WATER PERMEABILITY OF A CHARACEAN INTERNODAL CELL WITH SPECIAL REFERENCE TO ITS POLARITY

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Summary

Water permeability (hydraulic conductivity) of the *Nitella* internode was studied by means of transcellular osmosis with special reference to its polarity. It was shown that the change in turgor or in cell volume involved in transcellular osmosis is almost over within 5 sec after the onset of both forward and backward osmoses. What is different with respect to turgor between the two osmoses is its final level. In forward osmosis the turgor drops down to a definite level depending on the external concentration, while in backward osmosis the turgor invariably comes back to the normal level.

"Transcellular osmotic constant" (K) and "transcellular permeability constant to water" (K'), both of which are defined in the present paper, were determined by the rate of water flow measured at the initial phase of transcellular osmosis after the turgor change has been almost accomplished.

Osmosis experiments were performed using the cell wall tube freed from all the cell contents. Exosmosis induced by a mannitol solution outside the cell wall tube occurs almost linearly with osmotic concentration of the mannitol. Exosmosis induced both by the external sucrose solution and simultaneously by the internal hydraulic pressure has a rate that is just equal to the sum of the rates of each osmosis induced separately. This shows that the exosmosis caused by the external sucrose solution is not influenced by the outflow of water hydraulically induced.

The value of the polarity (ρ), or the ratio of endosmotic (k_{en}) to exosmotic (k_{ex}) water permeability constants, varied from cell to cell, values ranging from 1·2 to 2·7 with an average of 1·7. There is, however, hardly any correlation between ρ and the ratio in which the cell is partitioned.

The facts summarized in the preceding paragraphs show that the polarity is not explained in terms of the dilution effect ("sweeping-away effect"). We thus conclude that polar permeability is an intrinsic characteristic of the living cell.

Transcellular permeability constant to water (K') decreases with the increase in osmotic concentration of the external medium in forward osmosis, but not in backward osmosis. The decrease in K' in forward osmosis is more conspicuous the less the turgor level. When the osmotic concentration of the cell sap is made higher than normal, K' decreases, and when it is brought to a subnormal level, K' increases. The effects of turgor and of the osmotic concentration of cell sap on water permeability may be explained in terms of hydration and dehydration on the part of the cytoplasm, including membranes.

I. INTRODUCTION

Nearly a decade ago, we measured and characterized osmotic water permeability (hydraulic conductivity) of the *Nitella* cell taking advantage of transcellular osmosis (Kamiya and Tazawa 1956). Transcellular osmosis has outstanding merits as a means of studying water permeability in that measurement can be performed

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with a high accuracy and reproducibility and that the cell suffers least ill effect, least change in volume, and least change in surface area due to the measurement. Our previous experiments revealed that water permeability of the *Nitella* internode is not only extremely high but is distinctly polar in respect to the direction of osmosis. Our conclusion about the polarity of water permeability to the effect that water enters the cell with less resistance than when it goes out, however, has been much argued since then by Dainty and his associates.

Repeating our experiments using the internodal cell of *Chara australis*, Dainty and Hope (1959) reconfirmed the phenomenon we had observed. But there was a difference in the interpretation of the phenomenon. Dainty and Hope (1959) and Dainty (1963*a*) claimed that the external solution in the proximity of the cell surface on the exosmosis side is diluted by the outflow of water and that this lowers the net driving force of the transcellular osmosis. They believed that this "sweeping-away" effect of the solute from the cell surface would well account for the phenomenon without resorting to polarity in water permeability and that the polarity we postulated may not be inherent to the cell but only apparent. In the paper of Kamiya, Tazawa, and Takata (1962) a brief comment against the view of Dainty and Hope (1959) about the polar permeability was made. Recently Dainty and Ginzburg (1964) showed that the sweeping-away effect cannot be the major cause of the phenomenon. Thus they considered that there may well be a genuine polar permeability in the characean cell. The present paper deals with this problem in more detail on the basis of some new experimental facts.

II. Material and Methods

The material used was adult internodal cells of *Nitella flexilis* with a length of 5–10 cm and a diameter of 450–550 μ. The alga was collected from a nearby pond and stored in dechlorinated tap water in big jars. Before the experiment, an internodal cell was detached from the adjacent internodes and kept for more than 1 hr in a petri dish with the dechlorinated tap water.

(*a*) *Transcellular Osmometer*

Transcellular water movement was measured with the simple osmometer illustrated in Figure 1. The osmometer of this type is somewhat different from the closed-system type used previously by Kamiya and Tazawa (1956), but is the same in general construction as those used by Osterhout (1949), Tazawa and Nishizaki (1956), and Dainty and Hope (1959). An internodal cell is partitioned by means of a small glass cock into two chambers A and B which are filled with dechlorinated tap water. The glass cock is provided with a small groove just big enough for the internode to be fitted in to make a water tight seal with a small amount of Vaseline. When the water in B is replaced with a mannitol solution, the suction force of the cell half b becomes smaller than that of a with the result that water immediately starts moving from A to B through the cell. The volume of water transported was represented by a shift of the air bubble d in the capillary E, which was measured with a travelling microscope. Transcellular osmosis from A to B induced when water in B is replaced with a mannitol solution, or more generally, transcellular osmosis induced

by the osmotic gradient in the external medium is designated as "forward osmosis" (Kamiya and Tazawa 1956). The rate of forward osmosis is not kept constant under the same external osmotic gradient but decreases with time to about one-hundredth the initial rate in 15–20 min. This is because the osmotic concentration of the sap increases on side B and decreases on side A so that the suction forces of both cell halves become nearly equal. On replacing the solution in B with water, osmosis is induced in the opposite direction, or from B to A, by the intracellular osmotic gradient. This flow of water is referred to as "backward osmosis". The driving force of backward osmosis is almost the same as that of forward osmosis [cf. equations (10) and (11)].

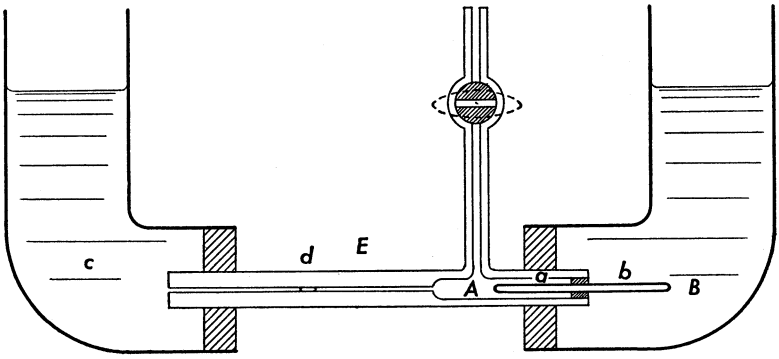

Fig. 1.—Double-chamber osmometer for the measurement of transcellular osmosis. Bent tube c to the left is provided in order to cancel the hydrostatic pressure difference between the two sides of the cell. Except for the open tubes the osmometer is immersed in a thermostat bath. For further explanation, see text.

