THE KINETICS OF PENETRATION

II. THE PENETRATION OF CO₂ INTO VALONIA

BY A. G. JACQUES AND W. J. V. OSTERHOUT

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

( Accepted for publication, September 19, 1929)

In a previous paper it was shown that in the cells of Valonia macrophysa the undissociated CO₂ very soon comes into equilibrium with that in the sea water outside. As this does not tell us in what form CO₂ penetrates it seemed desirable to study the time curves of penetration, for it has been shown that if they follow an equation of the first order the velocity constant, \( V_M \), which is found when molecules alone enter, should theoretically behave like the velocity constant, \( V_A \), found when the ion pair \( H^+ + HCO_3^- \) alone enters; furthermore if both molecules and the ion pair \( H^+ + HCO_3^- \) enter the velocity constant, \( V_M A \), should act like \( V_M \) and \( V_A \); but this is not true of \( V_N A \), found when only the ion pair \( Na^+ + HCO_3^- \) enters, or of \( V_M Na \), found when molecules of \( HA \) enter in addition to the ion pair \( Na^+ + HCO_3^- \). Similarly, if the internal pH value remains constant as the external pH value increases, \( V_M , V_A , \) and \( V_M A \) should, theoretically, remain constant, but \( V_N A \) and \( V_M Na \) should increase. If the internal pH value rises, \( V_M , V_A , \) and \( V_M A \) should fall off but \( V_N A \) should rise: and \( V_M Na \) should fall or rise, depending on whether molecules or ions penetrate more rapidly.

Furthermore, if we keep the concentration of undissociated CO₂

1 Osterhout, W. J. V., and Dorcas, M. J., J. Gen. Physiol., 1925-26, 9, 255. This paper contains references to previous work on the penetration of weak acids into living cells. For experiments on the penetration of CO₂ into Valonia see Brooks, M. M., Pub. Health Rep., 1923, 38, 1470.


constant while increasing that of HCO₃⁻ and CO₃²⁻ (by raising the pH value and concentration of total CO₂ in the sea water) we should expect an increase in the rate in case HCO₃⁻ or CO₃²⁻ enters. It was therefore decided to compare the time curves of penetration of CO₂ at high and low pH values, e.g., at pH 4.8 at which the CO₂ present in sea water is considered to be almost completely undisassociated, and at pH 6.8 where the dissociation is over 75 per cent.

The experiments were carried out on Valonia macrophysa in Bermuda at a temperature of 20° to 24°C.; the temperature did not vary more than 0.5°C. during any one run.

Samples of sea water were prepared in 2-liter Winchester bottles, uniform as to the sea water used, but differing as to content of CO₂: the pH value was either 4.8 or 6.8. All the samples for the series reported here were prepared at one time. They were protected from change in pH value or CO₂ content by being filled without gas space, and having the rubber stoppers wired down. The CO₂ was introduced by adding the required amount of NaHCO₃, and the pH value was adjusted by adding 0.1 N NaOH or HCl. The pH value was determined colorimetrically, using the indicators of Clark and Lubs, and Kolthoff's buffers (with allowance for salt error).

The experiments on penetration were carried out in wide-mouthed bottles of about 125 cc. volume. All the bottles required for one run were filled at one time. For this purpose one of the Winchester bottles mentioned above was unstoppered, and a rubber stopper carrying a wide siphon tube was inserted. About 100 cc. of the sea water was withdrawn to wash out the tube, and its place was taken by the same amount of "Nujol" drawn into the Winchester bottle as the sea water ran out. The small bottles were then quickly filled, the end of the siphon tube being allowed to dip below the surface of the entering liquid in order to cut down the loss of gas. Each bottle was stoppered with a rubber stopper as soon as it was filled, without gas space above the liquid. A certain amount of gas escaped from the main volume of sea water as its level fell in the Winchester bottle, but the layer of "Nujol" on top served to reduce this loss.

