IONIC RELATIONS OF CELLS OF *CHARA AUSTRALIS*

I. ION EXCHANGE IN THE CELL WALL

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[*Manuscript received April* 23, 1959]

Summary

Measurements of ion exchange were made between isolated cell walls of *Chara australis* and an external solution. Comparison between intact cells and cell walls showed that nearly all the easily exchangeable cations are located in the cell wall. The wall is shown to consist of "water free space" (W.F.S.) and "Donnan free space" (D.F.S.); the concentration of indiffusible anions in the D.F.S. is about 0·6 equiv/l. This finding is contrary to past suggestions that the D.F.S. is in the cytoplasm of plant cells.

The time course of cation exchange in the wall, measured with the aid of the radioactive isotopes ^{22}Na and ^{45}Ca, is shown to be complex. The cation exchange could be analysed into fractions with characteristic half-times ranging from about 100 sec for the "fast" fraction of sodium exchanging with ^{22}Na in the wall to many minutes for the "slower" fractions.

The fast fraction of both sodium and calcium exchange is shown to be diffusion-limited by a stationary film of the order of 100 μ thick outside the wall. It is suggested that the slowing of the remainder of the exchange is due to a combination of steric hindrance in micropores <100 Å in diameter in the wall, together with an electrostatic retardation in the electric double layers of the D.F.S.

I. INTRODUCTION

Recent studies of the ion-exchange processes in cells of *Nitellopsis* (MacRobbie and Dainty 1958) have revealed three processes separable on the basis of characteristic exchange half-time. These were (with respective half-times for K^+ exchange):

(1) Exchange with "free-space" ions ($\sim$1 sec).
(2) Exchange with "non-free-space" ions ($\sim$1 hr), said to be cytoplasmic.
(3) Exchange with vacuolar ions ($\sim 10^3$ hr).

The present study is an attempt to characterize in detail the free-space exchange in internodal cells of the alga *Chara australis* R. Br. var. *nobilis*.

Several papers have lately stressed the probability that the "Donnan free space" (D.F.S.) (Briggs, Hope, and Pitman 1958) is located in the cytoplasm with a rather larger "water free space" (W.F.S.) contributed by the cell-wall spaces and intercellular spaces (see Briggs 1957; Briggs and Robertson 1957). The possible contribution of the cell wall to the initial cation exchange, which is now widely regarded as adjustment of a Donnan system (Briggs, Hope, and Pitman 1958; Middleton and Russell 1958) has not been explored, although Sutcliffe (1957) states

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that the *cellulosic* components of the cell contributed very little to the initial ion exchange in his experiments with disks of red beet.

Earlier experiments (Hope and Stevens 1952; Hope 1953), sometimes quoted as evidence for including the cell cytoplasm in the free space, have been rightly criticized (Briggs 1957) as being inadequate. Further, the considerations of Walker (1957), whose experiments with *Nitella* point to the existence of a plasmalemma at the surface of the cell which hinders ion exchange in the cytoplasm, suggest that the situation should be reconsidered. The present material was chosen because of the

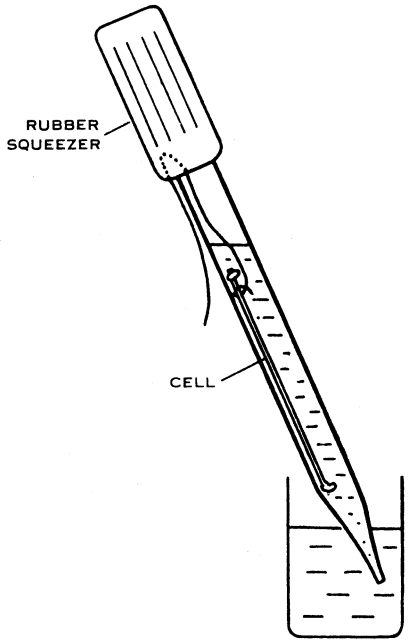

Fig. 1.—Method used to elute radioactivity from *Chara australis* cells and walls. A length of cotton was loosely tied around the cell and anchored between the rubber squeezer and glass tube.

possibility that various components of the cell could be studied separately. This first part of the series discusses the exchangeable Na^+ and Ca^{2+}, the iodide free space (I.F.S.) and mannitol free space (M.F.S.) of the cell wall, and the kinetics of the movement of these ions and mannitol between the cell wall and external solution.

II. Experimental Methods

(*a*) *Material*

Strands of *Chara australis* were collected from ponds and subcultured in a polythene-lined concrete tank containing tap water with a pond-mud base. Several weeks before they were needed for experiment individual strands were removed to a glass aquarium and placed in an artificial pond water (A.P.W.) of the following composition: NaCl, 1 m-equiv/l; $CaCl_2$, 0·5 m-equiv/l; KCl, 0·1 m-equiv/l. This corresponded roughly to the ion content of the field pond water.

Individual internodal cells together with the nodes, which contained a number of much smaller cells, were cut from a strand and placed in petri dishes in A.P.W. Cells in this condition survived many weeks with normal cytoplasmic streaming and appearance, and with the normal low rate of exchange of sap ions.

The cells were usually 4–7 cm long and 1–1·5 mm in diameter. The third, fourth, or fifth cell from the apex of the strand was chosen if possible.