(*b*) *Measurement of the Initial Rate of Transcellular Osmosis and Determination of the Transcellular Osmotic Constant, K*

According to Kamiya and Tazawa (1956) the rate of transcellular osmosis (dv/dt) is expressed in the following equation:

$$dv/dt = K(S_{en} - S_{ex}). \tag{1}$$

where S_{en} and S_{ex} are suction forces on endosmotic and exosmotic sides of the cell and K is what will be called the "transcellular osmotic constant" in the present paper. K is dependent not only upon the specific characteristics of the membrane, but also upon conditions involved in the experiment, such as surface areas of the endosmotic and exosmotic ends of the cell. S_{en} and S_{ex} are defined as follows:

$$S_{en} = p_{en} - P_{en} - T, \tag{2}$$

$$S_{ex} = p_{ex} - P_{ex} - T, \tag{3}$$

where p_{en} and p_{ex} are osmotic pressures of the cell, P_{en} and P_{ex} are those of external solutions, the subscripts "en" and "ex" designating "endosmotic" and "exosmotic" sides respectively, and T is turgor pressure.

Immediately after the start of osmosis (forward osmosis), p_{en} and p_{ex} are nearly equal to the original osmotic pressure, p_i, of the cell. Hence, at the moment when the water of one chamber is replaced with a mannitol solution of osmotic pressure P_{ex} while the other chamber is left filled with water ($P_{en} = 0$), we have $S_{en} - S_{ex} = P_{ex}$. The initial rate of osmosis $(dv/dt)_i$ is thus expressed as follows:

$$(dv/dt)_i = KP_{ex},$$

or

$$K = (1/P_{ex})(dv/dt)_i. \tag{4}$$

Although in our previous paper (Kamiya and Tazawa 1956) we obtained K from theoretical formulae of forward and backward osmoses, it is also possible to determine K if the initial rate of transcellular osmosis $(dv/dt)_i$ is measured accurately. The initial phase of transcellular osmosis is, however, inevitably accompanied by a change in turgor or by a slight change in cell volume. Hence it is important to note that a shift of the bubble at the very beginning of osmosis necessarily involves decrease in cell volume at a (Fig. 1). Thus, it is desirable to measure the osmosis after the change in cell volume is nearly over and yet p_{en} and p_{ex} can still be regarded as being equal to the original osmotic pressure p_i. As will be shown later, changes in turgor or in cell volume end almost within 5 sec. In practice, therefore, we have determined the initial rate $(dv/dt)_i$ in the time interval between 5 and 10 sec after the onset of osmosis, since the change in sap concentration within 5 sec at the very beginning of osmosis is small enough to be neglected.

For informing the observer of the time of measurement a light signal connected with an automatic timer was set with an interval of 5 sec.

(c) *Transcellular Permeability Constant* (K'), *and Endosmotic and Exosmotic Permeability Constants* (k_{en} and k_{ex})

In order to observe the effect of a substance admitted to both chambers (A and B, Fig. 1) on water permeability, it is enough to compare transcellular osmotic constants (K) of the same cell with and without test substance. However, in order to compare water permeabilities of cells having different lengths and diameters, it is convenient to partition them symmetrically and to get permeability constants expressed as volume of water per unit time per unit pressure per unit surface area. The constant in question, which we shall call "transcellular permeability constant" to water (K'), can be obtained simply by dividing transcellular osmotic constant K [cf. equation (4)] by one-half the surface area of the cell. According to Kamiya and Tazawa (1956) K is a function of endosmotic and exosmotic permeability constants (k_{en} and k_{ex}) and also of the surface areas of both sides (A_{en}, A_{ex}), i.e.

$$K = k_{en}k_{ex}A_{en}A_{ex}/(k_{en}A_{en}+k_{ex}A_{ex}). \tag{5}$$

For the case when the cell is partitioned equally ($A_{en} = A_{ex} = \frac{1}{2}A$),

$$K = \tfrac{1}{2}Ak_{en}k_{ex}/(k_{en}+k_{ex}),$$

or

$$K' = K/\tfrac{1}{2}A = k_{en}k_{ex}/(k_{en}+k_{ex}), \tag{6}$$

where A is the total surface area of the cell minus the surface area of the cell part

in the partition wall. It is easy to understand that K' is the reciprocal value of the sum of the two specific resistances against water flow which are equal to $1/k_{en}$ and $1/k_{ex}$.

A convenient method to obtain k_{en} and k_{ex} is to partition the cell asymmetrically and to subject the same cell to transcellular osmosis twice: in the first case, from the cell end with a larger area (A_a) to the other end with a smaller area (A_b); in the second case, in the reverse direction, or from A_b to A_a. If we call the larger surface area divided by the smaller surface area "partition ratio" and denote it by α, we have $\alpha = A_a/A_b$.

Representing k_{en}/k_{ex} by ρ, which is a measure of polarity in water permeability, the two transcellular osmotic constants for the above two cases, K_1 and K_2, are given by the following equations [cf. equation (5)], i.e.

$$K_1 = [\rho\alpha/(\rho\alpha+1)]k_{ex}A_b, \tag{7}$$

$$K_2 = [\rho\alpha/(\rho+\alpha)]k_{ex}A_b. \tag{8}$$

From equations (7) and (8) ρ can be calculated as follows:

$$\rho = (\alpha K_2 - K_1)/(\alpha K_1 - K_2). \tag{9}$$

If ρ is known, k_{ex} can be obtained either from (7) or (8), and then k_{en} is calculated as ρk_{ex}.

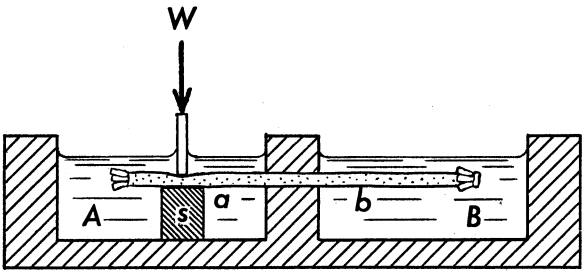

Fig. 2.—Double-pool vessel for measuring the change in turgor during transcellular osmosis. For explanation see text.

(*d*) *Measurement of Changes in Turgor during Transcellular Osmosis*

In order to follow changes in turgor pressure in the course of transcellular osmosis, a vessel (shown in Fig. 2) made of methacrylate resin was used. The vessel has two pools which are separated by the central septum with a narrow groove just wide enough for the internode to be inserted. One of the two pools (pool A) is equipped with a small supporting block (S) of resin at the centre. After the groove is loaded with the internode and is sealed with Vaseline, the two pools are filled with water. A part of the cell resting on the supporting block in pool A is then pushed down gently to a slight extent with an edge of the glass slide loaded with a certain weight (W). In practice the turgor balance (Tazawa 1957) was used, with which the weight imposed upon the cell can be controlled and the sink due to deformation of the cell can be magnified.

