THE KINETICS OF PENETRATION

XIV. THE PENETRATION OF IODIDE INTO VALONIA

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Former papers from this laboratory indicate that the penetration of a weak base, ammonia, and of a strong base, guanidine, is probably preceded by a chemical reaction with a constituent of the protoplasm. On the other hand the penetration of a weak acid, hydrogen sulfide, appears to take place by simple diffusion of molecular hydrogen sulfide through the non-aqueous protoplasm.

What is the method of penetration of a strong acid? The present paper is an attempt to throw light on this problem by an investigation of the rate of entrance of iodide into Valonia macrophysa, Kütz.

The choice of hydrogen iodide for this investigation was a practical necessity, since of the other available acids, nitric and hydrobromic, the former is already present in fairly large, but variable amounts, and the latter presents analytical difficulties in the presence of the high concentration of chloride ion in Valonia.

Hydrogen iodide presents some theoretical difficulties owing to the possibility of oxidation or reduction in the cell. The reversible reaction, $I_2 + 2 e \rightarrow 2I^-$, goes with the greatest ease in either direction according to conditions, while with much greater difficulty any free iodine produced may be oxidized to iodate. This will be discussed later.

On the other hand, hydriodic acid is as strong an acid as hydrochloric, and with proper care it and its salts can be determined acu-

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4 Unpublished results indicate that the concentration of nitrate ion in fresh Valonia sap is between 0.02 and 0.01 molar.
rately in very small amounts. Moreover with our method of analysis no iodide is detectable in normal Valonia sap.

EXPERIMENTAL

The rate of entrance of iodide was studied from the standpoint of the effect of concentration, pH, and light. In most cases the cells were exposed to the modified sea waters in 125 ml. bottles. Effective stirring was provided by means of stirrers described in previous papers. 5

The modified sea waters were made up by adding appropriate quantities of 0.6 molar NaI solution. The pH was adjusted by adding either 0.6 N HCl or 0.6 N NaOH and aerating, as described previously, 6 and the pH of sea water and sap was determined by means of indicators and the Hellige double wedge colorimeter with the aid of calibration curves, as reported in another paper. 7 After exposure the cells were washed in ordinary sea water, rinsed in distilled water, and dried on filter paper. The sap was then extracted by means of a tuberculin syringe with a steel needle.

The sap was then analyzed for iodide by oxidizing the iodide to free iodine and extracting the latter by chloroform. The concentration of iodine in the chloroform was then determined by means of the Zeiss-Pulfrich step-photometer, using the "S 53" or the "S 50" color filter. 8

In spite of the great amount of work which has been done on the determination of small amounts of iodine in biological materials, no standardized procedure which meets all objections has yet been worked out. For this reason it was considered necessary to develop for this investigation a method which would give accurate results for known amounts of iodide added to aqueous solutions resembling Valonia sap and to sap itself. Many of the objections to previous methods deal with the difficulty of incinerating the biological material in such a way as to avoid

8 Getman (Getman, F. H., J. Am. Chem. Soc., 1928, 50, 2883) finds that the absorption maximum for 0.0005 M iodine in chloroform lies between 540 and 530 mμ. Waentig (Waentig, P., Z. phys. Chem., 1910, 68, 513) finds the absorption maximum at about the same point but his results were criticized by Ley and von Engelhardt (Ley, H., and von Engelhardt, K., Z. anorg. Chem., 1911, 72, 55), who found that for 0.05 to 0.005 M solutions the maximum was at 500 mμ.

Recently for solutions between 0.005 and 0.3 molar, Chatellet (Chatellet, M., Ann. chim., Paris, 1934, 2, series 11,5) has located the maximum at 508 mμ. In our work with the Zeiss-Pulfrich spectrophotometer we have found about the same results with the light filters whose dominant wave lengths are 530 and 500 mμ. ("S53" and "S50" in the manufacturers' nomenclature). We have, however, found the latter more comfortable to use.
loss of iodine. But fortunately Valonia sap is sufficiently free of organic material to make incineration unnecessary. However, questions have also been raised on the following points, (a) the best agent to employ to oxidize the iodide to free iodine, particularly when other salts are present; (b) the solvent to be used to extract the free iodine; and (c) the effect of salts on the completeness of the extraction and on the titrimetric determination with Na$_2$S$_2$O$_3$.

With respect to (a), most authors seem to have preferred nitrous acid, as the oxidizing agent, but KMnO$_4$ has been used. Our own experience with nitrite-sulfuric acid mixtures and nitrite-hydrochloric acid mixtures was disappointing, probably because of the presence of a fairly high concentration, 0.6 M, of chloride in the sap. For this reason we selected another oxidizer, KIO$_3$. This, of course, has been used extensively in macroanalysis, but as far as we can ascertain not in micro-work.

The difficulty with this reaction which theoretically should go as follows,

$$5KI + KIO_3 + 6HCl \rightarrow 3H_2O + 6KCl + 3I_2$$

is that if the solution is strongly acid side reactions occur and part of the iodine from both the iodide and iodate appears as ICl, a compound which, according to Philbrick, is highly ionized in the presence of strong HCl, and is not extracted into carbon tetrachloride. It is therefore necessary to prevent the formation of ICl by keeping the hydrogen ion activity low. This was done by using Richard's plan of acidifying with tartaric acid as recommended by Kolthoff.

It seems not improbable that the large excess of chloride in the sap may favor side reactions when iodides are oxidized by KIO$_3$ in the presence of strong acids. The results given in Table I, dealing with the analysis of known iodide mixtures with and without the addition of sap salts, are significant in this connection. In

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11 The reverse procedure, whereby the iodide is transferred to iodate by a powerful oxidizing agent, and is subsequently reduced by the addition of an excess of potassium iodide, has, of course, been used extensively in determining very small amounts of iodide.


these analyses each solution contained 0.0400 mg. of iodine in the form of potassium iodide. Group 1 solutions contained the sap salts, KCl and NaCl, at the concentration found in Valonia sap, while Group 2 solutions were made up in distilled water. The iodide was oxidized to free iodine by the addition of KIO₃ and hydrochloric acid at the concentrations indicated at the heads of the vertical columns.

In contrast with these results we found that when tartaric acid was used to acidify the oxidation mixture, the same result accurate to 5 per cent was obtained whether the sample was made up with sap salts or in distilled water. Furthermore the concentration of the tartaric acid seemed to be unimportant. In most of our determinations we estimate that it was between 0.01 and 0.05 normal.

