

THE KINETICS OF PENETRATION
XIX. ENTRANCE OF ELECTROLYTES AND OF WATER INTO IMPALED
HALICYSTIS

By A. G. JACQUES

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

(Accepted for publication, February 10, 1939)

In a previous paper¹ it was shown that when cells of *Valonia macrophysa*, Kütz., are impaled on a capillary and immersed in normal sea water the rate of entrance of water and of electrolyte is about 15 times as great as with intact cells. In the present paper it is shown that similar results are obtained with impaled *Halicystis Osterhoutii* (Blinks and Blinks).

The experiments were carried out in Bermuda at the Bermuda Biological Station in the winter of 1936-37.

EXPERIMENTAL

The setup for the measurement of the increase in volume for single impaled cells has already been described in a previous paper,¹ and the same technique was followed here. Briefly it consists in impaling a cell on a very thin-walled capillary drawn on the end of a tube of capillary bore, exposing it to normal sea water, and determining the rate at which the volume increases from the increase in height of the sap in the capillary tube. The rise in height was determined by means of a micrometer caliper reading to 0.02 mm. and the volume was calculated from the formula

$$V = \pi r^2 h$$

where r is the radius of the capillary, and h is the capillary rise. The radius for each capillary tube was determined by direct measurement of the diameter with a microscope fitted with an ocular micrometer. The temperature of the sea water during the exposure was maintained at $17^\circ\text{C.} \pm 1^\circ\text{C.}$ by immersing the bottles

¹ Jacques, A. G., *J. Gen. Physiol.*, 1938-39, **22**, 147.

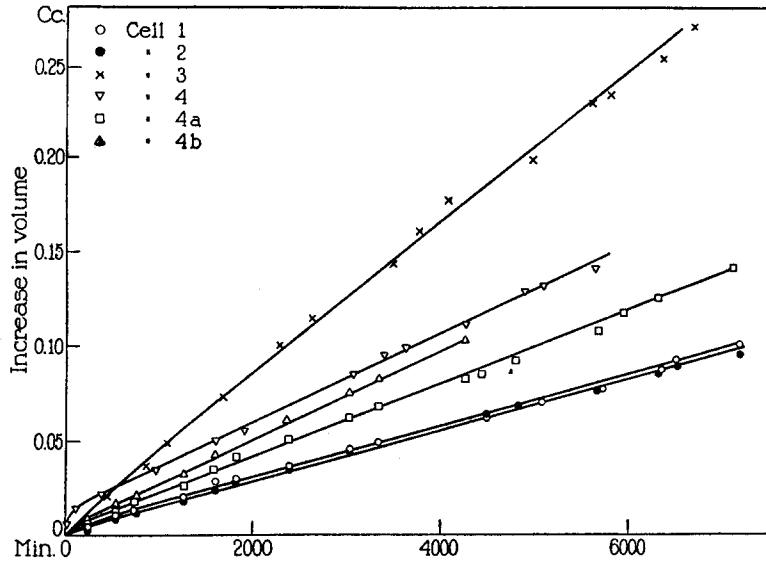


FIG. 1. Rate of increase of sap volume of impaled *Halicystis* in normal light (i.e. natural succession of daylight and darkness). The curve for Cell 4 (∇) starts immediately after impalement; the other curves start 24 hours or more after impalement.

