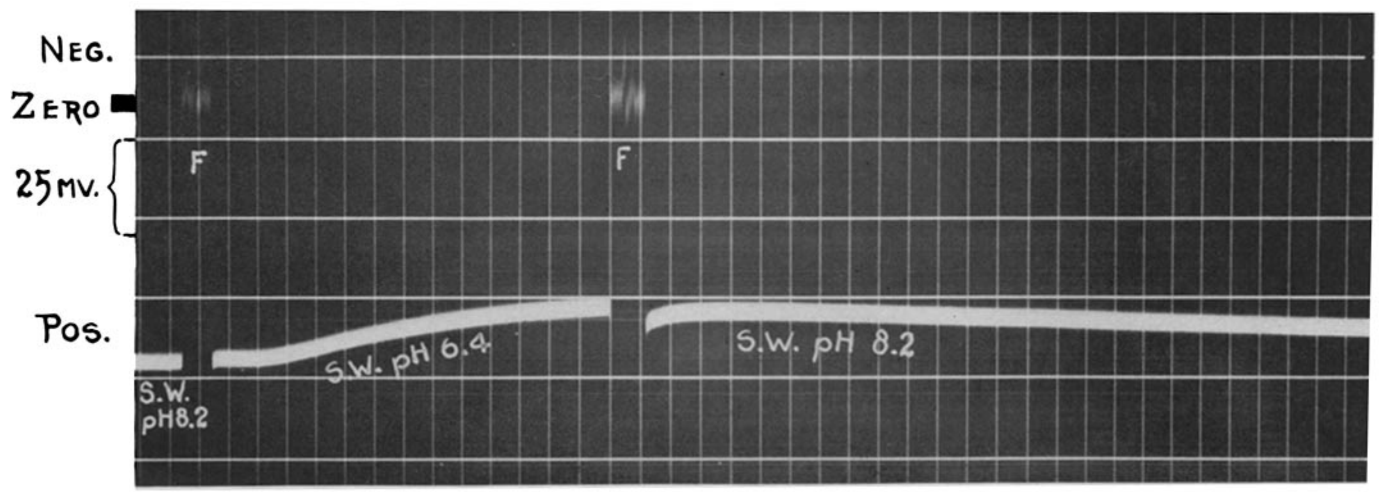

FIG. 1. Photographic record showing change of P.D. produced by lowering the pH of sea water from 8.2 to 6.4. At the start the cell in sea water at pH 8.2 had a positive P.D. of 68 mv. When it was lifted out of the sea water the curve jumped to *F*, the "free grid" of the amplifier. When it was placed in sea water at pH 6.4 the curve jumped back to its former position and after a few seconds (latent period) began to rise, the P.D. becoming less positive to the extent of 13 mv.

The cell was then replaced in sea water at pH 8.2 and the curve returned to the starting point. If the cell is left in sea water at pH 6.4 the P.D. remains at the maximum for at least 15 minutes (longer exposures were not made).

Time marks 5 seconds apart. Temperature 22°C.

on a factor which is at present unknown. Possibly the cell can exist in two or more physiological states. (Blinks[9] has described for *Valonia* two states known as "regular" and "delayed" in respect to polarization.)

How the lowering of external pH operates in decreasing the P.D. is not clear. We may recall in this connection the observation of Blinks[10] that when sufficient ammonia is present and the pH of the sea water is raised above a certain point the P.D. suddenly changes from positive to negative.[11] Lowering the external pH then produces positivity; *i.e.*, just the opposite of what happens in the experiments described in this paper. Since the concentration of hydrogen ions is very small in comparison with the other ions present Blinks has suggested that we are here dealing with isoelectric points or similar phenomena.

It is, of course, possible that isoelectric points may be involved in the changes seen in Fig. 1. A variety of ampholytes may be present with different isoelectric points.