Our attempts to use nitrite with hydrochloric acid gave very much poorer results even than the iodate-hydrochloric acid mixtures with or without salts.

| TABLE I |
| Effect of Strong Acid on the Determination of 0.04 M Iodide by Oxidation to I₂ by KIO₃ |
| | Group 1 | Group 2 |
| | Solutions made up with sap salts | Solutions made up in distilled water |
| | Conc. HCl, M | 0.0025 | 0.0092 | 0.0375 | 0.0417 | 0.0417 | 0.0392 | 0.0408 | 0.0400 | 0.0400 | 0.0400 |
| Iodine found, mg... | 0.0400 | 0.0400 |
| Av. | 0.04113 | Av. | 0.04023 |

But when tartaric acid was substituted for the strong acid fairly satisfactory results were obtained. However, in view of the fact that iodate has the slight advantage that only 5/6 of the total iodine comes from the unbound iodide, it seemed desirable to retain it as the oxidizer.

Other investigators have considered the effect of salts on the estimation of iodide. Thus Reith,¹⁴ investigating von Fellenberg's colorimetric method in which the nitrite-sulfuric acid mixture is used as the oxidizer, found that large negative errors might occur in the presence of SO₄²⁻, Cl⁻, NO₃⁻, Br⁻, BrO⁻, and ClO₄⁻. Reith indeed concludes that the method is only good for pure solutions of iodide. Maljaroff and Matskiewitsch¹⁵ have also had difficulty using the nitrite-hydrochloric acid mixture, but they have apparently attributed their losses to greater solubility of the iodine in the salt solutions, with consequent decrease in the partition coeffi-

cient between the aqueous phase and the chloroform. To us this seems very unlikely for while it is true that the solubility of iodine increases slightly in NaCl solution, the effect is certainly too small to account for 50 per cent or even 100 per cent losses of iodine. It seems much more probable that the failure of the method was due to side reactions.

Weil and Sturm also point out that they found low values for iodine when using von Fellenberg's method in the presence of large salt concentrations. With respect to (b), Blum has objected to the use of chloroform to extract iodine when the nitrite-sulfuric acid mixture is used on the grounds that the color fades rather rapidly. However, he states that this fading does not occur when pure iodine is dissolved in chloroform. Our experience has been somewhat similar to Blum's, but we have found that the iodine-chloroform mixture extracted from a mixture with iodate-tartaric acid as the oxidizer does not fade in 24 hours. Moreover most of our determinations of the color (light absorption) of the extracts were made within 30 minutes of the extraction, so that errors from this source could scarcely occur.

Settimi has strongly recommended the use of CS₂ in place of CHCl₃ or CCl₄ on the grounds that at low concentrations the color with CS₂ is more intense, and that the distribution ratio strongly favors CS₂. There are, however, certain objections to CS₂ when the analysis is made by means of a spectrophotometer. Thus it is clear from the results of Coblenz and Waentig that the extinction coefficient is not a linear function of the concentration of iodine, which behavior may possibly be connected with the fact that the distribution coefficient increases steadily with increasing concentration of iodine. It is inconvenient from the standpoint of the photometer because it does not permit the use of a linear curve.

In this connection, McClendon (McClendon, J. F., J. Biol. Chem., 1924, 60, 289) has suggested that in order to obtain uniform distribution of the iodine between the phases, the aqueous phase might be saturated with NaCl so that this salt might render negligible the effect of other salts which in most cases are present in much lower concentration.


These authors prefer to check the colorimetric method by a supplementary titration method (in which the iodide is oxidized to iodate, treated with an excess of KI, and titrated with Na₂S₂O₃) when the amount of iodine involved is less than 3 γ. In the present experiments, however, the amounts were usually above 10 γ and never below 5 γ.


for the interpolation of results. With chloroform, on the other hand, the distribution ratio appears to be fairly constant. At any rate it shows no trend. However, recently Chatelet has asserted that while solutions of iodine in carbon tetrachloride obey Beer's law those in chloroform do not. This leads him to the conclusion that there are present in the chloroform solution two forms of iodine. One of these, however, disappears when the solution is diluted sufficiently. This latter result is quite consistent with our own experience with iodine in chloroform.

We have studied the concentration range from 0.1 mg. per ml. down to 0.005 mg. per ml. with the Zeiss-Pulfrich spectrophotometer and have found that for the dominant wave length \( \lambda = 500 \text{ m} \mu \) (filter "S 50") the solutions follow Beer's law. A linear curve was therefore used in interpolating the results, and the size of the samples was so chosen that the amounts of iodine to be extracted into the chloroform fell between these limits.

After consideration of all the factors involved the following analytical procedure was adopted. Two equal samples of sap, usually colorless but occasionally colored green by chloroplasts, were delivered into two 12 ml. centrifuge tubes each of which contained 2 ml. of redistilled reagent grade chloroform. A drop each of 4 per cent tartaric acid solution and 1 per cent potassium iodate solution were then added to the test mixture. The second tube served as a blank. The test mixture was then shaken gently to mix, and both tubes were capped and allowed to stand 2 to 5 minutes to complete the oxidation. They were then shaken energetically for 30 seconds. In the blank, if the sap contained chloroplasts, the chloroform was colored slightly green, while in the test solution under these conditions the color was violet, modified slightly by the green color due to the extracted chlorophyll. Both chloroform solutions were cloudy due to the inclusion of droplets. Both samples were centrifugaled at 1500 R.P.M. for 3 to 5 minutes. The chloroform layers were then transferred by pipette to the absorption vessels. In order to get a 50 mm. layer for the determination of the light absorption with only 2 ml. of chloroform solution, micro vessels were used. The percentage absorption with the "S 50" filter was then determined, several settings being made for each determination and the vessels being interchanged to eliminate unequal lighting of the two halves of the photometer. From the "per cent absorption" the "extinction coefficient" was read off the table and the corresponding quantity of iodine read off the calibration curve. In a good many cases no chlorophyll was extracted so that the blank was colorless. In many others the rather feeble absorption of chlorophyll in the green in comparison with the strong absorption of the violet iodine made the error due to the chlorophyll negligible. It was only in the case of very dilute iodine solutions that the compensation was needed. It is possible to avoid the need for the chlorophyll compensation by first centrifugaling the sap to remove chloroplasts. And from time to time when extra amounts of sap were available this was done, and the analyses made on chlorophyll-free sap were compared with those made on the same sample with chlorophyll

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present. The results were invariably the same within the limits of error of the method.

The accuracy to be expected was determined by applying the method to known mixtures made up in artificial Valonia sap (i.e. a mixture of KCl, NaCl at the average concentrations found in sap), and also to known iodine mixtures in distilled water. The results indicate that the error is of the order of ±4 per cent. Analyses on sap samples containing known amounts of iodide and varied amounts of chlorophyll indicated that the "chlorophyll compensation" method is valid, and finally that the error is of the order of ±4.0 per cent.