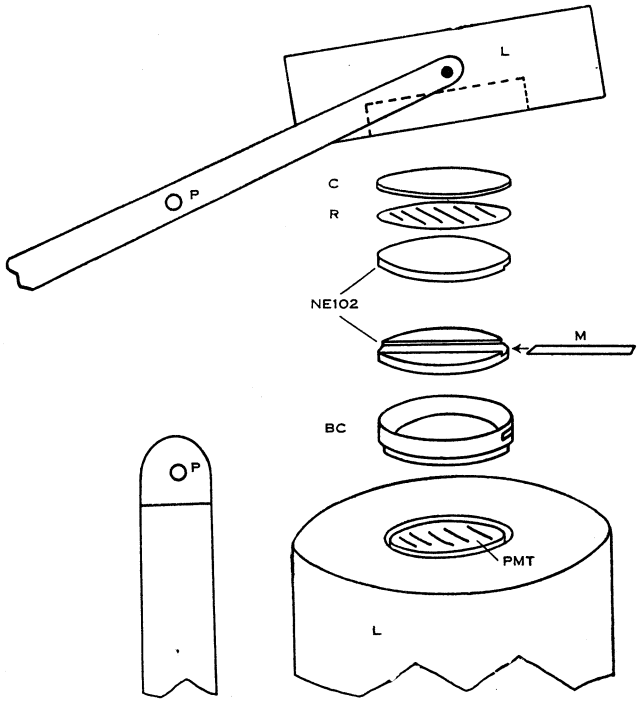

Fig. 2.—Expanded view of scintillation counter used to measure the radioactivity of 1 [^{14}C]-mannitol: *L*, lead castle, with lid pivoted at *P*; *PMT*, photomultiplier tube; *BC*, brass carrier for scintillating plastic; *NE*102, scintillating plastic slabs; *M*, mica slide carrying thin sample of mannitol; *R*, aluminium foil reflector; *C*, light-tight cap.

(*b*) *Experimental Procedures*

After periods of equilibration of up to 7 days in A.P.W., cells were placed in an A.P.W. of the same composition but labelled with the appropriate tracer ion, i.e. $^{22}Na^{+}$, $^{45}Ca^{2+}$, or $^{131}I^{-}$. The specific activity was usually 0·1–10 μc/ml. In experiments with internodal cells the nodes were kept free of radioactivity by means of "Perspex" stocks, the nodal few millimetres being bathed in inactive solution, frequently changed. After a certain time the cell was lightly blotted and placed in a drawn-out glass pipette and a rubber squeezer placed over one end (see Fig. 1). The radioactivity was then eluted into aliquots (10 ml for liquid counting; 2 ml, subsequently dried, for solid counting) of inactive A.P.W. by continually sucking up liquid around the cell and expelling it. This operation took a minimum time of

1 sec and was repeated several times in a given time interval. By this method the liquid medium was kept stirred, and, because the volume of the aliquot was large compared with that of the cell or wall, the transfer of radioactivity was not limited by increase in the external specific activity. The amounts of activity in the aliquots after various times were determined using conventional Geiger tubes and scalers, or the scintillation counter described below.

In experiments using ^{131}I as a tracer to determine anion free space,* the liquid aliquots were made alkaline and some solid sodium thiosulphate added to prevent oxidation of the iodide and subsequent loss to the atmosphere.

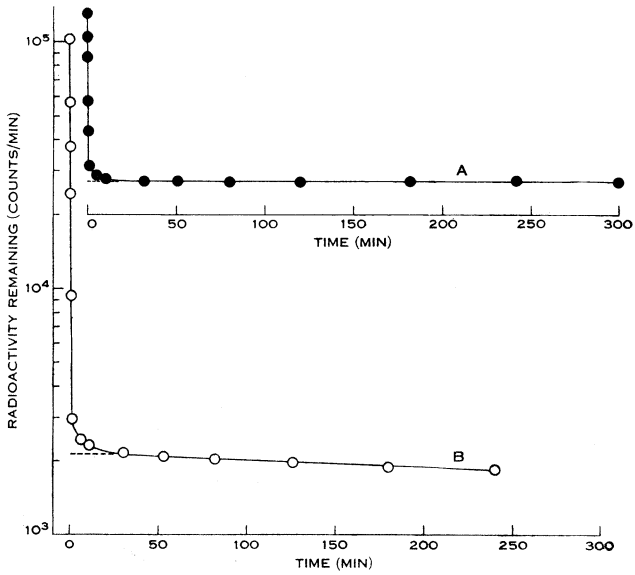

Fig. 3.—Radioactivity remaining in a *Chara australis* cell (*A*) and its isolated wall (*B*) plotted on a logarithmic scale against time of elution. Curves (*A*) and (*B*) have been separated by 10 min on the time axis to prevent overlapping.

Isolated cell walls were obtained by cutting the nodes from whole cells and scraping gently to remove the contents. Contamination by cytoplasmic interpenetration of the wall cannot be excluded. The time course of exchange in walls was measured in the same way as with the cells described above. When the total amount of an ion was to be measured, the activity of a cell wall was often counted directly after drying the wall on a planchette. The specific activity of the solution with which the wall had been in equilibrium was then determined by counting a sample of it, usually 20 μl, which was dried in the centre of the planchette together with a segment of inactive wall. Self-absorption and geometry complications were thus minimized.

*^{131}I was used instead of ^{36}Cl because the latter is available at such low specific activities that it could have been used only at very high external concentrations.

