THE KINETICS OF PENETRATION

XVI. THE ACCUMULATION OF AMMONIA IN LIGHT AND DARKNESS

By A. G. JACQUES

(From the Laboratories of The Rockefeller Institute for Medical Research, New York, and The Bermuda Biological Station for Research, Inc., Bermuda)

(Accepted for publication, December 30, 1938)

In a former paper 1 the accumulation of ammonia 2 by Valonia macrophysa, Kütz., was discussed. In the present paper we compare the rates, and steady states, in cells exposed to sea water containing ammonia in light 3 (regular succession of daylight and darkness) and in the dark. At the same time the pH changes have been examined by an improved method which avoids the loss of gas from the sap.

The experiments were carried out in Bermuda in the winter of 1937–38 at the Bermuda Biological Station for Research.

EXPERIMENTAL

In these experiments the cells were exposed in large jars to the sea water containing ammonia. In the dark experiments the glass jars were made dark by layers of adhesive tape covered with a thick layer of either black paint or a very opaque aluminum paint. In some cases, however, stoneware jars were used. In all cases the jars were stoppered and were immersed up to their necks in flowing salt water from the Biological Station salt water system. The temperature remained fairly constant at 17°C. ± 1°C. during the course of the experiments.

Analyses for potassium and sodium were each carried out on 0.1 ml. samples as described in previous papers 1 except that in place of the Kuhlmann micro balance, a Becker semi-micro balance was used. Weighings were made to 0.02 mg. which in most cases represented a weighing error of less than 0.5 per cent. This accuracy was quite sufficient in view of the variability of the material.

The ammonia analyses were carried out by means of Nessler's reagent using the method described in the previous paper. The quantity of sample varied from

---

2 To prevent misunderstanding we shall define ammonia as NH₃ + NH₄⁺ + NH₂OH; i.e., the total amount found by analysis. We shall speak of NH₃ as undissociated ammonia.

501
2 ml. to 0.1 ml., care being taken to operate always on the linear portion of the
extinction coefficient-concentration calibration curve. This required in many
cases dilution of the sample to 104 ml. in place of the usual 52 ml.

The volume was determined on selected groups of a few cells believed to be
representative of all the cells used in an experiment.

The pH of the sap was determined by a new method. It has been noticed
repeatedly in the past that the pH of the sap rises immediately after extraction.
This seems to be connected in part with the loss of carbon dioxide, and the process
is hastened by anything which furnishes nuclei on which the gas bubbles can form.
Metal surfaces fall in this class and mere passage of the sap through a stainless
steel hypodermic needle, during extraction, is sufficient to change the pH appreciably.
In the new method the loss of gas was avoided. Tuberculin syringes of
pyrex glass, usually of 0.25 ml. capacity, were used. These were fitted with
all-glass needles, and each syringe had a small pyrex glass bead in it to mix the
contents after the sap and indicator had been drawn in.

The procedure was as follows. With piston pushed in a cell was impaled and
its sap was forced into the syringe by squeezing the cell. This carried the small
amount of air in the needle into the syringe and also served to wet the barrel and
piston and to move the piston out. The piston was then pushed in, expelling
the air and filling the needle with sap, which might, however, have lost some of
its gas during its brief exposure to the air. Another charge of sap was then forced
into the syringe pushing the piston but no air ahead of it. This and one more
charge were used to wash out the syringe and the charge of sap to be tested was
then forced in. The required amount of indicator was then drawn into the needle
and was forced into the barrel by impaling another cell and squeezing the sap
into the needle. The charge of sap and indicator, still free of air bubbles was
then mixed by means of the glass bead. By this procedure we obtained a sample
of sap mixed with indicator, which had not been exposed either to air or to a metal
needle after extraction.

The pH could be determined by comparing the test syringes with buffers and
indicators in similar syringes, but we wished to keep on using the Hellige double-
 wedge colorimeter which has been so satisfactory. Accordingly a special carrier
was devised to hold the syringe in the colorimeter in approximately the position
usually occupied by the conventional glass cell. A simple plano-convex lens was
placed between the syringe and the eyepiece. This had the effect of spreading
the emergent beam from the syringe so that only a narrow band passing through
the middle of the syringe was viewed. Since the syringe is cylindrical the in-
tensity of the color of the band necessarily decreases from the center. But by
selecting only a narrow band the decrease in color to the edges of the field of view
was made inappreciable.

The ordinary tuberculin syringe has two other disadvantages. The figures
etched on the barrel must be kept out of the field by turning the syringe until
they are out of way, and the inner surface of the barrel is ground so that even
when it is filled with liquid some scattering occurs. This has the effect of slightly
reducing the brilliance of the color, but one soon learns to discount this slight effect. Doubtless the difficulty could be overcome by using syringes made of Jena KPG tubing which is so accurately fabricated that barrel and piston do not have to be ground to a fit. The pH's were read off a calibration curve constructed by means of buffers, treated in the syringe in the same way as the unknown sample.

RESULTS

Four experiments comparing the rates of accumulation in normal light\(^3\) and darkness are discussed.

