

## IS LIVING PROTOPLASM PERMEABLE TO IONS?\*

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This question, answered in different ways by opposing schools, has become a center of controversy. Although each side has assembled an imposing array of facts, on every important point the evidence is conflicting and the interpretation doubtful. It is evident that the most satisfactory way of attacking the problem is to turn aside from the indirect methods which have resulted in contradictions and to aim rather at accurate measurements of actual penetration by direct analysis of the contents of the cell. We may hope that this procedure will clear up the existing confusion and prepare a sound basis for a theory of permeability.

The writer has been fortunate in finding an organism which allows such direct determinations to be made. It is a marine alga, *Valonia macrophysa*, Kütz.,<sup>1</sup> whose large, multinucleate cells sometimes contain as much as 10 cc. of sap, forming a central vacuole, which is surrounded by a delicate layer of protoplasm, outside of which is a cellulose wall.

To prepare the material for experiments clusters of cells were brought into the laboratory and the individual cells carefully separated. They were then allowed to stand for a day or more in order that any effect due to the handling might become manifest. Cells which showed any sign of injury were discarded.

Cells as nearly alike as possible in respect to size and shape were selected (the average volume was about 0.33 cc.). When smaller cells were attached to larger ones, care was taken to have as much similarity in the absorbing surfaces as could be obtained. Cells

\* Contributions from the Bermuda Biological Station for Research. No. 151.

<sup>1</sup> Cf. Osterhout, W. J. V., *J. Gen. Physiol.*, 1922-23, v, 225.

to which other organisms had attached themselves were rejected. In this way the conditions for absorption were made as nearly uniform as possible.

In the experiments here described the penetrating substance was  $H_2S$ . Cells placed in sea water containing various amounts of this gas were left until equilibrium was established between the  $H_2S$  inside and outside the cell. The concentration of total sulphide in the cell sap was then compared with that in the sea water.

The  $H_2S$  was prepared by the action of dilute c.p.  $HCl$  on  $FeS$ . The gas was passed through a column of cotton. In some experiments c.p.  $Na_2S$  and c.p.  $CaS$  were used to discover if the less pure  $H_2S$  obtained from the crude  $FeS$  differed in its action from that of the uncontaminated gas. No difference was found in the effects of the  $H_2S$  obtained from the two sources.

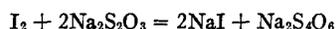
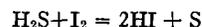
The  $H_2S$  was bubbled through sea water until the desired concentration was reached.<sup>2,3</sup> The cells were placed in bottles of 125 cc. capacity, filled to the brim with sea water containing  $H_2S$ : 12 cells were placed in each and the bottle immediately stoppered with a rubber stopper which was wired to hold it firmly in place. This eliminated any gas space, thereby helping to prevent oxidation of the sulphide during the experiment. When the pH value of the solution was varied the quantity of  $HCl$  or  $NaOH$  required to give the desired pH was placed in the bottle before pouring in the sea water. The stopper was inserted, the contents were then thoroughly mixed and a sample was taken for determination of the pH value. The bottles containing the cells were set aside until penetration was complete. This required about an hour for cells of this size, but in order to be sure that equilibrium was reached, the exposure was continued for

<sup>2</sup> The concentrations were varied in different experiments but were usually not allowed to go above .06 M. It was found that the relation between total sulphide inside and outside was little affected by the total outside concentration so long as the pH value was kept constant.

<sup>3</sup> The sea water became cloudy with particles of sulphur, due to the oxidation of the  $H_2S$  by oxygen in the sea water. There is no objection to this since these particles do not affect the titrations and these cloudy solutions were used in the treatment of the cells. When, however, the pH of the solution was raised to 8 or higher by the addition of  $NaOH$ , as happened in some experiments, there resulted a perfectly clear solution.

2 or  $2\frac{1}{2}$  hours. A longer exposure was avoided because of the possibility of injury to the cell.

The analysis was made by treating the sample with a measured volume of .01 N iodine, and then titrating back the excess iodine with .01 N  $\text{Na}_2\text{S}_2\text{O}_3$ , using starch as indicator.



This measures the total sulphide ( $\text{H}_2\text{S} + \text{HS}' + \text{S}''$ ).