It is desirable to have cells of uniform size and shape but as this was impossible we endeavored to have the same volume and surface exposed in each case. To get consistent results, the cells must be selected so that the same volume and surface...
are exposed in the determination of each point in a run, and the stirring must be uniform. To achieve the first condition we collected a large number of cells and divided them into four groups of the following approximate volumes, 0.75 to 0.6 cc., 0.5 to 0.4 cc., 0.4 to 0.3 cc., and 0.3 to 0.1 cc. For each small bottle we selected 1 cell from the first group and 2 cells from each of the other groups. And in each of these three groups we chose equal numbers of approximately spherical, and approximately ellipsoidal cells. The total volume of cells in each small bottle was thus about 2.7 cc. The second condition, uniform stirring, was secured by means of a special stirrer (Fig. 1). This consisted simply of a square brass shaft, A (Fig. 1), revolving on the bearings D and D₁, recessed on opposite sides. The six recesses, B, had curvatures which would fit the curvature of the type of bottle used throughout this work. Each recess was provided with a set of two brass rods, C, one on each side and so arranged that a bottle could be slipped easily between them (see the lower left recess of the figure). Sliding on each set of rods was a curved brass bar E (½ inch wide). This was fitted with a handle and it was pressed towards the shaft by means of steel springs F coiled around the rods. The bar E was prevented from approaching the shaft too closely by the brass pins, G, drawn through the rods. With no bottle in place the distance between the center of the bar and the center of the recess was about ¼ inch less than the diameter of a bottle. When it was necessary to insert a bottle the bar could be pulled out slightly by the handle and the bottle put in. On releasing the handle the bar held the bottle firmly against the shaft. It was possible to insert any bottle while the stirrer was in motion. Thus we could start the stirrer and without stopping it determine all the points required for one run. This contributed greatly to the uniformity of stirring throughout a run, and since the shaft was always revolved at the same rate (4 revolutions per minute) the stirring was quite uniform throughout the work. This was about the best rate since the bottles were turned over just fast enough to cause the cells to describe a figure of eight motion in the bottle. This served not only to keep the

That is, across the bottom, then ascending diagonally to the top, across the top, and descending diagonally to the bottom.
cells in motion, but also to keep the CO₂ content of the sea water uniform throughout.

In carrying out an experiment, i.e., the determination of a single point on a time curve, the stopper of a bottle was sharply jerked out (using a file with a sharpened point as a lever), the cells were at once dropped in, the stopper was reinserted tightly without gas space, and the bottle was immediately placed on the stirrer. A small loss of gas was inevitable when the stopper was removed, since there was a momentary vacuum created over the liquid. By jerking the stopper out quickly, this loss was reduced. The whole operation, from the selection of the cells to the commencement of stirring, could be carried out in from 5 to 10 seconds; so that, except in the case of the 1-minute experiments, the proportion of non-uniform stirring was small. About 2 seconds before the end of the experiment the bottle was taken from the stirrer and the cells were poured out onto a nichrome screen over a beaker. As quickly as possible the sap was extracted for analysis.

For the collection of the sap, pipettes were prepared from 5 mm. thick wall glass tubing. This was drawn down to a point about 2.5 mm. in diameter with an opening of approximately 0.5 mm. The point was then ground off at 60° to a sharp but slightly jagged edge. Three pipettes were used, delivering 1.1930 ±0.0015 cc., 0.8830 ±0.0008 cc., and 0.8857 ±0.0010 cc. of distilled water, the last drop being blown out in each case. It was found that the sap always left a thin film of gelatinous material in the pipette, so that to attain the accuracy mentioned it was necessary to treat the pipette with chromic acid solution between fillings.

The extraction of the sap was accomplished by bringing the cell against the sharp edge of the pipette and imparting a twisting motion to it. The saw-like edge instantly cut through the tough cellulose wall, and the sap could be expressed into the pipette by gentle squeezing. As the cell became deflated the protoplasm was detached, and mixed with the sap. To avoid driving this into the pipette the last drop of sap was not extracted. After extraction of the necessary amount of sap, those cells which were not punctured were returned to ordinary sea water for observation of injury and kept under observation for several days:¹⁰ when injury occurred the experiment was rejected.

In sampling, our routine was to proceed from the smallest cells up. An occasional cell softened during the experiment, and as we had no way of determining whether this indicated an increased permeability of the protoplasm we rejected all such cells. Sometimes on attempting to extract the sap from the smaller cells, the rupture spread beyond the rim of the pipette, and part or all of the sap

¹⁰ The best criterion of injury is microscopic observation (under the high power) which should be frequently carried out during the experiment as reversible injury may occur (i.e., injured spots may appear during the experiment which may become normal in appearance when the cell is returned to its normal environment).
was lost. For these reasons uniformity in the size of the cells taken for analysis was not attained.