*Experiment 1.*—Cells were exposed to sea water containing 0.00175 m ammonia at the normal pH of sea water.\(^4\) The results of this experiment are given in Table I and Fig. 1. The behavior of sodium and potassium will be considered in detail. In this experiment the pH was determined parallel with the ammonia changes by the method described in this paper. The results of the pH measurements together with the rate of increase of ammonia have been plotted in Fig. 3. Since the method is rather wasteful of cells the pH was not determined in Experiments 2, 3, and 4.

*Experiment 2.*—Cells were exposed in light and dark to sea water containing 0.0025 m ammonia at normal pH. The results are given in Fig. 1.

*Experiment 3.*—Cells were exposed in light and dark to sea water containing 0.0025 m ammonia at normal pH. The dark group was subjected in this experiment to extremely dark conditions. The results are given in Fig. 2.

*Experiment 4.*—As in Experiment 3, except that before exposure to sea water containing ammonia the cells of both groups were subjected to a preliminary period of darkness in ordinary sea water. (See Fig. 2.) Before considering the rates of entrance let us consider the pH measurements. From Fig. 3 it appears that soon after the cells were exposed in normal light to the sea water containing ammonia the pH rose by a rather small but quite definite amount (by 0.4 pH), and that the new pH level at pH 6.10 was maintained until the end of the experiment. In darkness also the pH rose promptly though not quite

\(^3\) Normal light means the regular daily succession of light and darkness.

\(^4\) The pH of the body of the sea water was 8.2 but it varied in the immediate neighborhood of the cells depending on illumination, etc.
so much as in the light. But in this case a slow decrease then followed and at the end of the experiment the pH of the sap was about that of normal sap, in spite of the fact that the sap now had 0.05 M

NH\textsubscript{4}\textsuperscript{+} and had lost a corresponding concentration of potassium. It appears then that there is little correlation between the concentration of ammonia in the sap and the pH. This is particularly clear in the

\begin{table}
\centering
\caption{Accumulation of Ammonia in Sea Water Containing 0.00175 M Ammonia (Experiment 1)}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Days & Volume & Concentration & \(P'_L\) & \(P''_L\) \\
& cc. & \textit{X} & \textit{X} & \textit{X} & \\
\hline
Light group & & & & & \\
0 & 1.98 & 0.0006 & 0.4846 & 0.1475 & \\
2.5 & 2.01 & 0.0274 & 0.4770 & 0.1520 & 0.0039 & 0.0027 \\
5 & 2.02 & 0.0493 & 0.4420 & 0.1576 & 0.0035 & 0.0026 \\
7 & 2.04 & 0.0600 & 0.4274 & 0.1600 & 0.0031 & 0.0023 \\
10 & 2.06 & 0.0850 & 0.4013 & 0.1649 & 0.0031 & 0.0026 \\
13 & 2.09 & 0.0978 & 0.3780 & 0.1786 & 0.0027 & 0.0024 \\
15 & 2.10 & 0.1178 & 0.3725 & 0.1692 & 0.0029 & 0.0028 \\
20 & 2.13 & 0.1339 & 0.3400 & 0.1779 & \\
26 & 2.16 & 0.1319 & 0.3143 & 0.1832 & \\
\hline
Dark group & & & & & \\
\hline
\multicolumn{2}{|c|}{Adjusted volume\textsuperscript{*}} & \textit{X} & \textit{X} & \textit{X} & \textit{X} \multicolumn{2}{|c|}{\textit{P'}_D \quad P''_D} \multicolumn{2}{|c|}{\textit{P'}_D \quad P''_D} \multicolumn{2}{|c|}{\textit{P'}_D \quad P''_D} \\
\hline
0 & 1.98 & 0.0006 & 0.4846 & 0.1475 & \\
2.5 & 1.97 & 0.0184 & 0.4766 & 0.1425 & 0.0097 & 0.0076 \\
5 & 2.00 & 0.0253 & 0.4628 & 0.1524 & 0.0067 & 0.0058 \\
7 & 1.94 & 0.0286 & 0.4729 & 0.1429 & 0.0077 & 0.0050 \\
10 & 1.97 & 0.0395 & 0.4760 & 0.1560 & 0.0054 & 0.0062 \\
13 & 2.01 & 0.0464 & 0.4515 & 0.1516 & 0.0049 & 0.0077 \\
15 & 1.98 & 0.0477 & 0.4509 & 0.1539 & 0.0043 & 0.0073 \\
20 & 2.02 & 0.0494 & 0.4361 & 0.1572 & 0.0035 & 0.0067 \\
26 & 2.02 & 0.0518 & 0.4366 & 0.1415 & \\
\hline
\textsuperscript{*} Adjusted by multiplying the measured volume in the dark group by 1.98 + 1.80 so as to make it possible to start light and dark group "moles" curves at the same zero point.
\end{tabular}
\end{table}
Fig. 1. Accumulation of ammonia in light and darkness. Experiments 1 and 2. The curves are drawn free-hand to give an approximate fit.
dark group where after the first rapid pH rise the ammonia concentra-
tion and the pH moved in general in opposite directions.
These results contradict effectively a suggestion made in a former
paper that the presence of a relatively high concentration of ammonia
in the sap results in the buffering of the sap by ammonium salts at a
somewhat higher level than that of normal sap. It now seems much
more likely that the change in pH may be associated with relatively
small amounts of basic ammonia in the sap.

