The large potential difference measurable across the protoplasm of impaled multinucleate cells of the alga *Halicystis* has been described in preceding papers. This is called "protoplasmic" since it is, to a large extent, independent of concentration gradients between sea water and the vacuolar sap. Thus a large P.D. persists when natural or artificial sap is applied externally, both in the species containing much KCl and in that containing little. Dilution and concentration of the sea water, or of many of its constituents, provided a reasonable physiological balance of salts is maintained, also have little effect, after certain transient changes have occurred. However, such applications at the outer surface may not immediately affect potentials at the inner surface, or within the protoplasm itself. To study these, internal changes must be produced. The effect of naturally differing cell saps on the vacuolar surface has already been noted. There remain as experimental changes: (1) direct alteration of the cell sap by injection or perfusion; (2) the penetration of substances into the cell from the sea water.

Both of these methods have been employed, with marked effects upon the observed P.D. The results with ammonia, representing the penetration of a substance into the cell, are reported in the present paper. Being technically simpler to produce than the changes by sap perfusion, they were completed first. The results, however, are inherently more complex, since they involve not only the changes known to occur in the sap, but also unknown changes in the protoplasm across which the ammonia must pass to reach the vacuole. Some of the results of direct perfusion which bear upon the interpre-

tation are therefore indicated, as in the paper on the effects of KCl. More complete description of the technique of perfusion and the principal results will appear in a forthcoming paper.

**Methods**

The cells were held rigidly between a glass ring below, and a glass tube, constricted to a capillary, inserted into the cell from above, as in Fig. 1. The tube and capillary, filled with artificial sap, formed the circuit from the vacuole to a KCl-agar bridge, communicating with a saturated KCl-calomel electrode. External solutions were changed without disturbing the cell by replacing vessels from below, or directing a stream of solution on the tube a short distance above the cell. This flowed down the tube and over the cell, dropping off below, making contact with a second KCl-agar bridge opening just beside or below the cell. A second saturated KCl-calomel electrode completed the electrical circuit to the measuring instruments, a potentiometer and galvanometer. The latter could be employed without prejudice to the results, since it was found that _Halicystis_ cells can withstand quite appreciable current drain without decrease of potential. Except during rapid changes of P.D., however, the circuit was kept compensated and no current passed through the cell. No different results were found when a vacuum tube electrometer, or a Compton electrometer, was used as a nullpoint indicator. Points of balance could easily be obtained at 15 second intervals; usually 1 minute intervals were adequate to follow the changes here described.

The potential plotted is that of the outer surface or solution (usually sea water). When this is positive, the positive current tends to flow outward across the protoplasm to the measuring instrument; when negative, it tends to flow inward. The positive sign is below the zero line, in conformity with the convention for _Valonia_ and _Nitella_ in this laboratory. This is the reverse of the plotting in the first paper of this series, but is followed in subsequent articles.

pH values of sap and sea water were determined colorimetrically with allowance for salt errors, the buffers used for comparison being checked with the quinhydrone electrode below pH 8.0. Ammonia estimations in the sap were made by Nessler test. The ammonia content of the sea water was regulated by adding measured quantities of NH₄Cl from a stock solution for each experiment, observing, and if necessary adjusting, the pH (by adding NaOH or HCl).

Sap was withdrawn from the cells by inserting a glass tube drawn into a fine point, rinsing and wiping the cells well to avoid contamination from sea water. The pH, determined directly in these tubes, with a minimum of exposure to air, was found to be very much lower than originally reported by Blinks and Jacques;³

namely, 5.0 instead of 6.1. The earlier determination was on a large mixed sample extracted from many cells and exposed to the air during collection. Hollenberg found the sap of *H. ovalis* to have a pH of 5.4; *Haliicystis* sap is thus considerably more acid than that of *Valonia*.

The temperatures varied from winter values of 18°C. to summer ones of about 25°C. This variation had very little effect upon the p.d. across the protoplasm at the different times, the summer values averaging possibly a millivolt or two lower than the winter ones. There may be other reasons for this, such as the onset of the reproductive period in the summer. The variation of temperature in a single experiment was seldom more than 1°C.