Immediately after extraction the sap was transferred to a Van Slyke constant pressure carbon dioxide apparatus, and the analysis was carried out according to the method of Van Slyke. In every case (regardless of whether the sample was slightly greater or less than 1 cc.) the total volume was made up to 2.5 cc. so that the tables given in the paper quoted could be used.

The accuracy of the analysis for CO₂ by the Van Slyke method can be calculated from the figures given by Van Slyke in the paper quoted above. The probable error of the mean is about 0.17 per cent of the mean for a concentration range comparable with that of our solutions. This error is assumed to include the error involved in measuring out the solution by pipette. The error produced by the loss of gas from the sap during transfer to the Van Slyke apparatus may be more serious than the formal error of analysis. But it is certainly small in comparison with the variation of the material. For this reason we did not adopt the method of transfer suggested by Van Slyke and Neill, by which the sample is delivered directly into the gas burette. Instead we kept this error as low as possible by transferring the sap rapidly.

A further source of error was the variation of the CO₂ content of the sea water in a single run. This was unavoidable in our method of filling the small bottles. The possibility of carrying out this operation by causing the sea water to displace an oil like "Nujol" upwards, or mercury downwards, was rejected because the oil could not be displaced cleanly and the cells became coated with grease, while mercury was rejected because of the toxic nature of its salts. To evaluate this error we filled a number of bottles with CO₂-containing sea water at pH 4.8, in the usual manner, and then unstoppered them at intervals during 2 hours, and analyzed for CO₂. The probable deviation from the mean was found to be 0.007 cc. or 1.1 per cent.

Unless otherwise stated the curves are drawn free-hand to give a rough fit.

The data for four time curves at pH 4.8 and four at pH 6.8 are given in Table I.

---

13 It may be of interest to compare the figures for penetration of CO₂ into living Valonia with those obtained by Northrop (Northrop, J. H., J. Gen. Physiol., 1928-29, 12, 435, Table III) for penetration through a (previously well dried) collodion membrane separating a solution of NaOH from a solution of CO₂. Since in his experiments CO₂ was dissolved in distilled water we may use for comparison our results at pH 4.8. If we consider that 0.1 mg. CO₂ penetrates in 1 minute per cubic centimeter of sap at 20°C. when the external concentration is 0.904 mg. CO₂ per cubic centimeter sea water the pressure at the start is 0.5 atmosphere (allowing 0.009 mg. CO₂ per cubic centimeter sap at the start) so that
If the penetration is a simple diffusion we might expect the time curve to obey an equation of the first order. The velocity constants calculated from the equation,

\[ K = \frac{1}{t} \log \frac{a}{a-x} \]

are shown in Fig. 2. Since they fall off from the start it might seem that the equation for a dimolecular reaction might give better values: accordingly calculations were made with the dimolecular equation

\[ K_1 = \frac{1}{t(a-b)} \log \frac{(a-x)b}{(b-x)a} \]

putting \( a = 1.00 \) and \( b = 1.35 \). Since this gives a more constant value of \( K_1 \) (as shown in Fig. 2) it might seem that the dimolecular equation should be employed, and that possibly a reaction occurs between the penetrating substance and some constituent of the protoplasm. We find, however, that when we multiply all the ordinates of a time curve by the same factor (varying the factor from curve to curve) so as to make the equilibrium value the same in all cases, the curves agree within the limits of experimental error (Fig. 3). Hence the times for half completion must be approximately the same in all cases. This would not be the case if they obeyed a dimolecular equation (unless

at 1 atmosphere 0.2 mg. \( \text{CO}_2 \) would penetrate per minute per cubic centimeter of sap and for a cell containing 0.41 cc. the amount penetrating would be 0.2 (0.41) \( = 0.082 \text{ mg.} \). Such a cell may be regarded as having a surface of 2.8 sq. cm. If the surface were 1 sq. cm. the amount penetrating per minute would be 0.082 (2.8) \( = 0.2029 \text{ mg.} \) and the amount penetrating per day would be 42 mg. \( \approx 21.2 \text{ cc.} \). The figure given by Northrop is \( 8.6 \times 10^{-4} \text{ cc. per square centimeter per day for a membrane 1 cm. thick.} \) If we consider the protoplasm to be in the neighborhood of 0.001 cm. thick this would give 0.86 cc. per day for collodion but as the non-aqueous part of the protoplasm is undoubtedly much thinner this figure is probably much too small. It would seem therefore that penetration may be of the same order of magnitude for such collodion membranes and for \textit{Valonia}.

