THE ACCUMULATION OF ELECTROLYTES

I. THE ENTRANCE OF AMMONIA INTO VALONIA MACROPHYSA

BY WM. C. COOPER, JR., AND W. J. V. OSTERHOUT

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

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Experiments on the penetration of NH₃ into Valonia macrophysa, carried out in Bermuda in 1924–27, are described in the present paper. The interpretation of these results will receive further discussion in subsequent articles.

In order to ensure uniformity of material and of sea water a large number of cells, collected at the same time, were kept under the same conditions and all the sea water used for these cells and for the experiments was collected at the start and kept in carboys. The average volume of the cells was about 0.5 cc.

The experiments were carried out in stoppered bottles each containing 2400 cc. of solution with about 50 cells in each bottle. When the bottles were placed in the ocean they were fastened beneath a specially constructed raft so that the light received was mostly reflected from the whitish coral sand of the bottom. In the experiments in the laboratory direct sunlight was avoided. Growth and absorption of ammonia were more rapid in the ocean.

The temperature in the ocean varied from 20° to 25°C., but in the course of any one experiment the variation was not more than 2° or 3°C., except in those lasting several weeks. In experiments carried on in the laboratory the variation was much greater.

The NH₄Cl was added to the sea water in the form of a solution, 0.6 molar or normal in NH₄Cl; the pH value of the sea water was adjusted by the addition of HCl or NaOH. After exposure to the sea water containing NH₄Cl, the sap was collected by means of a pipette having a sharp point which was thrust into the cell (in order to avoid loss of NH₃ by suction, the sap was forced into the pipette with the fingers). Care was taken in collecting the sap to use some large and some small cells so that the sap¹ could be considered as taken from an average cell of about 0.5 cc. When sufficient sap had been extracted, it was blown out into a small beaker and covered. Two 5-cc. samples (for zero point determinations there were three) were pipetted out for sodium-potassium determinations, two 1-cc. samples for

ammonia, two 1-cc. samples for halide, and a single 1-cc. sample for pH and sulfate. The samples for sodium-potassium analysis were stored in quartz, the others in pyrex vessels. The samples for ammonia determination were at once acidified to prevent escape of NH₃.

Analysis of the various samples was made according to standard procedures: potassium was determined as potassium perchlorate (accurate with quartz vessels, to within 0.1 per cent): sodium by indirect analysis as sodium chloride (accurate to within 1 per cent); ammonia (total) by colorimetric determination as NH₄I·H₂O after distillation of the ammonia from the strongly alkaline solution and subsequent Nesslerization (distillation was necessary because the turbid condition of the Nesslerized raw sap made color estimation exceedingly difficult); halide was determined by the Volhard method; the pH values were determined colorimetrically, allowing for the salt error by adding NaCl to the standard buffer solutions.

For the volume measurements, each cell was carefully dried with cloth and filter paper and weighed rapidly to milligrams, then placed in 0.005 M NH₄Cl sea water in a specific gravity bottle which was then immersed in a thermostat and allowed to come to a temperature of 25°C. The bottle was then dried and weighed to milligrams. From these weighings the cell volume was calculated. Such cells were photographed in groups (under water) to facilitate future identification, the volume of each being noted on the photograph.

When NH₄Cl is added to the sea water it soon appears inside the cell, where its concentration may become over 40 times as great as the sea water, thereby lessening the specific gravity of the sap so much that the cells, leaving their normal situation on the bottom, rise to the surface, where they continue to grow. The change in the specific gravity of the sap is shown in Fig. 1.

Experiments were made to determine the rate of penetration at

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4 The change is more rapid when the cells, enclosed in bottles, are placed in the ocean; this may be due in part to light (cf. Hoagland, D. R., and Davis, A. R., *J. Gen. Physiol.*, 1923-24, 6, 47; Hoagland, D. R., Hibbard, P. L., and Davis, A. R., *J. Gen. Physiol.*, 1926-27, 10, 121.
various pH values. Since the initial rate is most easily interpreted the experiments were limited to 10 minutes which is as short a period as is practicable.

In each bottle containing 120 cc. of solution were placed 6 cells (each cell containing about 0.2 cc. of sap). Cells were selected as far as possible of the same size and shape. The bottles were placed on a slowly revolving wheel to keep the contents stirred. Ordinarily this treatment produced no injury which could be detected during the experiment or by subsequent observation (during several days) of cells kept in sea water. The ammonia (i.e., $\text{NH}_3 + \text{NH}_2\text{OH} + \text{NH}_4^+$) of the sap of untreated cells (about 0.0006 $\mu$) was subtracted from the ammonia values of the sap of treated cells to give the values shown in Fig. 2. The pH value of the sea water was adjusted by adding NaOH or HCl; the total ammonia of the sea water was kept constant.