---

**Fig. 2.** Accumulation of ammonia in light and extreme darkness. Experiments 3 and 4. The curves are drawn free-hand to give an approximate fit.

In the accumulation of ammonia, tending to raise the pH of the sap,
we suppose that we have the entrance of ammonia as NH₄X, a form
equivalent to entrance as a base since each molecule of NH₄X is
decomposed at the sap-protoplasm interface by an acid stronger than
ILX (probably CO₂) thus,

\[ \text{NH}_4^+ + X^- + H^+ + HCO_3^- \rightarrow (\text{NH}_4)^+ + \text{HX} + \text{HCO}_3^- \]

---

\[ ^4 \] Osterhout (Osterhout, W. J. V., *Proc. Nat. Acad. Sc.*., 1935, 21, 125) has suggested that ammonia entrance is preceded by a reaction between ammonia in the sea water and an acid HX in the protoplasm.
Since $HX$ is by assumption a very weak acid nearly insoluble in water it does not figure in the pH of the sap. And tending to lower the pH we have (a) the loss of potassium as $KX$ which is equivalent to the loss of a base, since $KOH$ from the sap unites with $HX$ in the protoplasm to form the $KX$. (b) The elaboration in the protoplasm of water-soluble acids which pass into the sap. Carbon dioxide is the most conspicuous of these but others probably play a part. This process which goes on constantly is in part offset by the outward diffusion of $CO_2$ from the sap to the sea water. In the light photo-

![Figure 3](https://example.com/fig3.png)

**Fig. 3.** Change of pH of sap during accumulation in light and in darkness. The curves are drawn free-hand to give an approximate fit.

synthesis, which we assume to be confined chiefly to the sea water-protoplasm interface, removes the $CO_2$ there and as a result the gradient of concentration of $CO_2$ from the sap to the sea water is increased. This results in a more rapid loss of $CO_2$.

The pH of the sap is thus the resultant of several processes. The mixture of buffer systems is probably quite complex. But we may say confidently that the increase of free base will raise the pH and the decrease of base will decrease it. Such a change might possibly be brought about by the replacement of $KOH$ by an equivalent amount
of NH₃ with the system moving towards a higher pH due to the introduction of a new buffer system. Instead we believe that the results are more readily explained by assuming that the pH rise in both the light and dark groups depends on the rapid entrance of ammonia in a form equivalent to the base, without the loss of a corresponding amount of potassium as base. In the accumulation of ammonia we notice that in general at the very start of the process the total concentration of cations in the sap increases a little. We associate this in part with the gain of some ammonia without an equivalent loss of potassium.

In both groups the same process operates to raise the pH at the start, but in the dark group because the CO₂ diffuses out of the sap more slowly than in the light the effect of the entrance of the excess of ammonia over the potassium lost, tends to be nullified. Hence the slow decrease of pH.

We must now consider to what extent the pH measurements are trustworthy. We have to face the fact that the sap is poorly buffered. For example, probably the buffer system of normal sap is due to sodium bicarbonate and potassium bicarbonate. But the total carbon dioxide concentration of the sap, according to Osterhout and Dorcas,⁶ is only 0.0002 M of which, at pH 5.72,⁷ one third is salt and the rest free CO₂. The final concentration of indicator used was 0.0001 M, or about half that of the buffer system. It would not be surprising therefore if the buffer equilibrium should be seriously upset by the indicator. A practical test of this possibility was made in New York by determining without gas loss the pH of a sample of sap with two concentrations of indicator. The same value was obtained in both cases. But this might mean that the buffer capacity of the indicator is too great to be upset by the addition of sap. This could hardly have been so in the present case since there was an obvious change in the color of the indicator as it was added to the sap. On the whole therefore we believe that the measurements give a true picture of the trend of the pH changes, and roughly of their magnitude.

⁷ This value was obtained in the present paper. The proportion of salt to base was calculated by the Henderson-Hasselbalch equation, using for pK', the value of 6.02 according to the latest results of MacInnes and Shedlovsky.
Considering now the rate experiments, all four lead to the conclusion that the rate of entrance of ammonia is strongly influenced by light. Thus the rate of entrance of moles (concentration $\times$ volume) was about 2.5 times as fast in light as in darkness. This was hardly a question of the greater rate of growth of the cells in light. On a concentration basis in which we ignore the difference in growth rate the rate of concentration increase was about twice as fast in two experiments, and about three times as fast in the other two.

From Experiment 3 we can draw the additional conclusion that accumulation can go on even in total darkness. In this experiment in order to rule out the possibility that any daylight at all might get to the dark group the bottles in which the cells were exposed were covered with thick layers of adhesive tape painted black. These bottles were placed in sea water in a ten gallon stoneware crock with a cover. This was kept in a room sufficiently dark to be used as a photographic dark room. To keep the cells at about the same temperature as the light group, the stoneware crock was immersed in a bath through which a flow of salt water was maintained. In spite of the absence of light the cells accumulated ammonia and the rate was comparable with that observed in Experiment 2 in which the darkness was not so complete. The rate in darkness was about half that in normal light.