RESULTS

Rate of Entrance at Constant pH.—Table II and Fig. 1 give the rate of entrance into the sap of Valonia of iodide from solutions with different concentrations of iodide at the normal pH of sea water. The experiments were carried out in the rather feeble light of the laboratory and no change in pH due to photosynthesis was observed. Separate experiments under the same conditions indicated that growth was negligible. The curves have been drawn in this and other cases free-hand to give an approximate fit. Each point on a curve represents the average of two or more analyses made on different samples of sap and, as usual, when large numbers of cells were available, cells of approximately the same size (0.2 to 0.4 ml.) and shape were used. The cells used in any one experiment were from the same collection and had been seasoned in the laboratory (for 2 weeks or more) under the same conditions.

pH Effect.—Fig. 2 shows that the external pH has little or no effect on the rate of entrance of iodide. As in previous experiments, the pH was adjusted by the addition of 0.6 N HCl or carbonate-free 0.6 N...
Fig. 1. Rate of entrance, $dx/dt$, of iodide into *Valonia* at various external iodide concentrations.

Fig. 2. Rate of entrance of iodide into *Valonia* at various pH's.
NaOH, with aeration to bring the solutions into equilibrium with the CO$_2$ of the atmosphere. Some difficulty was experienced in keeping up the pH of the more alkaline solutions, probably because of the CO$_2$ production of the cells and absorption of CO$_2$ from the air, and to offset this the solutions were frequently renewed. Some falling off overnight, however, was inevitable.\textsuperscript{25}

![Graph](https://example.com/graph.png)

**Fig. 3.** Rate of entrance of iodide into *Valonia* in darkness and illuminated.

*Light Effect.*—Fig. 3 shows that light has little or no effect on the entrance of iodide. In these experiments the cells were exposed to the solutions in 125 cc. bottles. The "dark" bottles were first covered with two coats of "black brushing lacquer," then with a layer of adhesive tape, and finally a coat of lacquer over the tape. The light

\textsuperscript{25} The pH varied a little, particularly at the high pH, but by changing the sea water frequently the effect of this was minimized. If there had been any evidence of a pH effect we should have resorted to flowing solutions as in previous experiments. But as there was not, this refinement seemed unnecessary.
was provided by a 500 watt concentrated filament projection lamp, placed about 18 inches from the bottles. A half-inch layer of flowing water was interposed to absorb heat rays.

TABLE III

<table>
<thead>
<tr>
<th>Time</th>
<th>Molar concentration of iodide in sap</th>
</tr>
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<tr>
<td></td>
<td>0.0200</td>
</tr>
<tr>
<td>hrs.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.00054</td>
</tr>
<tr>
<td>65</td>
<td>0.00496</td>
</tr>
<tr>
<td>20</td>
<td>0.00094</td>
</tr>
<tr>
<td>69</td>
<td>0.00093</td>
</tr>
</tbody>
</table>

Effect of External Concentration.—Table III and Fig. 4 (a, b, c, and d) show the increase in rate as the external concentration increases. By means of several experiments, concentrations from 0.2 to 0.0014 m were tested.
As Fig. 2 shows, the rate of entrance of iodide is affected little or not at all by the external pH of the sea water between 7 and 9. This is in marked contrast with the entrance of H$_2$S, which has a "rate"$^{26}$ which is a linear function of the external concentration of undissociated H$_2$S and therefore of $f_a^{27}$ [H$^+$] [HS$^-$].$^{27, 28}$

We may therefore conclude that entrance by diffusion as HI through the protoplasm is negligible. It is true that a case might be made out for the hypothesis that the lack of pH effect is due to masking by other factors. For example, if as a necessary preliminary to entrance a reversible reaction HI + ZOH $\rightleftharpoons$ H$_2$O + ZI must occur (where ZOH is a basic constituent of the protoplasm), the importance of the increase in the concentration of HI in accelerating the rate will become less as ZOH decreases in concentration, so that finally ZOH may be so limited in amount that any increase in the value of [HI] or of $f_H [H^+] f_I [I^-]^{29}$ becomes ineffective. In sea water where the ionic strength is high compared with [H$^+$] and [I$^-$] as used in our experiments we may regard the activity coefficients $f_H$ and $f_I$ as constant, and we may compare the effect of changing the product $f_H [H^+] [I^-]$ (which is proportional to [HI]) by altering the pH, or by altering the iodide concentration.

At pH 8.2, when the iodide concentration is 0.06 M, the product $f_H [H^+] [I^-]$ is $10^{-9.25}$, and at pH 7.0 it is $10^{-8.22}$. This change had no effect on the rate. On the other hand in another experiment selected at random from the data of Table III, when the pH was 8.2

$^{26}$ "Rate" is used here to designate the function $\left(\frac{\partial x}{\partial a}\right)$, where x is the concentration of total sulfide in the sap and a the concentration of undissociated sulfide in the sea water.

$^{27}$ Guggenheim (Guggenheim, E. A., J. Phys. Chem., 1930, 34, 1758) has shown that the single ionic activity coefficient has no physical significance. We shall therefore replace it as far as possible by the mean activity coefficient $f_a$.

$^{28}$ In this expression and hereafter square brackets refer to concentration in moles per liter, round brackets to activities in the same limits, and f is the mean activity coefficient when the concentration is expressed as moles/liter.

$^{29}$ In this case it is convenient to speak of single ion activity coefficients, since the data available for calculation are the H ion activities, which with reservations as to the liquid junction potential, may be measured.
FIG. 4a

FIG. 4, a, b, c, d. Rate of increase of iodide in the sap plotted against concentrations of iodide in the external solution.
**Fig. 4c**

Molar concentration iodide in sea water

**Fig. 4d**

Molar concentration iodide in sea water
and the concentration of iodide 0.0580 \text{ m}, the product was equal to \(10^{-8.44}\), and when the concentration of iodide was 0.0400 \text{ m} the product was equal to \(10^{-9.40}\). This comparatively small change in the product had a pronounced effect on the rate. If the entrance were accompanied by combination with ZOH it should make no difference whether we increase the product by changing \(H^+\) or by changing \(I^-\).

Further evidence that HI is not an important factor in the entrance of iodide is obtained from a consideration of the gradients. We may assume that the rate of increase of iodide concentration in the sap depends on the flux of iodide-containing solutes in the inner and outer non-aqueous protoplasmic surface layers. And since the same principles apply to both\(^{20}\) for convenience we shall assume that there is only one layer and that the intervening watery layer may be neglected.

When steady state diffusion is established (for entrance solely as HI) we may write under certain conditions that

\[
\frac{dQ}{dt} = K_{HI} ([H^+]_i [I^-]_i) - ([H^+]_o [I^-]_o)
\]

where \(o\) and \(i\) refer to the sea water and sap, respectively, and \(Q\) is the quantity of HI which has passed into the sap in time \(t\). The derivation of this equation and the conditions involved will now be discussed.