At the close of the experiment the bottle containing the cells and sea water was shaken so as to mix the contents. The stopper was then removed and a specially constructed 3 cc. pipette was dipped into the sea water. This pipette was fashioned to reduce to a minimum the loss of  $\text{H}_2\text{S}$  in obtaining the sample for analysis. It consisted of 3 cc. bulb blown in a glass tube of 4 mm. bore, the bulb being drawn out at the lower end into a fairly large capillary and constricted at the upper end or graduation point. The tip of the pipette was ground with a file to a very sharp edge to facilitate pricking the cells in collecting sap. When the pipette was dipped in the sea water the finger was placed over the open end and the pipette taken out of the bottle, the stopper being replaced. The pipette was carefully wiped with a clean cloth and the water level allowed to fall to the graduation mark. The pipette was then dipped into a measured volume of iodine solution and its contents allowed to run out. Rinsing with distilled water followed. The flask was shaken and three drops of starch solution added. The excess iodine was then titrated with  $\text{Na}_2\text{S}_2\text{O}_3$ . The stopper was again taken from the bottle and the cells and sea water emptied into a finger-bowl. The cells were taken one by one from the sea water, a spot quickly wiped dry and this spot pricked by thrusting the sharp end of the pipette through the cell wall. Pressure applied to the cell by the fingers forced the sap up into the pipette. When filled slightly above the graduation mark it was carefully wiped, the water level allowed to fall to the graduation mark, and the contents of the pipette allowed to run into a second measured volume of iodine. Ordinarily it took 1 minute to collect 3 cc. of sap.

The question arises whether any  $\text{H}_2\text{S}$  is lost in obtaining the sap.

To decide this point, dead cells were placed in sea water containing  $H_2S$  and allowed to come to equilibrium. The samples of sap and of sea water were taken in the usual manner. Practically no difference was found between the sulphide content of the dead cell sap and that of the sea water, which indicates that little if any  $H_2S$  is lost in procuring the sap from the cells.

Since the sap has some reducing power it was desirable to determine its value. 3 cc. of sap from cells of ordinary size were treated with .01 N iodine and the excess titrated with .01 N  $Na_2S_2O_3$ . The reducing power ranged from .1 cc. to .2 cc. of .01 N iodine for 3 cc. of sap. A correction of .15 cc. .01 N iodine was therefore applied for each 3 cc. of sap (the titration usually consumed from 5 to 20 cc. of iodine solution, according to circumstances). The reducing action of the sea water was found to be negligible.

The pH values were determined colorimetrically; that of the external solution was taken as the figure obtained at the end of the exposure. The pH value of the sap of normal cells was found to average about 5.8. The pH value of the sap was not much affected during the time of the experiments by exposure to solutions with a pH range of 6.0 to 10.0. Outside of this pH range some influence of the external pH was observed, probably accompanied by injury.

The question arises whether the cells were injured by the treatment given them. It has been found that as soon as injury occurs the  $SO_4$  of the sea water begins to penetrate. No penetration of  $SO_4$  was observed during the time of the experiments unless the concentration of  $H_2S$  was very high and the pH value of the sea water fell below 6.0.<sup>4</sup>

At the end of each experiment all the cells that had been exposed to  $H_2S$  but which had not been pricked for the collection of sap were replaced in sea water and allowed to stand overnight. It was observed that after an exposure of 2 hours to  $H_2S$  there were often signs of injury the next day. It is not believed, however, that there was sufficient injury in any case to affect the results except possibly toward the end of the exposure in the highest concentrations at low pH values.

<sup>4</sup> When the cell is killed the concentrations of  $SO_4$  and total sulphide become the same in the sap as in the sea water.

The temperature varied somewhat from day to day (ranging from 20°C. to 22°C.) but there was very little variation during the time occupied by any experiment (on the average not more than one degree). The temperature coefficient is of the order characteristic of diffusion.

