

EFFECTS OF HYDROXYL ON NEGATIVE AND POSITIVE CELLS OF NITELLA

By W. J. V. OSTERHOUT

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

(Received for publication, May 1, 1945)

As OH^- has unique properties its action on protoplasm is of especial interest.

There appears to be little change in the potential of *Nitella* when the external pH is raised from 5 to 9 but interesting results are obtained by applying 0.01 M NaOH or KOH. The effects are reversible if the exposure is not too prolonged.¹

With KOH we find² that with some cells the more dilute solution is positive to the more concentrated solution (positive cells) but with others the more dilute solution is negative (negative cells): the latter can be converted to the former by sufficient exposure to KOH.

This might be accounted for on the ground that the protoplasm contains an organic acid which makes the cell negative and which is dissolved out by KOH.

¹The application of 0.01 M NaOH or KOH was not toxic if the exposure did not exceed 20 minutes and the same cell could be used in further experiments on subsequent days. It should be remembered that only a small portion of the cell (about 2 cm.) was covered by the reagent. When the whole cell was covered the effect was greater but when cells were entirely submerged in 0.01 M NaOH half the cells lived 6 hours and in 0.01 M KOH 4 hours.

²The cells, after being freed from neighboring cells, stood in the laboratory at 15°C. $\pm 1^\circ\text{C}$. in Solution A (cf. Osterhout, W. J. V., and Hill, S. E., *J. Gen. Physiol.*, 1933-34, 17, 87) for several days.

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 20-26°C. Regarding the amplifier see the reference just cited.

Two spots on the cell, *A* and *B*, were connected to a spot *C* through a recording galvanometer. At the end of the experiment, *A*, *B*, and *C* were killed (in this order) by applying chloroform, which reduced the p.d. at each spot to zero. During the experiments the solutions at *A* and *C* were changed but at *B* the solution was not disturbed: this was a relatively dilute solution.

It was then possible to ascertain the total potential across the protoplasm at *A* and *B* at any previous point on the record on the assumption that *C* had remained constant up to that point (or by correcting for any change). If *C* had changed the amount of alteration could be measured because it would appear as a simultaneous change at *A* and *B* (in the same direction at both).

We find that under certain conditions the mobility of OH^- may be intermediate between that of K^+ and Na^+ : hence the protoplasmic surface cannot be a pore system for in such a structure all cations must have a higher mobility than all anions or *vice versa*.

We may now consider some important measurements and their significance.

Concentration Effect of NaOH.—Measurements of the concentration effect were made in order to calculate the relative mobilities of the ions in the outer protoplasmic surface. This surface may be called *X* for convenience.

When 0.001 M NaOH in contact with *Nitella* is replaced by 0.01 M NaOH the p.d. becomes more positive.³ This indicates that the mobility of OH^- in *X* is greater than that of Na^+ . In this respect OH^- differs from the anions mentioned in previous studies on *Nitella* since they have in all cases had lower

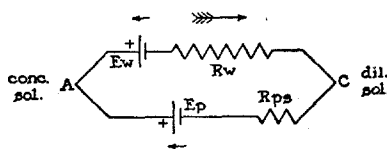


FIG. 1. Diagram of circuit in a negative cell when *A* is in contact with a concentrated solution of KOH and *C* is in contact with a more dilute solution of KOH. The diffusion potential in the cellulose wall is E_w and that in the protoplasm is E_p . The resistance in the cellulose wall is R_w and that in the protoplasm and sap is R_{ps} .

The direction of the E.M.F. of E_w and of E_p is shown by the small arrows. The direction of the current around the circuit is shown by the large feathered arrow. The dilute solution is negative in the external circuit: this is true whether E_p is greater than E_w or *vice versa* and hence it does not depend on the direction of the large feathered arrow.

mobilities⁴ than Na^+ . As already stated, there is ground for thinking that *X* contains an acid: this we may call HA and assume that the entering NaOH may be changed to NaA by the reaction $\text{NaOH} + \text{HA} = \text{NaA} + \text{H}_2\text{O}$. Hence we may be dealing chiefly with v_A rather than with v_{OH} in *X*. It is, however, more convenient to speak of v_{OH} with the understanding that it includes v_A and this will be done in the subsequent discussion.