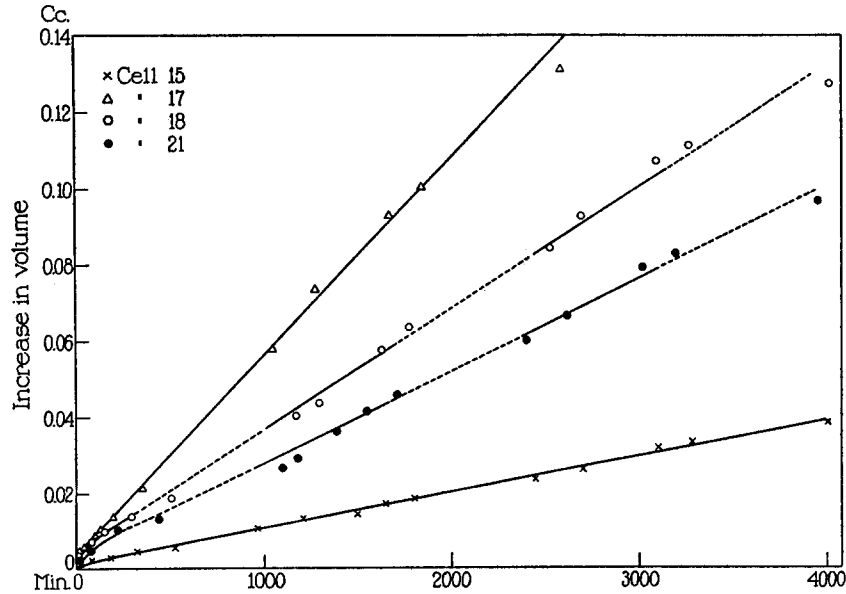


FIG. 2. Rate of increase of sap volume of impaled *Halicystis* cells in normal light (natural succession of daylight and darkness) from the moment of impalement. Note the initial rapid non-linear increase at the start. The curves for Cells 18 and 21 are drawn so as to show the falling off in rate during the hours of darkness and the gain in daylight. The broken portions represent 12 hours of darkness, the solid portions 12 hours of light. In drawing the curve these differences in rate have been disregarded so that the curve is approximately linear.

containing the cells in a large shallow tray through which passed a rapid current of sea water from the salt-water pumping system of the Biological Station.

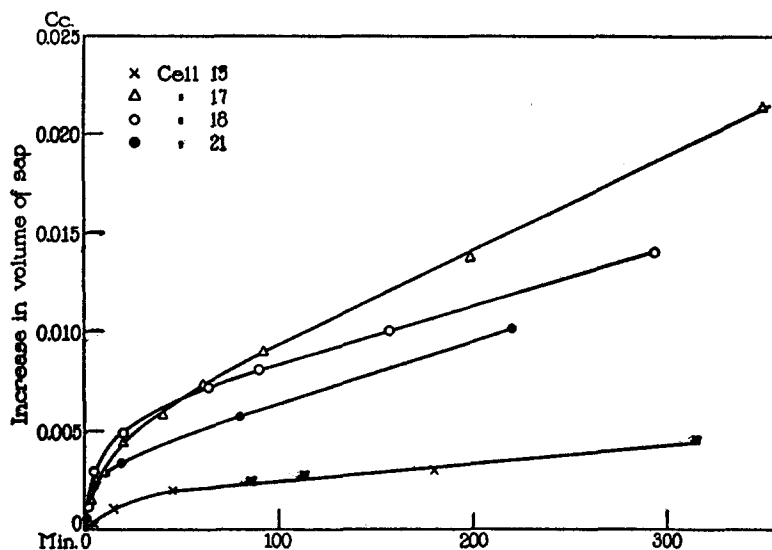


FIG. 3. A large scale representation of the early part of the curves of Fig. 2, illustrating the initial non-linear rate of volume increase.

TABLE I

Increase in Volume of Sap and in Moles of Halide in Impaled Cells of Halicystis

Cell No.	Days	Increase in	Increase in	Area of surface	Increase in	Increase in	
		volume of sap	volume of sap		moles halide	volume of sap	
		cc.	per cent	cm. ²	per cent	per cent	
1	5.0	0.095	33.0	2.108	6.6	6.6	
2	5.0	0.101	31.0	2.328	6.2	6.2	
3	4.6	0.269	45.7	3.394	9.4	9.9	
4	3.9	0.141	17.5	4.189	4.6	4.5	
Average						6.7	6.8

The increase in the moles of electrolyte in the sap (equal to molar concentration times volume increase in liters) was determined as the increase in the moles of halide. Since the cell sap of *Halicystis Osterhoutii* is chiefly a solution of sodium chloride, it was considered unnecessary to determine sodium and potassium as well in order to calculate moles of electrolyte.

The halide analyses were carried out electrometrically by titration with standard silver nitrate. The volume of each cell to be impaled was first determined by the method previously described and the number of moles of electrolyte originally present was calculated by using for the halide concentration in every case, the average halide concentration of a number of unimpaled cells from the same collection as the impaled cells. Since the natural variation in halide concentration is comparatively small this method gives sufficiently accurate results.

For the determination of the rate of increase in volume and in moles a group of unimpaled cells was kept under the same conditions as the impaled cells. The volume of these unimpaled control cells was determined in the manner previously described.²

A difficulty which arose constantly in these experiments was the decrease in the volume of sap within the cell vacuole at the moment of impaling. Blinks has already spoken of the difficulties associated with impalement of *Halicystis*, due to the tendency of the envelope to tear rather extensively. This means that in many cases some sap escapes round the capillary as the cell is pierced, hence it is hard to estimate the shrinkage of the vacuole. In a few cases cells were impaled without the loss except into the capillary tube and from measurements of this initial capillary rise in these cases it was possible to determine the shrinkage of the vacuole. It is variable, but appears to be between 15 and 25 per cent of the unimpaled volume.