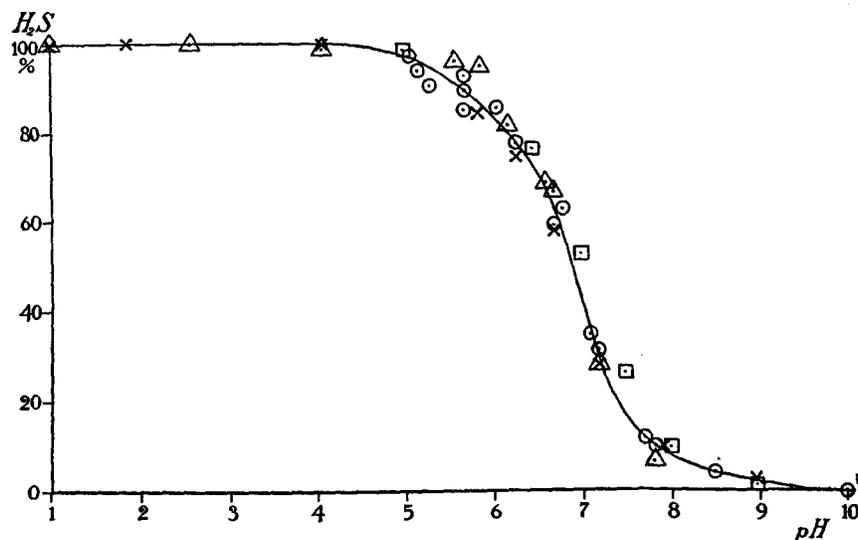


FIG. 1 demonstrates that the total sulphide in the sap corresponds with the undissociated  $H_2S$  in the external solution. The concentration of total sulphide ( $H_2S + HS' + S''$ ) in the cell sap (○) is expressed as per cent of the total sulphide in the outside solution. The values for the concentration of undissociated  $H_2S$  in the sea water as calculated from the dissociation constant (□) and as determined from the vapor tension (Δ) and from the rate of evaporation (×) at various pH values of the external solution are expressed as per cent of the corresponding values in the range pH 1 to 3 where all the  $H_2S$  is regarded as undissociated. Each point represents one determination.

The writer is indebted to Mr. William C. Cooper, Jr. for carrying out the experiments on penetration and on evaporation.

The results are shown in Fig. 1, in which the circles<sup>5</sup> denote the concentration of total sulphide ( $H_2S + HS' + S''$ ) found in the sap of living cells at various pH values after equilibrium is reached between

<sup>5</sup> Each circle represents the value obtained by mixing the sap of 12 cells and titrating.

the total sulphide outside and that inside. The curve is drawn free hand through these points to give an approximate fit. The concentration of total sulphide inside the cell corresponds approximately to that of undissociated  $\text{H}_2\text{S}$  in the sea water outside as determined by the vapor tension ( $\Delta$ ), the rate of evaporation ( $\times$ ), and as calculated from the dissociation constant ( $\square$ ).

The determinations of vapor tension were made by placing sea water containing  $\text{H}_2\text{S}$  in a bottle connected with a bottle of distilled water in such a way that a stream of gas could be made to circulate (bubbling through both solutions) by squeezing a rubber bulb. The whole formed a completely closed system, care being taken to make tight joints where the tubes passed through the stoppers, which were securely wired in place. The circulation of gas was continued until equilibrium was established between the  $\text{H}_2\text{S}$  in the sea water and that in the distilled water. The concentration of total sulphide in each was then determined by titration and the pH value was measured colorimetrically.

It was found that the lower the pH value of the sea water the higher was the relative total sulphide content of the distilled water as compared with that of the sea water; this was true down to pH 3 below which lowering of the pH value produced no increase in the relative concentration in distilled water. The  $\text{H}_2\text{S}$  was therefore regarded as undissociated at pH values below 3: the relative concentration of total sulphide in distilled water at these pH values was 116 per cent of that in sea water.<sup>6</sup> All the relative concentrations of total sulphide in distilled water were therefore multiplied by  $\frac{100}{116}$  and the resulting figures were taken as expressing the per cent of undissociated  $\text{H}_2\text{S}$  present. Thus at pH 1 to 3 the per cent of undissociated  $\text{H}_2\text{S}$  is 100, at pH 5.6 it is 96.5 per cent and so on. These are approximate determinations: it is probable that if care had been taken to keep the pH value of the distilled water solution low enough

<sup>6</sup> It is to be expected that  $\text{H}_2\text{S}$  will be less soluble in sea water than in distilled water. Cf. Hildebrand, J. H., Solubility, American Chemical Society monograph series, New York, 1924, 140.