The M.F.S. of cells and walls was measured by equilibrating the free space with 5 mM mannitol solution which was made radioactive by adding 1 μc/ml of 1 [^{14}C]-mannitol. After careful blotting the activity was eluted into 2-ml aliquots of distilled water or A.P.W. The radioactivity was then determined in a scintillation counter of special design (Fig. 2), as follows: The liquid samples were dried on sheets of white mica 20 mm by 45 mm by 20 μ thick and placed between two slabs of scintillating plastic† each 3 mm thick and 50 mm in diameter. These were optically

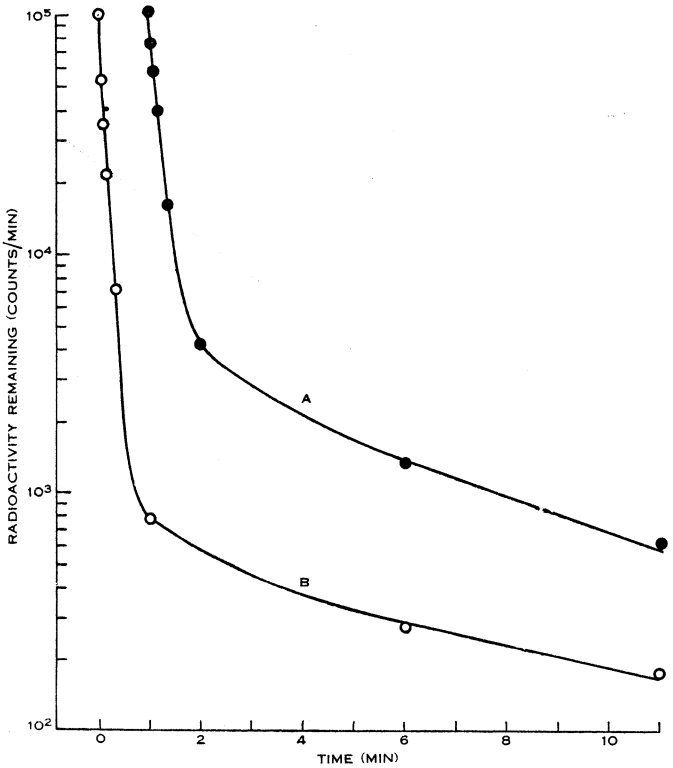

Fig. 4.—Points from Figure 3 replotted after subtraction of the extrapolates to zero time of the straight-line portions. The time scale covers only the initial 11 min. The time zeros have been separated by 1 min to prevent overlapping. *A*, cell; *B*, wall.

coupled to a type 6097 photomultiplier tube. A counting efficiency of approximately 22 per cent. for "infinitely thin" samples was obtained with the optimum setting of high tension voltage and discriminator bias, at which the background was usually 80 counts/min.

III. Results

(*a*) *Comparison of Exchange in Intact Cells and Walls*

The rate of loss of radioactivity from a cell which had been soaked for 24 hr in Na* (i.e. ^{22}Na) A.P.W. is shown in Figure 3, *A*. The activity remaining in the

†NE102, Nuclear Enterprises.

cell at various times is plotted on a logarithmic scale. The initial fast exchange was followed by a much slower loss of activity. The cell wall was then isolated and treated in the following way:

(1) Soaked for a period of 2 days in inactive A.P.W.

(2) Soaked for 2 days in Na* A.P.W.

(3) Blotted, and the activity eluted into A.P.W. as before.

Figure 3, *B*, shows the time course of the release of the exchangeable sodium in the wall. The difference between *A* and *B* (Fig. 3) (25,000 counts/min), after the

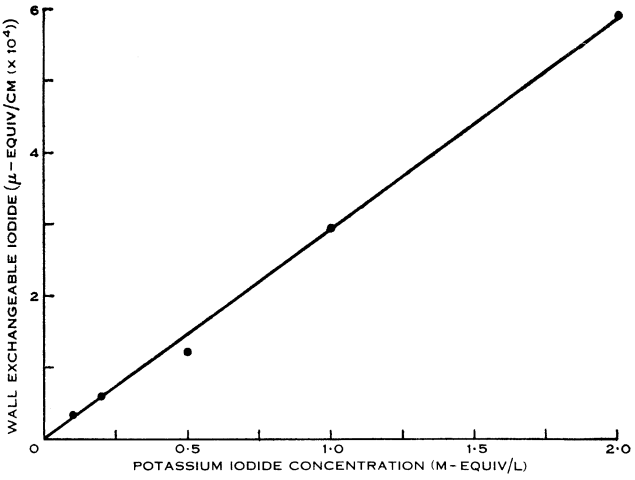

Fig. 5.—Exchangeable iodide in a *Chara australis* cell wall (in μ-equiv/cm ($\times 10^4$)) plotted against concentration (in m-equiv/l) of potassium iodide in the external solution.

initial fast exchange, was in the vacuole of the intact cell, as shown by counting a sample of extracted sap. If the extrapolates of the straight-line portions are subtracted from the respective total activities, the "quickly exchangeable sodium" in intact cell and wall is as shown in Figure 4, *A* and *B*. The amounts of sodium are very similar (104,000 counts/min in the cell and 102,000 counts/min in the wall) as are the rates of the exchange ($t_{\frac{1}{2}} = 6 \cdot 3$ sec for the cell and $5 \cdot 6$ sec for the wall). The amount of quickly exchangeable sodium was in each case $0 \cdot 03$ μ-equiv. The volume of the cell was 75 μl.

Similar results were obtained using Ca* (i.e. ^{45}Ca) A.P.W.—the intact cell and wall contained similar amounts of fairly quickly exchangeable calcium. However, the amount was greatly in excess of the exchangeable sodium, and the exchange rate much slower.

Though it is probable that there are some quickly exchangeable ions further in than the cell wall, it is clear that the majority are located in the latter and that the wall warrants a separate study.

(*b*) *Anion Exchange in the Cell Wall*

Walls were cut into 1-cm segments and one segment from each wall put into solutions of potassium iodide for 2 days. They were then transferred for 2 hr into the corresponding solution labelled with ^{131}I. The wall segments were blotted in the standard way and the activity eluted into 10 ml and counted in a liquid counter. In Figure 5 the amount of exchangeable iodide is plotted as a function of concentration.