The cells of Experiment 3 were transferred directly from a large collection which was kept under normal light. It seemed not impossible therefore that they might have stored energy during this period which would be available for accumulation for a considerable period after the withdrawal of the light. In order to minimize this factor, if it should exist, the cells of Experiment 4 were first subjected to a preliminary exposure to normal sea water in total darkness for 14 days. This treatment had no visible effect on the rate of accumulation in the dark. In the light accumulation took place somewhat faster than in Experiment 3. However, Experiments 3 and 4 are not directly comparable and we cannot say definitely that the preliminary dark treatment rendered the cells capable of accumulating ammonia faster in the light.

All the curves for the accumulation of ammonia in Fig. 1 flatten out towards the end of the exposure. In the light where the cells continue
to grow we should expect the moles of ammonia to continue to increase. However, at best the growth is slow and the error of the volume measurements and the natural variation of the sap samples are such that when the rate of ammonia entrance has become slow some uncertainty in the location of the points is to be expected. The simplest interpretation of the flattening is that the concentration of ammonia in the sap has reached a constant level, and that thereafter as the volume increases slowly, ammonia is taken in slowly to keep the concentration constant.

The approach to constant ammonia concentration during ammonia accumulation is also clearly foreshadowed in the curves for Experiment 3 (Fig. 2) and in a curve in a previous paper. But there is little or no flattening of the curves of Experiment 4. This may mean merely that the experiment was terminated before the onset of the flattening. On the other hand it may be connected with the preliminary dark treatment. This point will be the subject of further investigation.

The flattening of the curve suggests the approach to an equilibrium or rather, since we are dealing with a living system, to a “steady state.”

For equilibrium we may write that

\[ f_o^{NH_3} [NH_3]_o = f_i^{NH_3} [NH_3]_i \]

where \( f \) is the activity coefficient, \([NH_3]\) is the concentration of undissociated ammonia, and \( o \) and \( i \) refer to the sea water and sap respectively.

Assuming that \( f_o^{NH_3} = f_i^{NH_3} \) the equation reduces to

\[ [NH_3]_o = [NH_3]_i \]

It can be shown that with certain assumptions the same equation should fit the steady state.

The derivation is as follows. According to Osterhout ammonia may pass through the non-aqueous protoplasm as \( NH_4X \) where \( X \) is the anion of a weak acid elaborated in the non-aqueous layer of the protoplasm. It is assumed that

\[ \text{Jacques, A. G., and Osterhout, W. J. V., } J. \text{ Gen. Physiol.}, 1930-31, 14, 301 \text{ (Fig. 3).} \]

\[ \text{Cf. Zscheile, F. P., Jr., Protoplasma, 1930, 11, 481.} \]

\[ \text{This derivation is given in detail as it will be used as the foundation for calculations in subsequent papers.} \]
the reaction whereby NH₄X is formed takes place on the protoplasmic side of
the sea water-protoplasm or sap-protoplasm interface. We assume that the protoplasm has
the structure shown in Fig. 4. For purposes of discussion we may neglect the aqueous layer W and treat
the protoplasm as though it consisted of a single non-aqueous layer. Now at each interface there is a pair of adjacent
unstirred layers, one in the aqueous phase and the other in the non-aqueous
protoplasmic surface layer. And in each pair of such layers there are thin regions
immediately in contact where there is equilibrium across the interface. At the
sea water interface the aqueous unstirred layer is designated hereafter as op and
the non-aqueous layer as po. The thin equilibrium layers are called cop and apo.
At the sap-protoplasm interface the corresponding designations are
ip, pi, eip, and epi. (See Fig. 4.)

\[
\text{FIG. 4. Hypothetical structure of the protoplasm. } X \text{ and } Y \text{ are non-aqueous}
\text{layers; } W \text{ is an aqueous layer. The shaded areas represent unstirred layers.}
The very narrow stippled bands, bounding each interface, are extremely thin
layers which are in approximate equilibrium: some of these are labelled; i.e.,
epo, eop, epi, and eip.\]

Now at the steady state if we ignore the small amount of growth which occurs,
\[
[NH_4X]_{e_0} = [NH_4X]_{e_p}
\]
since in this condition the flux of NH₄X, which is assumed to be the only species
carrying ammonia, is zero.

But we assume that at each interface some of the NH₄X formed is transferred
to the adjacent aqueous layers, so that the following equilibria are set up
\[
[NH_4X]_{e_0} = S_{e_0}[NH_4X]_{e_0} \quad \text{and} \quad [NH_4X]_{e_p} = S_{e_p}[NH_4X]_{e_p}
\]
where \( S \) is the partition coefficient.