Another possibility is that the locus of the changes may not be the same in all cases. The changes shown in Fig. 1 may have their seat in the outer part of the protoplasm. But we must consider the possibility that the pH of the sap may be changed, for the lowering of the external pH would drive CO_2 into the cell.

Let us now consider the effects of guaiacol at pH 6.4 where the concentration of guaiacol ions is negligible.[12] In Fig. 2 we see the record of a cell which had at the start a positive P.D. of 59 mv. When the pH was lowered to 6.4 the P.D. became 13 mv. less positive. When the cell was transferred to sea water at pH 6.4 containing 0.01 M H*G* the P.D. became still less positive to the extent of 23 mv. (the average value was 20.2 $\pm$ 1 mv. in 21 observations). After standing for 1

[9] Blinks, L. R., *J. Gen. Physiol.*, 1935–36, **19**, 633.

[10] Blinks, L. R., *J. Gen. Physiol.*, 1933–34, **17**, 109; 1934–35, **18**, 409.

[11] This does not take place in *Valonia*.

[12] According to Shedlovsky and Uhlig (Shedlovsky, T., and Uhlig, H. H., *J. Gen. Physiol.*, 1933–34, **17**, 567) the pK of H*G* at 25°C. is 10.1. Subtracting 0.375 on account of the ionic strength of sea water gives pK = 9.725. Accordingly at pH 6.4 the degree of dissociation is 0.07 per cent, at pH 8.2 it is 3 per cent, and at pH 9.6 it is 42.9 per cent.