Variation of illumination has also small effect upon the normal potentials, which hold up for several days in complete darkness. In the presence of NH₄Cl in the sea water, however, illumination has a perceptible or even very marked effect.

Fig. 1. Diagram of arrangement for holding impaled cells of *Haliicystis*, with connection to calomel electrodes.

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effect under proper conditions. In ordinary diffuse north light of the laboratory
this was negligible, but on bright days, passing clouds often changed the P.D.
appreciably. This is evidently due to the photosynthetic extraction of CO₂
from the sea water around the cell, raising the pH and so influencing the dissociation
of ammonia. It is hoped to study this effect more fully, as a sensitive electrical
indicator of photosynthesis.

The results with Halicystis Osterhoutii, of Bermuda, are chiefly described in
the present paper, since it is with this species that the experiments with sap per-
fusion have been performed. It is also perhaps of greater intrinsic interest, since
its cell sap differs so slightly from the sea water; the potential is therefore almost
entirely protoplasmic, and not enhanced by a KCl gradient. In a few cases, the
almost identical results obtained with H. ovalis, of California, will be referred to.
The chief difference is the usually higher threshold of NH₄Cl necessary for poten-
tial reversal in the latter species.

The Reversal of P.D. by Ammonia

The normal concentration of total ammonia (NH₄⁺ + NH₂OH +
NH₃) in Bermuda sea water is below 0.00001 M. Up to about 0.0001
M NH₄Cl may be added, at the normal pH, 8.1, of the sea water, without appreciable effect upon the p.d. across the protoplasm of Halicystis
Osterhoutii which averages remarkably close to 68 mv., outside posi-
tive. But at a threshold varying between 0.0005 M and 0.002 M,
often at about 0.001 M NH₄Cl, a striking change occurs. The p.d.
rapidly reverses, to about 30 or 40 mv. negative, and remains reversed (with fluctuations) as long as the exposure to the ammonia continues. Higher concentrations increase the negative p.d. somewhat; a return to ordinary sea water causes recovery of positivity. The entire process is completely reversible, and may be repeated almost without limit if the ammonia exposures do not last too long; e.g., more than an hour or two at a time.

A characteristic time curve of the reversal and recovery process at
about the threshold concentration of NH₄Cl is shown in Fig. 2. When
the ammonia is first applied there is a small notch or cusp (a), during
which the p.d. decreases a few millivolts, then recovers nearly to its
original value (b). Here it remains a few minutes, then begins to
decrease slowly to about 40 mv. positive. After this the decrease
becomes much faster and the p.d. rapidly reverses (c) to about 40 mv.
negative. At this point there almost invariably occurs a reverse cusp
(d), then a return to an irregularly wavering negative value (e). When ordinary sea water is again replaced, a positive P.D. is quickly recovered (f), usually with a period of enhanced positivity (g) up to 75 mv. or more, before the normal value (h) is regained.

Fig. 2 is entirely typical of dozens of observed reversals at about the threshold concentration of NH₄Cl. The actual speed of reversal (and the negative P.D. attained) varies somewhat from cell to cell, as does the threshold itself. When higher concentrations of NH₄Cl are applied, the reversal becomes much quicker, as shown in Fig. 3. The curve becomes very abrupt and almost rectangular at high concentrations; the reversed P.D. may also become temporarily as high as 90 to 100 mv. negative; i.e., about as high, although with reversed sign, as the greatest positive values so far produced (with alkaline sea water). Usually, however, the negative P.D. does not greatly exceed
70 mv., again about the average for the positive values in normal sea water.

![Graph](image)

**Fig. 3.** Time course of p.d. change in cells of *Halicystis Osterhoutii* exposed to the indicated concentrations of NH₄Cl in sea water at pH 8.1. The rise becomes much more abrupt with increasing concentrations. The recovery curves in normal sea water are omitted to prevent overlapping but closely resemble the recovery in Fig. 2. Arrows indicate the application of sea water containing ammonia.