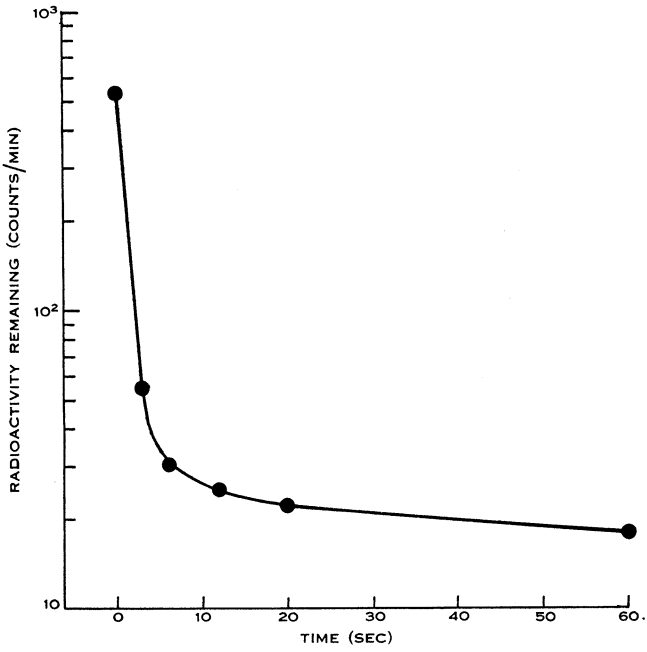

Fig. 6.—Time course of loss of radioactive iodide (in counts/min, log scale) from a *Chara australis* cell wall. The half-time for iodide exchange was 0·7 sec, and the I.F.S. 1·1 μl.

Since the amount of exchangeable iodide in equilibrium with various concentrations of anion is directly proportional to that concentration, we are dealing here with exchange with a W.F.S. (see Briggs, Hope, and Pitman 1958) or "outer space" (Epstein 1955). The mean volume of the I.F.S. in this experiment was 0·29 μl per cm length of wall, or about 0·48 μl per μl wall water.

The rate of equilibration of the wall iodide with external potassium iodide (10 m-equiv/l) is very rapid, as shown in Figure 6. A small quantity of iodide remains after 1 min but most of it leaves the wall with a half-time of about 0·7 sec. The I.F.S. was 1·1 μl (0·33 μl per μl wall water).

The fact that the I.F.S. averages about 46 per cent. of the wall water (Table 1) suggests that at least part of the wall contains a Donnan phase with a high concentration of indiffusible anions. The disposition of the I.F.S. in relation to the Donnan phase of the cell wall is discussed later.

Table 1 summarizes the data on anion exchange and I.F.S. of walls. The I.F.S. and M.F.S. have been expressed where possible in terms of μl per μl wall water by assuming a mean water content in blotted walls of 0·75 μl/mg.

TABLE 1

IODIDE FREE SPACE (I.F.S.) AND MANNITOL FREE SPACE (M.F.S.) AND HALF-TIMES FOR DIFFUSION EQUILIBRIUM OF IODIDE AND MANNITOL IN ISOLATED CELL WALLS OF CHARA AUSTRALIS

Expt. No.	I.F.S. (μl)	I.F.S. (μl per μl wall water)	M.F.S. (μl)	M.F.S. (μl per μl wall water)	$t_{\frac{1}{2}}$ (sec)	Blotted Weight (mg)	Length (cm)
1	2·2	0·45			1·2	6·5	6·4
			5·5	1·12	3·5		
2	1·35	0·44	—	—	1·0	4·1	5·3
3	1·1	0·49	—	—	0·7	3·0	5·0
4	1·45	(0·48)	—	—	—	—	5·0
5	—	—	3·6	1·04	—	4·6	5·1
6	—	—	3·2	0·85	4	5·0	6·0
7	—	—	2·6	(0·7)	3	—	6·5
8	—	—	3·0		2·5	—	—
Means		0·46		0·93			

(c) *The Mannitol Free Space of Cell Walls*

The release of labelled mannitol from walls into distilled water took place as shown in Figure 7 (a typical experiment). As with iodide, some label remained after 5 min but most of the mannitol diffused from the wall with a half-time of 3 sec.

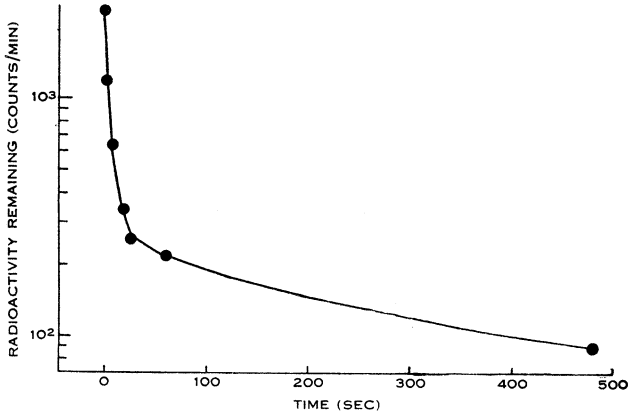

Fig. 7.—Time course of loss of radioactive mannitol (counts remaining/min, log scale), from a *Chara australis* cell wall. The half-time for mannitol diffusion was 3 sec, and the M.F.S. 5·5 μl.

The M.F.S., which is taken to correspond to the whole volume of the wall water penetrated by a molecule of this size, including the D.F.S., was 5·5 μl in this experiment. Table 1 also contains a summary of the M.F.S. of some walls, together with the half-times of equilibration.