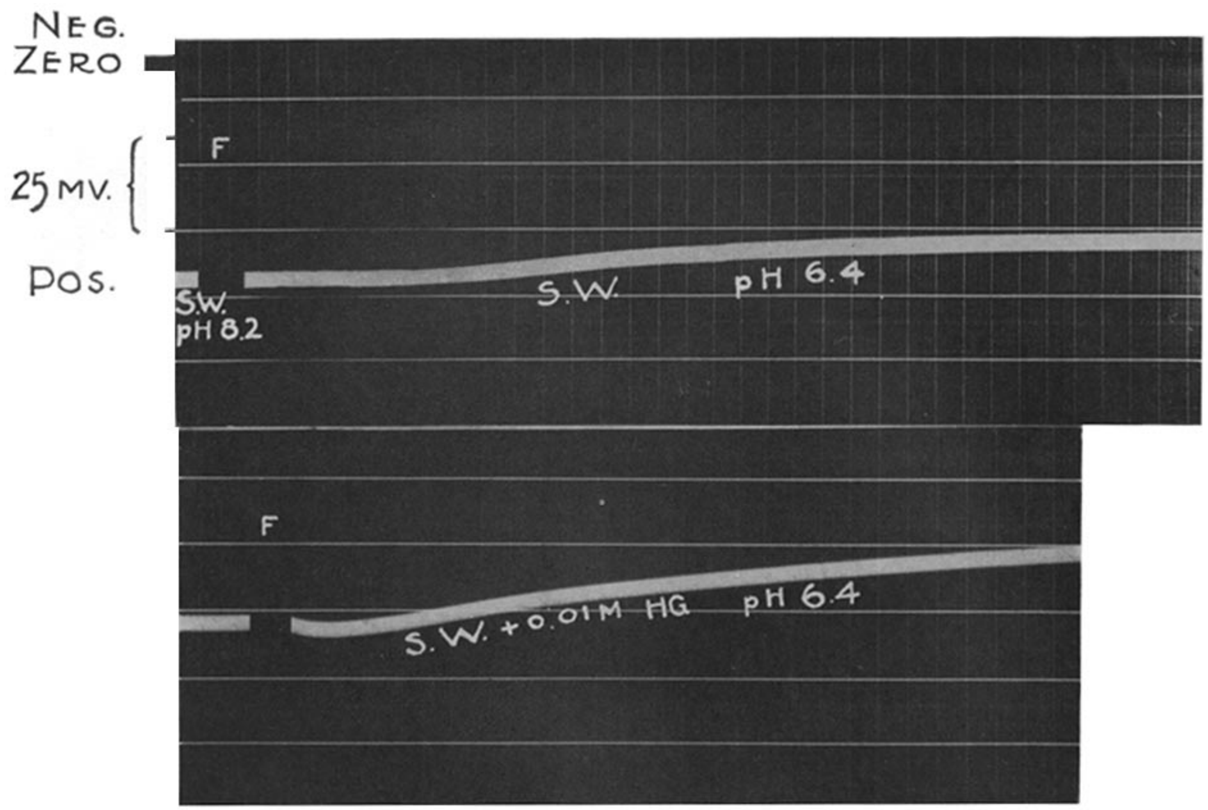

FIG. 2. Photographic record showing changes of P.D. produced by lowering the pH and adding guaiacol. At the start the cell in sea water at pH 8.2 had a positive P.D. of 59 mv.

The cell was placed in sea water at pH 6.4 and after a latent period the curve rose 13 mv. (the P.D. became less positive). The cell was then transferred to sea water at pH 6.4 containing 0.01 M H*G* and after a latent period the curve rose 22 mv. When the cell was returned to sea water at pH 8.2 the P.D. became normal (not shown in the figure).

Regarding *F* (which is invisible in the reproduction) see Fig. 1.

Time scale 40 seconds to the inch. Temperature 25°C.

For purposes of reproduction the record is cut in two and the latter part placed below the former part.

minute the value began to increase and in 5 minutes the P.D. was 40.7 ± 1.3 mv. (6 observations) less positive than before the application of H*G*.

On standing, even for 15 minutes, there is little or no further change and no recovery of the original P.D. such as occurs in *Valonia*.[1]

There is quite commonly a slight dip in the curve, soon after the start, as seen in Fig. 3 (which shows the effect of adding 0.01 M H*G* to sea water at pH 8.2). This form of curve is also found at pH 6.4 and intermediate forms between this and the one shown in Fig. 2 are

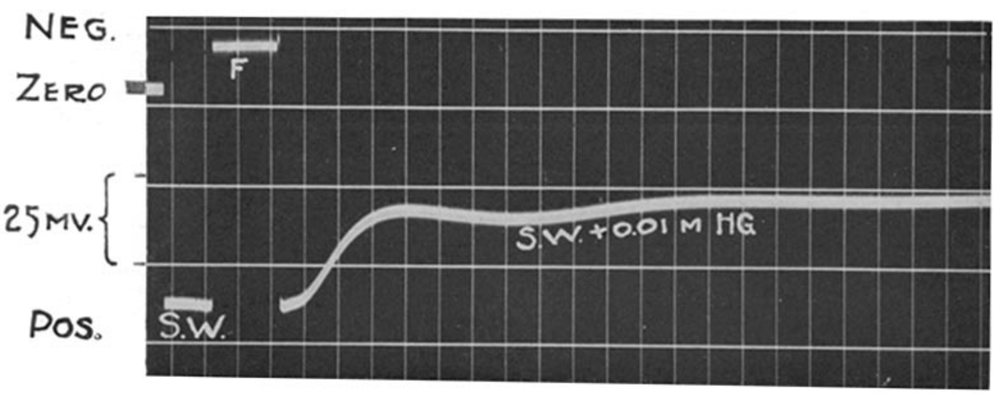

FIG. 3. Photographic record showing changes of P.D. produced by guaiacol at pH 8.2. At the start the cell in sea water at pH 8.2 had a positive P.D. of 62.5 mv. The cell was placed in sea water at pH 8.2 containing 0.01 M guaiacol and the curve rose 30 mv. (the P.D. became less positive). When the cell was replaced in sea water at pH 8.2 the P.D. became normal (not shown in the figure).

Regarding *F* see Fig. 1.