When the cell is in contact with 0.001 M NaOH at two spots, *A* and *C*, the diffusion potentials in *X* at *A* and *C* are equal and opposite and the p.d. between these spots is zero or nearly so. After allowing several minutes for diffusion to occur in *X* and in the cellulose wall we replace 0.001 M NaOH at *A* by 0.01 M NaOH and thus increase the diffusion potentials at *A*. The increase in *X*

³ The potential is called positive when the positive current tends to flow from the sap across the protoplasm to the external solution.

⁴ Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715.

may be called E_p and that in the cellulose wall E_w . We may assume that the new concentration gradient in the cellulose wall is between 0.01 M and 0.001 M NaOH and that in X is between S (0.01 M) NaOH and S (0.001 M) NaOH, where S is the partition coefficient of NaOH.⁵

A simplified diagram of the electrical circuit is shown in Fig. 1. The only potentials which appear in the diagram are E_w and E_p , both of which may be regarded as located at A . The potentials at other places may be supposed to cancel out.

The current I is given by

$$I = \frac{E_p - E_w}{R_w + R_{ps}} \quad (1)$$

Here R_w is the resistance in the cellulose wall and R_{ps} that in the protoplasm and sap.

For the P.D. between A and C we have

$$\text{P.D.} = E_p - IR_{ps} \quad (2)$$

By substitution and rearrangement we obtain from (1) and (2)

$$\text{P.D.} = \frac{E_p R_w + E_w R_{ps}}{R_w + R_{ps}} \quad (3)$$

On the basis of rough calculations we assume that $R_w \div R_{ps} = 11$. This is a larger value than is indicated by the experiments of Blinks⁶ but in the present case there were two regions between A and C where the cell was surrounded by air (to prevent short-circuiting). This gave an opportunity for the cell wall to dry out which would cause the salts in the cellulose wall to move⁷ into X thus increasing the resistance of the cellulose wall and decreasing that of X . The cellulose wall between A and C was imbibed with tap water or with Solution A.

If we assume a lower value for $R_w \div R_{ps}$ the calculated value of the concentration effects of NaOH and of KOH may become unduly high.

Since absolute values are not needed we may write $R_w = 11$ and $R_{ps} = 1$.

Taking the ionic mobilities in water as proportional to ionic conductivities we may write⁸ for 25°C. $u_{\text{Na}} = 50.1$ and $v_{\text{OH}} = 198$. Assuming that these

⁵ Osterhout, W. J. V., *J. Gen. Physiol.*, 1943-44, **27**, 91. In calculations we employ the activity partition coefficient which is constant.

⁶ Blinks, L. R., *J. Gen. Physiol.*, 1929-30, **13**, 495.

⁷ This is not only by simple diffusion but also by the forces which cause salts to move in and reach a higher concentration inside than outside.

⁸ See MacInnes, D. A., *The principles of electrochemistry*, New York, Reinhold Publishing Corporation, 1939, p. 342.

values hold for the cellulose wall which is imbibed with aqueous solution we have (at 25°C.)

$$\begin{aligned} E_w &= 59 \frac{v_{\text{OH}} - u_{\text{Na}}}{v_{\text{OH}} + u_{\text{Na}}} \log \frac{a_1}{a_2} \\ &= 59 \frac{198 - 50.1}{198 + 50.1} \log \frac{0.01(0.90)}{0.001(0.99)} \\ &= 34 \text{ mv.} \end{aligned}$$

here a_1 and a_2 are the activities in water⁹ (at 25°C.).

Replacing 0.001 M NaOH at A by 0.01 M NaOH gave a change in a positive direction of 36 mv.¹⁰

Substituting values in Equation 3 we have

$$36 = \frac{E_p(11) + 34(1)}{11 + 1}$$

whence $E_p = 36.2$ mv.