(*d*) *Cation Exchange in the Cell Wall*

In all the following experiments cell walls were pretreated for several days in frequently renewed NaCl or $CaCl_2$ solutions to try to saturate the native anionic groups with either sodium or calcium. Then followed an equal period in labelled solution and elution into aliquots of inactive solution as described above. In the following section, the notation "Na*/1 Na" etc. means an experiment in which Na* was exchanged for Na^+ of concentration 1 m-equiv/l in the external solution.

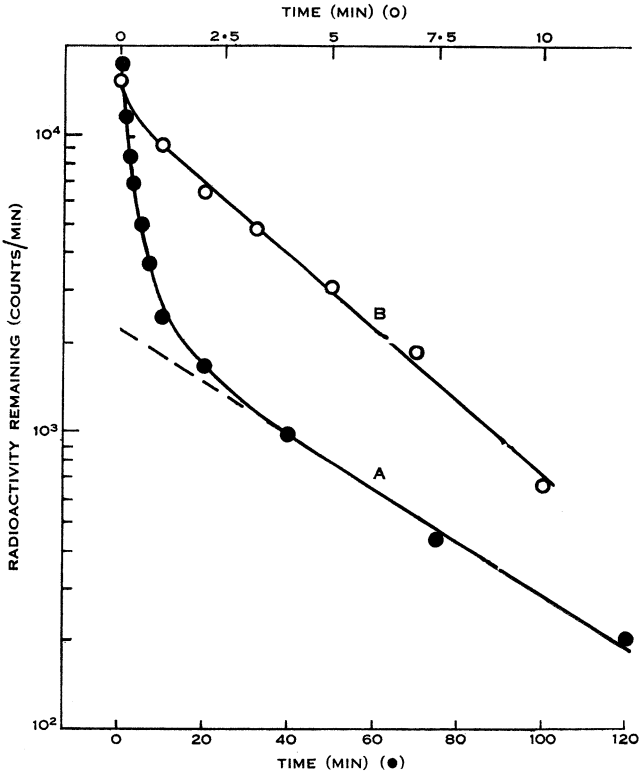

Fig. 8.—Time course of sodium exchange in a *Chara australis* wall equilibrated with sodium (counts remaining/min, log scale). *A*, total activity; *B*, fast exchange—points got by subtracting the extrapolate of the straight-line portion of *A* from the total activity at the respective times. The external concentration was 1 m-equiv/l.

(i) *Sodium Exchange.*—Figure 8 shows the results of an Na*/1 Na experiment. The total exchangeable cation was 0·225 μ-equiv. in a wall segment 2 cm long, or approximately 0·19 μ-equiv. per μl of wall water. The curve of log (radioactivity remaining) *v.* time has been divided into two parts by extrapolation of the tail of the curve (Fig. 8, *A*) to the *Y*-axis and subtraction of this extrapolate from the total. A very small fraction of the activity (10^{-3} μ-equiv.) remained in the wall after 120 min. The removal of this is not shown on the figure.

An appreciable fraction of the total activity, i.e. 0·028 μ-equiv. or 12 per cent. exchanged from the wall with a half-time of 34 min. The activity, after subtraction

of the "slow" component, is plotted, on a log basis, against time (Fig. 8, *B*). The resulting graph is nearly linear with time, indicating a single exchange rate. An amount of 0·195 μ-equiv., or 87 per cent. of the total, exchanged with a half-time of 144 sec. While we do not hold the view that two (or more) discrete "compartments" are necessarily involved in sodium exchange in the cell wall, the above procedure does enable characteristic half-times to be ascribed particularly to the fastest exchange, which depend only slightly on the way in which the total curve is divided

TABLE 2

MEAN AMOUNTS OF EXCHANGEABLE SODIUM IN ISOLATED CHARA AUSTRALIS CELL WALLS, AND MEAN HALF-TIMES OF ISOTOPIC EXCHANGE

Expt.	Total Exchangeable Sodium (μ-equiv/cm)	Fast Fraction		Other Fractions		No. of Observations
		Per Cent. of Total Exchanged	$t_{\frac{1}{2}}$ (sec)	Per Cent. of Total Exchanged	$t_{\frac{1}{2}}$ (min)	
Na*/1 Na	0·12±0·01	75±10	116±13	24±9 1	25±9 (500)	4
Na*/2 Na	0·12	70	(170)	26 4	40 (270)	1
Na*/5 Na	0·13±0·01	96±3	20±4	3±3 1	(3) (130)	3

up. The conclusion from this experiment is that while some (usually the greater part) of the sodium is exchangeable with a characteristic half-time of the order of 100 sec for 1 m-equiv/l Na^+, the remainder is much slower to exchange, surprisingly so in view of the fact that the walls are only 16 μ thick on the average.

Table 2 summarizes the half-times for Na*/Na exchange, at various concentrations. In some experiments, three segments, 2 cm long, of the same wall were put into different concentrations of inactive solution; in others, separate walls were used. The half-times are inversely proportional to the concentrations within the accuracy of the experiments. The values in parenthesis are very approximate, due to the small amounts of cation being measured, or doubtful because of some other experimental error.

(ii) *Calcium Exchange.*—Table 3 contains the results of Ca*/Ca experiments and of one Ca*/Na experiment. The isotopic exchange of calcium is somewhat slower than that of sodium, for the same concentration of the inactive (eluting) medium. The results of a typical experiment are plotted in Figure 9. The characteristic half-times of the fast fractions, which are almost unambiguous with regard to the splitting up of the total curve, are inversely proportional to the external calcium concentration.