\[^{14}\text{Common logarithms are used for convenience and } a \text{ is put equal to the final equilibrium value of the time curve: this value corresponds approximately to that of the undissociated } \text{CO}_2 \text{ in the sea water but is not equal owing to differences in solubility. (Cf. 1.)} \]

\[^{15}\text{Another way of looking at the matter is to say that if the curves agree when multiplied up (so as to have the same equilibrium value) the time for half com-} \]
TABLE I
Increase of Total CO₂ (Observed Value Less the Amount Present at the Start) in Cells
Unlike in Size and Shape. Each Figure Represents the Average of
20 or More Cells

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>pH of sea water 6.8</th>
<th>mg of total CO₂ per cc. of sap</th>
<th>pH of sea water 4.8</th>
<th>mg of total CO₂ per cc. of sap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. 1</td>
<td>Exp. 2</td>
<td>Exp. 3</td>
<td>Exp. 4</td>
</tr>
<tr>
<td>1</td>
<td>0.035</td>
<td>0.044</td>
<td>0.044</td>
<td>0.062</td>
</tr>
<tr>
<td>3</td>
<td>0.094</td>
<td>0.134</td>
<td>0.095</td>
<td>0.115</td>
</tr>
<tr>
<td>5</td>
<td>0.147</td>
<td>0.225</td>
<td>0.146</td>
<td>0.143</td>
</tr>
<tr>
<td>7</td>
<td>0.163</td>
<td>0.254</td>
<td>0.204</td>
<td>0.143</td>
</tr>
<tr>
<td>9</td>
<td>0.186</td>
<td>0.317</td>
<td>0.217</td>
<td>0.143</td>
</tr>
<tr>
<td>12</td>
<td>0.224</td>
<td>0.397</td>
<td>0.240</td>
<td>0.266</td>
</tr>
<tr>
<td>15</td>
<td>0.242</td>
<td>0.433</td>
<td>0.258</td>
<td>0.302</td>
</tr>
<tr>
<td>20</td>
<td>0.269</td>
<td>0.527</td>
<td>0.302</td>
<td>0.307</td>
</tr>
<tr>
<td>30</td>
<td>0.302</td>
<td>0.569</td>
<td>0.391</td>
<td>0.378</td>
</tr>
<tr>
<td>45</td>
<td>0.353</td>
<td>0.601</td>
<td>0.439</td>
<td>0.408</td>
</tr>
<tr>
<td>60</td>
<td>0.396*</td>
<td>0.649*</td>
<td>0.452</td>
<td>0.440</td>
</tr>
<tr>
<td>120</td>
<td>0.395</td>
<td>0.643</td>
<td>0.452</td>
<td>0.456</td>
</tr>
</tbody>
</table>

* This is taken as the equilibrium value.

Completion must be the same for all as is the case when they are of the first order and all have the same velocity constant. But if they are of the second order this is not true. For example, if the two reactants are equal at the start and if the velocity constant is 1 we have as the time for half completion, t, the following:

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

where \( a \) is the original concentration of each reactant or the final equilibrium value of \( x \) (\( x \) being the amount of substance formed by the reaction). Since \( x = 0.5 a \) at half completion, we have

\[ t = \frac{0.5a}{a^2 - 0.5a^2} = \frac{1}{a} \]

that is, \( t \) is inversely proportional to \( a \). Hence if with different concentrations of reactants the curves have the same velocity constant, it follows that the times for half completion cannot be the same and the curves will not be identical when the equilibrium values are made equal by multiplying the ordinates.

If the reaction is dimolecular we should expect the velocity constant to increase
FIG. 2. Monomolecular velocity constants for living and dead cells calculated from the equation \( K = \frac{1}{t} \log \frac{a}{a-x} \), where \( x \) is the increase of total CO\(_2\) in the sap and \( a = 1.00 \), the equilibrium value: also dimolecular constants for living cells calculated from the equation \( K_2 = \frac{1}{t(a-b)} \log \frac{(a-x)b}{(b-x)a} \), where \( a = 1.00, b = 1.35 \).