When the water in B (Fig. 2) is replaced with mannitol solution, the transcellular osmosis starts immediately. At the same time the turgor begins to decrease, which is indicated by a slight sink of the glass edge affixed to the turgor balance. In order to know the turgor change from the sink of the glass edge, it is necessary to correlate the amount of sink to cell turgor in advance. According to Kamiya, Tazawa, and Takata (1963) the cell wall of *Nitella flexilis* is so resistant to turgor that the cell volume changes by only a small percentage from the full (6–7 atm) to zero turgor state. In other words, the cell sap keeps the osmotic pressure nearly constant independently of the turgor. Since the turgor (T) is equal to the difference in osmotic pressure between the cell sap (P_i) and the outer medium (P_{ex}), i.e. $T = P_i - P_{ex}$, the turgor change is approximately equal to the change in osmotic pressure of the outer medium. Consequently, in order to correlate the sink of the glass edge to turgor, it is enough to measure the sink when the water in both pools (A and B) is replaced with a hypotonic mannitol solution of the same concentration. In practice, the concentration of mannitol solution was varied stepwise in the range between zero and 0·25M (cf. Fig. 3). The turgor of the cell is then to be obtained from the equation $T = P_i - P_{ex}$, where P_i was measured with the turgor balance (Tazawa 1957).

(*e*) *Modification of Osmotic Concentration of the Cell Sap*

At the steady state of forward osmosis [cf. Section II(*a*)] the osmotic concentrations of the cell sap on the endosmosis and exosmosis sides (C_{en}, C_{ex}) are given by the following equations:

$$C_{en} = C_i - [V_{ex}/(V_{en}+V_{ex})]c_{ex_o} \tag{10}$$

$$C_{ex} = C_i + [V_{en}/(V_{en}+V_{ex})]c_{ex_o}, \tag{11}$$

where C_i and c_{ex_o} are the original sap concentration and the concentration of the external mannitol (or sucrose) solution on the exosmosis side, and V_{en} and V_{ex} are the volumes of the cell on respective sides. By ligating the cell with strips of silk thread at two loci near the partition and by cutting the small central fragment mounted in the partition, two cell fragments having higher and lower osmotic concentrations are obtained (Kamiya and Kuroda 1956). For simplicity's sake, we shall hereafter call the cell fragment with a higher osmotic pressure "H cell" and that with lower osmotic pressure "L cell".

(*f*) *Measurement of Water Permeability of the Cell Wall*

The water permeability, or hydraulic conductivity, of the cell wall of *Nitella* internode was measured by the method developed by Kamiya, Tazawa, and Takata (1962). Cutting one end of the internode and squeezing out the cytoplasm and cell sap, a transparent cell wall tube free from all the cell content is obtained. After the cell wall tube, or so to speak, the "ghost" of the *Nitella* internode, is filled with water, a certain hydraulic pressure is applied to the interior of the tube through a glass capillary. Since the volume of water escaping from the cell wall tube is measureable in this case, the water permeability of the cell wall alone (volume of water/unit area/unit time/unit pressure) can be calculated.

III. Results

(*a*) *Turgor Change Accompanying Transcellular Osmosis*

When the concentration of the media in both pools (*A* and *B* in Fig. 2) is heightened stepwise and then lowered stepwise again, the cell undergoes corresponding changes in turgor which are indicated by the sink of the glass edge affixed to the turgor balance. Figure 3 shows the result of such an experiment. The osmotic value of the cell used was 0·32M (8·7 atm). Hence the turgor of the cell in the external media with various osmotic pressures (P_{ex}) can be calculated as 8·7 atm minus P_{ex}.

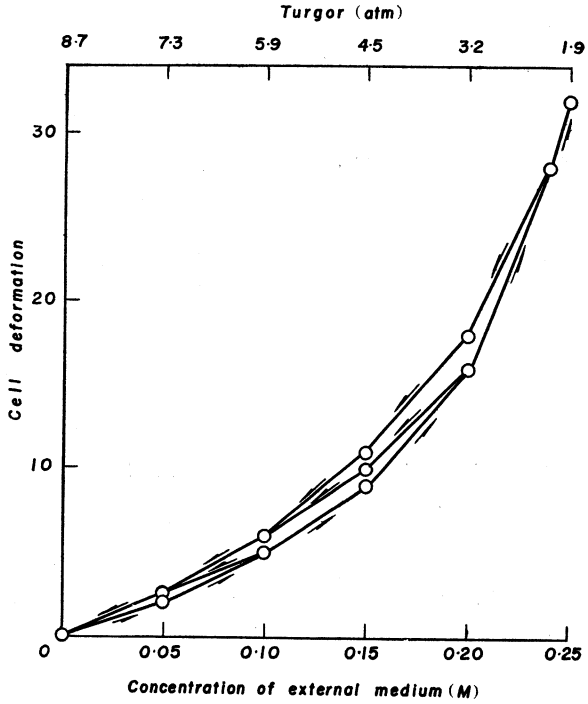

Fig. 3.—Deformation of the *Nitella* internode in relation to osmotic concentration of the external medium and to turgor. Deformation of the cell is expressed in an arbitrary scale of the eyepiece micrometer on which the deformation of the cell is shown magnified by means of a lever affixed to the turgor balance. The osmotic value of the cell is 0·32M sucrose equivalents (8·7 atm). Cell deformation takes different curves according to the direction of turgor change.

Next turgor change was measured during transcellular osmosis when the cell was partitioned into equal halves (Fig. 4). At first both halves of the cell were bathed in water (or in 0·2M sucrose). Then the medium in *B* (cf. Fig. 2) was replaced with 0·2M sucrose (or water). After 5 min the sucrose solution (or water) was substituted again for water (or the sucrose solution) in *B*. It is clear from Figure 4 that the turgor change in this case was almost over in a very short period of time after

the forward osmosis had started. Numerically speaking, 80–90% of the total turgor change have been attained within the first 5 sec. A further fact to be noticed is that the turgor change in forward osmosis is about one-half the change expected when the cell is surrounded in its entirety with the medium on the exosmosis side. In backward osmosis the turgor is brought back also in a very short period of time, but, unlike in forward osmosis it is brought back always to the original normal level irrespective of the degree of foregoing osmotic polarization.

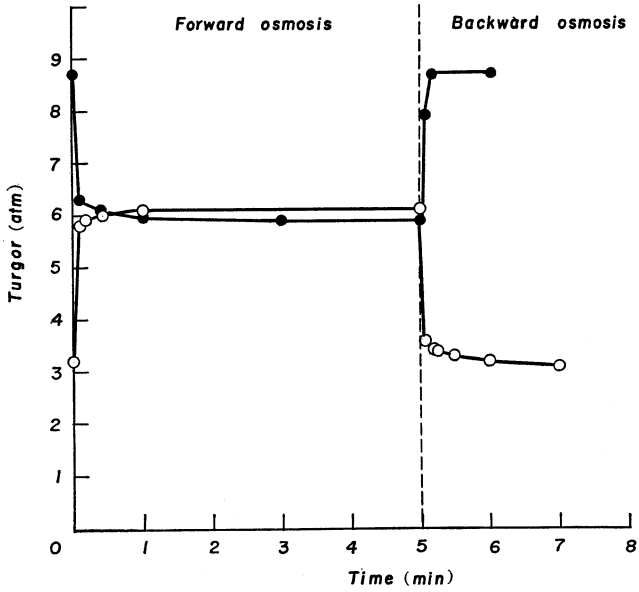

Fig. 4.—Changes in turgor of symmetrically partitioned *Nitella* internode during transcellular osmosis induced by 0·2M (≡ 5·3 atm) mannitol solution. The osmotic value of the cell is 0·32M (≡ 8·7 atm). Before the start of forward osmosis both cell halves are either in water (●) or in 0·2M mannitol solution (○).