The ratio between the concentrations of total sulphide in the two solutions at any given pH of the sea water solution was not much affected by the variations in the total sulphide content of the sea water.

to prevent all dissociation of  $\text{H}_2\text{S}$  and if the formation of polysulphides by oxidation had been completely prevented there would be less irregularity. Each point on the curve represents one determination. The writer is indebted to Mr. M. J. Dorcas for these determinations.

In order to determine the rate of evaporation dishes of the same size (with straight sides and flat bottoms) were filled to the same height with solutions having the same concentration of total sulphide but brought to different pH values by the addition of acid or alkali. The rate of evaporation was ascertained by titration and was taken as the reciprocal of the time required to lose a given fraction of the total sulphide: this was ascertained by constructing time curves and taking the reciprocals after the loss of a fraction which was so small that the change in pH value due to the evaporation could be neglected (or a suitable correction made). The amount of excess base varied in these experiments but not sufficiently to alter markedly the dissociation curve as calculated by Becking's equation.<sup>7</sup>

It was found that the rate of evaporation increased with increasing acidity until the neighborhood of pH 3 was reached. As in the experiments on vapor tension the results indicated that at pH values below 3 the  $\text{H}_2\text{S}$  is not dissociated and in consequence the rate of evaporation at this point was taken as 100 per cent and all other rates expressed as per cent of that found from pH 1 to 3.

The per cent of undissociated  $\text{H}_2\text{S}$  as indicated on the curve by square symbols ( $\square$ ) was calculated by means of the equation

$$\text{H}_2\text{S} = \frac{(H)(100 - \text{H}_2\text{S})}{K}$$

in which the total sulphide is taken as 100 and  $K$ , the dissociation constant, is  $0.91 \times 10^{-7}$ . The equation neglects the dissociation of  $\text{HS}'$  into  $\text{H}^+$  and  $\text{S}''$  but the dissociation constant of this step is so small (probably about  $10^{-15}$ ) that it may be neglected. We may also neglect the effect of excess base for such calculations as are here required. These factors are taken into account in the equation given by Becking.<sup>7</sup>

$$\text{H}_2\text{S} = \frac{[(B) + (H)](H)^2 - K_w(H)}{K_1(H) + 2K_1K_2}$$

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<sup>7</sup> Cf. Becking, L. B., *Proc. Soc. Exp. Biol. and Med.*, 1924-25, xxii, 127.

in which  $K_1$  is the dissociation constant for  $\text{H}_2\text{S} = \text{H}^+ + \text{HS}'$ ,  $K_2$  the dissociation constant for  $\text{HS}' = \text{H}^+ + \text{S}''$ ,  $K_w = (\text{H})(\text{OH}) = 10^{-14}$ , and  $B$  (excess base in relation to  $\text{H}_2\text{S}$ ) is defined by the equation  $(\text{B}) + (\text{H}) = (\text{OH}) + (\text{HS}) + 2(\text{S})$ . The values given by this equation do not differ sufficiently from those obtained by the simple equation employed above to make any marked difference in the curve.

Fig. 1 shows that the concentration of total sulphide inside the cell agrees very well with that of undissociated  $\text{H}_2\text{S}$  in the sea water outside, indicating that only undissociated  $\text{H}_2\text{S}$  can penetrate and that it does not become much dissociated after entering the cell.

This may be illustrated by a simple example. Let us consider an experiment in which the pH value of the outside solution at the start is the same as that of the sap, the excess base inside and outside being equal and the volume of sea water being very large as compared with that of the cell. Let us assume that only undissociated  $\text{H}_2\text{S}$  enters the cell and that it does not change its degree of dissociation after entering the cell (since the inside and outside pH values are the same). If the solubility of  $\text{H}_2\text{S}$  is the same in sap and sea water the concentration of  $\text{H}_2\text{S}$  inside and outside will be the same: this will also be true of the ions and consequently of the total sulphide (ions plus undissociated molecules). At the usual pH value of the sap (about 5.8)  $\text{H}_2\text{S}$  is about 5.4 per cent dissociated, so that if we call the total sulphide in the sea water 100 the concentration of undissociated  $\text{H}_2\text{S}$  in both sap and sea water is 94.6. Let us now raise the pH value of the outside solution to pH 7.05 at which the concentration of undissociated  $\text{H}_2\text{S}$  in the sea water is only 50: undissociated  $\text{H}_2\text{S}$  will move out of the cell until its concentration in the sap becomes the same as that in the sea water (50). The concentration outside will show practically no increase owing to the relatively large volume of sea water. We find that the pH of the sap does not rise noticeably, at least not during the time of the experiment, and we should have in the sap a concentration of 50 undissociated  $\text{H}_2\text{S}$  and  $(50) \cdot (5.4) = 2.7$  ionized molecules, so that the total sulphide in the sap is  $50 + 2.7 = 52.7$ , which would correspond fairly well with the concentration of undissociated  $\text{H}_2\text{S}$  outside (50). Proceeding in this way to higher pH values, keeping the total sulphide outside