As the external concentration of CO\(_2\) increases, but Fig. 3 shows that this is not the case (for example, when we compare experiments at the same external pH value).

If a compound is formed between the protoplasm and CO\(_2\), we should expect that in the case of (H\(^+\), HCO\(_3^-\)) ion pairs we should have to deal with two consecutive dimolecular reactions: H\(^+\) + HCO\(_3^-\) \(\rightarrow\) H\(_2\)CO\(_3\) at the surface, followed by H\(_2\)CO\(_3\) + protoplasm \(\rightarrow\) H\(_2\)CO\(_3\) protoplasm, and even a termolecular reaction: H\(^+\) + HCO\(_3^-\) + protoplasm \(\rightarrow\) H\(_2\)CO\(_3\) protoplasm. In either case the imposition of the second reaction on the reaction where molecules alone are involved ought to alter the form of the curve. The fact that it does not might be regarded as evidence for the view that (H\(^+\), HCO\(_3^-\)) ion pairs do not penetrate. And as we pointed out above there is also evidence for the view that (Na\(^+\), HCO\(_3^-\)) ion pairs do not penetrate.
their velocity constants happened to fall in just the right relation to the concentrations employed: this would require an extraordinary set of coincidences) but would if they followed the monomolecular formula. The question arises, How can they follow this formula

![Graph](image)

**Fig. 3**. Shows the result of bringing all the curves (for unlike cells) to the same final value by multiplying all the ordinates of a curve by the same factor (using a different factor for each curve).

To avoid crowding some symbols have been displaced to one side but these are connected by a line to the point where they belong.

when the velocity constants fall off as in Fig. 2? Now in the case of dead cells, where there would seem to be no question that the ordinary

16 These often show a slightly higher velocity constant at pH 4.8 than at pH 6.8 which is to be expected since the diffusion constant of CO₂ is greater than that
diffusion formula is followed, the constants often fall off in much the same way. It would therefore seem possible to regard the curves as of the first order but having a "velocity constant" which falls off from the first as would be the case, for example, if the temperature were falling. They can be fitted more or less closely\(^\text{17}\) by means of the empirical formulae\(^\text{18}\)

\[
K_3 = \frac{h_3}{t_3} \ln \frac{a}{(a-x) - \alpha} \quad \text{and} \quad K_4 = \frac{h_4}{t_4} \ln \frac{a}{(a-x) - \alpha}
\]

of Na\textsubscript{2}CO\textsubscript{3} (cf. Internat. Critical Tables, V, 63, 67) and the cell wall is so permeable that only ordinary diffusion would appear to be involved. We might apply here the equations employed elsewhere\(^\text{9}\) but this is not necessary since no protoplasm is present and consequently there is only one phase (the cell wall being so permeable that it need not be regarded as a distinct phase).

\(^\text{17}\) These may fit the latter part of the curve better than the earlier part.

\(^\text{18}\) In these formulae \(K_3\) is not independent of \(a\).
other empirical formulae may be used in similar cases, e.g.,

\[ K_3 = \frac{1}{t} \ln \frac{a}{a - x} \quad \text{and} \quad K_2 = \frac{1}{x^2} \ln \frac{a}{a - x} \]

Let us now consider the behavior of the "constants" more closely. When they are plotted against \( a - x \) (as in Fig. 4) the curve does not differ greatly from that with time as abscissae (Fig. 2). It is better to plot the constants calculated for short time intervals at various points along the curve; these may be called "momentary constants" for convenience. They are calculated from the smoothed curve

\[ \text{Table II} \]

Increase of Total CO₂ (Observed Value Less the Amount Present at the Start) in Cells Alike in Size and Shape. Each Figure Represents the Average of 20 or More Cells