\[\text{Osterhout, W. J. V., } \textit{Ergebn. Physiol.}, 1933, 35, 967.\]
In the aqueous layers we have the following hydrolytic reaction,
\[
\text{NH}_4X + H_2O \rightleftharpoons \text{NH}_3\text{OH} + HX
\]
for which we can write the thermodynamic mass action equation,
\[
K_{\text{hyd.}} = \frac{(\text{NH}_4\text{OH})_{\text{eop}} (HX)_{\text{eop}}}{(\text{NH}_4X)_{\text{eop}} (H_2O)_{\text{eop}}} = \frac{(\text{NH}_4\text{OH})_{\text{eip}} (HX)_{\text{eip}}}{(\text{NH}_4X)_{\text{eip}} (H_2O)_{\text{eip}}}
\]  
where parentheses indicate activities. But
\[
\text{NH}_3\text{OH} \rightleftharpoons \text{NH}_3 + H_2O
\]
for which the thermodynamic mass action equation is
\[
k_k = \frac{(\text{NH}_3)_{\text{eop}} (H_2O)_{\text{eop}}}{(\text{NH}_3\text{OH})_{\text{eop}}}
\]  
Now
\[
[HX]_{\text{eop}} = S^{HX}_{\text{eop}} [HX]_{\text{eop}} = \frac{S^{HX}_{\text{eop}} [HX]_{\text{eip}}}{S^{HX}_{\text{eip}}}
\]
where square brackets indicate concentrations. (A similar set of equations apply to the eip-epi interface.)

Whence for the second term of equation (2) we get
\[
(NH_4X)_{\text{eop}} = S^{NH_4X}_{\text{eop}} [NH_4X]_{\text{eop}} = \frac{S^{NH_4X}_{\text{eop}} [NH_3X]_{e^{\text{ip}}}}{S^{NH_4X}_{\text{eip}}}
\]
and for the third term of equation (2)
\[
S^{NH_4X}_{\text{eip}} [NH_4X]_{\text{eip}} = \frac{[NH_4]_{\text{eip}} [HX]_{\text{eip}}}{K_{\text{hyd.}} k_k S^{HX}_{\text{eip}}} \quad (4a)
\]
or collecting constants
\[
[NH_4X]_{\text{eop}} = K_{\text{coll}} [NH_4]_{\text{eop}} [HX]_{\text{eop}}
\]
and
\[
[NH_4X]_{\text{eip}} = K_{\text{coll}} [NH_4]_{\text{eip}} [HX]_{\text{eip}}
\]
Or assuming that corresponding activity and partition coefficients are equal in sap and sea water, at the steady state
\[
[NH_4]_{\text{eop}} [HX]_{\text{eop}} = [NH_4]_{\text{eip}} [HX]_{\text{eip}}
\]  
(5)
or if there are no concentration gradients of \([\text{NH}_3]\) across the \(op\) and \(ip\) layers, as is almost certainly the case,

\[
[\text{NH}_3]_o [\text{HX}]_{ip} = [\text{NH}_3]_i [\text{HX}]_{op} \quad (5a)
\]

Now if \(\text{HX}\) is distributed equally throughout the non-aqueous protoplasm
\([\text{HX}]_{ip} = [\text{HX}]_{op}\). For purposes of calculation we may regard \([\text{HX}]_{op}\) and
\([\text{HX}]_{ip}\) as constant. We then have for the steady state,

\[
[\text{NH}_3]_o = [\text{NH}_3]_i \quad (6)
\]

If a steady state has been attained we should be able to upset it by changing \([\text{NH}_3]_o\). An experiment to determine if this could be done was carried out by exposing cells which had ceased to accumulate ammonia to (a) sea water containing more ammonia at the normal pH of sea water and (b) to sea water containing the same amount of total ammonia at a higher pH. There were six groups in this part of this experiment.

(a) Light and dark sub-groups in which cells from the light and dark groups of Experiment 1 were exposed respectively in normal light and darkness to sea water at the normal pH* containing 0.00175 \(\text{m}\) ammonium chloride. Since this involved no change in conditions these cells were regarded as controls.

(b) Light and dark sub-groups in which cells as in (a) were exposed to sea water containing 0.0025 \(\text{m}\) ammonium chloride at normal pH.

(c) Light and dark sub-groups in which cells as in (a) were exposed to sea water containing 0.00175 \(\text{m}\) ammonium chloride at pH 9.5.

As Table II shows, the concentration of ammonia in the sap increased hardly at all in the control groups, but it did increase decidedly in the others. This suggests that real steady states had been attained in both light and dark groups of Experiment 1 during the exposure of the cells to sea water, at normal pH, containing 0.00175 \(\text{m}\) ammonium chloride, since in both groups increasing \([\text{NH}_3]_o\) caused the concentration of total ammonia in the sap to increase. It is noteworthy that in the dark sub-groups (b) and (c) there is a suggestion of an approach to new steady states. This was not the case in the light sub-groups, but it may have been that in these cases new steady states would have been attained if it had been possible to continue the experiments further.
We may now compare the steady state concentrations attained in Experiment 1 with values calculated on the assumption that at the steady state \([\text{NH}_3]_o = [\text{NH}_4]^+\).

The normal pH of the sea water is 8.2 and when \([\text{Am}]_o = \text{total ammonia in sea water} = 0.00175 \text{ M}, [\text{NH}_3]_o = 5.9 \times 10^{-5}\), according to the Henderson-Hasselbalch equation.\(^{12}\) But the pH of the sap according to the measurements discussed on pages 503 to 508 was about 5.8 at the steady state. At this pH in order for \([\text{NH}_4]^+\) to be equal to \([\text{NH}_3]_o = 5.9 \times 10^{-4}],[\text{Am}],\) would have to be approximately 0.4 M.