**The Relation of NH₄Cl Concentration to P.D.**

It is evident that although the p.d. has a very definite relation to the NH₄Cl concentration in the sea water, it is distinctly not proportional, but rather in the nature of an “all or none” response: below a critical concentration, there is very little change of p.d.; at the threshold a reversal amounting to a change of 100 mv. or more; and then again above the threshold only relatively small increases of p.d. This is shown for a single cell with a series of increasing concentrations in Fig. 4. Furthermore when the concentrations are
decreased, it is seen that the threshold for recovery is at a lower NH₄Cl concentration than for original reversal. Thus the reversed potential is maintained with only half the NH₄Cl concentration necessary originally to cause reversal; only at about one-fourth of the original threshold value is the positive P.D. recovered, and then with a slower curve than in ordinary sea water.

Fig. 4. Time course of P.D. change in cells of Halicystis Osterhoutii exposed to sea water at pH 8.1 containing first increasing and then decreasing concentrations of NH₄Cl. Curve A is for concentrations below the threshold (in this case below 0.001 M). Curve B shows the reversal of P.D. at 0.001 M and the effect of successively doubled NH₄Cl concentrations, followed by halving to 0.0005 M. Curve C shows recovery of positive P.D. at 0.0002 M NH₄Cl. Arrows indicate change of solution.

This is something in the nature of hysteresis, giving two curves, one for increasing, the other for decreasing NH₄Cl concentrations. For the reversed P.D. there are also two points, at least, for each NH₄Cl concentration: the maximum reached first on reversal, before the cusp (d of Fig. 2) and an average of the wavering plateau (e). No single curve of P.D. against NH₄Cl concentration can thus be plotted. There
are three which could be used: (A) the highest values reached during increase of concentration; (B) the lowest values reached during increased concentrations; and (C) the steady values reached after decreased concentrations. The latter are probably the most reliable values. All of these are plotted, for the cell of Fig. 4, in Fig. 5. Such a plot for a single cell is more significant than an average or composite plot for many cells, as showing more sharply the reversal at a critical concentration. It is, moreover, quite characteristic for any other...
cell, with a slight shift of the critical concentrations and of actual P.D. values.

The Relative Roles of Ammonium Ions and Undissociated Ammonia

It might be concluded from Fig. 5 alone that we are here dealing with no ordinary ionic concentration effects, so different is this relation from the usual one relating P.D. to salt concentration, i.e. in straight logarithmic ratio (cf. Damon's results with Na and K in Valonia6). There is, however, still more direct evidence as to the slight effect of ammonium ions on the P.D. of Halicystis. The basic
dissociation constant of ammonia being approximately $10^{-4.7}$, ammonium salt will be 50 per cent dissociated at pH 9.3. At the ordinary pH of sea water, 8.1, it will therefore be over 95 per cent dissociated,

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6 This is the value commonly assumed for the dissociation constant of ammonia in dilute solution. The greater ionic strength of sea water will tend to increase the ionization of ammonia, but the change was found (Cooper, W. C., Jr., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1930–31, 14, 117) to be slight, shifting the $pK_a$ from 9.3 to 9.5. This, as well as the difference between concentrations and activities of the substances concerned, may be neglected for the purposes in hand.
and at pH 5, nearly 100 per cent dissociated. For a given NH$_4$Cl concentration the concentration of ammonium ions will be increased about 5 per cent when sea water is acidified from pH 8.1 to pH 5; but the concentration of undissociated ammonia (NH$_3$ or NH$_3$OH) will be 1000 times as high at pH 8 as at pH 5, and at pH 10.3 will be over 100 times as great as at pH 8.1. Changing the pH over this range with definite NH$_4$Cl concentrations should therefore give information on the relative rôle of ammonium ion and undissociated ammonia. Two such experiments are shown in Fig. 6. When reversal is obtained with 0.001 M NH$_4$Cl at pH 8.1, lowering the pH to

![Graph showing variations of P.D. across the protoplasm of Halicystis Osterhoutii.](image)

**Fig. 7.** Variations of P.D. across the protoplasm of Halicystis Osterhoutii. Sea water of pH 5 is first applied, producing a characteristic cusp. Sea water containing 0.1 M NH$_4$Cl at pH 5 is then applied. There is no reversal of P.D. pH 5 sea water and pH 8 sea water then follows, with positive P.D. throughout. Arrows indicate change of solution.