A further feature of both sets of results is that the amount of cation in the fastest exchanging fraction tends to increase as the concentration of eluting solution is increased.

IV. Discussion

The evidence given above, on the nature of the "free space" of *Chara* cell walls, can be summarized and discussed as follows:

Mannitol probably diffuses into all the wall water since the mean M.F.S. is 93 per cent. of the calculated wall water (Table 1). The I.F.S., 46 per cent. of the wall water, is less than the M.F.S. This immediately suggests some exclusion of mobile anions from part of the wall by fixed negative charges, i.e. by a Donnan

Table 3

Mean amounts of exchangeable calcium in isolated Chara australis cell walls and mean half-times for isotopic exchange

Expt.	Total Exchangeable Calcium (μ-equiv/cm)	Fast Fraction		Other Fractions		No. of Observations
		Per Cent. of Total Exchanged	$t_{\frac{1}{2}}$ (sec)	Per Cent. of Total Exchanged	$t_{\frac{1}{2}}$ (min)	
Ca*/0·5 Ca	0·14	66	1110	34	345	2
Ca*/1 Ca	0·20±0·01	52±8	321±16	39±6 10±2	54±13 (800)	4
Ca*/2 Ca	0·17	73	180	24 3	25 (700)	1
Ca*/5 Ca	0·20±0·01	87±3	84±16	8±3 5±1	22±4 (550)	4
Ca*/10 Ca	0·20	93	22	3 4	9 (270)	1
Ca*/1 Na	(0·2)	26	18000	74	15 days	1

system. However, the exclusion is not as great as would be expected if the complete wall were a homogeneous Donnan phase; for, from the total exchangeable cation, the average concentration of the fixed negative charge is about 0·3 μ-equiv. per μl of wall water and this would result in a mobile anion concentration of about 4×10^{-6} μ-equiv. per μl, whereas the measured mobile anion concentration is about 10^{-3} μ-equiv. per μl for an outside concentration of 1 m-equiv/l. This must mean that the wall is not a homogeneous Donnan phase but consists of a W.F.S. and a D.F.S., i.e. it must contain regions (wide pores for example, see below) which are unoccupied or only partly occupied by the electric double layer composed of the indiffusible negative ions and counterions. The suggestion that the I.F.S. exerts little Donnan effect is confirmed by the results given in Figure 5 where the exchangeable wall iodide increased linearly with external iodide concentration. This is characteristic of a W.F.S. (cf. Briggs, Hope, and Pitman 1958).

The wall is represented schematically in Figure 10. It is possible that the I.F.S. is on the surface of the wall, i.e. consists of a film of water which is not removed by our blotting technique. This is regarded as unlikely for the film would have to be some 5 μ thick and the walls were blotted so firmly that such a film thickness could not have remained; further, the results were too consistent to be due to such an effect. From the calculated widths of the electric double layer (treated as a Gouy-Chapman layer) for the concentrations in these experiments it can be shown that the width of the macropores (W.F.S.) must be considerably greater than 100 Å to allow the presence of mobile anions. However, the wall need not contain such large-diameter pores if, in an alternative picture, one were to envisage a system of pores,

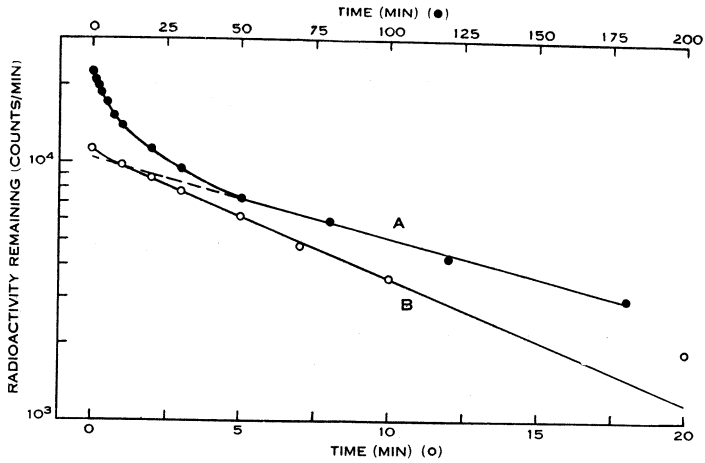

Fig. 9.—Time course of calcium exchange from a *Chara australis* wall equilibrated with calcium, plotted on a log scale. *A*, total activity; *B*, fast fraction, calculated as for Figure 8. The external concentration was 1 m-equiv/l.

all of much the same diameter, but having various amounts of fixed negative charge per unit area of pore "wall". We will usually assume, for the purposes of this discussion, a macro- and micropore system as illustrated in Figure 10 and as suggested by Northcote (1958).

Since the wall calcium can exchange completely for sodium, and from other evidence that the ion distribution obeys the Donnan distribution equations (Dainty, Hope, and Denby, unpublished data 1959), it is supposed that the forces between the cations and the exchange sites are purely electrostatic and that there is no chemical selectivity other than that due to electric charge. No chelation or other chemical bonds are envisaged.