### TABLE II

<table>
<thead>
<tr>
<th>Light group</th>
<th>Dark group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sub-group</td>
<td>Concentration ammonia in sap</td>
</tr>
<tr>
<td>(a) Control</td>
<td>(a) Control</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>(b)</td>
<td>(b)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>(c)</td>
<td>(c)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Actually it was about 0.05 M. In the light group \([\text{Am}]_i = 0.13 \text{ M} which is 6/10 of the theoretical value of \([\text{NH}_3]_o = 5.9 \times 10^{-4}\) when pH, is taken as 8.2. As a matter of fact it is almost certain that during the period of illumination at least, pH\(_{\text{app}}\) is greater than pH\(_o\) since by photosynthesis CO\(_2\) is removed from the sea water, and although with a large body of sea water in proportion to the cell volume, as was used in our experiments, this effect does not show up in the bulk of sea water, some increase of pH in the unstirred layer of

\(^{12}\)In this calculation pK\(_i\) was taken as 4.34. The reason for this choice is given in a previous paper (Jacques, A. G., J. Gen. Physiol., 1935–36, 19, 397).
sea water in the cellulose wall adjacent to the protoplasm seems inevitable in light.

Crozier\textsuperscript{13} has shown that in aquaria the photosynthesis of Valonia may raise the pH to 9.5 in sunlight and this agrees with our results when the volume of cells to sea water is not too great. Apparently at 9.5 the precipitation of calcium or magnesium carbonates or both serves to buffer the system somewhat by removing CO\textsubscript{3}\textsuperscript{2-} ion so that 9.5 is the limit to which pH\textsubscript{sap} can rise during illumination.

At this pH the maximum value of [NH\textsubscript{3}]\textsubscript{eap} neglecting loss of NH\textsubscript{3} from eap to o by diffusion would be 7.0 \times 10\textsuperscript{-4}.

But pH\textsubscript{i} according to our measurements, in the light group, was 6.10 at the end of the experiment. Hence [Am], should be 2.5 M in order for [NH\textsubscript{3}]\textsubscript{i} to be equal to [NH\textsubscript{3}]\textsubscript{eap} = 7.0 \times 10\textsuperscript{-4}. The steady state value of [NH\textsubscript{3}]\textsubscript{i}, corresponding to [NH\textsubscript{3}]\textsubscript{o}, in normal light must be somewhere between 0.21 M and 2.5 M and in any case it is greater than 0.13 M, the value found.

These calculations are only approximate since they depend on the somewhat arbitrarily selected value of 4.34 for pK\textsubscript{f}. However, calculating for the dark group where complications due to photosynthesis are absent the pH which the sap would have to have in order to make [NH\textsubscript{3}]\textsubscript{o} = [NH\textsubscript{3}]\textsubscript{i} when pH\textsubscript{o} = 8.2 and pH\textsubscript{i} = 5.8, we get pH\textsubscript{i} = 6.7. The actual value was 5.80 and the discrepancy can scarcely be explained away even by making all possible allowances for error in the pH measurements and error in the selection of the value for pK\textsubscript{f}.

It will be recalled (p. 512) that the conclusion that [NH\textsubscript{3}]\textsubscript{o} = [NH\textsubscript{3}]\textsubscript{i} at the steady state is based on the assumptions that (a) corresponding activity coefficients in sap and sea water are equal; (b) corresponding partition coefficients in sap and sea water are equal, and (c) [HX]\textsubscript{eap} = [HX]\textsubscript{eap}.

The first is almost certainly valid since the ionic strengths of sap and sea water are not far apart. In general we have assumed that (b) also is valid, but our information in this respect is vague. But in regard to (c) it is not improbable that [HX]\textsubscript{eap} > [HX]\textsubscript{eap}. Such a situation could arise if HX is elaborated only at the sap-protoplasm

\textsuperscript{13} Crozier, W. J., J. Gen. Physiol., 1918-19, 1, 581.
interface. In this case the equation governing the steady state is equation (5a) (p. 513); i.e.,

$$[\text{NH}_3]_o [\text{HX}]_{e0} = [\text{NH}_3][\text{HX}]_{e0}$$

Now according to the calculation for the dark group (p. 514), where complications due to photosynthesis are absent, $[\text{Am}]_o$ is only 1/8 of what it should be in order for $[\text{NH}_3]_o$ to equal $[\text{NH}_3]_e$. But if $[\text{HX}]_{e0}$ is only 1/8$[\text{HX}]_{e0}$, equation (5a) will be satisfied; i.e., the ratio $[\text{HX}]_{e0} / [\text{HX}]_{e0} = 0.125$.

It is assumed that all the ammonia passing through the protoplasm travels as $\text{NH}_4\text{X}$ whence the flux for cells with surface of unit area and thickness is given by

$$\frac{d[\text{Am}]}{dt} = D\text{NH}_4\text{X} \left( [\text{NH}_4\text{X}]_{e0} - [\text{NH}_4\text{X}]_{e0} \right)$$

where $[\text{Am}]$ means concentration of ammonia. $D$ is a constant for the movement of $\text{NH}_4\text{X}$ in the protoplasm which is the analogue of a diffusion constant.