<table>
<thead>
<tr>
<th>Time in minutes</th>
<th>1</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>7.5</th>
<th>10</th>
<th>13</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live cells ( \text{pH}) of sea water (= 6 )</td>
<td>0.159</td>
<td>0.489</td>
<td>0.510</td>
<td>0.573</td>
<td>0.649</td>
<td>0.709</td>
<td>0.990</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live cells ( \text{pH}) of sea water (= 7 )</td>
<td>0.075</td>
<td>0.171</td>
<td>0.193</td>
<td>0.236</td>
<td>0.266</td>
<td>0.305</td>
<td>0.337</td>
<td>0.469</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dead cells ( \text{pH}) of sea water (= 8.2 )</td>
<td>0.181</td>
<td>0.501</td>
<td>0.669</td>
<td>0.777</td>
<td>0.844</td>
<td>1.112</td>
<td>1.240</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(A, Fig. 5) obtained by averaging all the points shown in Fig. 3. For this purpose we employ the formula

\[ K_m = \frac{1}{t_2 - t_1} \log \frac{a - x_1}{a - x_2} \]

where \( x_1 \) is the total CO₂ penetrating in the time \( t_1 \) and \( x_2 \) is that penetrating in the time \( t_2 \). The results of these calculations, shown in Figs. 6 and 7, offer nothing essentially new.

It is therefore clear that the constants really fall off and it might be natural to ascribe this to differences in size and shape as the result of which some cells would have a relatively greater surface and

\[ ^{19} \text{In the first part of the curve the interval between} \ t_1 \text{and} \ t_2 \text{is in seconds but in the latter longer intervals are used.} \]
consequently reach equilibrium sooner than the others so that when the whole group is considered penetration appears relatively rapid at the start.

To test this suggestion would require a quantity of cells uniform in size and shape which there seemed to be no immediate prospect of obtaining. Eventually, however, a supply was secured and the experiments were carried out. The results obtained are shown in Table II and Fig. 5; the velocity "constants" fall off as is shown in Figs. 2, 4, 6, and 7.

The experiments were carried out as before except that a Van Slyke constant volume apparatus was used and in order to avoid loss of CO2 higher pH values (6 and 7) were used. The temperature varied between 18°C and 20°C.

It is evident that if some of the cells were more permeable than others the effect would be the same as if their surface were relatively large and this would cause a falling off of the "constants." We are inclined to accept this explanation.

Experiments on dead cells bear out this idea and are in harmony with investigations on the penetration of KIO3 made several years ago by Mr. W. C. Cooper, Jr. (in collaboration with the senior author). Studying the penetration of KIO3 into dead cells of the same size and
shape it was found that the velocity "constants" sometimes fell off and sometimes remained practically constant. The latter condition was favored by using cells whose permeability was similar because they were freshly killed. When such cells were mixed with others that had stood for a long time in sea water after killing (so that their walls were partially disintegrated) the constants fell off much more markedly. This was doubtless due to the fact that some of the dead cells were more permeable than the others.

Since under the most favorable conditions the velocity constants do not fall off when dead cells of the same size and shape are employed, we are inclined to attribute the falling off with cells of the same size and shape, whether alive or dead (for dead cells see Table II and

20 These experiments were carried out with sea water at pH 8.2.
Figs. 2, 6, and 7), to differences in permeability which cause a more rapid penetration on the part of certain cells: for when these have reached equilibrium penetration continues in the others so that the process for the whole group seems relatively rapid at the start.21

There is always a small amount of CO₂ present in the sap even before its exposure to the CO₂-bearing sea water, which would tend to make the velocity constant too high at the start. We have determined this amount a number of times on freshly gathered cells which may be assumed to be growing, and on cells which have been kept in the laboratory and have practically ceased to grow. Very little difference was found between the two groups and the average amount was 0.009 mg. of CO₂ per cubic centimeter of sap. This was subtracted from the observed figures before calculating the velocity constants. It may

21 It might be thought that if there is much difference in permeability an occasional sample would consist largely of cells of high or of low permeability. The experiments with cells of the same size and shape are not extensive enough to settle this point: the figures for one set of determinations are given in Table III.
be added that the production of CO$_2$ during the experiment is too small to affect the result.