6 causes recovery of positive values; conversely when 0.0001 M NH$_4$Cl is applied at pH 8.1 it is insufficient to cause reversal, but if the pH is raised to 10.3 good reversal occurs. The relative inactivity of ammonium ions in altering the P.D. of Halicystis is perhaps most strikingly seen in Fig. 7. Here the sea water (without NH$_4$Cl) was first acidified to pH 5, giving a typical cusp, presumably due to the diffusion of hydrogen ions into the protoplasm. Then 0.1 M NH$_4$Cl in sea water of pH 5 was substituted; the additional effect is very slight and reversal does not occur. Indeed, concentrations of NH$_4$Cl as high as 0.5 M, i.e. NH$_4$Cl entirely substituted for NaCl in van't Hoff artificial sea water, at pH 5 have been applied to some cells of rather
high threshold without reversing the p.d. On the other hand the threshold at pH 10.3 lies between 0.00005 and 0.0001 M NH₄Cl. Since at this pH 95 per cent of the ammonium salt is present as undisso-
ciated ammonia these concentrations may be taken as essentially the actual threshold values for NH₄(or NH₄OH). They are in good agree-
ment with the values at pH 8.1 where only about 1/20 as much un-
dissociated ammonia exists, and the threshold of total NH₄Cl con-
centration lies between 0.001 and 0.002 M; i.e., about 20 times as high. We may conclude that the reversal of potential depends upon the concentration of undisso-
ciated ammonia rather than that of ammonium ions in the sea water applied to the cells. But it is again not proportional to such ammonia; for the threshold “all or none” effect still holds. We must therefore ask what internal changes may be produced by the entrance of undisso-
ciated ammonia, which might account for the abrupt reversal of p.d. at a particular NH₃ con-
centration.

Internal Effects

The ease with which ammonia enters living cells is one of the well established facts of permeability studies.\(^7\) Not only its tendency to
enter more at high pH values, but its observed effects (increase of internal pH in the cell) indicate that it enters as base (NH₄OH) rather than as salt (NH₄Cl), and probably as undisso-
ciated ammonia (NH₃) although it could of course enter as the ion pair (NH₄) and (OH). \(\textit{Haliycystis}\) shows no exception to this tendency; with the increase of undisso-
ciated ammonia in the sea water applied to cells, both the pH and the ammonia content of \(\textit{Haliycystis}\) sap increase.

(a) Increase of Ammonium Salts.—It seems unlikely that the in-
crease of ammonium salts as such in the cell produces the observed effect on p.d. If the p.d. were due to the mobility of ammonium ions it is difficult to imagine any mechanism or system which would give rise at the threshold to any very sudden increase of ammonium inside (necessarily nearly 50-fold to give 100 mv. potential change). Nor is such a sudden rise detected in the sap. Instead, the total ammonium increases in a regular manner, apparently much as in

\(^7\) \textit{Cf.} Cooper and Osterhout,\(^6\) and previous experiments of others cited in foot-
note 2 of their paper.
**Valonia**. Significant figures for the increase in *Halicystis* cannot be given, because in the cells available for such experiments a considerable and variable amount of ammonium salt (up to 0.005 or even 0.01 M by Nessler test) was already present in the sap, rendering any accumulation study uncertain. But this natural presence of ammonium salts in the sap is in itself an indication that ammonium ions are not concerned in the reversal. If it be assumed that the p.d. suddenly reversed above a critical concentration of ammonium salt in the sap, this should occur sooner, and at a lower threshold concentration of NH₄Cl outside, in those cells already containing considerable ammonium. This is not the case, the threshold being no lower for such cells than for those containing little or no ammonium. If anything, it is a little higher.

Finally, the ammonium salt content of the sap may be experimentally increased, by the method of vacuolar perfusion which will be described in a later paper. Concentrations of NH₄Cl as high as 0.1 M or even 0.5 M have been thus produced in the sap, without causing a reversal of potential, as long as the normal pH of the sap was maintained. In fact the positive p.d. was slightly increased, as with perfusion of KCl. P.D. reversal does not therefore seem to be due to the increase of ammonium ions in the sap.