The results are shown in Fig. 2 and it is evident that as the concentration of the undissociated base (i.e., $\text{NH}_3 + \text{NH}_2\text{OH}$) increases the

![Graph showing the decrease in specific gravity of the sap during the penetration of ammonia from sea water at pH 8.3 containing 0.005 $\mu$ NH$_4$Cl.](image)
rate of penetration of ammonia (total) also increases. But it is not easy to determine just how closely they agree. If we knew the rate of penetration when the total ammonia (i.e., NH$_3$ + NH$_4$OH + NH$_4^+$) is present as the undissociated base (NH$_3$ + NH$_4$OH) it would be easy to see whether the rate is reduced to one half when the concentration of

![Graph showing the increase in concentration of total ammonia in the sap after an exposure of 10 minutes (at 21° ± 1°C.) to sea water containing 0.01 M NH$_4$Cl at various pH values. Broken line, observed values; continuous line, calculated curve for dissociation of NH$_4$OH + NH$_3$ in distilled water \( \frac{(NH_4^+)(OH^-)}{(NH_4OH) + (NH_3)} \approx K_B = 10^{-1.74} \). Since it happens that the inflection points of both curves lie at approximately pH 9.26 they have been made to coincide there: at that point the ordinate of the observed curve is 0.00275 M and this point is taken as 50 per cent of the calculated dissociation curve (whose inflection point and 50 per cent dissociation point coincide).

the undissociated base is reduced to one half, but unfortunately we cannot safely perform experiments at a sufficiently high pH value to ensure that practically all of the ammonia is present in undissociated form (even at pH 10.1, which is as high as we have gone, less

5 Experiments with dead cells show little or no effect of pH value on penetration.
than 87 per cent of the total ammonia is regarded as present as the undissociated base).

It seems probable that in the absence of complicating factors the curve might resemble the equilibrium curves for H₂S and CO₂, so that the inflection point would occur where the rate of entrance and the per cent of undissociated base was one half the maximum. We find that the point of inflection of the rate curve occurs at pH 9.25, which corresponds fairly well to the point (9.26) at which one half the total ammonia is in the form of the undissociated base (NH₃ + NH₄OH) as calculated from conductivity data of solutions of ammonia in distilled water. This would suggest that the initial rate of entrance reaches 50 per cent of its maximum when the undissociated base reaches 50 per cent of its maximum concentration.

In order to compare the two curves we have made them coincide at their inflection points as shown in Fig. 2.

We should expect the point of inflection of the dissociation curve to occur at a somewhat higher pH value in sea water on account of the greater ionic strength. In order to determine approximately this value for sea water NH₃ was circulated in a closed glass vessel (designed by Dr. Grinnell Jones to whom our thanks are due for allowing us to use his invention in advance of publication), containing on one side ammoniacal sea water and on the other, distilled water to which sufficient NaOH had been added to bring the pH value above 12 under which conditions all of the ammonia exists as the undissociated base (NH₃ + NH₄OH). The total ammonia was kept constant and the pH value of the sea water was varied by adding HCl or NaOH; after equilibrium had been attained at any given pH value a sample was taken of the ammoniacal water in equilibrium with the ammoniacal sea water and the ammonia determined by colorimetric comparison. We therefore made measurements which gave a curve indicating that the inflection point, i.e., the point where half the total ammonia is in the form of the undissociated base, is in the neighborhood of pH 9.5.

If the rate of entrance of ammonia is proportional to the external concentration of the undissociated base we might expect the curves to coincide, but this could be true only if the internal pH value remained constant. If, for example, the pH of the sap were kept constant at 12

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7 This gives \( K_A = 10^{-14} \), \( K_B = 9.26 \) and \( K_B = 4.74 \).
so that the entering ammonia did not dissociate inside, there would be no complications, but with sap at lower pH values a part of the entering \( \text{NH}_3 \) would be transformed to ions and, therefore, the back pressure, or the tendency of the undissociated base to return to the sea water, would be diminished. In other words, the rate of penetration of ammonia would be a function not only of the external pH but of the internal pH as well.\(^9\)

Our experiments show that the entrance of \( \text{NH}_3 \) raises the internal pH value rapidly (Fig. 3), this effect being greater the higher the concentration of undissociated ammonia in the sea water. Hence we expect the rate to be relatively higher at the lower pH values of the sea water because under these conditions there is a smaller penetration of ammonia and consequently less effect on the pH of the sap (normally about 5.8). This would increase the ordinates of the curve progressively toward the left\(^9\) and consequently make the slope of the curve less steep, i.e., like that of the observed curve.\(^11\)