We may calculate the relative mobilities in X by the usual formula (for 25°C.).

$$E_p = 36.2 = 59 \frac{v_{\text{OH}} - u_{\text{Na}}}{v_{\text{OH}} + u_{\text{Na}}} \log \frac{a_1}{a_2}$$

where $a_1 \div a_2$ is the ratio of ionic activities of NaOH in X which is taken as equal to the ratio of concentrations in the external solution (as in previous papers¹¹). Putting $u_{\text{Na}} = 1$ we obtain $v_{\text{OH}} \div u_{\text{Na}} = 4.2$ (in water $v_{\text{OH}} \div u_{\text{Na}} = 198 \div 50.1 = 3.95$).

These calculations, like most others in this paper, may be regarded as merely approximate.

It will be shown later that the concentration effect of NaOH can be reversed by exposure to KOH (p. 52).

Concentration Effects of KOH.—When we apply KOH to cells not previously subjected to experimental treatment we find that with some the dilute solution is negative to the more concentrated (as with NaOH). These may be called negative cells for convenience. We also find some which behave in the opposite fashion, with dilute solution positive; these may be called positive cells. In

⁹ MacInnes, D. A., The principles of electrochemistry, New York, Reinhold Publishing Corporation, 1939, p. 155.

¹⁰ This is usually reversible; *i.e.*, when the opposite change is made the p.d. changes in the opposite direction by approximately the same amount. But when the change is in a negative direction an action current may result, giving too large a value.

¹¹ It has been found in studying models that the ratio of concentrations in the external solutions is approximately the ratio of activities in the non-aqueous phase. Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1942-43, **26**, 293.

the same lot both positive and negative cells may occur. The reason for this will be discussed later (p. 52).

The values for negative cells are as follows. Taking, as before, conductivities in water³ as proportional to mobilities we may for convenience (at 25°C.) put $u_K = 73.5$ and $v_{OH} = 198$.

Hence when 0.001 M KOH at *A* is replaced by 0.01 M KOH the change in the cellulose wall is¹²

$$E_w = 59 \frac{198 - 73.5}{198 + 73.5} \log \frac{0.01(0.90)}{0.001(0.99)}$$

$$= 26 \text{ mv.}$$

On replacing 0.001 M KOH at *A* by 0.01 M KOH the average change was 44 mv. in a positive direction.

Inserting these values in Equation 3 we have

$$44 = \frac{E_p(11) + 26(1)}{11 + 1}$$

whence $E_p = 45.6$ mv.

We may now write for the mobilities in *X* (see p. 46)

$$45.6 = 59 \frac{v_{OH} - u_K}{v_{OH} + u_K} \log \frac{a_1}{a_2}$$

whence (taking $a_1 \div a_2 = 10$) we have $v_{OH} \div u_K = 7.8$ (in water we have $v_{OH} \div u_K = 198 \div 73.5 = 2.7$).

If all the mobilities were constant we could calculate $u_K \div u_{Na}$ but we find that they are not constant in cells exposed to OH.

In positive cells, 0.001 M KOH is positive to 0.01 M KOH and the current has the direction shown in Fig. 2. We have

$$I = \frac{E_p + E_w}{R_w + R_{ps}} \quad (4)$$

For the p.d. between *A* and *C* we have

$$\text{p.d.} = E_p - IR_{ps} \quad (5)$$

By substitution and rearrangement we obtain from (4) and (5)

$$\text{p.d.} = \frac{E_p(R_w) - E_w(R_{ps})}{R_w + R_{ps}} \quad (6)$$

In a typical lot of cells the change in a negative direction when 0.001 M KOH was replaced by 0.01 M KOH was 46 mv.

Hence we have

$$46 = \frac{E_p(11) - 26}{11 + 1}$$

¹² The activity coefficients are here taken as equal to those for NaOH, see p. 46.

whence $E_p = 52.5$.

For the mobilities in X we have

$$52.5 = 59 \frac{u_K - v_{OH}}{u_K + v_{OH}} \log \frac{a_1}{a_2}$$

Proceeding as with NaOH for mobilities in X , we obtain $u_K \div v_{OH} = 17.2$.