The relation between changes in volume and length of a *Nitella* internode being known (Kamiya, Tazawa, and Takata 1963), decreases both in length of the cell in pool *A* (cf. Fig. 2) and in cell turgor were measured in the same cell in order to correlate the volume change to turgor change. The cell used had the osmotic value of 0·28M and the external medium applied to pool *B* was 0·3M mannitol. The result is shown in Figure 5, where we see that the cell length ceased to decrease also within a very short period of time after the onset of the osmosis.

Next were measured changes in turgor and cell length in the course of transcellular osmosis partitioning asymmetrically the same cell as used for the experiment shown in Figure 5. In one case 0·3M mannitol solution was applied to the longer cell end (Fig. 6) and in the other case to the shorter cell end (Fig. 7). In either case the major changes in cell turgor and cell length were accomplished within the first 5 sec, but the absolute amounts of the changes were different in the two cases.

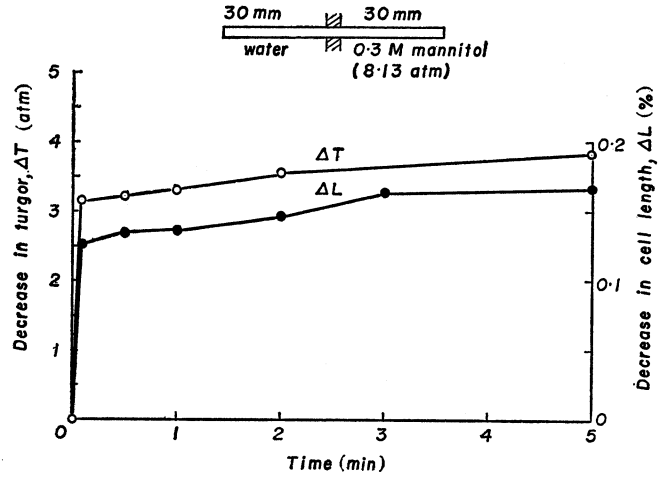

Fig. 5.—Decrease in turgor (ΔT) and in cell length (ΔL) during transcellular osmosis induced by 0·3M mannitol. The cell, having the osmotic value of 0·28M (7·6 atm), is partitioned symmetrically.

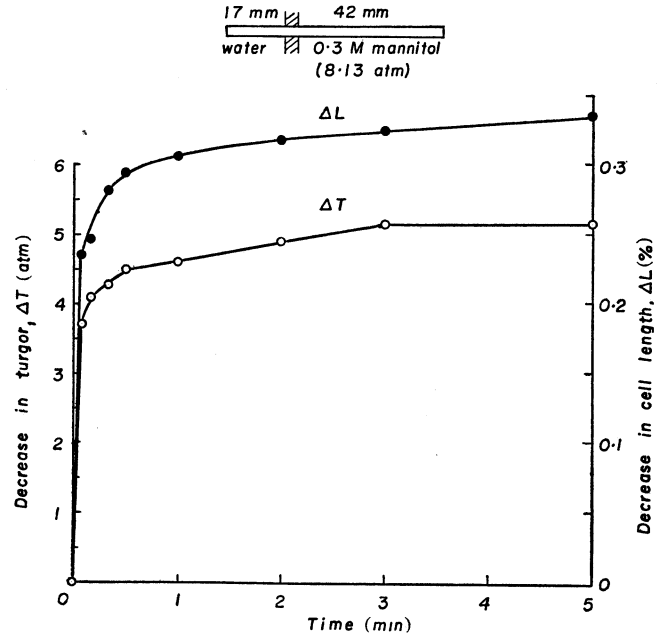

Fig. 6.—As for Figure 5, but with the cell partitioned asymmetrically.

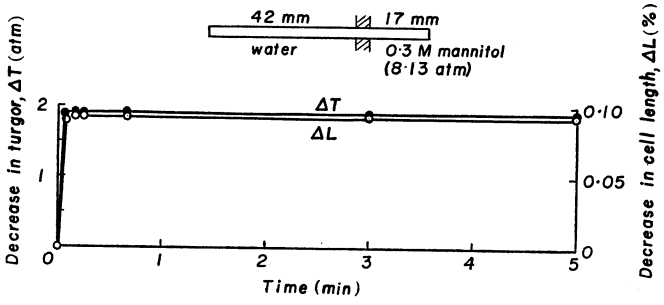

Fig. 7.—As for Figure 5, but with the cell partitioned asymmetrically and opposite to Figure 6.

From the results shown in Figures 4–7 it has been made clear that within a very short lapse of time the turgor reaches a steady value which is given by the following relation:

$$T = p_i - [V_{ex}/(V_{en}+V_{ex})]P_{ex} \tag{12}$$

$$= [p_i V_{en} + (p_i - P_{ex})V_{ex}]/(V_{en}+V_{ex}), \tag{13}$$

where T is turgor, p_i initial osmotic pressure of the cell, P_{ex} osmotic pressure of the external medium in pool B, and V_{en} and V_{ex} are volumes of the cell part on the endosmotic and exosmotic sides. At the start of transcellular osmosis, p_i must be equal to the turgor potential (T_{en}) of the cell part a. In the same manner ($p_i - P_{ex}$) must be the turgor potential (T_{ex}) of the cell part b. Therefore, equation (13) can be rewritten:

$$T = (T_{en}V_{en} + T_{ex}V_{ex})/(V_{en}+V_{ex}). \tag{14}$$

This situation is analogous to the following simple model. Suppose two balls with solid walls and of different volumes, V_1 and V_2, are under different internal gas pressures, P_1 and P_2. If the two balls are connected by a small pipe to each other, the pressures are rapidly equalized, giving a new pressure P, which is calculated easily by the following relation:

$$P = (P_1V_1 + P_2V_2)/(V_1+V_2). \tag{15}$$

Since the volume flow v, which is represented by the shift of the index bubble, is actually the sum of the true transcellular water movement (v_{tr}) and the change in volume of the cell part in A (ΔV_{en}) (cf. Fig. 1), it is important to calculate $\Delta V_{en}/v$ at the initial phase of transcellular osmosis. Thus we have

$$v = v_{tr} + \Delta V_{en}, \tag{16}$$

or

$$v/V = v_{tr}/V + \Delta V_{en}/V, \tag{17}$$

where V is the total cell volume ($V = V_{en}+V_{ex}$)*.