In calculating the per cent increase in volume and in moles this has been taken into account by making a correction of the initial volume of 20 per cent. It is interesting that the difficulty just discussed is almost entirely absent in *Valonia*. In the first place, on impalement the vacuole shrinks only a very little, and, in the second, in nearly all cases of successful impalement the cell wall clings to the capillary so closely that there is no spurting.

In most cases spurting occurred with *Halicystis* cells, and before measurements of the increase in volume were started the cells were allowed to recover in normal sea water for 24 hours or more. The volume time curves for a number of these cells are given in Fig. 1. The curves are typical and the cases chosen were selected so as to avoid too many overlapping points.

In a number of cases we were successful in impaling cells without loss around the capillary. In some of these cases measurements were started at once, in order to determine the shape of the curves at the start. The results for some of these experiments are given in Fig. 2 and on a larger scale in Fig. 3 for the early part of the runs. One of these cells, No. 4, has been included in Fig. 1 for comparison.

Table I gives the data for the beginning and end of four experiments for which sap analyses are available. In 17 other cases not reported

² Jacques, A. G., and Osterhout, W. J. V., *J. Gen. Physiol.*, 1931-32, **15**, 537.

in detail the cells were transferred to other experiments and no sap analysis is available.

The control cells (unimpaired) gained 6.71 per cent in volume of sap in 8 days and 17.76 per cent in 18 days. The average daily increase was taken as 0.99 per cent.

DISCUSSION OF RESULTS

Figs. 1, 2, and 3 show that generally the behavior of the *Halicystis* cells was like that of the *Valonia* cells. Immediately after impalement there was a rapid increase in volume which was succeeded after a few hours by a slower increase, approximately linear with time, which lasted until the end of the experiment. The longest experiments described in this paper lasted 5 days, after which the cells were used in other experiments, and it seems not improbable that in the case of *Halicystis*, barring accidents, the linear increase in volume might go on indefinitely.³ In the case of *Valonia* the experiments were all terminated by the end of 48 hours because of the difficulty of keeping the capillary from plugging for long periods. But it seems probable that with *Valonia* also, as long as the capillary was kept free, the linear rate of increase of volume would continue.

In the case of *Halicystis*, although the general course of the curve after the first rapid uptake of water is linear, the curves are actually a series of waves. This is clearly brought out in Fig. 2 where a rather large scale is used. Some of the deviations from the linear curve are undoubtedly the result of experimental error but there is a certain regularity about them which we are able to identify definitely with the illumination. During the dark hours, the rate dropped off appreciably but it recovered promptly in daylight so that the net result was nil and the average curve is a straight line.

In order to bring out the periodic nature of the process under the influence of light two of the curves have been roughly divided into light and dark periods, the dotted portions representing 12 hours of darkness, roughly from 7 P.M. to 7 A.M., and the solid portions each

³ As a matter of fact cells used in other experiments lasted up to 20 days on the capillary and it is our impression that but for the unavoidable disturbances incident to the measurement of the capillary rise they might have lasted much longer.

representing 12 hours of light. The decrease in darkness and the increased rate in the light is fairly evident. These findings are in agreement with other light and dark experiments to be described in another paper.

Referring to Table I we find that the rate of increase of volume and of moles of halide (equivalent to moles of electrolyte) were parallel. This means that the halide concentration remained practically constant. The average percentage increase in volume per day in this group was 6.8 per cent, or if we apply a correction of 20 per cent to the initial volume of the cells to account for the shrinkage on impalement, the daily percentage increase in volume was 8.5 per cent.

This may be compared with the average daily increase of volume of 9.08 for 14 cells all drawn from an old collection, or on the corrected basis of 11.35 per cent. We have elected to compare these 14 cells which include the 4 of Table I because these were of the same collection as the unimpaled cells. The remaining 7 cells were of a much more recent collection and this apparently has some effect on the rate, since the corrected daily volume increase in this case was 19.9 per cent or rather less than twice as much as with the older cells. This difference may have some significance.

Neglecting these last younger cells for the reason that we have no figures for the rate in young unimpaled cells we see that volume increase and hence electrolyte increase was between 9 and 11 times as fast in the impaled cells as in the intact cells. This may be compared with the 15-fold increase observed when *Valonia* cells were impaled. We may say that the increase in rate produced by impalement is approximately the same in *Valonia* and in *Halicystis*.

In the case of *Valonia* we were not able to compare rates with the surface area, because the cells vary so much and are frequently so irregular in shape that we cannot relate the surface to the volume. In the case of *Halicystis* we can assume that the cells are spherical, particularly if they are small cells or have been kept a long time in the laboratory, under which condition they tend to round out to spheres. And if they are spheres we can calculate A , the surface area, from the volume.