constant at 100 and the pH value inside constant at 5.8, we should obtain a result like that given in the figure, but the curve showing the concentration of total sulphide in the sap would be ~~5.4~~ per cent higher than that showing the concentration of undissociated H<sub>2</sub>S in the sea water.

Instead of raising the outside pH value from the starting point (5.8) we might lower it, for example to pH 5.5, at which the concentration of undissociated H<sub>2</sub>S (if we keep the total sulphide outside constant at 100) would be 97.2. If the inside pH value remained constant the inside concentration of total sulphide would be 97.2 + ~~(97.2 × 5.4)~~ = 102.5; if the outside pH value were low enough (pH 3 or lower) the concentration of total sulphide outside would be 100 and that inside would be ~~100 + 5.4~~ = 105.4.  $100 + \frac{5.4}{94.6} = 105.7$

We might expect the concentration inside to be even higher because H<sub>2</sub>S might be more soluble in sap than in sea water since the sea water contains Mg, Ca, and SO<sub>4</sub> which would tend to make H<sub>2</sub>S more soluble in the sap.<sup>8</sup> In order to test this idea an artificial sap<sup>9</sup> was made by dissolving NaCl and KCl in water in the proportions in which they occur in sap. A current of air was passed in succession through sea water containing H<sub>2</sub>S and through the artificial sap, the whole forming a closed system as in the vapor tension experiments. It was found that at equilibrium the artificial sap contained about 13 per cent more total sulphide than the sea water.

We should therefore expect to find an excess of undissociated H<sub>2</sub>S in the sap as compared with the sea water, but this excess would be lessened if the cell produced enough CO<sub>2</sub> to make its concentration higher in the sap than in sea water (photosynthesis would act in the opposite direction); this would tend to diminish the solubility of H<sub>2</sub>S in the sap.<sup>8</sup> Such a diminution was actually observed when CO<sub>2</sub> was added to artificial sap.<sup>9</sup> The concentration of CO<sub>2</sub> in the sap might be subject to some fluctuation: it is usually less than in sea water.

The presence of organic matter in the sap might affect the solubility

<sup>8</sup> Cf. Hildebrand, J. H., Solubility, American Chemical Society monograph series, New York, 1924, 140.

<sup>9</sup> This was made by mixing 86.24 cc. of KCl .6 M with 15.08 cc. of NaCl .6 M.

$$\frac{4}{6} = 5.7$$

$$2 \times \frac{5.4}{94.6} = 102.8$$

of  $\text{H}_2\text{S}$  in either direction but the amount of organic matter is very small.<sup>10</sup>

As a matter of fact we do not find an excess of total sulphide in the sap as compared with the undissociated  $\text{H}_2\text{S}$  of the sea water. The reason for this is not wholly clear but at all the pH values below 5.8 it might be due in part to the fact that the penetrating  $\text{H}_2\text{S}$  lowers the pH of the sap (as is actually observed) which would tend to diminish the excess of total sulphide inside.<sup>11</sup>

Our present problem concerns itself with the entrance of ions into the protoplasm rather than with the extent to which substances accumulate in the cell. We therefore wish to know whether there is a general correspondence between the undissociated  $\text{H}_2\text{S}$  in the sap and in the sea water. If such a correspondence exists we shall get similar curves when we plot the total sulphide in the sap (expressed as per cent of total sulphide outside) and at the same time plot the per cent of undissociated  $\text{H}_2\text{S}$  in the outside solution. This has been done in Fig. 1 and the result is very striking. The concentration of undissociated  $\text{H}_2\text{S}$  inside agrees so closely with that outside that we can hardly escape the conclusion that it is only the undissociated molecules which penetrate.<sup>12</sup> If the protoplasm is practically impermeable

<sup>10</sup> The organic matter amounts to 1.433 parts per thousand.