Time marks 5 seconds apart. Temperature 21°C.

found at both pH values. The average change due to 0.01 M H*G* at pH 8.2 at the end of 1 minute was 33 ± 1.3 mv. (17 observations).

Increasing the concentration of guaiacol (at pH 8.2 or 6.4) increases the effect but the value of the P.D. appears to approach zero as a limit, *i.e.* the P.D. which in sea water is about 67 mv. positive[13] becomes less as the concentration of guaiacol increases but it seldom passes the zero mark so as to become negative (never more than a few millivolts in any case).

[13] Blinks, L. R., *J. Gen. Physiol.*, 1932–33, **16,** 147.

In the experiments described in this paper the effects were fully reversible. For example, if the P.D. had been lowered to zero by adding guaiacol it would promptly return to the normal value when replaced in sea water (even after an exposure of 15 minutes to H*G*). There was no sign of injury in these experiments except with 0.03 M H*G* or higher concentrations.

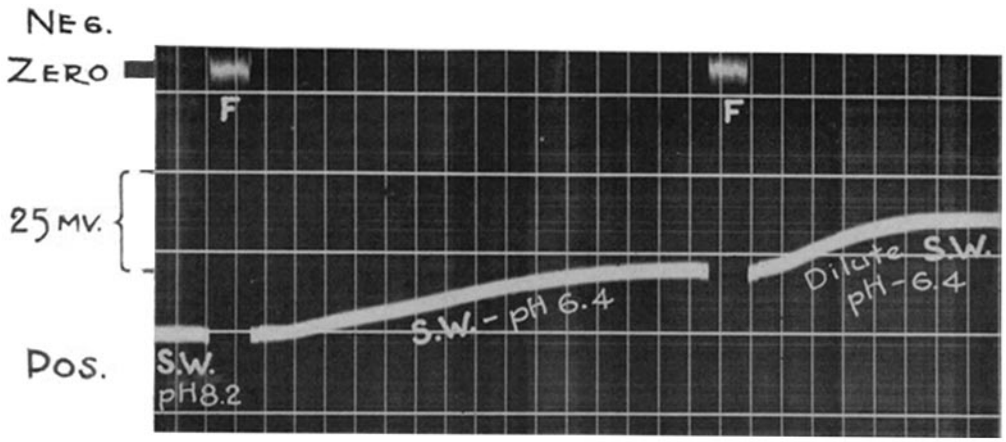

FIG. 4. Photographic record showing changes of P.D. produced by lowering the pH and diluting the sea water.

At the start the cell in sea water at pH 8.2 had a positive P.D. of 67.5 mv. When the pH was lowered to 6.4 the curve rose 16 mv. (the P.D. became less positive).

The cell was then placed in dilute sea water (sea water plus an equal volume of a solution containing 1.1 M mannite + 0.02 M $CaCl_2$ + 0.012 M KCl) and the curve rose 14 mv. When the cell was replaced in sea water at pH 8.2 the P.D. became normal (not shown in the figure).

Regarding *F* see Fig. 1.

Time marks 5 seconds apart. Temperature 21°C.

Let us now consider the effect of the guaiacol ion. In *Valonia* it was found that at first it made little difference whether H*G* was in molecular form or was largely dissociated. But after standing for about 5 minutes in sea water + 0.01 M H*G* the cell became sensitized to G^-. This did not happen with *Halicystis*. Neither at the start nor after exposures up to 15 minutes to sea water (at pH 8.2) containing 0.01 M HG did a rise of pH from 8.2 to 9.6 (increasing [12] the concentration of G^- about 14 times)[14] have much effect[15] on the P.D.

There is another aspect of the action of guaiacol which is of considerable interest and which seems to be similar in *Valonia* and in *Halicystis*. After sufficient exposure to HG an application of dilute sea water to *Valonia* made the P.D. more positive (instead of more negative, as happens when the cells have not been exposed to HG).