From the calculations it appears that there is very little correlation between the surface area and the rate of uptake of water and electro-

lytes. However, when different cells are compared the average rate of increase of volume per day per square centimeter of surface for the first 14 cells was 0.013 cc. and for the other 7 cells 0.022 cc., or nearly twice as great. In the latter case, as pointed out already, this may be correlated with the time the cells remained in the laboratory.

In the case of the unimpaled cells the total surface area for 4 cells from the original volumes was 9.658 cm.² And ignoring the small increase in surface area during the volume increase this means that the average volume increase per day per square centimeter of surface was 0.015 cc., so that on this basis the rate is increased somewhat less than 9-fold due to impalement. In the calculation of the surface no correction was made for volume shrinkage on impalement. Such an allowance would raise the figure somewhat, possibly to nearly 10.5-fold, but we need not, in view of other uncertainties, make the calculation here. On either volume or surface basis therefore we can conclude that the rate of uptake of electrolyte and of water increases about 10-fold when the restriction due to the cellulose wall is removed by impalement.

As in the case of *Valonia* the osmotic concentration⁴ of the sap in the intact cell is slightly greater than that of the sea water. The freezing point of the sea water passing through the Biological Station pumping system varies slightly from time to time, but in comparison the freezing point of the sap of *Halicystis* kept in a flow of this sea water always has a slightly greater freezing point depression. For example, in one case the freezing point of the sea water was -2.030°C . and the freezing point of the sap of cells kept in it was -2.059°C . The usual difference of freezing point between sea water and the sap of *Valonia* is considerably greater than this. For example, in one case when the freezing point of the sea water in contact with the cells was -2.021°C . the freezing point of the *Valonia* sap was -2.150°C . Qualitatively this difference can be observed in the turgidity of the cells. *Halicystis* cells are invariably soft and rubbery to the touch, *Valonia* cells are firm and hard. In the case of *Valonia* we were unable to determine by calculation if the early more rapid entrance of

⁴ The osmotic concentration is unity when the freezing point depression equals 1.864; *i.e.*, the freezing point depression of 1 mole of non-electrolyte dissolved in 1 liter of aqueous solution.

water immediately after impalement represents the more rapid entrance of water than electrolyte with a corresponding adjustment of the osmotic concentration of the sap to that of the sea water. But it seems probable that this is the case.

In the case of *Halicystis* the difference in osmotic concentration between sap and sea water is much less. The osmotic concentration of sap is $2.059 \div 1.864 = 1.1046$ M. Now in the case of cell 17 (Fig. 3) the initial period of rapid rise lasted about 92 minutes during which time the volume increased by 0.0088 cc., but we may suppose that only part of this was pure water, since electrolytes could enter also and it is a reasonable assumption that the rate of electrolyte entrance might be constant throughout the experiment. Therefore part of the increase in volume may be considered as the addition of undiluted sap to the volume. Interpolating from the straight part of the curve which apparently represents the increase in volume without dilution we find that in 92 minutes the amount of undiluted sap entering would be approximately 0.0045 cc. The original volume of the cell was 0.348 cc. Hence the calculated osmotic concentration should be approximately 1.0913 M. But the osmotic concentration of the sea water in contact with the cell was approximately $2.030 \div 1.864 = 1.0891$ M, a negligible difference.

On the other hand, similar calculations in the case of cell 15 gave for the probable osmotic concentration of the sap at the end of the rapid initial volume increase 1.0986 which suggests that the complete osmotic adjustment did not occur in this case. The evidence in this connection from halide analysis is also not decisive because the calculated change of halide concentration corresponding to complete osmotic adjustment is smaller than the natural variations in halide concentration among normal cells.

On the whole, therefore, the evidence while pointing to some osmotic adjustment does not support decisively the view that complete osmotic equality is attained. From a theoretical viewpoint, however, we think it desirable to assume that osmotic equality does occur on impalement.

We shall assume at first, that, just as in the case of *Valonia*, the entrance of sodium and potassium occurs by the diffusion of NaX and KX in the aqueous layer of the protoplasm, where X is the anion

of a weak acid HX elaborated by the protoplasm. In the present case, since the concentration of potassium in the sap is very small, we shall discuss sodium entrance only.⁵ In a previous paper we have suggested that the flux of M (M may be sodium or potassium) across a unit area of protoplasm of unit thickness is given by the equation

$$\frac{dM}{dt} = D^{MX} \{ [MX]_{epo} - [MX]_{epi} \}$$

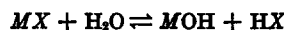
and this with suitable simplifications reduces to

$$\frac{dM}{dt} = k_{\text{coll.}} D^{MX} \{ [M]_o (\text{OH})_o - [M]_i (\text{OH})_i \}$$

where

$$k_{\text{coll.}} = \frac{f_o^{\text{Na}} f_{epo}^{\text{HX}} S_{eop}^{\text{NaX}} [HX]_{epo}}{f_{eop}^{\text{NaX}} S_{eop}^{\text{HX}} k_{\text{hydrolysis}}^{\text{NaX}}}$$