<sup>11</sup> This effect of the penetrating  $\text{H}_2\text{S}$  would depend on the buffer action of the sap which is apparently small. If the exposure is prolonged at pH values below 5 injury occurs as shown by the entrance of  $\text{SO}_4$ .

<sup>12</sup> If we suppose that  $\text{H}^+$  ions can diffuse in and out of the cell (an improbable assumption in view of the fact that the inside pH remains about 5.8, while that of sea water is about 8.2) and if indiffusible ions are present and Donnan equilibrium is set up with  $\text{HS}'$  ions passing in freely, but not undissociated  $\text{H}_2\text{S}$ , we should expect the relation (ignoring the formation of  $\text{S}''$  ions).

$$\frac{\text{H}^+ \text{ inside}}{\text{H}^+ \text{ outside}} = \frac{\text{HS}' \text{ outside}}{\text{HS}' \text{ inside}}$$

For example when the inside pH value is 5.8 and the outside is 5.8  $\frac{\text{H}^+ \text{ inside}}{\text{H}^+ \text{ outside}} = 1$  and we should expect to find  $\frac{\text{HS}' \text{ outside}}{\text{HS}' \text{ inside}} = 1$ . As a matter of fact we find the value to be close to 1. When  $\frac{\text{H}^+ \text{ inside}}{\text{H}^+ \text{ outside}} = 10$  we find  $\frac{\text{HS}' \text{ outside}}{\text{HS}' \text{ inside}} =$  about 10 and so on.

If, however, it is  $\text{HS}'$  and not undissociated  $\text{H}_2\text{S}$  which penetrates we should expect to find the rate of penetration highest when the outside concentration of  $\text{HS}'$  is highest; *i.e.*, at high pH values. But this is not necessarily the case for

to ions but allows undissociated molecules to enter freely we should expect precisely the result we have found.<sup>13</sup>

In this connection it may be noted that the work of Beerman<sup>14</sup> on H<sub>2</sub>S and that of Loeb, Harvey, Crozier, Haas, Jacobs, Brooks, Smith, Clowes, and others<sup>15</sup> (on various weak acids) indicates that undissociated molecules penetrate although the methods employed do not enable us to decide positively whether ions enter or not. Those who have concluded that ions cannot penetrate have done so on purely theoretical grounds or as the result of indirect evidence.

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when the outside concentration of HS' is highest the concentration which it finally reaches in the sap is lowest and hence the rate of penetration might be proportionally low. Since we actually find that the rate of penetration is highest when the outside concentration of undissociated H<sub>2</sub>S is highest; *i.e.*, at low pH values, it seems probable that only undissociated H<sub>2</sub>S can penetrate. Moreover, certain unpublished results make it difficult to assume that ions can penetrate while undissociated molecules are unable to do so.

In general we cannot assume that Donnan equilibrium is the primary factor since in that case the relation of K<sup>+</sup> and Na<sup>+</sup> inside to K<sup>+</sup> and Na<sup>+</sup> outside should be the same as that of H<sup>+</sup> inside to H<sup>+</sup> outside. This is approximately true for K<sup>+</sup> under normal conditions, but not for Na<sup>+</sup> which has a much higher concentration in the sea water than in the sap. If Donnan equilibrium prevailed the concentration of Cl<sup>-</sup> ions should be about a hundred times as great outside as inside but it is actually somewhat greater inside. If any of these ions are not diffusible, as such, these remarks would not apply to them.

<sup>13</sup> When the pH was varied but the concentration of undissociated H<sub>2</sub>S in the outside solution was kept constant by adjusting the concentration of total sulphide it was found that the concentration of total sulphide in the sap at equilibrium remained unaltered despite changes in the pH value of the outside solution.

<sup>14</sup> Beerman, H., *J. Exp. Zool.*, 1924-25, xli, 33.