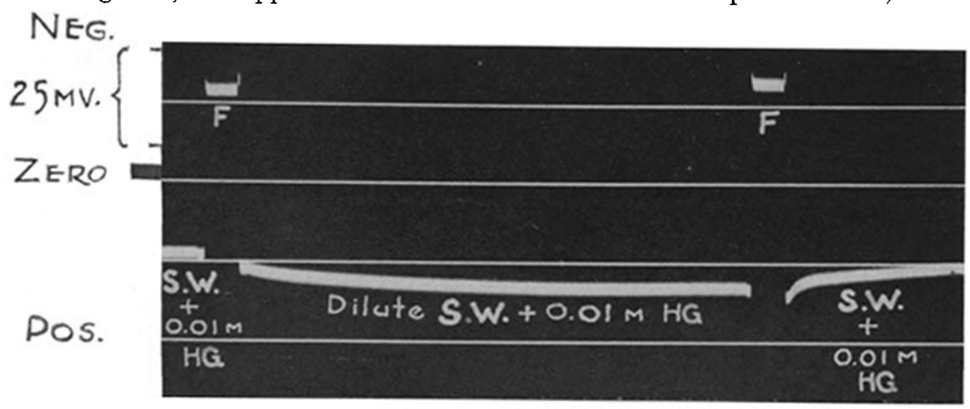

FIG. 5. Photographic record showing changes of P.D. produced by diluting the sea water (all solutions at pH 6.4). At the start of the record the cell had been in sea water containing 0.01 M guaiacol for 15 minutes and had a positive P.D. of 20 mv. (before the addition of guaiacol the P.D. was 65 mv.).

The cell was placed in dilute sea water (sea water containing 0.01 M guaiacol plus an equal volume of a solution containing 1.1 M mannite + 0.02 M $CaCl_2$ + 0.012 M KCl + 0.01 M guaiacol) and the curve fell 8 mv.; *i.e.*, the P.D. became more positive. (In dilute sea water without HG the curve rises and the P.D. becomes more negative: compare Fig. 4.)

Regarding F see Fig. 1.

Time scale 40 seconds to the inch. Temperature 23°C.

This also occurs in *Halicystis*. Fig. 4 shows the normal effect of dilute sea water.[7,16] Fig. 5 shows the effect after an exposure of 15

[14] It has already been stated that in the absence of HG such a rise has little or no effect. In the presence of 0.01 M HG a rise from pH 6.4 to 8.2 or to 9.6 makes the P.D. more positive to about the same extent as in the absence of HG: hence we may suppose that it is not due to G^-.

[15] If any change occurred it was at most 3 mv., sometimes in a positive and sometimes in a negative direction.

[16] This value was about the same as for *Valonia* (*cf.* Damon, E. B., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 445).

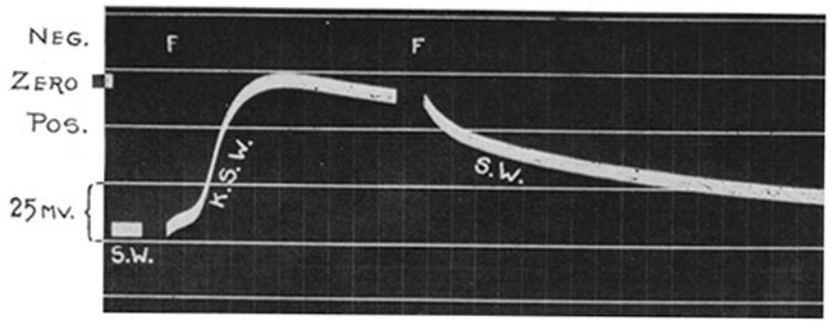

FIG. 6. Photographic record showing changes of P.D. produced by potassium (all solutions at pH 8.2). The cell in sea water at the start had a positive P.D. of 65 mv. It was placed in sea water containing 0.52 M KCl + 0.012 M NaCl (the other constituents of the sea water had the normal concentration): this is labelled K.S.W. in the record. With no latent period the curve rose to 1 mv. negative and then began to drop as the P.D. became more positive. When the cell was replaced in sea water the P.D. became normal.