In these equations D is the diffusion constant, S is the partition coefficient, f is the activity coefficient, and $k_{\text{hydrolysis}}^{\text{NaX}}$ is the thermodynamic hydrolysis constant of the reaction



Round brackets indicate activities and square brackets concentrations. The subscripts o and i refer to the bulk of the sap and sea water respectively, and the subscripts epo and eop refer to a pair of adjacent unstirred layers in the protoplasm and sea water respectively in which the solute and solvents are in equilibrium across the interface: epi and eip refer to similar layers at the sap-protoplasm interface.

In the derivation of this equation it was assumed that corresponding activity coefficients and partition coefficients in the sap and sea water are equal. HX is also considered to be distributed equally throughout the protoplasm.

The rate of entrance of water is given by the flux of water across the non-aqueous protoplasm, *viz.*

$$\frac{dH_2O}{dt} = D^{H_2O} \{ [H_2O]_{epo} - [H_2O]_{epi} \}$$

⁵ The situation is complicated by the fact that halide is entering the cell simultaneously. A mechanism whereby halide may enter will be discussed elsewhere.

Now the entrance of water and electrolyte goes on under steady state conditions. Hence the ratio of the rates of entrance of electrolyte to water remains constant while only the volume of the sap increases. There is good evidence that the rate of entrance of water takes place much more rapidly than the rate of electrolyte entrance. Thus, as we showed in a previous paper, even when the sea water is diluted extensively the adjustment of the osmotic pressure between the sea water and the sap of an impaled *Halicystis* cell takes place almost entirely by entrance of water into the sap and hardly at all by loss of electrolyte.⁶

If this is so then in the impaled cell we should expect the osmotic concentration of the sap at the steady state to be very close to that of the sea water, for when the cell takes in electrolyte and thereby raises the osmotic concentration by a small amount water can enter at once, since there is no volume restriction due to the cell wall. This would tend to abolish the osmotic gradient.

When the cell is intact we expect a different steady state concentration in the sap, for then water can enter only when the cell wall grows and so is able to enclose more space. Hence the rate of water entrance is decreased, and in order to decrease the rate of electrolyte entrance $\{[Na]_i(OH)_o - [Na]_i(OH)_i\}$ must decrease. This can only happen by $[Na]_i$ increasing since the other concentrations are fairly well fixed. We may note that the situation is somewhat different from the situation in Osterhout's guaiacol model, where the rate of uptake of water is increased as the concentration of electrolyte in the inner phase increases. In the present case the rate of uptake of water cannot increase as the concentration of the electrolyte in the sap increases, because of the cell wall restriction. Consequently all the adjustment to the new steady state depends on $[Na]_i$ increasing. We need not predict at what point the steady state will be established but it is clear that it must be much higher than 0.6 M for $(OH)_o$ is at least 100 times $(OH)_i$; hence $[Na]_i$ may increase many fold without decreasing the gradient appreciably.⁷

⁶ Jacques, A. G., *J. Gen. Physiol.*, 1938-39, **22**, 743.

⁷ Let us consider a cell containing 1 ml. of sap. In the impaled cell the cell sap gains in about 10 days (10 per cent increase in volume per day, the concentration of electrolyte being 0.6 M) 1 ml. of water and 0.6 millimole of electrolyte. Now suppose an impaled cell could be suddenly made intact. The cell would

It was because practically the same steady state was obtained in both intact and impaled *Valonia* cells, even though there was a 15-fold change in the rate of entrance of water and electrolyte, that we were led to suggest a regulatory mechanism which operates by changing the partition and diffusion coefficients of MX in the non-aqueous protoplasm. It was suggested that the mechanism operates about as follows. In the intact cell a little electrolyte enters thereby raising the osmotic concentration of the sap. As a result water enters and stretches the cellulose wall to the limit. Electrolyte continues to enter but water cannot until more cellulose is produced. Then as the osmotic pressure of the sap increases, water is withdrawn from the protoplasm. This does not require any change in the volume (sap + protoplasm) inside the cellulose envelope, but it does change the properties of the non-aqueous protoplasm, by lowering the partition coefficient of MX , so that $\{MX_{eop} - MX_{epi}\}$ is decreased.⁸ At the same time the viscosity of the protoplasm is increased and D^{MX} is decreased.