(b) Increase of pH.—At first glance, the S shape of the NH₄Cl-p.d. curve (Fig. 5) suggests that it might be explained as an electro-

In this respect these cells differed remarkably from those used for the sap analysis previously reported (Blinks and Jacques) which showed practically no ammonia. The reason for this is apparently the age and condition of the cells. Those used for the previous sap analyses were all very young, small cells collected early in the spring before reproduction had occurred and probably of the same year's growth. Those available for the present work were collected later in the spring, and in the fall, and had all undergone several or many reproductive periods. In this species the gametes are frequently not discharged normally to the exterior, but escape into the vacuole, where they swim for a while in the sap, then sink to the bottom in a dark mass. This mass apparently breaks down, with ammonia as one of its products, since cells containing such masses almost invariably have ammonia in the sap. The presence of this ammonia seems in no way toxic; cells from cultures containing it have lived normally in the laboratory for well over a year, growing greatly in size, and reproducing again in the spring at exactly the same day as freshly collected cells (although entirely isolated and without change of sea water for months).
metric titration curve, on the assumption that the inner surface of the protoplasm acts like a hydrogen electrode or is a membrane like a glass electrode, responding directly by changes of p.d. to changes of pH produced by the entrance of ammonia. Each increase of NH₃ outside might be like an increment of base in a titration, entering and neutralizing a certain portion of the cell's acids. The great change of p.d. at the reversal point would correspond to the neutralization point, the flatter ends to the smaller changes of pH at either side of neutralization.

Similarly, on this assumption, Fig. 2 could be interpreted as the time course of such a titration performed with a regularly increasing amount of base—in this case the NH₃ diffusing constantly into the cell as a result of its concentration gradient.

However, neither theory nor facts bear out this suggestion. In the first place we are not performing a titration when we increase the concentration of NH₄Cl in the sea water. We are correspondingly increasing the concentration of undissociated base but the total amount depends on the volume of sea water. In these experiments this volume is so much greater than that of the cells that it may be considered infinite; the sea water is also renewed from time to time, or a constant flow is maintained. Therefore at any given concentration NH₃ will continue to enter the cell until its activity is as great inside as outside. In both sap and sea water the equilibrium formula for this would be

\[
\text{NH}_3 = \frac{(\text{NH}_4^+)(\text{OH})}{K_b}
\]

or

\[
\log (\text{NH}_3) = \log (\text{NH}_4^+) + \text{pH} - pK_{ab}
\]

where \(\text{NH}_4^+\) is the activity of ammonium salt or ions; and \(pK_{ab} (= pK_w - pK_b)\) for ammonia lying at pH 9.3. For any constant \(\text{NH}_4\) concentration, therefore:

\[
\text{pH} \propto \log (\text{NH}_3).
\]

In other words, when \(\log (\text{NH}_3)\) increases in the sap, the pH will rise proportionally.

The experiments are in good agreement with this expectation.
Fig. 8 shows the actual course of pH change in the sap of Halicystis cells exposed to varying concentrations of NH₄Cl over the range well above and below the reversal threshold. For each concentration of NH₄Cl the pH of the sap rises to a nearly constant value during the course of 60 to 120 minutes. When the constant or apparent equilibrium pH value is plotted against the log of outside NH₄Cl concentration (or NH₃, taken as 5 per cent of NH₄Cl at pH 8.1) the essentially straight line of Fig. 9 results.

The plot does not have the full proportionality of 1 to 1 demanded by Equation 2, but is more nearly 4 to 5, the pH of the sap not increasing as fast as the NH₃ outside. Several factors might contribute to this. Probably the sampling of the whole sap, even after 2 hours' penetration of NH₃, does not truly represent the pH just within the protoplasm, which really governs the equilibrium; the same applies to the supposed pH of the sea water just outside the cell. The NH₃ 

There is a corresponding reversal of the pH change when cells are replaced in normal sea water. In a very regular time course the original low pH is regained although somewhat more slowly than the rise to higher values. This slower exit may account for the apparent hysteresis in the recovery of P.D.
in entering the cell must leave a more acid region of sea water just outside the protoplasm; with the best of stirring this would probably extend the thickness of the cell wall, and therefore decrease the outside \( \text{NH}_3 \) concentration. These combined effects, more acid sea water outside, more alkaline sap inside, than are shown by the gross determinations, would greatly reduce the supposed gradients. The influence of such unstirred films on accumulation has been pointed out by Osterhout.\(^9\)