A similar result was obtained at pH 6.4.

Regarding the record at *F* (invisible in the figure) see Fig. 1.

Time marks 5 seconds apart. Temperature 25°C.

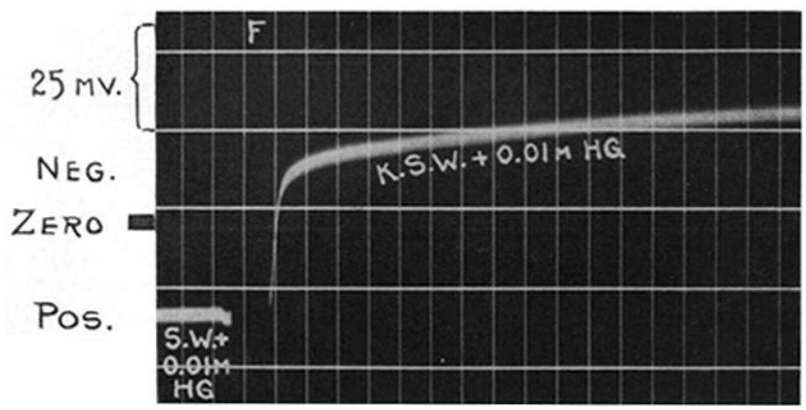

FIG. 7. Photographic record showing changes of P.D. produced by potassium after exposure of the cell for 10 minutes to 0.01 M guaiacol (all solutions at pH 6.4). (Before exposure to guaiacol the P.D. in sea water at pH 8.2 was 65 mv. positive.) At the start of the record the cell in sea water containing 0.01 M guaiacol had a positive P.D. of 23 mv. The cell was placed in sea water containing 0.01 M *HG* + 0.52 M KCl + 0.012 M NaCl (the other constituents of the sea water had the normal concentrations): this is labelled K.S.W. + 0.01 M *HG* on the record. The curve rose (with no latent period) and the P.D. became 23 mv. negative.

Dilution of this solution (not shown in the record) with an isotonic solution of mannite (as in Fig. 5) made the P.D. 10 mv. positive.

Regarding the record at *F* (invisible in the figure) see Fig. 1.

Time marks 5 seconds apart. Temperature 23°C.

minutes[17] to sea water containing 0.01 M *HG* at pH 6.4 (the greatest change found in 12 trials was 9 mv.[18]).

As in the case of *Valonia* we may[1] interpret this to mean that H*G* greatly increases the apparent mobility of Na^+ (as compared with that of Cl^-) in the outer non-aqueous protoplasmic surface. In normal cells (Fig. 4) the apparent mobility of Na^+ is much less than that of Cl^- but in Fig. 5 it is much greater than that of Cl^-.

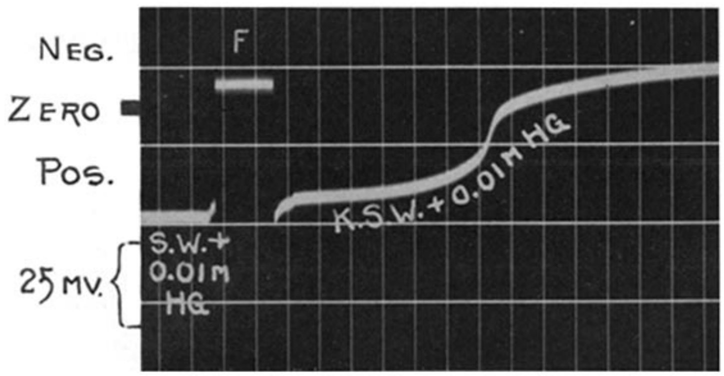

FIG. 8. Photographic record showing changes of P.D. produced by potassium after exposure of the cell to guaiacol (all solutions at pH 8.2). The cell in sea water containing 0.01 M H*G* had at the start of the record a positive P.D. of 29 mv. (originally in sea water it had a positive P.D. of 66 mv.). The cell was placed in sea water containing 0.01 M H*G* + 0.52 M KCl + 0.012 M NaCl (the remaining constituents of sea water had their normal concentrations): this is labelled K.S.W.+0.01 M H*G* on the record. The curve, with no latent period, rose to 13 mv. negative.