On the whole we think that the same explanation may apply in the present case. Before discussing it let us consider an alternative mechanism by which sodium is assumed to enter the cell as sodium chloride.

According to the analysis of Bermuda sea water and *Halicystis* sap⁹ $[Na]_o = 0.498$ M, $[Cl]_o = 0.580$ M, $[Na]_i = 0.557$ M, and $[Cl]_i = 0.603$ M. Since the ionic strengths of the sap and sea water are quite close to each other the activity coefficients may be taken as equal.¹⁰

now gain 1 ml. of water in about 100 days under these conditions, and if the rate of entrance of electrolyte remained unchanged it would gain 6 millimoles of electrolyte in the same time and the concentration would become $6.6 \div 2 = 3.3$ M. But if the concentration were 3.3 M the gradient would be reduced from approximately 5.94×10^{-7} to 5.67×10^{-7} . This very approximate calculation is made as follows, $[Na]_o = 0.6$ M, $[OH]_o = 10^{-6}$ M, $[Na]_i = 0.6$ M, $[OH]_i = 10^{-8}$ M, $[Na]_o \times [OH]_o = 6 \times 10^{-7}$, and $[Na]_i \times [OH]_i = 0.06 \times 10^{-7}$, whence the difference is 5.94×10^{-7} . When $[Na]_i$ is taken as 3.3 M, $[Na]_i \times [OH]_i = 0.33 \times 10^{-7}$ and the difference becomes 5.67×10^{-7} . That is, it would decrease less than 10 per cent. We need not attempt to fix the steady state concentration more closely.

⁸ Regarding *epo*, *eop*, *epi*, and *eip* see p. 765.

⁹ Osterhout, W. J. V., *J. Gen. Physiol.*, 1922-23, **5**, 225. Blinks, L. R., and Jacques, A. G., *J. Gen. Physiol.*, 1929-30, **13**, 733.

¹⁰ The ionic strength of Bermuda sea water, according to the calculation of

Hence the chemical potential of sodium chloride is greater in the sap than in the sea water. Consequently NaCl as such cannot diffuse from the sea water to the sap without the cell supplying energy. If sodium chloride diffuses as such from the sea water to the sap we may write for the flux of NaCl

$$\frac{d[\text{NaCl}]}{dt} = \frac{D^{\text{NaCl}}}{h} \{[\text{NaCl}]_{epo} - [\text{NaCl}]_{epi}\} \quad (1)$$

or

$$= \frac{D^{\text{NaCl}}}{h} \{S_{eop}[\text{Na}]_o[\text{Cl}]_o - S_{eip}[\text{Na}]_i[\text{Cl}]_i\}^* \quad (2)$$

* The assumption and steps by which we arrive at equations similar to the present one have been applied in several forms in another paper (Jacques, A. G., *J. Gen. Physiol.*, 1938-39, **22**, 147) and they need not be considered in detail here.

In order for the right hand term of the gradient to become less than the left hand in *Halicystis* S_{eip} must be less than S_{eop} . But in any diffusion system to which no energy is supplied S_{eop} and S_{eip} must obviously come closer together as $[\text{Na}]_o[\text{Cl}]_o$ and $[\text{Na}]_i[\text{Cl}]_i$ come closer together until when these products are equal the partition coefficients are also equal. In a system such as *Halicystis* it seems not impossible that the required energy may be available.

Suppose the energy of the cell were directed to the production of a substance which depresses the solubility of sodium chloride in the non-aqueous phases. This would lower the partition coefficients. But in order for such a substance to lower S_{eip} differentially it would be necessary for it to be present in higher concentrations at the sap-protoplasm interface than at the sea water-protoplasm interface. This seems possible, for example, if the substance is produced only at the sap-protoplasm interface and has a low rate of diffusion in the non-aqueous protoplasm. Thus we can imagine a "steady state"

Zscheile (Zscheile, F. P., Jr., *Protoplasma*, 1930, **11**, 481) is 0.7212, and the ionic strength of *Halicystis* sap by our calculation is 0.6326. By reference to the data of Harned (Harned, H. S., in Taylor, H. S., *A treatise on physical chemistry*, New York, D. Van Nostrand Company, Inc., 2nd edition, 1931, **1**, 772) we see that between these two ionic strengths the activity coefficient of sodium chloride changes hardly at all.

in which the "depressant" agent is being produced rapidly at the sap-protoplasm interface and is diffusing slowly to the sea water-protoplasm interface, so that a rather steep but constant gradient of the substance is set up across the protoplasm. S_{eip} then remains continuously less than S_{eop} and the flux of NaCl is inward in spite of the fact that $[Na]_i [Cl]_i > [Na]_o [Cl]_o$.