It can be shown\(^{20}\) for the diffusion of a non-electrolyte that

\[
v = D \frac{c}{v}
\]

where \(c\) is the concentration, \(v\) is the velocity of the solute, \(D\) is the diffusion constant, and \(C_1\) and \(C_2\) are respectively the concentrations of the solute at the boundaries of the diffusion layer. But \(v = \frac{Q}{t}\), where \(Q\) is the quantity of solute diffusing through a plane in unit time, whence

\[
\frac{Q}{t} = D (C_1 - C_2)
\]

This in terms of HI diffusing as molecules in the non-aqueous protoplasm becomes

\[
Q_{HI} \int_{HI} \int_{o,p} = D_{HI} \left( \int_{HI} [HI]_{o,p} - \int_{HI} [HI]_{i,p} \right)
\]

where \(o,p\) and \(i,p\) refer to the sea water-protoplasm and sap-protoplasm interfaces and \(\rho\) to any plane in the protoplasm where the measurement is made. But

owing to the inevitable presence of unstirred layers at the interfaces, we may write
for the sea water-protoplasm interface

\[ \int_{\text{HI}}^{o-p.} [\text{HI}]_{o,p.} = S_o \int_{\text{HI}}^{o} [\text{HI}]_o \]

and for the sap-protoplasm interface

\[ \int_{\text{HI}}^{i-p.} [\text{HI}]_{i,p.} = S_i \int_{\text{HI}}^{i} [\text{HI}]_i \]

where \( S \) is the partition coefficient and \( o \) and \( i \) refer to the sea water and sap respectively. Because of the stirring of the sap and sea water it may be assumed that the unstirred aqueous layers in contact with the protoplasm are at approximately the same concentration as the stirred aqueous solutions of which they are a part.

But

\[ K \cdot S_o \int_{\text{HI}}^{o} [\text{HI}]_o = S_o \left( \int_{\text{HI}}^{o} [\text{H}^+] o [\text{I}^-] o \right) \]

and

\[ K \cdot S_i \int_{\text{HI}}^{i} [\text{HI}]_i = S_i \left( \int_{\text{HI}}^{i} [\text{H}^+] i [\text{I}^-] i \right) \]

and substituting these values above we have

\[ Q_{\text{HI}} \int_{\text{HI}}^{o} = \frac{D_{\text{HI}}}{K} \left( \left( S_o \left( \int_{\text{HI}}^{o} [\text{H}^+] o [\text{I}^-] o \right) - \left( S_i \left( \int_{\text{HI}}^{i} [\text{H}^+] i [\text{I}^-] i \right) \right) \right) \]

In these equations under the experimental conditions \( f_{\text{HI}} \), the activity coefficient of HI in the non-aqueous layer of the protoplasmic surface may be regarded as a constant. And since the ionic strength of the sap and sea water are so nearly alike (about 0.7 \( \text{cm} \)) the mean activity coefficients \( f_{\text{HI}}^o \) and \( f_{\text{HI}}^i \) may also be regarded as equal and constant, and \( S_o \) and \( S_i \), the partition coefficients, may also be regarded as equal and constant, so that collecting all constant terms we have

\[ Q_{\text{HI}} = K'_{\text{HI}} [\text{H}^+]_o [\text{I}^-]_o - [\text{H}^+]_i [\text{I}^-]_i ] \]

or for diffusion with a gradient decreasing with time

\[ \frac{dQ}{dt} = K'_{\text{HI}} [\text{H}^+]_o [\text{I}^-]_o - [\text{H}^+]_i [\text{I}^-]_i ] \]

In this equation \( K'_{\text{HI}} \) may be called the "permeability constant" since it includes in addition to the diffusion constant of Fick's law, a variety of other terms which according to the above discussion may also be regarded as constants under the experimental conditions.
In these experiments also, since the cells did not grow, and since
\( Q = VX \), where \( V \) = volume and \( X \) is the concentration of iodide in
the sap, we may write \( K = K' + V \) and
\[
\frac{dx}{dt} = K_{HI} ([H^+]_o, [I^-]_o - [H^+], [I^-])
\]
Unfortunately we cannot calculate either product from the available
data, and we adopt the best possible expedient by using the equation
in the form
\[
\frac{dx}{dt} = K_{HI} \left( \int_{H^+}^{H^+} [H^+]_o, [I^-]_o - \int_{H^+}^{H^+} [H^+], [I^-] \right)
\]
which introduces the indefinite single ion activity coefficient of \( H^+ \).
We do not have to know this value since we have from the measured
pH of the sea water and sap the value of \( (H^+) \), which with reservations
with regard to the liquid junction involved in the measurement is
equal to \( f_{H^+} [H^+] \).
Fortunately this equation introduces practically no error since we
are in all cases comparing these products in sap and sea water both of
which have nearly the same ionic strength and hence the same
value of \( f_{H^+} \).
From an experiment where \( [I^-]_o = 0.06 \, \text{m} \), Fig. 2, we derive the
following values for the products \((H^+)_o [I^-]_o\)
at pH 7.0, \( 10^{-4.23} \)
at pH 8.2, \( 10^{-3.43} \)
at pH 9.0, \( 10^{-2.63} \)
The pH of the sap is approximately 6.0. Therefore when the external
pH is 7.0 the gradient should vanish when \([I^-]_o \) becomes equal to
0.0060 \( \text{m} \); at pH 8.2, when \([I^-]_o \) equals 0.00038 \( \text{m} \); and at pH 9.0, when
\([I^-]_o \), equals 0.00006 \( \text{m} \). But actually at pH's 8.2 and 9, iodide con-
tinued to enter even after the limiting concentrations were reached,
and further, the rates at all pH's were the same in spite of the great
difference in the gradients.
If the iodide moves into the sap by a process involving the product
\((H^+) [I^-] \), our experiments indicate that accumulation has taken place
at pH 8.2 and 9 against a gradient. This can only happen if the cell
itself supplies energy in some form capable of causing a predominantly
one-way flow of the diffusing substance. There is a tendency to consider this possible for the living cell because of its abundant supply of energy due to metabolism. In the case of the entrance of \( \text{Cl}^-, \text{NO}_2^-, \text{Br}^-, \) and \( \text{I}^- \) into \textit{Nitella}, for example, Hoagland and Davis\(^1\) have expressed the opinion that the energy of the cell metabolism is involved and that in some way light aids in the necessary transfer of energy, since the accumulation of these ions is greatly accelerated by light. Our own experiments with the entrance of iodide into \textit{Valonia} indicate that light is not an important factor, since we obtained no appreciable difference in rate between cells in total darkness and those strongly illuminated (Fig. 3). In this case it seems unnecessary to refer the entrance directly to the cell metabolism since, as will be shown later, favorable gradients for the entrance of iodide exist, so that in all probability diffusional energy only is involved. These will be discussed later.