Concentration Effects of NaCl and of KCl.—Here the dilute solution is always positive indicating that v_{Cl} is less than u_{Na} or u_K . Taking, as before, the ion

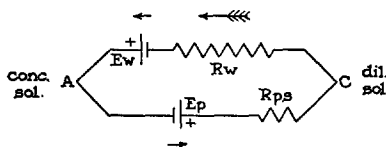


FIG. 2. Diagram of circuit in a positive cell when A is in contact with a concentrated solution of KOH and C is in contact with a more dilute solution of KOH. The diffusion potential in the cellulose wall is E_w and that in the protoplasm is E_p . The resistance in the cellulose wall is R_w and that in the protoplasm and sap is R_{ps} .

The direction of the E. M. F. of E_w and of E_p is shown by the small arrows. The direction of the current around the circuit is shown by the large feathered arrow. The dilute solution is positive in the external circuit. This is true only when $E_p R_w > E_w R_{ps}$ for when the P.D. is zero we have

$$P. D. = 0 = \frac{E_p R_w - E_w R_{ps}}{R_w + R_{ps}}$$

$$0 = E_p R_w - E_w R_{ps}$$

$$E_p R_w = E_w R_{ps}$$

mobilities as proportional to the ionic conductivities we may write (at 25°C.) for E_w in the case of NaCl.

$$E_w = 59 \frac{76.3 - 50.1}{76.3 + 50.1} \log \frac{0.01(0.90)}{0.001(0.99)}$$

$$= 11.7 \text{ mv.}$$

Replacing 0.01 M NaCl by 0.01 M NaCl gives a change in a negative direction of 29 mv.

Hence we have

$$29 = \frac{E_p(11) - 11.7}{11 + 1}$$

whence $E_p = 32.7$.

For the mobilities in X we obtain $u_{Na} \div v_{Cl} = 3.5$.

The concentration effect of KCl resembles that of NaCl in that the dilute solution is always positive so that u_K is greater than v_{Cl} no matter whether we have negative cells, where $v_{OH} > u_K$, or positive cells, where $u_K > v_{OH}$.

On replacing 0.01 M KCl by 0.001 M KCl the change for a typical lot of cells was 39 mv.¹⁰ Taking, as before, ionic mobilities in water as proportional to ionic conductivities we may write (for 25°C.)

$$E_w = 59 \frac{76.3 - 73.5}{76.3 + 73.5} \log \frac{0.01(0.90)}{0.001(0.99)}$$

$$= 1.1 \text{ mv.}$$

We therefore have

$$39 = \frac{E_p(11) - 1.1}{11 + 1}$$

whence $E_p = 42.6$.

We then obtain $u_K \div v_{Cl} = 6.2$. We have already found (p. 48) that in positive cells $u_K \div v_{OH} = 17.2$ and if the value of v_{OH} is constant it would seem that v_{Cl} is greater than v_{OH} , but in that case we should expect a change in a negative direction when 0.01 M KCl is replaced by 0.01 M KOH. The fact that this does not always happen in positive cells may be due to differences in the partition coefficients, S_{KCl} and S_{KOH} .

Change from Negative to Positive Behavior and Vice Versa.—Negative cells may become positive under the influence of KOH. An example is shown in Fig. 3. At the start the recorded spot, A, was in contact with 0.001 M KOH with a p.d. of 90 mv. When 0.001 M KOH was replaced by 0.01 M KOH the p.d. changed in a positive direction to the extent of 50 mv. and the spot was clearly negative; *i.e.*, dilute solution negative in contact with KOH.

When 0.01 M KOH was replaced by 0.001 M KOH the curve moved upward slowly for a time and then rose abruptly; this was apparently accompanied by an action current, as often happens¹³ when there is a decided slow rise.

The next change was from 0.001 M to 0.01 M KOH, causing a change in a negative direction of 40 mv. The cell had now become positive (*i.e.*, with dilute solution positive in contact with KOH). This is verified by the next change from 0.01 M to 0.001 M KOH which produced a change of 40 mv. in a positive direction. The spot continued to behave as positive throughout all the subsequent changes of solutions (not shown in the figure).