<sup>15</sup> Loeb, J., *Biochem. Z.*, 1909, xv, 254; 1910, xxiii, 93. *Arch. ges. Physiol.*, 1897-98, lxi, 1; 1898, lxxi, 457. Artificial parthenogenesis and fertilization, Chicago, 1913, 143. *J. Gen. Physiol.*, 1922-23, v, 231. Harvey, E. N., *Internat. Z. physik.-chem. Biol.*, 1914, i, 463. *Carnegie Institution of Washington, Pub. No. 212*. 1915. Crozier, W. J., *J. Gen. Physiol.*, 1922-23, v, 65, with references to earlier papers. Haas, A. R. C., *J. Biol. Chem.*, 1916, xxvii, 225. Jacobs, M. H., *Am. J. Physiol.*, 1920, li, 321; liii, 457. *Biol. Bull.*, 1922, xlii, 14. Brooks, M. M., *Pub. Health Rep., U. S. P. H.*, 1923, xxxviii, 1449, 1470. Smith, H. W., and Clowes, G. H. A., *Am. J. Physiol.*, 1924, lxxviii, 183. Smith, H. W., *Am. J. Physiol.*, 1925, lxxii, 347.

If it should turn out to be generally true that ions are unable to penetrate how shall we regard the evidence for the contrary view? This evidence rests chiefly on experiments with plasmolysis and electrical conductivity. It is found that many cells recover after plasmolysis when left in the plasmolyzing salt solutions (provided they are not too concentrated). Since these salts are largely ionized this may be regarded as evidence of permeability to ions. It is, however, quite possible that in these experiments the cells are permeable to ions only because they are abnormal. It is well known that plasmolysis produces injury and that injury is accompanied by changes in permeability. It is also possible that the cell may subsequently recover from such injury and appear to be normal: in this case the permeability to ions would be only a temporary one. Injury might affect only a portion of the cell surface, possibly numerous small areas. Experiments on large multinucleate cells (*Valonia*, *Nitella*, *Caulerpa*, *Bryopsis*) have convinced the writer that a portion of the cell surface may be greatly altered while the remainder remains in normal condition for a long time afterward.

If recovery from plasmolysis in salt solutions depends on alterations of permeability we should expect the rate of recovery from plasmolysis to correspond somewhat with the amount of alteration. If the alteration goes too far the cell may become so permeable that no recovery is possible, but up to a certain point increase in permeability would increase the rate of recovery from plasmolysis if exosmosis were not greater than endosmosis. From this standpoint we might expect the recovery in NaCl to be more rapid than in a balanced solution of NaCl + CaCl<sub>2</sub> (or in sea water) since alterations in permeability would be more rapid in NaCl. We find that recovery is more rapid in NaCl than in balanced solutions. When recovery occurs in balanced solutions it is possible that it is also due to alterations in permeability, since it is well known that hypertonic balanced solutions may cause injury.

If ions are unable to penetrate normal protoplasm how are we to regard the experiments which indicate that marine plants bathed in sea water allow ions to enter the protoplasm and thus conduct the electric current under conditions which seem to ensure that the cells are in a normal state?

It is possible that if the cell normally opposes a high resistance to the passage of ions this resistance may be overcome under electric stress so that ions may be forced through the surface of the protoplasm, although they would not enter if the electric potential were absent. In this case the measurement of electrical conductivity would reveal changes in resistance to the passage of ions brought about by various conditions, but the passage of the electric current would not mean that ions could penetrate to an appreciable extent in the absence of an applied potential. From this standpoint we may say that the general conclusions derived from electrical experiments would not be changed except that the normal cell would not be regarded as permeable to ions. The measurement of changes in resistance to the passage of ions brought about by abnormal conditions and the conclusions drawn from these measurements would still be valid.

It is also possible that the measurements of conductivity do not indicate the passage of ions through the protoplasm, as has been supposed. If the cell acts as a condenser an alternating current may seem to pass without actual transfer of ions through the protoplasm as indicated, for example, by the recent experiments of McClendon.<sup>16</sup> Experiments to test this were carried out in the writer's laboratory by Mr. M. J. Dorcas several months before the appearance of McClendon's article and have been continued by Mr. L. R. Blinks. If this turns out to be true the increase in conductivity (as measured by the alternating current) which occurs when a cell is injured may be regarded as analogous to the change by which a condenser becomes a conductor. If the cell surface is covered with a non-conducting substance injury might result in the alteration of this substance in certain places, so that the conductivity would increase. If this view should turn out to be correct we should still regard the measurements of the electrical conductivity of living tissues by means of the alternating current as of great value in detecting changes of permeability.