In Figures 5–7, only the ratio of the change in cell length ($\Delta L/L$) was measured. From the work of Kamiya, Tazawa, and Takata (1963) the ratio of the volume

* The volume of the cell part mounted in the partition wall is not considered here. Hence what is meant with the total cell volume in this case is the actual total cell volume minus the volume of the cell part in question.

change ($\Delta V/V$) is approximately four times as large as $\Delta L/L$. Table 1 shows the estimated values of $\Delta V_{en}/V_{en}$ and $\Delta V_{en}/V$ and the value of v/V 5 sec after the onset of transcellular osmosis. From Table 1 it is clear that at the start of osmosis the change in cell volume (ΔV_{en}) is involved to some extent (14–19%) in the volume flow (v) which is actually measured by the shift of the index bubble. However, change in cell volume has little effect on the bubble shift after 5 sec.

TABLE 1

PARTICIPATION OF THE CHANGE IN CELL VOLUME IN THE SHIFT OF THE INDEX BUBBLE IN 5 SEC AFTER THE ONSET OF OSMOSIS

	V_{en}/V_{ex}		
	1 : 1	1 : 2·5	2·5 : 1
$\Delta L_{en}/L_{en}$* (%)	0·17	0·34	0·10
$\Delta V_{en}/V_{en}$ (%)	0·68	1·36	0·40
$\Delta V_{en}/V$ (%)	0·34	0·40	0·29
v/V* (%)	2·5	2·1	1·7
$\Delta V_{en}/v$ (%)	13·6	19·0	17·0

* Actually measured.

(b) Concentration of the Cell Sap and its Bearing on Transcellular Water Permeability Constant (K')

Before obtaining a H cell and L cell pair from a normal internode the osmotic value and the transcellular water permeability constant K' of the normal internode were determined [cf. equations (4) and (6)]. Figure 8 shows the values of K' of the normal cell, H cell, and L cell. When the osmotic concentration of the cell sap is heightened (H cell), K' decreases; when the osmotic concentration of the cell sap is lowered (L cell), it increases. In Figure 9 K' is shown as a percentage of K' of the normal internode plotted against the deviation of osmotic concentration of cell sap from the normal level. It is shown that permeability change is more sensitive toward dilution than toward concentration of the cell sap.

The effect of osmotic concentration of cell sap on endosmotic and exosmotic permeabilities, k_{en} and k_{ex}, has not yet been studied separately.

(c) Transcellular Water Permeability (K') in Relation to Osmotic Gradient

(i) *K' for "Forward Osmosis".*—Figure 10(a) represents one example showing the water flow in forward osmosis through an internode with the normal sap concentration under different osmotic gradients. The flow increases nearly linearly or with a slight decline with the increase of osmotic gradient. This tendency is also clearly shown in Table 2. The turgor decreases with the increase of the osmotic gradient between A and B. The table shows that from full turgor down to zero turgor K' decreases by about 20–30%.

The dependence of K' on turgor is more clearly demonstrated on cells having higher (H) and lower (L) osmotic pressures. Comparing H cells in Table 3 with the

normal cells in Table 2, we see that depression of K' of H cells is less pronounced than in the normal cells under the same osmotic gradient. On the other hand, K' of the

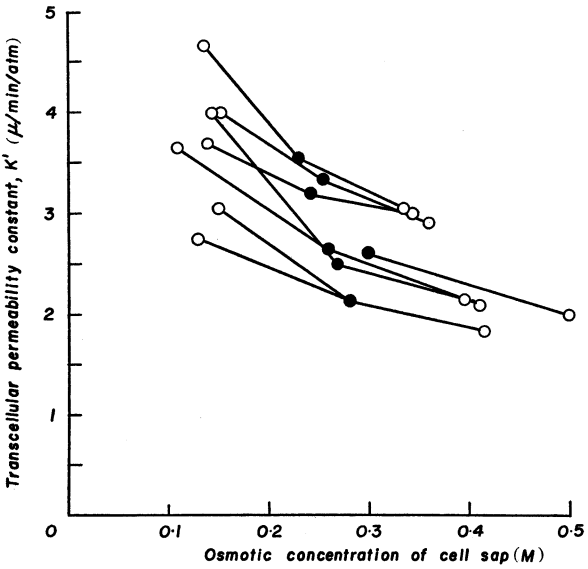

Fig. 8.—Transcellular permeability constant to water (K') in relation to the osmotic concentration of the cell sap. ● K' of the internodes at their normal sap concentration.

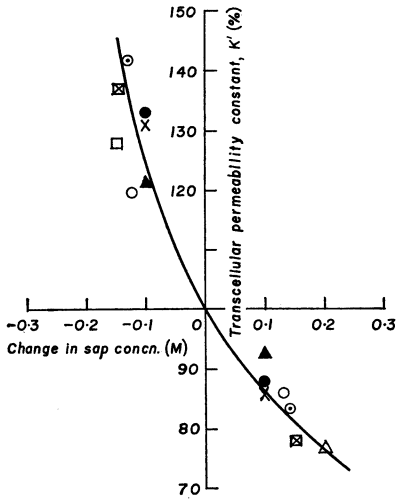

Fig. 9.—Transcellular permeability constant (K') in relation to the deviation of osmotic concentration of the cell sap from the normal level.

L cell decreases significantly under the osmotic gradient as low as 0·3M where K' of the normal internode is hardly affected.

(ii) *K′ for "Backward Osmosis"*.—Fifteen minutes after forward osmosis set in, mannitol solution was replaced with water to induce backward osmosis. In this case the osmotic drive is caused by the gradient of intracellular sap concentration established during forward osmosis. Figure 10(*b*) shows the rate of backward osmosis under different intracellular osmotic gradients. There the water flow increases quite linearly with the increase in osmotic gradient unlike the case in the forward osmosis.

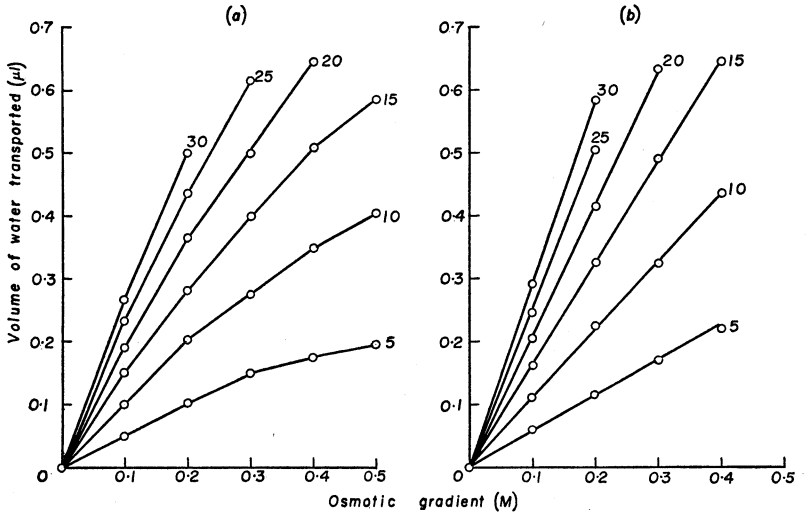

Fig. 10.—Transport volume of water in relation to the osmotic gradient in various lapses of time after the onset of forward (*a*) and backward (*b*) osmoses. The osmotic gradient for the forward osmosis was established by the difference in osmotic concentration of the external medium between two chambers and that for the backward osmosis was established by the difference in sap concentration between the two cell halves. Numbers alongside each curve represent the time (in sec) elapsed after the onset of osmosis.