Another example is seen in Fig. 4. The recorded spot showed a change of 160 mv. in a positive direction when 0.01 M KCl was replaced by 0.01 M KOH: hence $v_{OH} > u_K$. On standing in KOH the curve rose to a little above zero showing that the spot had changed and the effect of KOH had become practi-

¹³ Blinks, L. R., *J. Gen. Physiol.*, 1936-37, **20**, 229. Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 541.

cally the same as that of KCl so that $u_K > v_{OH}$ (just as $u_K > v_{Cl}$), and the cell had become positive, as seen in subsequent changes (not shown in the figure) from 0.01 M to 0.001 M KOH and *vice versa*.

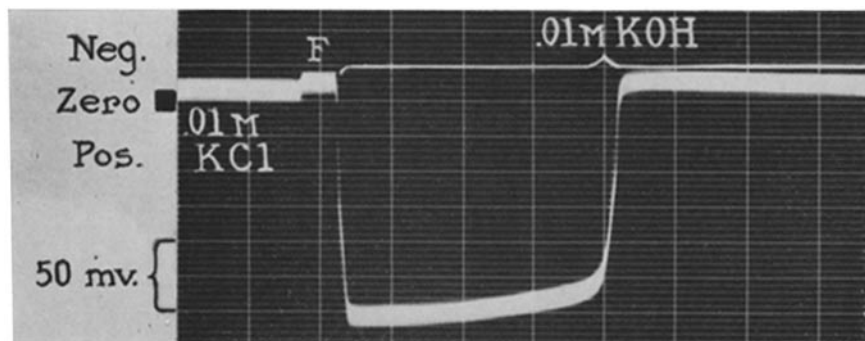


FIG. 4. Record of a spot whose behavior changed under the influence of KOH. At the start the order of mobilities was $v_{OH} > u_K > v_{Cl}$ so that when 0.01 M KCl was replaced by 0.01 M KOH the p.d. changed in a positive direction.

The recorded spot *A* was connected through the recording galvanometer to another spot *C* in contact with 0.01 M KCl which kept its p.d. constant approximately at zero. At first *A* was in contact with 0.01 M KCl with its p.d. near zero. When 0.01 M KCl was removed so that the spot was out of the electrical circuit, the curve jumped to *F*, the free grid potential of the amplifier. When 0.01 M KCl was replaced by 0.01 M KOH the p.d. changed in a positive direction to the extent of 160 mv. On standing in KOH the curve rose, showing that the value of $v_{OH} \div u_K$ was becoming smaller: v_{OH} became less than u_K so that when 0.01 M KOH was replaced by 0.001 M KOH (not shown in the figure) the change of p.d. was in a positive direction. Hence the spot had become positive.

The rise of the curve in the figure was not due to injury: this was shown by its subsequent behavior (not shown here).

Regarding changes in the value of $v_{OH} \div u_K$ see p. 52.

The cell was freed from neighboring cells and kept in Solution *A* for 4 days at $15^\circ\text{C.} \pm 1^\circ\text{C.}$ The temperature was raised to 25°C. an hour before the experiment was started.

Time marks 15 seconds apart.

To change negative to positive behavior (as judged by the concentration effect of KOH) usually requires an exposure to 0.01 M KOH of from 2 to 5 minutes or a longer exposure to 0.01 M NaOH. Such an alteration signifies that the order of mobilities in *X* has changed from $v_{OH} > u_K$ to $u_K > v_{OH}$. After such an exposure to 0.01 M KOH we find that the order $v_{OH} > u_{Na}$ has been changed to $u_{Na} > v_{OH}$ but this change is not produced by exposure to 0.01 M NaOH although the value of $v_{OH} \div u_{Na}$ (or of $S_{NaOH} \div S_{NaCl}$) may be somewhat lessened, as shown by the behavior of the curve; for when 0.001 M

NaOH is replaced by 0.01 M NaOH the curve drops sharply (like the curve in Fig. 4 when 0.001 M KOH was replaced by 0.01 M KOH) and later may rise slowly to the extent of 30 mv.

It would appear that exposure to 0.01 M KOH can lessen the value of v_{OH} and change the order $v_{OH} > u_K, u_{Na}$ to $u_K, u_{Na} > v_{OH}$ and exposure to 0.01 M NaOH can change the order to $u_K > v_{OH} > u_{Na}$ but not to $u_K, u_{Na} > v_{OH}$. In other words, the concentration effect of KOH can be reversed by KOH and by NaOH but to reverse the concentration effect of NaOH requires exposure to KOH. We also find in some cases $u_K > v_{OH} > u_{Na}$ so that a spot may have the concentration effect of KOH reversed but not that of NaOH. Such a spot shows dilute solution positive if tested with KOH but dilute solution negative if tested with NaOH.