When $[\text{HX}]_{e0}$ is considered to be equal to $[\text{HX}]_{e0}$ and constant this reduces to

$$\frac{d[\text{Am}]}{dt} = D\text{NH}_4\text{X} K_{\text{coll}} \left( [\text{NH}_4\text{X}]_{e0} - [\text{NH}_4\text{X}]_{e0} \right)$$

We now proceed to calculate the permeability constants from equation (8). Putting this in a familiar form for simplicity, we have,

$$\frac{dx}{dt} = P'(a - x)$$

where $a = [\text{NH}_3]_{e0}, x = [\text{NH}_3]_{e0}$, and $P'$ contains all the constants of equation (8). On integration

$$P' = \frac{2.3}{l} \log \frac{a}{a - x}$$

This is, of course, only an approximation in many cases, cf. Jacobs, M. H., Ergebn. Biol., 1935, 12, 1. According to this equation the rate is directly proportional to $\text{NH}_3 - \text{NH}_3$. But this is not necessarily true when the initial rates with different concentrations of $\text{NH}_3$ are compared (see footnote 5).
In the present case we have first applied equation (10) to the dark group of Experiment 1 (Table I). a has been calculated on the assumption that the pH\textsubscript{o} = pH\textsubscript{w} = 8.2 and [Am]\textsubscript{w} = [Am]\textsubscript{o}, whence [NH\textsubscript{3}]\textsubscript{w} = a = 5.9 \times 10^{-5} (see p. 514). x has been calculated from [NH\textsubscript{3}]\textsubscript{w} on the assumption that pH\textsubscript{w} = pH\textsubscript{i} = 5.90. The result is given in Table I under P\textsubscript{D}. It will be seen that this "constant" decreases steadily with time.

If [HX]\textsubscript{w} \approx [HX]\textsubscript{w}, we must put
\[
\frac{d[Am]}{dt} = D^{NH\textsubscript{3}X}\frac{K^{NH\textsubscript{3}X}}{[NH\textsubscript{3}]\textsubscript{w} + [HX]}\frac{[NH\textsubscript{3}]\textsubscript{w} [HX] - [NH\textsubscript{3}]\textsubscript{w} [HX]}{[NH\textsubscript{3}]\textsubscript{w} [HX] - [NH\textsubscript{3}]\textsubscript{w} [HX]}
\]
Putting this in the simplified form
\[
\frac{dx}{dt} = P''\left[\frac{b}{c} - x\right]
\]
where b = [HX]\textsubscript{w} and c = [HX]\textsubscript{w}. Since we do not know the absolute values of either b or c we multiply the right hand by c/c to get
\[
\frac{dx}{dt} = P''\left[\frac{b}{c} - x\right]
\]
where P'' = cP''. On integration we get
\[
P''' = 2.3 \frac{b}{c} \frac{\ln \frac{b}{c} - x}{a - x}
\]
This equation has been used to calculate P\textsubscript{D}'. The most natural assumption to make in calculating the coefficient b/c is that at the end of the experiment when the steady state was attained
\[
b/c[NH\textsubscript{3}]= [NH\textsubscript{3}]
\]
Assuming that pH\textsubscript{i} = 5.90 and [Am]\textsubscript{i} at the steady state was 0.0518, [NH\textsubscript{3}]= 9.0 \times 10^{-4} and this value was used to calculate the permeability constant for the dark group. By trial we found that the value 8.83 \times 10^{-6} \textsuperscript{15} for b/c [NH\textsubscript{3}]\textsubscript{i} = [NH\textsubscript{3}]\textsubscript{o}, gave a slightly better series of values for the permeability constant. These values are

\textsuperscript{15} We may not assume that at the steady state b/c \textsubscript{a} \textsubscript{a} <x, but the slight correction from 9.0 \times 10^{-4} to 8.83 \times 10^{-4} is within the limits of the natural variations of the cells.
given as $P''_D$ in Table I. Although the values are erratic there is no
definite trend and the deviations may be associated with faulty
sampling of the sap and errors in analysis.

On using the value $1.0 \times 10^{-4}$ for $b/c [\text{NH}_3]_o$, the values of the permeability constant had a marked trend. Hence we conclude that $8.83 \times 10^{-4}$ is a significant value.\(^\text{16}\) And the ratio $b/c$ is therefore approximately

\[
\frac{8.83 \times 10^{-4}}{5.9 \times 10^{-4}} \approx 0.15
\]

In the light group, Experiment 1 (Table I), we may not assume that $\text{pH}_{ap} = \text{pH}_o$ at least not during illumination. According to Crozier\(^\text{18}\) the maximum pH which can be expected due to the photosynthetic removal of CO$_2$ from the sea water is 9.5. Using this value for $\text{pH}_{ap} [\text{NH}_3]_{ap} = 1.1 \times 10^{-4}$. But the cells were, in our experiments, illuminated not more than half the time due to the onset of darkness, and in the dark period $\text{pH}_{ap}$ may be taken as equal to $\text{pH}_o = 8.2$, whence $[\text{NH}_3]_o = 5.9 \times 10^{-4}$. The average of these two values $= 3.85 \times 10^{-4}$ has been taken as the value for $a$ in calculating $P'_L$ from equation (10) and $x$ has been calculated on the basis that $\text{pH}_i = 6.15$. As Table I shows, $P'_L$ calculated in this way has an obvious drift.