Another factor which might affect the velocity of penetration is the change in the pH of the sap as the CO$_2$ concentration increases. We have investigated this change experimentally. Fig. 8 gives our results. Our experiments were not exhaustive but it seems safe to say that the rate of change in pH of the sap is dependent chiefly on the rate of accumulation of CO$_2$ in the sap, and that it is independent of the pH of the sea water, except as a lower pH of the sea water means that a greater proportion of CO$_2$ is in molecular form. Assuming that when the pH of the sap has fallen to 4.7 the CO$_2$ is present almost entirely as molecules, we see that this condition of association may be reached early in the runs (and might be reached earlier in the protoplasm than in the sap). We are inclined to think that this means that there

![Fig. 8. Changes in the pH value of the sap during the entrance of CO$_2$.](image-url)
is little or no penetration of the ion pair $Na^+ + HCO_3^-$ for the entrance of NaHCO$_3$ would raise the pH, opposing the drop produced by the entrance of molecules of CO$_2$.

If it is chiefly the molecules which enter, the decrease of internal pH value during penetration would tend to make the velocity constant appear greater and greater as long as it continued, but as a matter of fact, the value of $K$ is found to fall off at the start and it continues to fall off after the internal pH value has presumably become constant.

In case the outside volume is small compared to that of the cells the external concentration will decrease as time goes on, but this should not cause a falling off in the velocity constant since the calculation is referred to the equilibrium value of CO$_2$.

The essential point is not the constancy of $K$ but its behavior at different pH values. If we regard the time curves as belonging to the first order (but with $K$ falling off from the start) we may conclude that the velocity constant is practically the same at high and low pH values. For reasons pointed out elsewhere this would be expected if the chief substance penetrating were undissociated CO$_2$ or the ion pair $H^+ + HCO_3^-$, but not if NaHCO$_3$ entered to any noticeable extent.

The question whether the time curve is of the first order need not be raised if we consider only the initial rate $\left( \frac{dS}{dt} \right)_i$ (i.e., the rate when $t$ is very small) for then all time curves approximate the zero order (i.e., a straight line) since the outside concentration remains approximately constant and practically none of the penetrating substance moves outward. It has been shown elsewhere that when both un-

22 As the pH value of the sap decreases the per cent of loss during manipulation increases because more of the CO$_2$ is in volatile form but this would not cause a falling off in the velocity constant because it affects the equilibrium value even more than the early values.

23 The case is similar to that of a reversible monomolecular reaction $A \rightleftharpoons B$ where $A$ decreases but the time curve of increase of $B$ is monomolecular (cf. Mellor, J. W., Higher mathematics for students of chemistry and physics, London 1922, p. 228) when the calculation is based on the equilibrium value.

In these experiments the volume of cells was relatively small in comparison with that of the sea water and in no case did the external concentration of total CO$_2$ fall off more than 2 per cent during an experiment.
dissociated molecules and the ion pairs $H^+ + HCO_3^-$, $Na^+ + HCO_3^-$ and $K^+ + HCO_3^-$ enter we may write

$$\left(\frac{dS_i}{dt}\right)_b = P_H M_o + P_H A_o + P_{Na} Na A_o + P_{K} K A_o$$

where $M_o$ is the molar concentration of undissociated CO$_2$ outside (including H$_2$CO$_3$), $H_o$ the external concentration of $H^+$, $Na_o$ that of $Na^+$, $K_o$ that of $K^+$, and $A_o$ that of HCO$_3^-$; $S_i$ is the total CO$_2$ inside, $P_H$ is the amount of $M_o$ entering in unit time through unit surface under unit pressure, $P_A$ is the corresponding value for the ion pair $H^+ + HCO_3^-$, $P_{Na}$ that for the ion pair $Na^+ + HCO_3^-$, and $P_{K}$ that for the ion pair $K^+ + A^-$. 

The results here given (together with unpublished figures) show that when the equilibrium values are made the same by multiplying the ordinates the time curves agree closely. For example, when all the curves are brought (by multiplication of the ordinates) to the equilibrium value 1.00, the average for the first minute is 0.0965 for pH 4.8 and 0.0960 for pH 6.8. With uniform cells (which furnish the most decisive test) the values for the increase at pH 6 at the end of the first minute are 0.1568, 0.1582, and 0.1605, giving an average of 0.1585. In order to bring the equilibrium value (0.990) to the standard (1.00) we must multiply it by 1.01 and we accordingly multiply 0.1585 by the same factor giving 0.160. How does this compare with the result at pH 7? The values at the end of the first minute are 0.0728, 0.0724, 0.0738, giving an average of 0.073. Since the equilibrium value 0.469 must be multiplied by 2.132 to bring it to the standard (1.00) we multiply 0.073 by the same factor, giving 0.156 which is very close to the value at pH 6 (0.160).