From the mean fresh weight/cm (0·8 mg) and the data in Tables 1, 2, and 3 it can be calculated that the average amount of exchangeable sodium was 0·19 μ-equiv. per μl cell wall water, in walls in which an attempt was made to replace all the calcium by sodium. In walls in which all the counterions were made Ca^{2+}, the average amount of exchangeable calcium was 0·32 μ-equiv/μl. This difference is probably due to incomplete replacement of calcium by sodium or to less complete

ionization of the indiffusible anions when Na^+ is the counterion (Briggs, Hope, and Pitman 1958; Dainty, Hope, and Denby, unpublished data 1959). If the larger value is taken as the better estimate, the average concentration of indiffusible anions in the wall is $0 \cdot 32$ μ-equiv. per μl of wall water. However, 46 per cent. of the total wall water is W.F.S. and therefore the true concentration of the fixed negative charge in the D.F.S. is $0 \cdot 32/0 \cdot 54 = 0 \cdot 6$ μ-equiv/μl. This value is very similar to that obtained by Briggs, Hope, and Pitman (1958) for beet storage tissue. These authors were unable to specify the location of the D.F.S. in the tissue, though they suggested

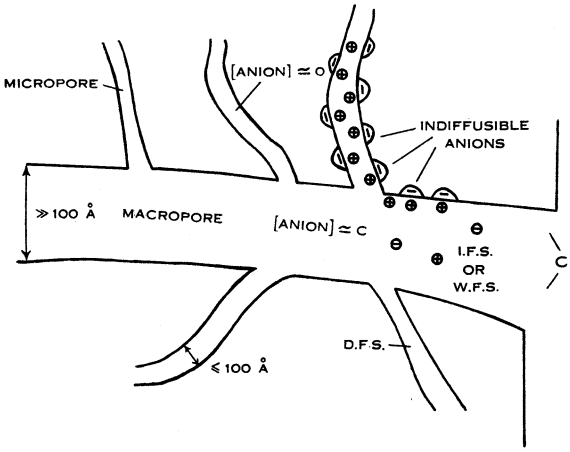

Fig. 10.—Schematic diagram of the macropore–micropore system thought to explain qualitatively the experimental results. *C*, external concentration.

the cytoplasm. In the present experiments it is quite certain that the great majority of the quickly exchangeable cations (and therefore the D.F.S.) is in the cell wall* (Figs. 3 and 4).

It is clear from Figures 3–9 that the time course of cation exchange is complex. This complexity and the existence of an appreciable W.F.S. can be explained qualitatively by considering the wall as a system of macropores ($\gg$100 Å in diameter) with a system of micropores ($\leqslant$100 Å in diameter) leading into them, or by considering it as a micropore system with pore walls of widely differing charge densities or as a combination of both. Figure 10 illustrates the suggested macro- and micropore system. We can roughly identify the macropores (or, with alternative picture, the uncharged pores) with the W.F.S. and the micropores (or, alternatively, the highly charged pores) with the D.F.S. One would expect parts of the micropore (highly charged pore) system to be more difficult of access than other parts and, because of the greater hydrated size of the Ca^{2+} ion and its greater charge, calcium would have greater difficulty than sodium in getting into these parts: thus a greater fraction of the exchangeable calcium would be rate-controlled by diffusion and exchange processes in the cell wall than would be the case for sodium. This is borne out by the

*Recent experiments by Pitman (private communication) have shown that in beet tissue, too, the D.F.S. is mostly in the cell wall.

experimental results, which show a more complex exchange curve for calcium than for sodium. The "fast" fraction of the exchange curves is explained later as due to those fairly accessible counterions which can diffuse, in exchange for similar ions, to the surface of the cell wall faster than they can be removed to the stirred part of the external solution, by diffusion in a "stationary" film of solution. The "slowly" exchangeable ions must therefore have considerable difficulty in diffusing through the micropore (or highly charged pore) system. This can partly be explained by steric hindrance to diffusion in very narrow pores, but there also seems to be an electrostatic effect. The cations in the electric double layer due to the negatively charged walls have a smaller potential energy than those outside the double layers (this effect will be twice as large for calcium as for sodium). The activation energy for diffusion of a cation from inside to outside a double layer will therefore be greater than in free solution, i.e. the diffusion coefficient will be lower. This extra activation energy depends on the electric potential in the double layer. Our results show some experimental support for this idea for, as the external concentration is increased, the mean electric potential in the double layer is decreased and more counterions should be enabled to diffuse readily. In Tables 2 and 3 it can be seen that the percentage of total exchangeable cation in the fast fraction increases with increase in external concentration. For sodium of 1 m-equiv/l, 75 per cent. exchanges quickly, and for sodium of 5 m-equiv/l, 96 per cent. Overbeek (1956) mentions a retardation of counterions in a Donnan phase.

A further possibility, which is also difficult to discuss quantitatively, is that some micropores are narrow enough to cause the filing effect discussed by Harris (1956), the counterions having to wait for a series of favourable collisions before moving along the pore. It is apparent that, under these conditions, a counterion will take longer to diffuse a given distance than when in the bulk phase.

Considering now the fast fraction, i.e. the proportion (50–96 per cent. depending on concentration) exchanging with a single rate constant, we can reasonably make the assumption that the exchange is diffusion-limited (Kitchener 1957). Now ions have to diffuse through the cell wall and then through a stationary film* of external solution before reaching the stirred solution in which we can assume that the concentration of radioactive isotope is zero. The complete solution of this problem is quite complicated (Grossman and Adamson 1952), but results can be adequately discussed by considering the two limiting cases in which the loss of the radioactive isotope is rate-controlled by (1) diffusion in a stationary film of external solution, or (2) diffusion in the cell wall itself.