The part played by the aqueous phases may be important. Since the depressant agent is able to displace the polar sodium chloride from the non-polar protoplasm, it must itself be relatively more non-polar. Hence its partition coefficient must be high. At the sap-protoplasm interface we have the relationship

$$S_{eip}^a [a]_{eip} = [a]_{epi}$$

where a is the depressant agent. In the steady state it seems likely that $[a]_{eip} = [a]_i$ (where $[a]_i$ is the concentration in the body of the sap) because the volume of the sap is limited.

At the sea water-protoplasm interface, however, $[a]_{eop} > [a]_o$ since the sea water is not limited in volume. Hence there is a loss of the agent to the sea water, and in the steady state this may take place at such a rate that the decrease in the *epo* layer is just made up by the diffusion of the agent from the sap-protoplasm interface. This steady loss is important since if the depressant were produced continuously and did not escape from the protoplasm no steady state would be possible. Instead the concentration of the depressant would eventually become equal at the two interfaces when, of course, its differential effect on the partition coefficients of sodium chloride at sea water and sap interfaces would disappear.

Osterhout¹¹ has suggested that the protoplasm of such large plant cells as *Halicystis* consists of two non-aqueous layers, X and Y , adjacent to the sea water and sap respectively, and W , an aqueous layer, between them. It might be supposed that if the X and Y layers have different properties S_{eop} and S_{eip} must necessarily be different, and that accumulation of sodium chloride as NaCl could occur on this account alone. It is easy to see that this could not be so thermodynamically. As a matter of fact the system does not

¹¹ Osterhout, W. J. V., *Ergebn. Physiol.*, 1933, **35**, 1014.

differ fundamentally from a system with only one non-aqueous layer separating sea water and the sap.

Suppose we have a three layer system in which the diffusion coefficients are different and suppose the thicknesses of the layers are h_x , h_w , and h_y . The total thickness of the system is small so that "steady state diffusion" prevails. The flux in all parts of the system is therefore the same. Jacobs¹² has shown for a two layer system of this type that the rate of transfer of solute is proportional to the gradient across the whole system. The separate gradients across each layer need not be considered. Extending Jacobs' reasoning to our three layer system we have for the flux across a unit area

$$\frac{d[\text{NaCl}]}{dt} = \frac{D_x D_w D_y}{D_y D_w h_x + D_y D_x h_w + D_x D_w h_y} \{[\text{NaCl}]_{epi} - [\text{NaCl}]_{epo}\} \quad (3)$$

which is very similar to the equation (1) set up for the flux in a single layer.

It might be suggested that the supposed diffusion of the depressant from the sap to the sea water resembles the movement of an ionogenic "diffusing agent" which Teorell¹³ has shown is capable of bringing about accumulation in non-living systems. The resemblance is superficial since the depressant is not supposed to be ionogenic and hence cannot furnish the ions of different mobility required to produce the electrical stress which according to Teorell's theory is the force which brings about the redistribution of other ions in the system.

In an average *Halicystis* cell $[\text{Na}]_o [\text{Cl}]_o = 0.289$ and $[\text{Na}]_i [\text{Cl}]_i = 0.336$. Hence if the ratio $S_{epi} \div S_{epo}$ could be maintained at a value slightly greater than 1.16 sodium could enter the cell as sodium chloride. Let us suppose for the moment that this condition is met in the intact cell. When the cell is impaled the rate increases about 10-fold hence if D^{NaCl} remains unchanged $S_{epo} [\text{Na}]_o [\text{Cl}]_o - S_{epi} [\text{Na}]_i [\text{Cl}]_i$ must increase 10-fold. Since there is only a very slight decrease of $[\text{Na}]_i [\text{Cl}]_i$ in the impaled cell the change must be effected through the partition coefficients. However, it is difficult to see

¹² Jacobs, M. H., *Ergebn. Biol.*, 1935, **12**, 70.

¹³ Teorell, T., *Proc. Nat. Acad. Sc.*, 1935, **21**, 152; *J. Gen. Physiol.*, 1937-38, **21**, 107.

how the results we observe could come about through the depressant a . There is no reason to suppose that its rate of production would be increased by impalement. But since the volume of the sap increases faster in the impaled cell, the loss of a to the sap will be greater, hence $[a]_{epi}$ will be smaller.