We now consider the nature of the function \( \frac{\partial a}{\partial t} \) whose \( x \) and \( a \) are the concentrations of the diffusing substance in the sap and in the sea water. For the case of simple diffusion of a molecular species in the protoplasmic surface layer, with equal partition coefficients \( S \) at both interfaces and a linear gradient, the equation

\[
\frac{\partial x}{\partial t} = k (a - x)
\]

where \( k \) is a constant which includes the partition coefficient, should apply. Actually, in the case of the entrance of sulfide, where apparently only the diffusion of molecular \( \text{H}_2\text{S} \) is involved, the relationship did not hold: instead the value of \( k \) diminished with time. A number of reasons for this were suggested, and to these may now be added the possibility that diffusion gradients exist also in the sea water and in the sap. These will be discussed later. At the moment it is necessary to realize that the simple relationship can be applied only when \( a \) and \( x \) are known. In the case of iodide we know the external and internal concentration of iodide at any time \( t \), but we do not know the diffusing species in the protoplasm. However, a calculation has been made, using for \( a \) and \( x \) the concentrations of iodide in the sea water and sap,

\(^1\) Hoagland, D. R., and Davis, A. R., \textit{J. Gen. Physiol.}, 1923–24, 6, 47.
and the data of Table IV for the case when \( a \) was 0.04 M. The result shows that \( k \) is falling steadily with time.

It is unlikely that the diffusing species in the non-aqueous protoplasmic surface is iodide ion. It is probably an undissociated molecule and we therefore consider the gradients of these. If the diffusing species is sodium iodide we may assume that the rate of its flux through the protoplasm or, what amounts to the same thing, the rate of gain of NaI by the sap, is proportional to the gradient \([\text{NaI}]_{\text{o.p.}} - [\text{NaI}]_{\text{i.p.}}\) where o.p. and i.p. represent the layers of protoplasm in immediate contact with the sea water and the sap respectively. The direction of the flow of NaI between sea water and sap will be determined (in

<table>
<thead>
<tr>
<th>Time (hrs.)</th>
<th>([I^-]_s)</th>
<th>(k_{\text{NaI}} = \frac{2.3}{I} \log \frac{[\text{NaI}]_s [I^-]_s}{[\text{Na}^+]_s [I^-]_s - [\text{Na}^+]_s [I^-]_s})</th>
</tr>
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<tr>
<td>416</td>
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</tbody>
</table>

the absence of the supply of energy from the cell) by the activity product gradient \((\text{Na}^+)_s (I^-)_s - (\text{Na}^+)_s (I^-)_s\). But under the conditions set above for the diffusion of HI in the protoplasm we may write for the rate of NaI

\[
\frac{d[I^-]}{dt} = K_{\text{NaI}} ([\text{Na}^+]_s [I^-]_s - [\text{Na}^+]_s [I^-]_s)
\]

which may be integrated to give

\[
K_{\text{NaI}} = \frac{2.3}{I} \log \frac{[\text{Na}^+]_s [I^-]_s}{[\text{Na}^+]_s [I^-]_s - [\text{Na}^+]_s [I^-]_s}
\]

The constant calculated on this basis was also found to decrease steadily with time (Table IV).
But if NaI can diffuse in the protoplasm as such it seems possible that KI can also. \([K^+]_o = 0.012 \text{ m} \) in Bermuda sea water, and \([K^+]_i = 0.5 \text{ m} \) for the average cell, hence, assuming that the activity coefficients of \([K^+]_o\) and \([K^+]_i\) are equal in the experiment under discussion, where \([I^-]_o = 0.04 \text{ m}\), when \([I^-]_i\) becomes 0.00096 m the concentration products are equal. Thereafter as \([I^-]_i\) increases, KI should come out of the cell. As Fig. 1 shows the exit of KI should occur comparatively early in the process.

For the situation when NaI is entering the cell and KI is emerging we may make the following calculation

\[
\frac{d[NaI]_i}{dt} = k_1 ([Na^+]_i [I^-]_i - [Na^+]_o [I^-]_o) \tag{2a}
\]

and

\[
\frac{d[KI]_i}{dt} = k_2 ([K^+]_o [I^-]_o - [K^+]_i [I^-]_i) \tag{2b}
\]

We now assume that all the iodide which enters or leaves the cell does so as NaI or KI.

\([Na^+]_o = 0.5 \text{ m} \) in Bermuda sea water, \([I^-]_o = 0.04 \text{ m}\).

\[
\frac{d[NaI]_i}{dt} = k_1 (0.02 - [Na^+]_o [I^-]_o) \tag{2c}
\]

\[
\frac{d[KI]_i}{dt} = k_2 (0.00048 - [K^+]_i [I^-]_i) \tag{2d}
\]

\[
\frac{d[I^-]_i}{dt} = \frac{d([NaI]_i + [KI]_i)}{dt} = 0.02 k_1 + 0.00048 k_2 - (k_1 [Na^+]_o + k_2 [K^+]_o) [I^-]_i \tag{2e}
\]

In the sap, as NaI enters and KI leaves, \([Na^+]_i\) must increase and \([K^+]_i\) decrease. However, \(\Delta[Na^+]_i\) and \(\Delta[K^+]_i\) are small compared with \([Na^+]_o\) and \([K^+]_o\), and may be disregarded.

In the average cell \([Na^+]_i = 0.1 \text{ m}\). Then

\[
\frac{d[I^-]_i}{dt} = 0.02 k_1 + 0.00048 k_2 - (0.1 k_1 + 0.5 k_2) [I^-]_i \tag{2f}
\]

Integrating

\[
\ln \left( \frac{0.02 k_1 + 0.00048 k_2} {0.02 k_1 + 0.00048 k_2 - (0.1 k_1 + 0.5 k_2) [I^-]_i} \right) = \frac{(0.1 k_1 + 0.5 k_2) t}{0.02 k_1 + 0.00048 k_2} \tag{2g}
\]
Attempts to apply this equation by giving various values to \(k_1\) and \(k_2\) have proved to be unsuccessful. A pair of values which fit the upper part of the curve show large deviations at the lower part. This is scarcely surprising in view of the fact that the theory is oversimplified, particularly in neglecting the reversal of direction of movement of KI which should occur if this salt moves through the protoplasm at all.

We now consider the "rate curve" defined as

\[
\left( \frac{d[I^-]}{d[I^-]_0} \right)_t
\]

Two possible diffusion processes will be considered.