Table 4 summarizes the values of K' obtained from backward osmosis of several internodes at different internal osmotic gradients. The cells were the same as those used for forward osmosis in Table 2. In the backward osmosis, as already mentioned, the turgor, once decreased to whatever level by the foregoing forward osmosis, invariably regains the same original turgor in several seconds (cf. Fig. 4). From the Table 4 it is clear that K' values are also nearly the same* independently of internal osmotic polarization. Comparing Table 4 with Table 2, we further realize that K'

* In the backward osmosis the sap concentration is high on the endosmosis side and low on the exosmosis side. In the foregoing it has been shown that K' increases or decreases when the sap is diluted or concentrated. Assuming the same rate of increase and decrease in water permeability for exosmosis (k_{ex}) and endosmosis (k_{en}) as in K' (cf. Fig. 9), and the polarity ($\rho = k_{en}/k_{ex}$) as 1·5, the relative value of K' for the backward osmosis can be calculated as 103 and 106 at 0·2M and 0·3M osmotic gradient respectively if K' is given as 100 at 0·1M osmotic gradient. Actually K' increases by a small percentage in some cases for higher osmotic gradient (0·2M, 0·3M, 0·5M Table 5).

values for the backward osmosis are larger than those for forward osmosis. It is therefore probable that maintenance of turgor at a high level is responsible for high K' value in backward osmosis.

(*d*) *Polar Water Permeability*

(i) *Dilution Effect in Transcellular Osmosis.*—Dainty and Hope (1959) maintained that the polar permeability found by Kamiya and Tazawa (1956) on an unequally partitioned *Nitella* internode is accounted for in terms of the dilution effect

TABLE 2

TRANSCELLULAR PERMEABILITY CONSTANT TO WATER (K') FOR FORWARD OSMOSIS IN NORMAL INTERNODES UNDER DIFFERENT OSMOTIC GRADIENTS

Cell No.	Temp. (°C)	Osmotic Gradient (M)	Osmotic Gradient (atm)	Turgor (atm)	K' (μ/min/atm)	K' (%)*
1	25	0	0	6·98		
		0·10	2·64	5·66	6·2	100
		0·20	5·29	4·33	5·8	94
		0·30	8·13	2·91	5·0	81
		0·40	11·1	1·43	4·9	80
		0·50	14·3	−0·17	4·4	71
2	23	0	0	7·35		
		0·10	2·64	6·03	5·3	100
		0·20	5·29	4·70	5·2	98
		0·30	8·13	3·29	4·8	92
		0·40	11·1	1·80	4·4	83
		0·50	14·3	0·20	4·2	80
3	26	0	0	6·70		
		0·05	1·32	6·04	9·0	98
		0·10	2·64	5·38	9·2	100
		0·20	5·29	4·05	8·8	95
		0·30	8·13	2·63	8·1	88
4	25	0	0	7·40		
		0·10	2·64	6·08	6·1	100
		0·20	5·29	4·75	6·1	100
		0·30	8·13	3·34	5·5	90
		0·40	11·1	1·85	5·0	82
		0·50	14·3	0·35	4·9	81

* As percentage of value under an osmotic gradient of 0·10M.

and hence polarity in water permeability is merely apparent. This is because the dilution effect should be different according to whether the shorter end or the longer end of the cell is in a sucrose solution. In order to check whether the dilution effect is actually involved to a measurable extent in transcellular osmosis, the following experiments were done on the empty cell wall tube of *Nitella* internode. The apparatus used is originally so constructed as to measure the flow of water through the cell

wall tube on application of the hydraulic pressure inside the cell wall tube (Kamiya, Tazawa, and Takata 1962). This apparatus can be used also for measuring the osmotic flow of water across the cell wall. Namely, when the inside of the cell wall tube is water and the outside medium is a sucrose or mannitol solution, exosmosis occurs. In the reverse situation endosmosis takes place.

Table 5 shows the cell wall permeabilities to water, one for the pressure flow (k_w^p) induced by hydraulic pressure applied inside the cell wall tube and the other for the osmotic flow induced by sucrose either outside or inside the cell wall tube

TABLE 3

TRANSCELLULAR PERMEABILITY CONSTANT TO WATER (K') FOR FORWARD OSMOSIS IN H AND L CELLS UNDER DIFFERENT OSMOTIC GRADIENTS

Cell No.	Temp. (°C)	Osmotic Gradient (M)	Osmotic Gradient (atm)	Turgor (atm)	K' (μ/min/atm)	K' (%)*
5H	21	0	0	10·50		
		0·10	2·64	9·18	4·7	100
		0·20	5·29	7·85	4·6	99
		0·40	11·1	4·95	4·3	91
		0·50	14·3	1·60	3·8	81
6H	21	0	0	10·20		
		0·10	2·64	8·88	4·0	100
		0·20	5·29	7·55	4·0	100
		0·40	11·1	4·75	3·6	91
		0·50	14·3	1·30	3·0	76
7H	21	0	0	9·72		
		0·10	2·64	8·40	4·4	100
		0·20	5·29	7·07	4·2	95
		0·40	11·1	4·17	3·6	82
		0·50	14·3	0·82	3·0	69
7L	21	0	0	3·06		
		0·10	2·64	1·74	5·6	100
		0·20	5·29	0·41	5·4	97
		0·30	8·13	−1·00	4·0	71

* As percentage of value under an osmotic gradient of 0·10M.

($_{ex}k_w^s$, $_{en}k_w^s$). In order to study a possible polarity of the osmotic flow through the cell wall, we were successful in an attempt to turn the *Nitella* cell wall tube inside out. The osmotic effectiveness (Ray 1960) or the reflection coefficient (Dainty 1963*b*). can be given by k_w^p/k_w^s and this is found to be 0·25–0·5 of sucrose for the cell wall of the adult *Nitella* internode.