We now apply equation (11) using $b/c = 0.15$ as in the dark group. $b/c a$ then equals $5.78 \times 10^{-4}$ m.

The permeability constant has been calculated on this basis. It was found to be nearly without trend but with a slight upward drift at the end. A slightly better constant was obtained by using for $b/c a, 5.9 \times 10^{-4}$. This is $P''_L$ in Table I.

Since by using the factor $b/c = 0.15$ we obtain fairly satisfactory permeability constants we assume that this ratio may have some physical significance. Provisionally we suppose that it is the ratio of $[\text{HX}]_{ap}/[\text{HX}]_{re}$. The actual concentration of each is unknown.

\(^{16}\) It might be suggested that since the pH value of the sap used in these calculations, viz. 5.90, is a compromise value, being the apparent average value of the pH during the entrance of ammonia, that we could correct $[\text{NH}_3]$ from $9.0 \times 10^{-6}$ to $8.83 \times 10^{-4}$ by assuming a slightly lower value for pH. But if this corrected value is used in calculating $x$ the constants are the same as those obtained by using $9.0 \times 10^{-6}$ and $\text{pH}_i = 5.99$.\(^\text{16}\)
If $b/c$ has the significance suggested at the steady state $x$ should be equal to $b/c\ a$. In the dark group $x_e$, where $e$ signifies the steady state, was found to be equal to $9 \times 10^{-4}$, but $b/c\ a$ was taken as $8.83 \times 10^{-4}$. This slight discrepancy is of no importance. In the light group conditions are more complicated, because owing to photosynthesis $a$ is not well known. But by taking $a$ as the probable average value of $[\text{NH}_3]_{eop}$ in light and in darkness a value for $b/c\ a = 5.9 \times 10^{-4}$ was obtained. This value gave a good series of values for the constant but $x_e$ in the light was only $4.1 \times 10^{-4}$. Although the two values are of the same order there is some discrepancy here not yet explained.\footnote{A slight uncertainty in the determination of pH$_e$ at the steady state would account for the discrepancy, but since $x$ and $x_e$ are calculated from the same value of pH$_e$, the calculated value of the constant would again show large deviations if pH$_e$ were increased enough to make $x_e = 5.9 \times 10^{-4}$.}

It appears that $P''''$ is about 3 times $P''''$. Assuming that $P''''_o = P''''_o$ we may suppose that $[\text{HX}]_{eop}$ is 3 times as great in the dark. This seems possible for we must remember that some of the HX is being lost to the sea water and this loss may well be much greater in light than in darkness due to the higher pH at the sea water-protoplasm interface when the cell is photosynthesizing actively. We assume that $[\text{HX}]_{eop}$ is also 3 times as great in the dark.

Hitherto we have assumed that the reaction is a simple reversible neutralization

$$\text{NH}_2\text{OH} + \text{HX} \rightleftharpoons \text{NH}_4\text{X} + \text{H}_2\text{O}$$

But it might be much more complex than this. This will be discussed in a forthcoming paper.

**SUMMARY**

The accumulation of ammonia takes place more rapidly in light than in darkness. The accumulation appears to go on until a steady state is attained. The steady state concentration of ammonia in the sap is about twice as great in light as in darkness. Both effects are possibly due to the fact that the external pH (and hence the concentration of undissociated ammonia) outside is raised by photosynthesis.

Certain "permeability constants" have been calculated.
indicate that the rate is proportional to the concentration gradient across the protoplasm of \( \text{NH}_4X \) which is formed by the interaction of \( \text{NH}_3 \) or \( \text{NH}_4\text{OH} \) and \( \text{HX} \), an acid elaborated in the protoplasm. The results are interpreted to mean that \( \text{HX} \) is produced only at the sap-protoplasm interface and that on the average its concentration there is about 7 times as great as at the sea water-protoplasm interface. This ratio of \( \text{HX} \) at the two surfaces also explains why the concentration of undissociated ammonia in the steady state is about 7 times as great in the sea water as in the sap. The permeability constant \( P'''' \) appears to be greater in the dark. This is possibly associated with an increase in the concentration of \( \text{HX} \) at both interfaces, the ratio at the two surfaces, however, remaining about the same.

The pH of sap has been determined by a new method which avoids the loss of gas (\( \text{CO}_2 \)), an important source of error. The results indicate that the pH rises during accumulation but the extent of this rise is smaller than has hitherto been supposed.

As in previous experiments, the entering ammonia displaced a practically equivalent amount of potassium from the sap and the sodium concentration remained fairly constant.

It seems probable that the pH increase is due to the entrance of small amounts of \( \text{NH}_3 \) or \( \text{NH}_4\text{OH} \) in excess of the potassium lost as a base.