(a) Diffusion of sodium iodide alone leading to

\[
\frac{d[I^-]}{dt} = k_{NaI} ([Na]^+_0 [I^-]_0 - [Na]^+_t [I^-]_t)
\]

which on integration gives

\[
[I^-]_t = [Na]^+_0 [I^-]_0 (1 - e^{-k_{NaI} t})
\]  

(b) Simultaneous diffusion of NaI and KI. Under the simplest assumption discussed above this leads to

\[
\frac{d[I^-]}{dt} = k_1[Na]^+_0 + k_2[K]^+_0 [I]_0 - k_1[Na]^+_t - k_2[K]^+_t [I^-]_t
\]

Putting

\[
k_1 [Na]^+_0 + k_2 [K]^+_0 = m\text{ and } k_1 [Na]^+_t + k_2 [K]^+_t = n
\]

on integration we get

\[
[I^-]_t = \frac{m [I^-]_0}{n} (1 - e^{-n t})
\]  

Each of these equations differentiated with respect to \([I^-]_t\) and \([I^-]_0\) at constant \(t\) gives

\[
\left( \frac{\partial [I^-]}{\partial [I^-]_t} \right) = \text{const.}\]

* It is assumed, of course, that the concentrations of K and Na do not change seriously in either sap or sea water.

In the penetration of H\(_2\)S the relationship

\[
\frac{\partial [H_2S]}{\partial [H_2S]_t} = \text{const.}
\]
was true. But in the present case the relationship does not appear to hold at lower concentrations. However, after a certain concentration of $[I^-]_e$ is passed each curve becomes linear. The concentration at which this break occurs is not the same in all cases, but in general we may say that it lies between an external concentration of 0.01 and 0.02 molar.

Several ways of considering the observed facts suggest themselves as follows. (a) The entrance of iodide takes place by diffusion of a molecular species in the protoplasm but part of the iodide in the cell is immobilized by combination with a constituent of the sap which is present in limited amount. Under these conditions the rate of entrance will be favored more at lower concentrations of $I_o$, since a greater portion of the entrance will take place according to the equation

$$\frac{d[I^-]}{dt} = k_e$$

i.e., without any back pressure. However, this explanation is untenable in the present case for, as reference to Fig. 4 shows, the points of inflection of the curves in a single experiment do not show any tendency to lie on a line parallel to the axis of abscissae, as would be required by the immobilization hypothesis.

(b) The entrance of iodide is by diffusion of molecular species in the protoplasm but in addition to the gradients of the diffusing substances in the protoplasm there are also gradients in the aqueous solutions in contact with the protoplasm. The effect of these diffusion gradients has been studied by Lewis and others for the absorption of gases and by Roughton for the uptake of $O_2$ by blood corpuscles. The cell and its surroundings include the following regions, the sea water, the cellulose envelope, the protoplasm, and the sap. Fig. 5 shows schematically the possible unstirred regions in which concentration gradients may exist.

For the sake of simplicity Regions II and III in Fig. 5 are considered as one layer. Since in the case under discussion the sea water was

---

24 There are practical grounds for this, since all the bioelectric experiments on Valonia and Nitella indicate that the envelope is as permeable to electrolytes as the aqueous solution. Thus when a cell is transferred from one solution to another the P.D. associated with its new environment is established within a few seconds.
stirred the thickness of Region II may be very small and hence the diffusion gradient in it may be negligible. Region III, however, can scarcely be stirred under any conditions. Similarly we have no control over the amount of stirring in the protoplasm so that the thickness of IV and VI are uncontrolled. Bioelectric measurements indicate that these two layers are dissimilar. Finally the thickness of Region VII depends on the amount of stirring in the sap. This depends on the stirring secured by the rolling of the cells as they fall through the sea water in the bottles, which was sufficient to keep in

![Diagram of diffusion layers](image-url)

FIG. 5. Schematic representation of diffusion layers involved in iodide entrance.

35 The picture of the protoplasm which best fits the numerous bioelectric measurements of Osterhout and his coworkers (Osterhout, W. J. V., *Ergeb. Physiol.*, 1933, 35, 967) consists of at least two dissimilar non-aqueous layers between which lies an aqueous layer. This might mean the introduction of more regions between IV and V, and V and VI, but inasmuch as the introduction of new layers does not alter matters essentially they are disregarded.

brisk motion random dark particles occasionally seen in the vacuoles, and on the stirring due to convection. This may be considerable. Nevertheless it is not inconceivable that the thickness of VII is appreciable and that a concentration gradient exists in it.

Jacobs\textsuperscript{37} has indicated the method of deriving equations for the rate of flow \( \frac{dQ}{dt} \) in the steady state of a solute through a series of dissimilar layers.

According to Fig. 5 we have in the present case four possible layers, II–III, IV, VI, and VII. Because V is adequately stirred, IV and VI are considered to be adjacent layers, there being no concentration gradient in V. Moreover the customary assumption is made that the layers are so thin that the time for the establishment of steady state conditions is an inappreciable fraction of the total diffusion time.

Let the diffusion layers in the cell be \( D_1, D_2, D_3, D_4 \), the layer thicknesses \( h_1, h_2, h_3, h_4 \), and the partition coefficients be \( S_1, S_2, S_3, \) and \( S_4 \),\textsuperscript{88} and \( a \) and \( x \) the concentrations of the diffusing solute in the sea water and the sap. Then

\[
\frac{dQ}{dt} = \frac{S_1D_1S_2D_2S_3D_3S_4D_4 (a - x)}{h_1S_1D_2S_2D_3S_4D_4D_4 + h_2S_1D_2S_2D_3D_3D_4 + h_3S_1D_2S_3D_3D_3D_4 + h_4S_1D_2S_3D_3D_3D_4}
\]

This shows that the form of the diffusion curve is independent of the number of layers even when the thicknesses, the diffusion constants, and the partition coefficients are different. \( \left( \frac{\partial x}{\partial a} \right) \) remains constant.

Hence if the entrance of I\textsuperscript{−} takes place by diffusion under steady state conditions, the presence of diffusion gradients in several dissimilar layers cannot account for the observed shape of the curve where I\textsubscript{i} is plotted against I\textsubscript{o}.

If, however, the sap is completely unstarred, the vacuole becomes part of the diffusion layer VII. Up to now we have assumed the total thickness of the diffusion system to be so small that the areas of the inner side of VII and the outer side of II–III are equal. This is not


\textsuperscript{88} The partition coefficients are as follows: \( S_1 \) between Regions I and II + III (Fig. 5), \( S_2 \) between II + III and IV, \( S_3 \) between IV and VI, \( S_4 \) between VI and VII. Region V is, for convenience, regarded as absent. \( S_1 \) and \( S_3 \) are equal to 1 and \( S_2 \) might be equal to \( S_4 \). Moreover \( D_1 \) might equal \( D_4 \) but we put the equation in its most general form.
true because diffusion is taking place into an enclosed space, but when the sap is adequately stirred we may deal with the diffusion as if it were taking place in a volume bounded by plane parallel surfaces. When the vacuole is wholly unstirred it is necessary to take the shape of the cell into account. The natural cell is unsymmetrical but for convenience it may be regarded as a sphere with the diffusion region extending in a series of concentric layers from the stirred mass of the sea water to the center of the vacuole.