When the cell was replaced in sea water + 0.01 M H*G* the P.D. returned to the starting point (not shown in the figure).

Regarding *F* see Fig. 1.

Time marks 5 seconds apart. Temperature 21°C.

In some cases we find after exposures to H*G* up to 15 minutes that no change occurs when dilute sea water is applied. In such cases we may assume (as in the case of *Valonia*) that the mobility of Na^+ has become equal to that of Cl^-.

[17] This effect may occur after an exposure of only 30 seconds to H*G*.

[18] This was after an exposure of 11.5 minutes to 0.01 M H*G* in sea water at pH 6.4.

Another interesting aspect of the action of H*G* is seen in its effect on the apparent mobility of K^+. In *Valonia* this mobility is decreased by H*G* until KCl instead of making the P.D. less positive actually makes it more positive.[1]

Halicystis shows no such change in its behavior toward KCl. An application of sea water in which Na^+ has been replaced[19] by K^+ makes the P.D. much less positive whether the cell has been exposed to H*G* or not. But the form of the curve[20] showing the action of KCl is altered by the exposure to H*G*, as shown in Figs. 6, 7, and 8. We see that the P.D. can become negative under the combined action of KCl and H*G*.

DISCUSSION

Changes of pH have already been considered. The effect of H*G* remains to be discussed.

In *Valonia*, H*G* makes the P.D. more positive and this occurs before any change in the apparent mobility of K^+ or Na^+ can be detected. It would therefore seem that the effect of H*G* concerns their partition coefficients or the organic ions in the cell. It might conceivably change the nature or the concentration of these organic ions or alter their mobilities or partition coefficients in the non-aqueous protoplasmic surfaces.

In *Halicystis* the effect of H*G* on the apparent mobility of Na^+ may occur very early, *e.g.* after an exposure of 30 seconds or less to H*G*, which may account, to some extent at least, for the change of P.D. in a negative direction. The apparent mobility of Na^+ is normally less than that of Cl^- and this tends to make[21] the P.D. more positive. Hence in so far as H*G* increases the apparent mobility of Na^+ it will make the P.D. less positive.

It would seem, however, that organic ions in the protoplasm may play a part in determining the P.D. Blinks finds that when the solutions at the inner and outer protoplasmic surfaces are identical much of the P.D. persists.[22] This seems to be due to unlike apparent mo-

[19] This contains 0.52 M KCl and 0.012 M NaCl. The other constituents of the sea water remained unchanged.

[20] Osterhout, W. J. V., *J. Gen. Physiol.*, 1937–38, **21**, 631.

[21] *I.e.*, if it is diffusing inward, as it is in the case of a growing cell.

[22] Blinks, L. R., *J. Gen. Physiol.*, 1934–35, **18**, 409.

bilities of the same ions in the inner and outer surfaces. To what extent these are inorganic is not clear but organic ions in the protoplasm and sap may well play a part. Hence it would seem that such organic ions may be involved when H*G* lessens the P.D.

In *Valonia* the P.D. does not retain the positivity produced by guaiacol but gradually returns to its normal value. This is known as "recovery." It has been interpreted[1] to mean that guaiacol penetrates to the inner protoplasmic surface *Y* and there sets up changes which are of opposite sign to those set up in the outer protoplasmic surface, *X*. Failure to set up these changes in *Y* would prevent recovery and this explanation may apply to *Halicystis*.