The fact that the initial rate of penetration increases when the concentration of undissociated molecules increases suggests that it is chiefly undissociated molecules which penetrate, but it has been pointed out elsewhere that this does not necessarily follow.\(^12\) It does indicate that the penetrating substance is \( \text{NH}_3 \) or \( \text{NH}_4\text{OH} \) or the ion pair \( \text{NH}_4^+ + \text{OH}^- \), rather than \( \text{NH}_4\text{Cl} \) whose rate of entrance would not be expected to increase as the external pH value increases.

That the substance penetrating at the start is largely \( \text{NH}_3 \) (or

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\(^10\) We should expect a similar effect if penetration depended on chemical combination of undissociated molecules with some constituent of the protoplasm (or on absorption), since the amount of the compound formed would determine the rate of penetration and this would increase more and more slowly as the external concentration of undissociated molecules increased.

\(^11\) This can easily be seen by making the ordinates of the dissociation curve progressively larger as we go toward the left and then multiplying all the ordinates by the same factor so as to make the maximum ordinate the same as for the dissociation curve.

\(^12\) This results from the fact that if the ion pair \( \text{NH}_4^+ + \text{OH}^- \) enters, its penetration is proportional to the ionic activity product \( (\text{NH}_4)^+ \times (\text{OH}^-) = K(\text{NH}_4\text{OH}) \), so that as the penetration of the indissociated base increases that of the ion pair will also increase. Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1929–30, 13, 261.
Fig. 3. Time curves showing the increase of pH value of sap during penetration of ammonia from sea water, at pH 8.1, containing 0.005 M NH₄Cl.

Fig. 4. Time curves showing increase of total ammonia during penetration from sea water (at pH 8.3) containing various amounts of NH₄Cl.
NH₄OH) rather than NH₄Cl is rendered quite certain by the data given in Fig. 3, showing the rise in pH value as the total NH₃ in the sap increases (for it is evident that the penetration of NH₄Cl would not produce such an effect). The pH value of the sap soon reaches a maximum¹³ and may subsequently fall off in a gradual manner.

As has been said already, the increase of undissociated ammonia does not raise the rate of penetration of ammonia as much as would be expected if there were no change in the internal pH value. The time curves in Fig. 4 bear out this statement. These curves do not reach a true equilibrium, for the cell keeps on growing; furthermore, when the concentration of NH₃ in the sap reaches a certain point (less than 0.3 M) injury causes it to come out.¹⁴

¹³ With 0.001 M NH₄Cl in the sea water this is 6.6, with 0.01 M NH₄Cl it is 8.2, the pH value of the sea water being 8.0.
Fig. 5 shows that as the total ammonia in the sap increases, the concentration of potassium in the sap falls off, and measurements of volume\textsuperscript{18} indicate that the decrease was not due merely to the absorption of water but that some potassium actually diffused out of the cell. The behavior of Na is not yet certain: further experimentation is in progress.

Fig. 5 also shows that the initial entrance of ammonia is accompanied by an increase in the concentration of halide, which soon reaches a fairly constant value dependent on the external pH. In the absence of ammonia in the sea water, there is no such variation in the halide of the sap with change in the external pH.

It may be added that the growth of the cells is increased by the addition of NH\textsubscript{4}Cl to the sea water.

**SUMMARY**

When 0.005 M NH\textsubscript{4}Cl is added to sea water containing cells of *Valonia macrophysa* ammonia soon appears in the sap and may reach a concentration inside over 40 times as great as outside. It appears to enter as undissociated NH\textsubscript{3} (or NH\textsubscript{4}OH) and tends to reach a pseudoequilibrium in which the activity of undissociated NH\textsubscript{3} (or NH\textsubscript{4}OH) is the same inside and outside.

When ammonia first enters, the pH value of the sap rapidly rises but it soon reaches a maximum and subsequently falls off. At the same time there is an increase of halide in the sap which, however, does not run a parallel course to the ammonia accumulation, but it comes to a new equilibrium value and remains constant.

The increase in NH\textsubscript{3} in the sap is accompanied by a decrease in the concentration of K.

As NH\textsubscript{3} enters the specific gravity of the sap decreases and the cells rise to the surface and continue to grow as floating organisms. The growth of the cells is increased.

\textsuperscript{18} The cells grow faster when NH\textsubscript{3} is added to the sea water.