For a homogeneous spherical system Jacobs has derived the equation

\[
\frac{x}{a} = 1 - \frac{6}{\pi^2} \left( e^{-\frac{a^2 d t}{\pi^2}} + \frac{1}{4} e^{-\frac{4 a^2 d t}{\pi^2}} + \ldots \right)
\]  

(6)

where \(a\) is the constant concentration at the surface of the sphere, \(x\) is the average concentration in the sphere, and \(R\) is the radius of the sphere. The cell is not homogeneous but as has been shown the presence of several dissimilar layers affects the rate of diffusion but not the form of the diffusion equation.

In this case also \(t\) is an exponential function of \(x\) and \(\left(\frac{\partial x}{\partial a}\right) = \text{const}\). Under unstirred conditions, therefore, for a single cell the form of the curve, when \(x\) is plotted against \(t\) or against \(a\), is unchanged. But in our experiments the vacuole was stirred so that the problem in our case becomes roughly that of diffusion through a thin closed curved membrane of spherical shape. As Fig. 5 shows, this is probably composed of several layers but, as Equation 5 (p. 759) indicates, this does not affect the form of the diffusion equation. Moreover if in Equation 5 one of the terms \(S_n D_n\) is much smaller than any of the others it will obviously control the rate and the diffusion system can be treated as homogeneous. The external concentration of the diffusing solute is \(a\) and the internal concentration in the vacuole at time \(t = x\); and if the concentration gradient is linear so that \(\frac{dQ}{dt}\) is a constant, we may write

\[
dQ = V_1 \, dx = \frac{D' \, 4\pi r s (a - x)}{n_2 - n_1} \, dt
\]  

(7)


---

** Some cells are rather more cylindrical but the same principles apply.
where $r_1$ and $r_2$ are the radii of the inside and outside respectively of the spherical shell and $V_1$ is the volume of the stirred portion of the system in the vacuole. It should be noted that $D'$ is not the $D$ of Fick's equation but is a "permeability constant" including diffusion constants in the protoplasm, partition coefficients, etc.

On integration

\[ \ln \frac{a}{a-x} = \frac{4\pi r_1 r_2 D'}{V_1 (r_2 - r_1)} - \frac{3D' r_1^2}{r_1^2 (r_2 - r_1)} \]  \(8\)

But $r_2$ and $r_1$ are nearly equal. Hence, as the equation shows, the time required for any degree of "saturation" of the sap is directly proportional to the radius of the cell and the thickness of the diffusion region. This gives a mathematical basis for the well authenticated fact that the rate of diffusion into dead cells of unequal sizes is not "exponential." 5

From the data of Table IV we can calculate by the use of the above equation values for $D'$ for cells of various sizes and with different thicknesses of the diffusion layer. 40 Under the following conditions: $a = [Na^+]_o [I^-]_o = 0.02, x = [Na^+]_i, [I^-]_i = 0.00020, t = 13, r_1 = 0.25$ cm., and $r_2 = 0.2505$ corresponding to a thickness of 5 microns, $D = 3.22 \times 10^{-8}$ cm.²/hr. If $r_2 = 0.255$ corresponding to a thickness of 50 microns, $D' = 3.1 \times 10^{-8}$ cm.²/hr. With the other conditions the same and $r_1 = 0.5$ cm., $r_2 = 0.5005$ cm., $D' = 6.4 \times 10^{-8}$ cm.²/hr. 41

None of the cells used in the experiment was over 0.50 cm or less than 0.25 cm in radius. The thickness of the protoplasm was less than 10 microns. 42 For this reason the extremely low value of $D'$, the permeability constant, is probably significant, for the diffusion constant of NaI in water at 0.04 º is of the order of 0.03 cm.²/hr. 43 Assuming

40 Since in the experiment the rate of entrance of iodide took place with a falling value for the "monomolecular" constant, we have selected for the calculation values at the beginning of the experiment when the falling off was proportionally less.

41 In case the vacuole is not stirred $r_2 - r_1$ becomes greater and this would affect the value of $D'$.

42 Doyle's results with Valonia macrophysa of Tortugas indicate that the protoplasm is from 5 to 8 µ thick. Doyle, W. L., Papers from the Tortugas Laboratory of the Carnegie Institution, 1936, 28, 13.

that this value in water corresponds to the permeability constant of NaI in the living cell it is clear that its entrance into the cell is impeded very greatly. The impediment must be either in the cellulose wall, or in the protoplasm. But the cellulose wall when wet with sea water appears to be so permeable that diffusion in it is as rapid as in water. Thus, as Blinks\textsuperscript{44} found, a freshly killed cell of \textit{Valonia ventricosa}, a closely related form, has the low resistance corresponding to the specific resistance of the extracted sap. The protoplasm must therefore be responsible for the slow entrance of iodide.

In these calculations the cell is considered to be a sphere but other shapes give similar results.\textsuperscript{45}

We may sum up by saying that if we take the value of \( D' \) to be of the order of \( 10^{-8} \) the passage of NaI through the protoplasm is about a million times as slow as it would be through water. The protoplasm is almost impermeable\textsuperscript{46} to MgSO\(_4\), CaSO\(_4\), and CsCl. Since the protoplasm is mostly water we may suppose that the hindrance to the passage of these substances lies almost entirely in the non-aqueous surface layers. It does not seem probable that they could act thus if they were unimolecular.

An interesting question now arises in connection with the penetration of H\(_2\)S which was studied previously.\textsuperscript{3} In that case the rate of entrance was very high compared with that of iodide. Assuming, however, that the penetration involved only diffusion, use of data in

\begin{equation*}
\ln \frac{a}{a - x} = \frac{ttl}{H} \left( \frac{3}{2a} + \frac{3}{2eb} \arcsin \varepsilon \right)
\end{equation*}

where \( H \) is the thickness of the diffusion layer, \( a \) and \( b \) are respectively the major and minor semi-axes of the ellipse which generates the solid, and \( \varepsilon \) is its eccentricity = \( \sqrt{a^2 - b^2} / a \). Taking an average cell \( a = 0.700 \text{ cm.}, b = 0.255 \text{ cm.}, \) and \( H = 0.0005 \text{ cm.} \) which has almost the same volume as the sphere of radius 0.5 cm. \( D = 3.58 \times 10^{-8} \). For the same volume of sap the calculated value of \( D \) will be inversely proportional to the area of the surface which encloses it.

\textsuperscript{44} Blinks, L. R., \textit{J. Gen. Physiol.}, 1929-30, \textbf{13}, 361.