In Figure 11(*a*) curve 1 shows the time course of the exosmosis when 0·2M sucrose was applied to the outside under an internal hydraulic pressure of less than 2 cm of water, which is too small to bring forth any appreciable pressure flow. The

TABLE 4

TRANSCELLULAR PERMEABILITY CONSTANT TO WATER (K') FOR BACKWARD OSMOSIS IN NORMAL INTERNODES UNDER DIFFERENT INTRACELLULAR OSMOTIC GRADIENTS

Cell No.	Temp. (°C)	Osmotic Gradient (M)	Osmotic Gradient (atm)	Turgor (atm)	K' (μ/min/atm)	K' (%)*
1	25	0·10	2·64	6·98	6·4	100
		0·20	5·29		6·6	103
		0·30	8·13		6·5	102
		0·40	11·1		6·5	102
2	23	0·10	2·64	7·35	5·8	100
		0·20	5·29		6·1	105
		0·30	8·13		6·1	105
		0·40	11·1		5·5	95
3	26	0·10	2·64	6·7	9·2	100
		0·20	5·29		9·5	103
		0·30	8·13		9·1	98
4	25	0·10	2·64	7·4	6·7	100
		0·20	5·29		7·0	104
		0·30	8·13		6·7	100
		0·40	11·1		6·6	98
		0·50	14·3		7·2	107

* As percentage of value under an osmotic gradient of 0·10M.

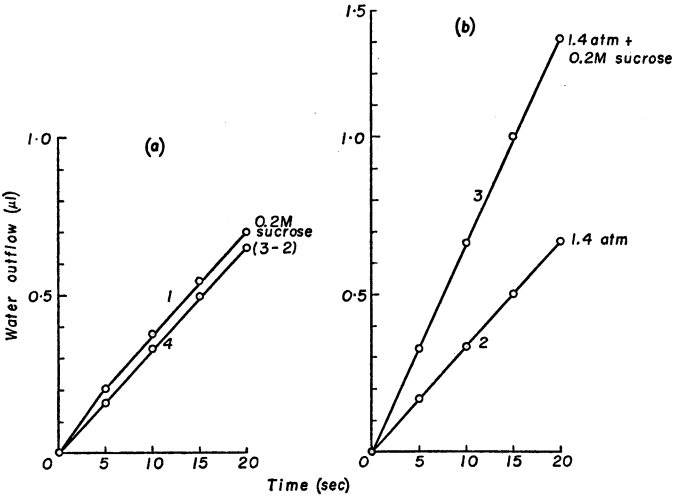

Fig. 11.—(*a*), (*b*): Outflow of water (v) through the cell wall tube. *1*, Osmotic flow induced by 0·2M sucrose applied to the outside of the tube. *2*, Pressure flow induced by 1·4 atm hydraulic pressure to the inside of the tube. *3*, Flow induced by 0·2M sucrose plus application of 1·4 atm hydraulic pressure. *4*, Flow obtained by subtracting the pressure flow (curve *2*) from combined flow (curve *3*).

flow velocity of water across the cell wall was 0·021 μl/min/mm^2 in this case. The same outflow velocity of water was obtained, on application of hydraulic pressure of 1·4 atm to the inside of the cell wall tube when the inside and outside are both water (Fig. 11(*b*), curve 2). The next step is to substitute 0·2M sucrose solution for the water outside the tube while hydraulic pressure of 1·4 atm is kept applied to the

TABLE 5

WATER PERMEABILITY OF CELL WALL TUBES

Cell Wall Tube No.	k_w^p* (μ/min/atm)	${}_{ex}k_w^s$† (μ/min/atm)	${}_{en}k_w^s$† (μ/min/atm)	Wall Thickness (μ)	Temp. (°C)
73	20	10·4		8·0	15·5
14	10·8	5·1		10·0	9·5
15	14·9	3·9		13·0	17·5
16	16·5	4·8	4·8	8·5	14·0
17‡	21·0	4·3	4·0	9·0	18·0

* Measured by the outflow of water caused by the hydraulic pressure applied to the interior of the cell wall tube.

† Determined for exosmosis induced when 0·2M sucrose was outside the cell wall tube or for endosmosis induced when 0·2M sucrose was inside the tube, under the assumption that 0·2M sucrose had developed a full osmotic pressure of 5·3 atm across the cell wall.

‡ Determinations made after the tube was turned inside out.

interior. The rate of water outflow in this case, which is actually a summation of the rate of pressure flow and that of osmotic flow, is given by curve 3 in Figure 11(*b*). The net osmotic flow can therefore be obtained by subtracting the pressure flow (curve 2) from the combined flow (curve 3). Curve 4 in Figure 11(*a*) shows this.

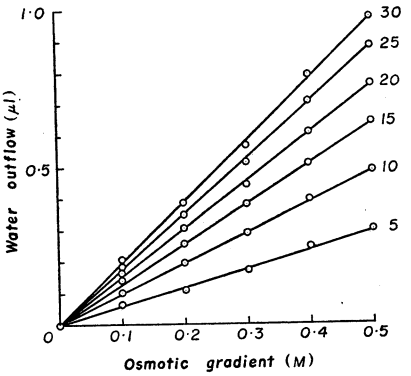

Fig. 12.—Outflow volume of water through the cell wall tube in relation to osmotic concentration of the external medium. Mannitol solutions were applied to the outside of the cell wall tube, while water was inside the tube. Numbers alongside each curve represent the time (in sec) elapsed after the onset of osmosis.

Comparing curve 1 with curve 4, it can be said that the rates of the two osmotic flows, or inclinations of the two curves, are practically the same. What this fact implies is that the rate of osmotic water flow is not influenced by the pressure flow of water. In other words, a possible dilution of the sucrose solution immediately outside the cell wall tube does not play a significant role in this case.

A further fact showing that the dilution effect needs not be taken into account in osmosis across the cell wall is that the rate of osmosis across the cell wall increases linearly with the increase of the osmotic gradient (Fig. 12). The initial flow velocities per unit area of the cell wall tube were 0·013, 0·021, 0·034, 0·049, and 0·059 μl/min/mm^2 for 0·1, 0·2, 0·3, 0·4, and 0·5M mannitol solution respectively. These values are of the same order as the rates of transcellular osmosis through the intact cell at the same osmotic gradients.*

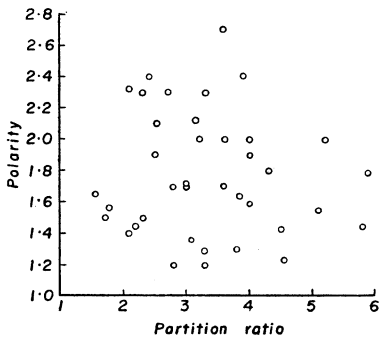

Fig. 13.—Polarity of water permeability against the partition ratio of the cell. The transcellular osmosis was induced by 0·2M mannitol or 0·2M sucrose solution.

(ii) *Water Permeability of Asymmetrically Partitioned Internodes.*—The polarity (ρ) of water permeability [cf. equation (9)], which is represented by the ratio between the inward and outward water permeabilities, was obtained under different partition ratios (Fig. 13). From Table 6 it is clear that the ρ is nearly the same irrespective

TABLE 6

POLARITIES OBTAINED UNDER DIFFERENT PARTITION RATIOS (a)

Numbers in parentheses represent the number of cells used

	Osmotic Gradient 0·2M		Osmotic Gradient 0·1M
a*	$1·5 \leqslant a \leqslant 3·5$	$3·5 < a \leqslant 5·9$	$1·6 \leqslant a \leqslant 3·6$
ρ†	1·77 (22)	1·75 (17)	1·57 (12)
ρ_P‡	2·0 (6)	1·8 (8)	1·8 (8)

* Ratio of the larger surface area to the smaller surface area.