Another factor which might make the pH rise less than expected would be an increase of \( (\text{NH}_4^+) \); this would cause \( (\text{NH}_3) \) to reach equilibrium at a lower pH, in accordance with equation (2). Presumably \( \text{NH}_3 \) would combine with acid when it entered the sap, and with a given concentration of acid, the amount of \( \text{NH}_4^+ \) formed could be calculated from equation (2) by the deviation of expected from observed pH, at equilibrium. Since there is already a large amount of ammonium salt in the sap of these cells, the relative amount of its increase is evidently not great, or there would be larger deviations than those found.

However, we are probably not dealing with equilibria, nor with concentration gradients alone. The continuous production of acid

by the cell tends to keep down the internal pH, and this may be stimulated to an even higher rate by the entrance of ammonia. Such a compensation has been reported by Cooper and Osterhout in the case of Valonia, where the pH of the sap eventually fell from its first high value, over longer periods in the presence of ammonia. In Halicystis there is evidence of an even prompter response of this sort, 

possibly accounting for the temporary lag of pH rise between 20 and 30 minutes exposure to ammonia, shown in Fig. 8; for the cusp following p.d. reversal (Figs. 2 to 4); and finally for the positive overshooting which occurs in the recovery from ammonia exposures (Fig. 2). All of these could be accounted for by a compensatory gush of acid production following the rise of pH produced by the entrance of NH$_3$. This would no doubt occur in the protoplasm rather than

Fig. 10. Variation of p.d. in Halicystis ovalis showing extreme exaggeration of the cusp on reversal (with 0.004 M NH$_4$Cl in sea water at pH 8.1), causing only temporary reversal, followed by recovery of positivity. This may be due to a gush of acid production by the cell, compensating the rise of pH caused by entering NH$_3$. Arrows indicate change of solution.
in the sap, and it might be in the nature of glycolysis, rather than of increased respiration. An extreme case of its possible effect is shown in Fig. 10 (for *H. ovalis*) where some such influence caused complete recovery of P.D. after a brief reversal, a lasting reversal occurring only at the next higher NH₄Cl concentration. The almost invariably occurring cusp (d of Fig. 2) might be taken as an incomplete response of this sort, but insufficient to cause complete recovery.¹¹

Some of these relations are of more interest from the viewpoint of salt accumulation than of potential difference. They are discussed at this length not so much for their bearing upon the pH and salt concentration in the sap, which is after all accessible both to analysis and to direct alteration *in vivo*, but rather as showing how any aqueous phase, separated from the sea water by a non-aqueous phase permeable to NH₃, might be expected to behave. The actual pH values of aqueous phases in the protoplasm we cannot directly determine, but if the sap can in any way be taken as representative, such pH will depend upon the original amount of ammonium salts and the buffer capacity of those phases when further ammonia enters. When the sap pH is 6.1, the pH of any given aqueous phase of the protoplasm may not be 6.1, probably is not. But we may be reasonably sure that it will bear some regular and probably linear relation to the NH₃ outside, and hence also to the NH₃ and to the pH of the sap.

Fig. 11, a combination of Figs. 5 and 9, summarizes at a glance the relation of the change in sap pH, which is proportional to log (NH₄Cl), or log (NH₃), in sea water; and the P.D., which passes through an abrupt inflection when the sap pH increases from 6.0 to 6.5.

We may now ask if in practice, such a change of pH could give rise to the S-shaped P.D. curve. The answer, based upon perfusing new solutions directly through the vacuole, is in the affirmative. When freshly extracted natural sap is made more alkaline, e.g. brought to