It is of interest to note that if the cell is originally negative we can change any part to positive by exposure to KOH while the untreated part remains negative.

This might be explained on the ground that the apparent high mobility of v_{OH} is in reality due to the high mobility of A^- , the anion of a fatty acid HA in X as previously mentioned (p. 43), on the assumption that KOH entering X combines with HA according to the reaction $KOH + HA = KA + H_2O$.

It is possible that the acid HA is dissolved out more rapidly by KOH than by NaOH as is the case with the fatty acids in ordinary soaps where the potassium soap is much more soluble in water than the sodium soap.

There can be little doubt that the cell produces organic acids, including fatty acids, and it therefore seems possible that after the cell has been made positive by dissolving out some fatty acid it might become negative again owing to further production of acid. A few cases have been observed in which the cell after being transformed from negative to positive afterward became negative again.

It also seems possible that the anion with the high mobility is really OH^- at least in part or that the mobility of OH^- is greater when HA is present.

Concentration Effects in Relation to Pore Structure.—Positive cells show a change in a positive direction when 0.01 M KOH is replaced by 0.001 M KOH (dilute solution positive). Hence $u_K > v_{OH}$.

These same individual cells may show a change in a negative direction when 0.01 M NaOH is replaced by 0.001 M NaOH (dilute solution negative). Hence $v_{OH} > u_{Na}$.

Hence we have $u_K > v_{OH} > u_{Na}$. This shows that the outer protoplasmic surface cannot be a pore system for in such a system all cations must have a greater mobility than all anions and *vice versa*.

Chemical Effects.—Of importance also are chemical effects; *i.e.*, changes due to replacement of one compound by another. When 0.01 M NaCl is replaced

by 0.01 M NaOH the p.d. becomes more positive. The average value¹⁴ in one lot of cells was 120.¹⁰ In another lot it was 89.

If we calculate partition coefficients by means of Henderson's equation, as in former papers,¹⁵ we obtain a high value for S_{NaOH} (the concentration of NaOH in X divided by that in the external solution) but as this involves mobilities which may not be constant in cells exposed to OH the quantitative significance of S_{NaOH} is doubtful. A high value for S_{NaOH} is not surprising if X contains an organic acid as will appear later (p. 54).

Let us now consider similar experiments with KOH. With negative cells (where the dilute solution is negative with KOH) we find that replacement of 0.01 M KCl by 0.01 M KOH gives a change in a positive direction of 145 mv.¹⁰

Although it is not feasible to calculate the partition coefficient of KOH (because mobilities are not constant) it is evident that it is greater than that of KCl. For when 0.01 M KCl is replaced by 0.01 M KOH the change in p.d. occurs promptly but when the opposite change is made the alteration of p.d. is very slow. Similar considerations apply to NaCl and NaOH.

When we turn to positive cells (which show dilute solution positive with KOH) we see a different picture. On replacing 0.01 M KCl by 0.01 M KOH the change in p.d. is small and may be in a positive or in a negative direction. It would thus appear that the effects of KCl resemble those of KOH in these cells.

When 0.01 M NaCl is replaced by 0.01 M KOH we might expect a change in a negative direction (since u_{K} is greater than u_{Na}) unless the value of v_{OH} is so much greater than that of u_{K} as to produce a change in a positive direction. We find that in negative cells the change is always in a positive direction up to 150 mv.¹⁰ In positive cells it is in a negative direction (up to 50 mv.).

The Influence of OH⁻ on the Total Potential and on the Potassium Effect.—When a cell in contact with 0.01 M NaCl is exposed for a time to KOH (or in some cases to NaOH) and again placed in contact with 0.01 M NaCl it may be found that the potential has increased by an amount varying from 10 to 80 mv. An increase in the potassium effect is often observed after exposure to OH⁻. (The potassium effect is the change in a positive direction observed when 0.01 M KCl is replaced by 0.01 M NaCl.¹⁶)

¹⁴ In many cases the process was reversible (see footnote 10).