If the protoplasmic surface were a pore system in the sense of Michaelis we should be obliged to say that its charge had been changed from negative to positive in *Halicystis* by the action of guaiacol if we are considering NaCl but not if we are considering KCl.

If we regard the surface as a non-aqueous layer without pores we may suppose that guaiacol alters mobilities or partition coefficients in this layer. It is quite possible that chemical compounds[23] are formed or complexes in the sense of Kraus and of Fuoss.[24]

We have no explanation at present for the fact that guaiacol changes the sign of the response to KCl in *Valonia* but not in *Halicystis*.

Another difference between the two organisms is seen when the pH is lowered from 8.2 to 6.4. This has little or no effect on *Valonia* but usually makes the P.D. of *Halicystis* less positive.

In this respect the cells of *Halicystis* are variable and such variability is highly characteristic. In fact it may be said that both plants are variable in many respects. Certain cells fail to show an expected change in P.D. in response to external changes such as alterations of pH, dilution, application of KCl, or of guaiacol, although evidently not dead, as shown by the P.D. in sea water and by the response to certain other external stimuli. It would seem that different physiological states are possible, perhaps analogous to those described by Blinks for *Valonia*.[9]

[23] Guaiacol forms compounds with both acids and bases.

[24] *Cf.* Kraus, C. A., *Tr. Electrochem. Soc.*, 1934, **66**, 179. Fuoss, R. M., *Chem. Rev.*, 1935, **17**, 27.

It may be added that although it has been assumed for purposes of discussion that the P.D.'s observed are chiefly due to diffusion potential this is done merely because it has been found in previous work[25] that the equations for diffusion potential enable us to predict results with sufficient accuracy. Other explanations may be possible, such as those discussed in a recent paper.[26]

The facts brought out in this paper add to the list of striking differences between *Valonia* and *Halicystis*. These differences are of interest as showing the possibilities of protoplasmic systems which were once regarded as very similar.

The fact that organic substances can change the apparent mobilities (as shown by the concentration effect) of inorganic ions (*e.g.*, K^+ and Na^+ in *Valonia* and Na^+ in *Halicystis*) is of considerable interest. It is shown not only by the action of guaiacol[1] but also by the fact that removal of organic substances from *Nitella* greatly lowers the apparent mobility[27] of K^+ so that the cell ceases to act in the fashion of a potassium electrode. The cell regains this power when treated with guanidine and certain other organic substances.[28]

SUMMARY

Lowering the pH of sea water from 8.2 to 6.4 lowers the positive P.D. of *Halicystis* reversibly (this does not happen with *Valonia*).

Exposure to sea water at pH 6.4 does not affect the apparent mobility of Na^+ or of K^+ (this agrees with *Valonia*).

Guaiacol makes the P.D. of *Halicystis* less positive (in *Valonia* it has the opposite effect).

Exposure to guaiacol does not reverse the effect of KCl in *Halicystis* which in this respect differs from *Valonia*.

The P.D. can be changed from 66 mv. positive to 23 mv. negative by the combined action of KCl and guaiacol.

Exposure to guaiacol affects *Halicystis* and *Valonia* similarly in respect to their behavior with dilute sea water. Normally the dilute

[25] Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, **13,** 715.

[26] Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24,** 75.

[27] Osterhout, W. J. V., *J. Gen. Physiol.*, 1934-35, **18**, 987.

[28] Osterhout, W. J. V., and Hill, S. E., *Proc. Soc. Exp. Biol. and Med.*, 1934-35, **32**, 715.

sea water makes the P.D. more negative but after sufficient exposure to guaiacol dilute sea water either produces no change in P.D. or makes it more positive. In the latter case we may assume that the apparent mobility of Na^+ has become greater than that of Cl^- as the result of the action of guaiacol. (Normally the apparent mobility of Cl^- is greater than that of Na^+.)

In *Halicystis*, as in *Valonia* and in *Nitella*, an organic substance can greatly change the apparent mobilities of certain inorganic ions (K^+ or Na^+).