\textsuperscript{45} A large number of cells are roughly prolate spheroids. The corresponding equation for the calculating of the permeability constant is

\textsuperscript{46} Osterhout, W. J. V., \textit{Ergebn. Physiol.}, 1933, \textbf{35}, 981 (Table I); also Cooper, W. C., Jr., Dorcas, M. J., and Osterhout, W. J. V., \textit{J. Gen. Physiol.}, 1928-29, \textbf{12}, 427.
Table II of the H₂S paper gives $a = 0.00625; x = 0.0009; t = 1$ minute. If we put $r_2 - r_1 = 0.0005$ cm., and $r_1 = 0.5$ cm. in Equation 8 (p. 761) we arrive at $1.29 \times 10^{-3}$ cm.$^2$/min., as the value of the permeability constant $D'$. But the diffusion constant of H₂S in water⁴⁷ is of the order of $1.0 \times 10^{-3}$ cm.$^2$/min. From these results we may be confident that in the penetration of H₂S also the protoplasmic layer is the deciding factor.⁴⁸

A calculation for CO₂ leads to similar results. In this case $D' = 1.46 \times 10^{-3}$ cm.$^2$/min. and $D$ for the diffusion of CO₂ in sea water is of the order of $1.0 \times 10^{-3}$ cm.$^2$/min. We conclude therefore that in this case also the protoplasm controls the rate of entrance.

It is interesting to find that Collander and Bärlund⁴⁹ have come to similar conclusions for the penetration of certain non-electrolytes into the cylindrical cells of Chara ceratophylla. They have compared the times required to half saturate the sap of dead cells with the calculated time required to half saturate “by diffusion” cylinders of the same dimensions as the cells. The two sets of figures agreed fairly well in that the actual time was about 4 times the theoretical time so that some impediment is offered by the cell wall. However, the times for half saturation in living cells were with two exceptions 70 to more than 50,000 times the theoretical.

It is evident that H₂S enters Valonia at a rate enormously greater than that of NaI (according to the figures just given it enters more than 10,000 times as fast). Although permeability to H₂S and to CO₂ appears to be much greater than to NaI (and to HI) it might be misleading to generalize this by saying that weak electrolytes always enter more rapidly for this idea is contradicted by experiments with models which show that a strong electrolyte with a high partition coefficient may pass through a non-aqueous layer more rapidly than a weak electrolyte or a non-electrolyte with a lower partition coefficient.

⁴⁸ In view of this we need not raise the question whether H₂S is more soluble in the non-aqueous layer than in the aqueous part of the protoplasm.
⁵⁰ Methyl alcohol and trimethyl citrate penetrated almost as rapidly into living cells as into dead cells.
But a weak acid or base may be expected to penetrate more rapidly than its salt.\textsuperscript{41}

So far it has been assumed that steady state (linear gradient) diffusion between the sea water and sap is established at once. But obviously it requires finite time. Without knowing the value of the diffusion constant for NaI in the protoplasm we cannot evaluate the time required for the establishment of linear gradient diffusion by the recognized methods.\textsuperscript{52}

The time required for approximate attainment of the steady state is given by the relationship

\[ t = \frac{H^2}{\pi^2 D} \ln \frac{2}{\epsilon} \]

where \( \epsilon \) is the fraction by which the system varies from the steady state.

Putting \( H = 10 \) microns and \( D' = 0.0004 \) cm.\(^2\) per hour and \( \epsilon = 0.0001 \), \textit{i.e.} \( 1/100 \) of \( D \) in aqueous solutions

\[ t = 9 \text{ seconds} \]

Thus the time required to establish the steady state is so brief, even when the absolute rate of entrance is very slow, as to be negligible,\textsuperscript{53} and can have no bearing on the observed falling off in the value of \( k \) for NaI penetration or the deviation of the \( (\frac{\partial x}{\partial \alpha})_t \) curve from the linear form.

\( (c) \) Finally the shape of the \( (\frac{\partial x}{\partial \alpha})_t \) curve inevitably suggests that two processes are involved in iodide entrance. For example, as Fig. 6 shows, the iodide may be entering in part by diffusion, a process which gives a linear relationship between \( x \) and \( \alpha \) (Curve I) and in part by a process which becomes less effective with time (Curve II). The

\textsuperscript{41} Osterhout, W. J. V., \textit{Bot. Rev.}, 1936, 2, 303 (footnote 27).


\textsuperscript{53} It is not necessary to take into account the partition coefficient in calculating the time for the attainment of steady state diffusion since \( S \) is only concerned with the concentration of the diffusing substance in the unstirred protoplasmic layers in contact with the sea water and sap, and these terms do not enter into the equation.
combination yields Curve III which is of the shape found. We can only speculate as to the nature of the process responsible for Curve II. Osterhout has shown that for the entrance of ammonia into *Valonia* a curve which has the required shape is obtained and that this is plausibly explained by assuming that a reversible reaction between NH$_4$OH and HX, an acidic constituent of the protoplasm which is present in limited amounts, precedes penetration. In the present case, however, it is difficult to see what the reaction can be, for as has been pointed

![Diagram](image)

**FIG. 6.** Hypothetical curves for iodide entrance; Curve I, entrance by diffusion; Curve II, entrance by combination; Curve III, the resultant of the two processes.

out above, there is very little reason to assume that a reaction between HI and a basic constituent of the protoplasm can occur. The nature of the reaction if it takes place is therefore entirely unknown.

**SUMMARY**

When 0.1 M NaI is added to the sea water surrounding *Valonia* iodide appears in the sap, presumably entering as NaI, KI, and HI. As the rate of entrance is not affected by changes in the external pH we conclude that the rate of entrance of HI is negligible in comparison
with that of NaI, whose concentration is about $10^7$ times that of HI (the entrance of KI may be neglected for reasons stated).

This is in marked contrast with the behavior of sulfide which enters chiefly as H$_2$S. It would seem that permeability to H$_2$S is enormously greater than to Na$_2$S. Similar considerations apply to CO$_2$. In this respect the situation differs greatly from that found with iodide.

NaI enters because its activity is greater outside than inside so that no energy need be supplied by the cell.

The rate of entrance (i.e. the amount of iodide entering the sap in a given time) is proportional to the external concentration of iodide, or to the external product $[N^+][I^-]$, after a certain external concentration of iodide has been reached. At lower concentrations the rate is relatively rapid. The reasons for this are discussed.

The rate of passage of NaI through protoplasm is about a million times slower than through water. As the protoplasm is mostly water we may suppose that the delay is due chiefly to the non-aqueous protoplasmic surface layers. It would seem that these must be more than one molecule thick to bring this about.

There is no great difference between the rate of entrance in the dark and in the light.