¹⁵ Regarding partition coefficients see Hill, S. E., and Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1938, **24**, 312; Osterhout, W. J. V., *J. Gen. Physiol.*, 1939-40, **23**, 171; Some models of protoplasmic surfaces, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8**, 51.

¹⁶ Osterhout, W. J. V., *J. Gen. Physiol.*, 1929-30, **13**, 715; 1939-40, **23**, 429; 1944-45, **28**, 23.

DISCUSSION

The relatively high mobility of OH^- in X in negative cells is very striking. We find that in such cells the apparent value of $v_{\text{OH}} \div u_{\text{Na}}$ is 4.2 (p. 46) and the value of $v_{\text{OH}} \div u_{\text{K}}$ is 7.8 (p. 47).

This might be explained on the ground that X contains a weak organic acid, possibly a fatty acid, which tends to raise the value of v_{OH} , perhaps in the same way that guaiacol raises the mobility of certain ions in X .¹⁷ Furthermore, if an acid HA is present so that KOH entering X reacts to form KA and if v_{A} has a high value this would have the same effect as if v_{OH} had a high value: hence the apparent high value of v_{OH} may include the value of v_{A} , as already stated (p. 44). This would imply that $v_{\text{A}} > u_{\text{K}}, u_{\text{Na}}$ but this might be explained on the ground that in place of K^+ and Na^+ in X we have complex ions of the type $\text{KX}_I, \text{KX}_{II}$, etc., as assumed by Kraus and by Fuoss.¹⁸

We find that on standing in KOH the apparent value of v_{OH} falls off (p. 51); this does not happen to the same extent with NaOH (p. 49). This suggests an analogy with ordinary soaps where the fatty acids are more rapidly washed out by KOH than by NaOH . On this basis we might expect the fatty acid to dissolve out and lower the apparent value of v_{OH} much more rapidly in KOH than in NaOH .

If X contains an acid it is not surprising that the partition coefficients for NaOH and for KOH appear to be high (p. 53). An analogy is found, for example, when $\text{Ba}(\text{OH})_2$ is shaken with isoamyl alcohol and only a small amount of barium enters but when 0.01 M oleic acid is added to the isoamyl alcohol a relatively large amount of barium is taken up, as shown in a previous paper.¹⁹ In our calculations we have considered only the partition coefficients of NaOH and KOH but it is evident that we have to do also with NaA and KA if an acid, HA , is present in X .

It has been found necessary to assume the presence of an acid in X to account for the rate of entrance of NH_3 ²⁰ and of guanidine²¹ into *Valonia*.

Furthermore, the fact that basic dyes readily penetrate X while acid dyes do not may be taken to indicate the acid nature of X .

The presence of a fatty acid in X might explain the increased resistance observed by Blinks²² in *Valonia* after the application of acetic acid. A model

¹⁷ Osterhout, W. J. V., *J. Gen. Physiol.*, 1939-40, **23**, 171, 749.

¹⁸ Cf. Kraus, C. A., *Tr. Electrochem. Soc.*, 1934, **66**, 179. Fuoss, R. M., *Chem. Rev.*, 1935, **17**, 27.

¹⁹ Hill, S. E., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, **21**, 553 (footnote 29).

²⁰ Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*, 1935, **21**, 125.

²¹ Jacques, A. G., *Proc. Nat. Acad. Sc.*, 1935, **21**, 488.

²² Blinks, L. R., The relations of bioelectric phenomena to ionic permeability and to metabolism in large plant cells, in Cold Spring Harbor symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, **8**, 204.

of this has been constructed by applying acetic acid to a layer of soap which sets free oleic acid and thus increases the resistance.²³

The fact that KOH is more effective than NaOH in changing the behavior of the protoplasm falls in line with other observations. Cells live longer²⁴ in 0.01 M NaCl than in 0.01 M KCl.