† Polarity of the entire membrane system consisting of the cell wall and living cytoplasmic layer.

‡ Polarity of the living cytoplasmic layer without the cell wall.

* In the case of the cell wall tube, water permeability must be much higher than that of the intact cell, since there is no plasmalemma, mesoplasm, and tonoplast, but, the cell wall being only incompletely semipermeable, the solute leaks into the tube during exosmosis. This is why osmotic outflow of water is smaller in rate than pressure flow. In the case of the living cell, there is no leakage of mannitol into it, but the resistance against passage of water must be larger. Therefore it is to be understood that a lower resistance of membrane with some leakage and a higher resistance of the membrane with no leakage will bring about a similar result in respect to the net flow of water.

of the partition ratio. This fact shows again that the polarity cannot be accounted for by the dilution effect, since, if there were increasing asymmetry in dilution effect, ρ must become larger with partition.

As mentioned in the foregoing, the cell wall is equally permeable for both endosmosis and exosmosis. Therefore, if we assume that the resistances of the cell wall and the protoplasmic layer act additively, it is possible to calculate the polarity due to the living part without the cell wall from the following equation (Kamiya, Tazawa, and Takata 1962), i.e.

$$\rho_p = \rho(k_w - k_{ex})/(k_w - k_{en}), \tag{18}$$

where ρ_p is the polarity of the protoplasmic layer and k_w ($= k_w^p$ in the previous section) is water permeability of the cell wall alone. In Table 6 ρ_p was obtained only for 14 cells on which k_w was measured. ρ on the average in these cells is 1·6, and k_{en} and k_{ex} are 11·5 and 7·2 μ/min/atm respectively at 18·5°C. ρ_p is calculated to be 1·9 for these cells, and the endosmotic and exosmotic permeability constants of the protoplasmic part proper are found to be 19·0 and 10·0 μ/min/atm. These values coincide well with those we obtained previously (cf. Table 1 in the paper by Kamiya, Tazawa, and Takata 1962).

IV. Discussion

In the present paper we have considered some basic problems involved in transcellular osmosis. Dainty and Hope (1959) and Dainty (1963*a*) tried to explain the polar permeability in terms of the decrease in net driving force due to the "sweeping away" effect of the solute from the cell surface on the exosmosis side. If this were the actual case, the polarity in water permeability deduced by us would be apparent. In this connection it is important to check to what extent the dilution of the surface concentration in transcellular osmosis should be taken into consideration.

If the dilution effect is operative to any significant extent in transcellular osmosis, the decrease in net driving force should be more conspicuous when the shorter cell part of the asymmetrically partitioned internode is in sucrose solution than when the longer cell part is in sucrose solution. However, there is no correlation between the value of polarity (ρ) and the partition ratio. There is also the fact pointed out already (Kamiya and Tazawa 1956) that keeping sucrose solution flowing has no effect on transcellular osmosis. Further the experiments on cell wall tubes described in Section III(*d*) prove that the dilution effect does not participate in transcellular osmosis in any significant manner. In the light of this evidence we feel it safe to say that the dilution of the external solution, or decrease in the net driving force, postulated by Dainty and Hope (1959), cannot explain the phenomenon which we pointed out and from which we inferred the polar nature of water permeability in the *Nitella* cell (Kamiya and Tazawa 1956).

It is true that transcellular permeability constant K' measured by "forward osmosis" tends to decrease when osmotic concentration of the external solution becomes higher (Dainty and Hope 1959; Dainty and Ginzburg 1964). However, our experiments with cell wall tubes show that the speed of exosmosis through a simple

cell wall tube increases linearly with the osmotic concentration of the external solution (Fig. 12) and further that the exosmosis caused by the external sucrose solution is not affected by the outflow of water hydraulically induced (Fig. 11). These facts seem to show that the decrease in K' in forward osmosis with the increase in osmotic gradient should be accounted for by conditions other than the dilution of the medium adjacent to the cell. As shown by Kamiya, Tazawa, and Takata (1963) the shrinkage of the *Nitella* cell per unit decrease in turgor is more conspicuous on the lower level of turgor than on the higher level. The same tendency is shared by K' [cf. Fig. 10(*a*)]. The decrease in K' observed in forward osmosis is not observed in backward osmosis. In forward osmosis turgor decreases to a definite level corresponding to the external osmotic concentration. In backward osmosis turgor is brought back to the normal level irrespective of the internal osmotic gradient. The above facts imply first that the dilution effect is negligible also in the vacuole, and second, that the decrease in cell volume or rather a possible change in the physical properties of the membranes accompanying turgor decrease may be responsible for the decrease in K' observed in forward osmosis.

Dainty and Ginzburg (1964) explain the decrease in water permeability on the basis of dehydration or shrinkage of the plasmalemma of the exosmotic cell part which is brought into contact from water to a solution of low water potential. According to their view, the inhibition of water permeability only at the exosmotic cell part will be responsible for the polarity in water permeability or difference between endosmotic and exosmotic water permeabilities. This inhibition of water permeability depends on the external osmotic concentration.

The water permeability, however, is modified also by sap concentration. When the sap is diluted K' becomes larger and when the sap is concentrated K' becomes smaller. In order to establish the osmotic equilibrium, it is expected that in the former case the protoplasm swells and that in the latter case it shrinks. The change in water permeability may therefore be related to the change in hydration of the protoplasm especially of the membranes (tonoplast or plasmalemma or both).

At the very beginning of the transcellular osmosis more water escapes from the protoplasm to the outside than is supplied from the vacuole, since the driving force should be produced at first across the plasmalemma. Consequently, the protoplasm on the exosmosis side contracts or dehydrates more or less. This initial loss of water will decrease the turgor, inducing the osmotic drive on the endosmosis side. The inflow of water will cause the swelling of protoplasm on the endosmosis side, because the osmotic drive should be localized, also in this case, only across the plasmalemma at the first moment. In view of the fact revealed by us that K' is lowered when the sap concentration is made higher, and that it is heightened when the sap concentration is made lower (Figs. 8 and 9), it is quite possible that hydration and dehydration of the protoplasm will increase and decrease water permeability. Thus, endosmotic permeability becomes higher than exosmotic permeability as far as water permeability is measured by an osmotic method. From this standpoint it is important to decide whether or not water permeability is polar when it is measured by diffusion method.

The fact that water permeabilities obtained in many plant cells by means of plasmolysis (cf. Kamiya and Tazawa 1956; Stadelmann 1963) are much lower than the water permeability of *Nitella* obtained by transcellular osmosis may in part be due to the difference in conditions of the experiment to which the living cell is exposed. In plasmolysis the cytoplasm including the membranes are supposed to be dehydrated significantly.

V. Acknowledgment

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