It would seem that the value of $\left(\frac{dS_i}{dt}\right)_b$ is practically the same at low and at high pH values. As the equilibrium values are the same the values of $M_o$ must also be the same (provided the internal pH values are approximately the same, as seems to be the case). Now at pH 4.8 the concentration of HCO$_3^-$ approaches zero so that the value of $P_H H_o A_o + P_{Na} Na A_o + P_{K} K A_o$ reduces almost to zero and when $\left(\frac{dS_i}{dt}\right)_b$ and $M_o$ have the same values at pH 6.8 and at 4.8 the value of
the expression \( P_{\text{H}_2\text{A}_2} + P_{\text{Na}_2\text{A}_2} + P_{\text{K}_2\text{A}_2} \) must be nearly zero at pH 6.8 even though the concentration of \( \text{HCO}_3^- \) and of \( \text{A}_2 \) is relatively large. This can only mean that there is little penetration of the ion pairs \( \text{Na}^+ + \text{HCO}_3^- \) and \( \text{K}^+ + \text{HCO}_3^- \).

In other words, the results indicate that adding \( \text{NaHCO}_3 \) to the external solution has little effect on the rate of penetration provided we do not at the same time change the external concentration of undissociated \( \text{CO}_2 \) so that we conclude that there is very little penetration of \( \text{HCO}_3^- \) or \( \text{CO}_3^- \).

It may be objected that since the value of \( \left( \frac{dS}{dt} \right)_b \) is not directly
determined there may be some uncertainty in this procedure. We have tested this matter experimentally in the following way.

Two samples of sea water, one at pH 6 and the other at pH 7, containing approximately the same amount of free \( \text{CO}_2 \) were prepared and placed in 125-cc. bottles. Then by means of an apparatus similar to that used by Osterhout and Dorcas\( ^3 \) gas was circulated between the two solutions for 2 hours, at the end of which time it was assumed that the \( \text{CO}_2 \) tension was the same in each. The two solutions were then used in penetration experiments on cells of about the same size and shape. The time of exposure was 2 minutes. The results of six pairs of determinations are given in Table III.

It will be seen that the amount of \( \text{CO}_2 \) penetrating during the 2 minutes of exposure in these experiments is much smaller than that penetrating in the same time in the experiments reported above. The reason for this is that the smaller amounts of \( \text{CO}_2 \) can be distributed equally between the two samples of sea water.

<table>
<thead>
<tr>
<th>pH of sea water</th>
<th>mg. of total ( \text{CO}_2 ) per cc. of sap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual experiments</td>
</tr>
<tr>
<td>6</td>
<td>0.0111</td>
</tr>
<tr>
<td>7</td>
<td>0.0130</td>
</tr>
</tbody>
</table>

Differences between the columns are due to differences in the concentration of \( \text{CO}_2 \) in the sea water in the different experiments but in each column the concentration of undissociated \( \text{CO}_2 \) in the sea water was the same for both pH values.
in a reasonable time, while at the same time the losses due to diffusion through the rubber part of the distributing system, and during the penetration experiments are very much cut down.

In spite of the small amount of CO$_2$, by reducing the gas to the smallest volume permitted by the Van Slyke constant volume apparatus, we have measured it with an error which does not exceed twice the error of the previous measurements.

Table III shows that the average values of 6 pairs of experiments check within 2 per cent which is well within the experimental error which we believe to be not greater than 6 per cent. It would therefore seem that the rate of penetration depends only on the concentration of undissociated CO$_2$ and not on that of HCO$_3^-$ or CO$_3^{2-}$ since in these experiments the concentration of ions could be greatly varied without affecting the rate of penetration as long as the concentration of undissociated CO$_2$ remained unaltered.

**SUMMARY**

The rate of penetration of CO$_2$ into living cells of *Valonia* has been studied at high and low pH values.

The time curve of penetration appears to be of the first order but with a "velocity constant" which falls off from the start.

The evidence indicates little penetration of ions. This is shown by (a) the similarity of velocity "constants" at high and low pH values, (b) the rate of penetration, which remains constant as long as the external concentration of undissociated CO$_2$ remains constant no matter how much the concentration of ions varies.