If film-control is operative, then the concentration C of radioactive isotope in the wall is given by:

$$C = C_0 \exp(-D^s t/d\delta k), \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

where C_0 is the concentration in the wall at time $t = 0$, D^s is the self-diffusion coefficient of the ion concerned in the *external* solution, d is the half-thickness of

*Such a film is better thought of as a boundary layer of laminar flow, i.e. a region where flow in a direction normal to the cell wall surface is either very small or absent. Across such a region mass flow must take place by diffusion (see Bircumshaw and Riddiford 1952).

the flattened wall (it is assumed that the wall is washed on both sides), δ is the thickness of the stationary film, and k is the ratio of the concentration of the ion in the wall to the concentration of the ion in the external solution. Clearly log C is a linear function of time (thus justifying, in this particular case, the log-linear plotting of the results) and the half-time is given by:

$$t_{\frac{1}{2}} = 0{\cdot}69\frac{d\delta k}{D^s}. \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

For the cations Na^+ and Ca^{2+}, k is inversely proportional to the external concentration, as the cell wall is a Donnan system with a rather high fixed negative charge. Thus with film-control, the half-time should be inversely proportional to the concentration.

Tables 2 and 3 show that this is so in the present experiments for the fast fractions, within the limits of experimental accuracy. Thus most of the cation exchange between the cell wall and the external solution is, under our experimental conditions, rate-controlled by diffusion in a stationary film of external solution. A further check on this conclusion can be obtained by calculating the value of δ, the stationary film thickness, from the experimental data. Taking an average value for d, the cell wall half-thickness, of 16 μ, the average sodium concentration in the cell wall as 130 m-equiv/l of cell wall and the calcium concentration as 217 m-equiv/l of cell wall, the values of δ calculated from equation (2) are 90 μ from the sodium results and 106 μ from the calcium results. The order of magnitude of these results is reasonable for the stirring conditions used (Bircumshaw and Riddiford 1952; Tetenbaum and Gregor 1954) and the agreement between the two values of the stationary film thickness is strong confirmation of the film-control hypothesis. A further deduction can be made; if the sodium exchange is film-controlled, the half-time for exchange of sodium between an untreated cell wall and its normal environment (A.P.W.) can be calculated from the results on "pure sodium" walls. This half-time works out to be 10 sec, in rough but adequate agreement with the experimental value of 5·6 sec (wall with rest of cell) or 6·3 sec (wall isolated)—see Section III(a). The exchange of calcium between the untreated cell wall and A.P.W. proceeds, of course, at the same rate as between calcium-treated cell wall and pure 0·5 m-equiv/l $CaCl_2$ solution, because Ca^{2+} is the chief counterion in the D.F.S. of the untreated wall. Thus in the normal cell wall in A.P.W., calcium exchanges about 25 times more slowly than sodium.

The average half-time for exchange of iodide between A.P.W. and the cell wall was 1·0 sec and that for loss of mannitol was 3·3 sec. While film-control is probably operative, equation (1) does not apply to the diffusion of iodide and mannitol, since the capacity of the laminar layers is comparable with that of the I.F.S. or M.F.S. (The capacity of the laminar layers for cations has been neglected in comparison with that of the D.F.S.) Thus it is not possible to deduce any quantitative information about iodide and mannitol exchange. However, from Figures 6 and 7 it can be seen that not all the iodide and mannitol leaves the wall with a single rate constant. The small amounts slow to exchange may be hindered in the micropores (mannitol) while the same may apply to iodide provided it penetrates some of the larger micropores.

To summarize, the results of this study thus indicate that the apparent free space of *Chara australis* is located in the cell wall and comprises both a W.F.S. and a D.F.S.; the exchange of ions, particularly cations, between free space and external solutions of "physiological" concentrations is mainly rate-limited by diffusion in a stationary film of external solution of effective thickness about 100 Å. Presumably the free space in *Nitellopsis obtusa*, studied by MacRobbie and Dainty (1958), is also entirely confined to the cell wall; they identified in this species an apparent free space, a protoplasmic non-free-space, and the vacuole as three separate compartments, but were unable to say whether or not part of the protoplasm might be in the apparent free space compartment. A recent study by Diamond and Solomon (1959) on "intracellular potassium compartments in *Nitella axillaris*" is in general agreement with the results of MacRobbie and Dainty (1958) and, in addition, proves that the potassium free space is entirely in the cell wall, which constitutes a Donnan system. They have further proved that the cell wall, the protoplasm, and the vacuole are, kinetically, "in series".

In our experiments on *Chara australis*, it has so far not been possible to distinguish unambiguously a protoplasmic non-free space in the time course of exchange of sodium or calcium in an intact cell. This is partly because of the complex nature of the cation exchange in the cell wall, but studies with ^{42}K—when it becomes available in Australia—should resolve this difficulty. In the past, the cytoplasm has been included in the free space, notably by Briggs (1957), Briggs and Robertson (1957), and Briggs, Hope, and Pitman (1958); others have considered that a plasmalemma exists at the surface of the cytoplasm, and this controls ion transport into the cytoplasm (Levitt 1957; Walker 1957; MacRobbie and Dainty 1958). It would appear from the present study that the free space, including the D.F.S. of *Chara australis* is located entirely in the cell wall, which must contain substances, presumably "pectins", which can ionize to give rise to fixed (Donnan) anions (Dainty, Hope, and Denby, unpublished data 1959). The protoplasm also, of course, contains indiffusible anions, but it does not seem to contribute to the D.F.S.; the reason for this may lie in the control of ion transport into the protoplasm by a plasmalemma.

V. Acknowledgments

Thanks are due to Dr. R. N. Robertson, Mr. E. W. Hicks, and Dr. J. H. B. Christian, Division of Food Preservation and Transport, C.S.I.R.O., for their helpful criticisms of the manuscript and to Dr. J. R. Vickery, Chief, Division of Food Preservation and Transport, C.S.I.R.O., and Professor R. L. Crocker, Professor of Botany, University of Sydney, in whose Laboratories the work was carried out.

One of us (J.D.) gratefully acknowledges receipt of a Royal Society–Nuffield Commonwealth Bursary.

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