Returning now to Fig. 4 it may be said that it shows a general resemblance to the curves produced by other reagents in *Valonia* and in *Nitella*. But these do not seem to depend on the same factors as the curve in Fig. 4. For example, the application of guaiacol to *Valonia* causes the curve to fall and then rise, and leaves the cell in an altered state.²⁵ But guaiacol does not produce such effects in *Nitella*.²⁶ Somewhat similar curves are produced in *Valonia* by aniline,²⁷ benzene,²⁸ nitrobenzene,²⁸ and hexylresorcinol.²⁹ The curves in Figs. 3 and 4 resemble somewhat those obtained by applying KCl plus formaldehyde¹⁶ or plus mercuric chloride²⁴ where the value of P_x (potential at X) at the start is equal to that of P_y (potential at Y , the inner protoplasmic surface) and P_x falls off at the start more rapidly than P_y . Since this is not the case with Figs. 3 and 4 of this paper the resemblance cannot be regarded as significant. In all these cases the underlying causes may be quite different from those involved in Figs. 3 and 4 of this paper.

It might be suggested that the change from negative to positive cells under the influence of OH^- is due to a shift whereby an ampholyte in X passes to the opposite side of the isoelectric point. Whether this is the case must remain undecided for the present.

One of the most interesting effects of KOH is the change from $v_{\text{OH}} > u_{\text{K}}$, u_{Na} to $u_{\text{K}} > v_{\text{OH}} > u_{\text{Na}}$ which, as already stated (p. 52), shows that in this case the protoplasmic surface is not a pore structure. It has been found that in *Valonia*³⁰ and in *Halicystis*³¹ the normal order is $u_{\text{K}} > v_{\text{Cl}} > u_{\text{Na}}$ and this can be changed to $u_{\text{Na}} > v_{\text{Cl}} > u_{\text{K}}$ in *Valonia*³² by guaiacol.³³

The observations reported in this paper indicate that the protoplasmic surface in *Nitella* is acid in nature and that it is not a pore system.

²³ Osterhout, W. J. V., Some models of protoplasmic surfaces, in Cold Spring Harbor^F symposia on quantitative biology, Cold Spring Harbor, Long Island Biological Association, 1940, 8, 51.

²⁴ Unpublished results.

²⁵ Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, 20, 13, 685.

²⁶ Osterhout, W. J. V., *J. Gen. Physiol.*, 1938-39, 22, 417; 1939-40, 23, 171, 749.

²⁷ Osterhout, W. J. V., *J. Cell. and Comp. Physiol.*, 1941, 18, 129.

²⁸ Osterhout, W. J. V., *J. Gen. Physiol.*, 1940-41, 24, 699.

²⁹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1940-41, 24, 311.

³⁰ Damon, E. B., *J. Gen. Physiol.*, 1938-39, 22, 819.

³¹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1939-40, 23, 53.

³² Osterhout, W. J. V., *J. Gen. Physiol.*, 1936-37, 20, 13.

³³ Guaiacol can change $v_{\text{Cl}} > u_{\text{Na}}$ to $u_{\text{Na}} > v_{\text{Cl}}$ in *Halicystis* (Osterhout, W. J. V., *J. Gen. Physiol.*, 1937-38, 21, 707).

I wish to thank Mr. Harry Bodner for the care and skill he has shown in carrying out these experiments.

SUMMARY

Remarkable changes are brought about by KOH in transforming negative cells of *Nitella* (showing dilute solution negative with KOH) to positive cells (showing dilute solution positive with KOH). NaOH is less effective as a transforming agent.

This might be explained on the ground that the protoplasm contains an acid (possibly a fatty acid) which makes the cell negative and which is dissolved out more rapidly by KOH than by NaOH, as happens with the fatty acids in ordinary soaps.

Part of a negative cell can be changed to positive by exposure to KOH while the untreated portion remains negative.

After exposure to KOH the potential the protoplasm has when in contact with NaCl may increase. At the same time there may be an increase in the potassium effect; *i.e.*, in the change of P.D. in a positive direction observed when 0.01 M KCl is replaced by 0.01 M NaCl.

In some cases the order of ionic mobilities is $u_K > v_{OH} > u_{Na}$. This shows that the protoplasmic surface cannot be a pore system: for in such a system all cations must have greater mobilities than all anions or *vice versa*.