This effect may be small and the increase in S_{eip} due to the decrease in the concentration of the agent may in part be compensated by the smaller concentration of a in the epo layer which will cause S_{oop} to increase. The net effect on the partition coefficient ratio will probably be nearly zero. And in any case there seems to be no possibility that it can be so increased by the depressant that the gradient term increases 10-fold.

Thus even if sodium diffuses in by the mechanism suggested above it seems necessary to assume that there is an additional mechanism whereby the gradient is altered when the cell is impaled.

As pointed out above we assume that the additional mechanism operates by withdrawing water from the non-aqueous protoplasm to reduce both S_{oop} and S_{eip} and at the same time by increasing the viscosity of the non-aqueous phase to reduce the diffusion coefficients. According to this view in the intact cell some of the energy of the cell is directed towards decreasing the rate of uptake of electrolyte. When the cell is impaled the energy is no longer expended in this way and without the need for an increase in the energy output of the plant the rate of uptake of electrolyte can increase.¹⁴

However, recent results of Blinks, Darsie, and Skow¹⁵ on the Pacific Coast *Halicystis ovalis* indicate that on impalement the cell consumes oxygen at a more rapid rate than normal and this keeps up for some time "even for several days." That is to say on impalement the en-

¹⁴ An alternative mechanism for keeping the osmotic concentration of the cell sap from increasing much above that of the sea water in the intact cell has been suggested to us by Dr. T. Shedlovsky. He suggests that when the cell is stretched to the limit the protoplasm ruptures momentarily and as a result sap escapes and the vacuole volume decreases. Then the rupture heals and as the cellulose wall is not now stretched to the limit, water can enter to stretch it, thereby reducing the osmotic concentration. This process is supposed to be repeated an indefinite number of times.

¹⁵ Blinks, L. R., Darsie, M. L., Jr., and Skow, R. K., *J. Gen. Physiol.*, 1938-39, **22**, 255.

ergy output of the cell is increased. Hence in contrast to the view expressed above it might be suggested that the increased rate of uptake of electrolyte is in some way associated with this. However, although in the present paper, the longest time during which the rate was followed was 5 days, in a forthcoming paper¹⁶ we shall describe experiments in which the accelerated rate of uptake over that of the intact cell continued for periods up to 15 days and it scarcely seems likely that the accelerated rate of oxygen consumption would continue so long.

Possibly, however, the more rapid non-linear rate of increase in volume during the first few hours is associated with increased energy output. But it seems just as likely that it is associated with the partial dissipation of the excess osmotic energy of the sap over that of the sea water.

Since in the case of the impaled *Valonia* cell the experiments were terminated in 48 hours or less, two questions arise. First, does impalement result in increased energy output for several days in *Valonia* as it does in *Halicystis ovalis*, and second, is the increased rate of uptake of electrolyte associated in any way with increased energy output? We think that the last question should be answered negatively, for the reason that *Valonia* and *Halicystis Osterhoutii* on impalement behave qualitatively and quantitatively nearly alike, and, as pointed out already, in the latter case it seems unlikely that the increased energy output would persist as long as the increased rate of uptake lasted.

In the case of *Valonia*, measurements on the sap made in two cases after 48 hours of increased electrolyte uptake due to impalement, failed to show any definite change in the pH. Yet if the cell were to continue to respire more rapidly on impalement we might possibly expect to find the pH of the sap lower than normal.

Between the two alternatives entrance as NaX or as NaCl by the aid of a depressant, there is little to choose as far as the evidence goes, but in the case of certain plants such as *Nitella*, where the osmotic pressure and the concentrations of most of the ions in the sap are greater than in the surrounding medium, it seems possible that favor-

¹⁶ Jacques, A. G., *J. Gen. Physiol.*, 1939-40, **23**, in press.

able gradients may depend on substances present in larger concentration at the sap-protoplasm interface, which depress the solubility of the salts in the non-aqueous phases.

SUMMARY

When cells of *Halicystis* are impaled on a capillary so that space is provided into which the sap can migrate, the rate of entrance of water and of electrolyte is increased about 10-fold. In impaled *Valonia* cells the rate is increased about 15-fold.

After a relatively rapid non-linear rate of increase of sap volume immediately after impalement (which may possibly represent the partial dissipation of the difference of the osmotic energy between intact and impaled cells) the volume increases at a linear rate, apparently indefinitely.

Since the halide concentration of the sap at the end of the experiment is (within the limits of natural variation) the same as in the intact cell, we conclude that electrolyte also enters the sap about 10 times as fast as in the intact cell.

As in the case of *Valonia* we conclude that there is a mechanism whereby in the intact cell the osmotic concentration of the sap is prevented from greatly exceeding that of the sea water. This may be associated with the state of hydration of the non-aqueous protoplasmic surfaces.