The principle that only the undissociated molecules can enter the protoplasm has far reaching implications. Among those we may mention the general question of equilibrium relations. In the case of a weak acid it is evident from what has been said that at equilibrium

<sup>16</sup> McClendon, J. F., *Science*, 1924, ix, 204.

when the internal pH value is greater than the external the total concentration of the weak acid (ionized plus unionized) will be greater inside than outside, and the reverse will be true when the internal pH value is less than the external. In the case of a weak base these relations will be reversed. It might be thought that a base penetrating into acid cell sap would soon raise the inside pH value to that found outside but this may be delayed by the buffer action of the sap or by the continuous production of acid (*e.g.* carbonic) within the cell. In this way ammonia might continue to accumulate in the sap, being changed into ions (or changed in some other way) as it enters and thus rendered incapable of escape, so that its concentration would become much greater inside the cell than outside. This apparently happens in the case of *Valonia*. We should not expect acids from the outside solution to accumulate in the sap, unless the outside pH value is lower than that in the sap; under such conditions the cell soon dies. Moreover, we should not expect to find a continuous production of alkali in the cell such as would be needed to ensure a great accumulation of acid. But if organic acids are produced inside the cell they might not be able to escape rapidly and hence the sap might remain acid. As a matter of fact we find the concentration of  $H^+$  ions in the sap to be as a rule about 100 times as great as in the sea water.

We may extend this hypothesis to include not only weak acids and bases but all other substances, organic or inorganic, which are able to change with changes in the concentration of  $H^+$  and  $OH^-$  ions. Such changes (including tautomerism, formation of complex salts, hydration, hydrolysis, etc.) may affect substances to a greater extent than is at present suspected and may explain the accumulation in the cell sap of substances whose behavior now seems unaccountable. (When we say that a substance accumulates we mean that it becomes more concentrated in the sap than in the sea water.)

Substances which accumulate in this way should come out when the external pH is made equal to the internal, provided the cell has not in the meantime produced additional acid or base. The writer's experiments indicate that when the outside pH is made equal to that of the sap injury occurs and accumulated substances begin to come out after a few hours.

It is to be expected that accumulation could also occur as the result of combination with organic constituents of the cell. This has recently been emphasized by Miss Irwin as the result of experiments on dyes.<sup>17</sup>

If ions are unable to penetrate (except possibly to a slight extent) how shall we account for the presence of KCl and NaCl inside the cell? The experiments so far carried out suggest that these substances enter so slowly that their penetration may be accounted for by supposing that only the undissociated molecules enter or that the ions enter with extreme slowness. These experiments will be discussed in a subsequent paper.

It is not to be expected that all undissociated molecules can enter the protoplasm with the same readiness and in many cases it appears as though they cannot penetrate into the sap unless they can combine chemically with some constituent of the protoplasm.

If we adopt the hypothesis that ions enter normal protoplasm very slowly or not at all it is evident that injury and death are accompanied by increased permeability to ions. There is good evidence that this is the case.<sup>18</sup>

The results described above might be thought to be explainable on the hypothesis that the protoplasm is permeable to kations but not to anions, or *vice versa*, as appears to be the case with some membranes.<sup>19</sup> Unless ions of like sign were exchanged no ion could penetrate unless accompanied by one of opposite charge, and in case such exchange were very slow penetration would be practically confined to undissociated molecules. Under ordinary conditions there seems to be little or no exchange of ions: otherwise it is difficult to see how the differences between sap and sea water in respect to ionic concentration can be maintained. These differences are found in anions as well as in kations.<sup>1</sup>

Numerous questions suggested by what is said above must be deferred to subsequent papers for discussion. The object of the

<sup>17</sup> Irwin, M., *J. Gen. Physiol.*, 1925-26, viii, 147.

<sup>18</sup> Cf. Osterhout, W. J. V., Injury, recovery, and death, Monographs on experimental biology, Philadelphia and London, 1922.

<sup>19</sup> Cf. Michaelis, L., *J. Gen. Physiol.*, 1925-26, viii, 33.

writer is merely to present certain facts with a tentative outline of some theoretical matters which will be taken up later as occasion offers.

SUMMARY.

The experiments indicate that under normal conditions little or no  $H_2S$  enters the cell sap of *Valonia macrophysa* except as undissociated molecules.