¹¹ The cusps might be interpreted as the somewhat similar ones caused by applying KCl to *Valonia* were interpreted by Damon: ⁵ as due to an advancing concentration boundary striking a surface of the protoplasm and then passing across it. Such an ammonium ion boundary might be formed within the protoplasm, due to the entrance of HN₃, but the absence of very large ammonium ion effects either on the outer or vacuolar surface of the protoplasm seems to rule out this explanation.
pH 7 or 8 by the addition of a trace of NaOH, and then perfused into
the vacuole of an impaled cell, practically the same results are ob-
tained as when NH₄Cl above threshold concentrations is applied
outside. The P.D. rapidly reverses from positive to negative, and
stays reversed as long as the pH is kept this high. But since natural
sap contains, as we have seen above, varying amounts of ammonium
salt (often well above 0.001 M, the average outside threshold) NH₃
might have passed out from the sap into the protoplasm when the
pH was raised, and produced its effects within the protoplasm exactly

![Combined plot of the relations between concentration of NH₄Cl in
sea water, log (NH₃) in sap and sea water, pH of sap, and P.D. across
protoplasm of Halicystis Osterhoutii. The abrupt inflection of the P.D. curve
is seen to be reflected in none of the other variables.](image)

as if derived from the sea water. Fortunately it was eventually found
possible to perfuse artificial sap and even sea water through the
vacuoles. The P.D. remained positive and nearly normal as long
as these were maintained at pH 5; and they could be circulated for an
hour or more, in order to make sure that most of the original sap had
been washed out, with its ammonium salt. Then when more alkaline
artificial sap or sea water was perfused, the typical reversal of P.D.
still occurred. Furthermore, the pH at which the reversal occurred
coincided remarkably well with that produced inside by the applica-
tion of threshold concentrations of NH₄Cl outside; namely, between pH 6.0 and 6.5. Thus in one case the P.D. stayed normally positive with perfusion of sea water buffered at pH 6.0, but reversed with continued perfusion at pH 6.2. The ammonia effects seem therefore to be accounted for, qualitatively at least, by the pH change occurring in the sap. A complete description of these perfusion experiments, and a discussion of the possible systems upon which such a change of pH might operate to reverse the potential, will appear in a forthcoming paper.

SUMMARY

The nature and origin of the large "protoplasmic" potential in Halicystis must be studied by altering conditions, not only in external solutions, but in the sap and the protoplasm itself. Such interior alteration caused by the penetration of ammonia is described. Concentrations of NH₄Cl in the sea water were varied from 0.00001 M to above 0.01 M. At pH 8.1 there is little effect below 0.0005 M NH₄Cl. At about 0.001 M a sudden reversal of the potential difference across the protoplasm occurs, from about 68 mv. outside positive to 30 to 40 mv. outside negative. At this threshold value the time curve is characteristically S-shaped, with a slow beginning, a rapid reversal, and then an irregularly wavering negative value. There are characteristic cusps at the first application of the NH₄Cl, also immediately after the reversal.

The application of higher NH₄Cl concentrations causes a more rapid reversal, and also a somewhat higher negative value. Conversely the reduction of NH₄Cl concentrations causes recovery of the normal positive potential, but the threshold for recovery is at a lower concentration than for the original reversal. A temporary overshooting or increase of the positive potential usually occurs on recovery. The reversals may be repeated many times on the same cell without injury.

The plot of P.D. against the log of ammonium ion concentration is not the straight line characteristic of ionic concentration effects, but has a break of 100 mv. or more at the threshold value. Further evidence that the potential is not greatly influenced by ammonium ions is obtained by altering the pH of the sea water. At pH 5, no
reversal occurs with 0.1 M NH₄Cl, while at pH 10.3, the NH₄Cl threshold is 0.0001 M or less. This indicates that the reversal is due to undissociated ammonia.

The penetration of NH₃ into the cells increases both the internal ammonia and the pH. The actual concentration of ammonium salt in the sap is again shown to have little effect on the pD. The pH is therefore the governing factor. But assuming that NH₃ enters the cells until it is in equilibrium between sap and sea water, no sudden break of pH should occur, pH being instead directly proportional to log NH₃ for any constant (NH₄⁺) concentration. Experimentally, a linear relation is found between the pH of the sap and the log NH₃ in sea water. The sudden change of pD must therefore be ascribed to some system in the cell upon which the pH change operates. The pH value of the sap at the NH₃ threshold is between 6.0 and 6.5 which corresponds well with the pH value found to cause reversal of pD by direct perfusion of solutions in the vacuole.