

## INTRACELLULAR POTASSIUM COMPARTMENTS IN NITELLA AXILLARIS\*

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### ABSTRACT

Three intracellular compartments for potassium exchange have been observed in intact cells of the giant-celled alga, *Nitella axillaris*. These compartments have been compared with the exchange properties of isolated subcellular structures. The smallest and fastest compartment (apparent half-time, 23 seconds) appears to involve passive absorption on the cell wall. The next largest (apparent half-time, 5 hours) may represent exchange with the cytoplasmic layer through the plasma membrane, the chloroplasts being in rapid equilibrium with the surrounding cytoplasm. The largest and slowest compartment (apparent half-time, 40 days) has been identified with the central vacuole. The vacuolar membrane and the plasma membrane have similar properties with respect to K permeability. Thus, the experimental data from the whole cell can be accounted for by a structural model of the compartments. Cyanide in concentrations up to  $10^{-3}$  M causes no net loss of K. The fastest compartment in *Nitella* and in higher plants is compared, and the ecological significance of the slow rate of potassium transport in *Nitella* is discussed.

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The alga, *Nitella axillaris*, found in fresh water ponds which are poor in ions, normally accumulates its major physiological ions against steep concentration gradients. Potassium concentration is maintained at an intracellular level of 0.13 M against an extracellular environment in which it is some 2000 times more dilute. In addition to the major ions, the Characeae family, which includes *Nitella*, can also concentrate a variety of other ions such as Li, Rb, Sr, Mn, Cs, and Ca, as shown by Collander (1). In 1938 Brooks (2) initiated the use of radioactive potassium in the study of the transport of this ion by *Nitella*. The low specific activities then available forced him and subsequent workers (Brooks (3, 4), Hoagland and Broyer (5), Holm-Jensen *et al.* (6), and Mullins (7)) to employ extracellular potassium concentrations of about 0.01 M, some 200 times more concentrated than the normal environment. The present experiments were initiated to study K transport in *Nitella* under more nearly physiological conditions, made possible by the higher spe-

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cific activities now available. In addition, the large size of individual *Nitella* cells (up to 8 cm. in length and 0.6 mm. in thickness) permitted comparison of the several intracellular ionic compartments with specific subcellular structures.

### Methods

#### 1. Growth and Handling of Cells.—

FIG. 1 is a schematic cross-section of a single *Nitella axillaris* cell. Cells were grown by soil culture, full size being reached within 3 weeks. The medium consisted of 40 ml. loam-leaf mold (sterilized in an autoclave) per liter distilled water. The cells

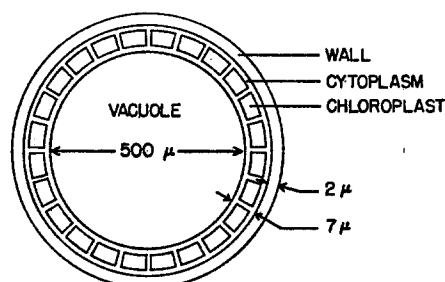


FIG. 1. Cross-section of *Nitella* cell. A single cell is a long, thin cylinder of nearly constant cross-section. On the outside is a cellulose wall  $2 \mu$  thick. Just inside the wall is the gelatinous cytoplasm containing a single layer of green chloroplasts. Each chloroplast is a flat body around  $7 \mu$  in diameter, and there are roughly two million of them in an average cell. Finally, the whole center of the cell—by far the largest part of its volume—is occupied by the vacuole, filled with a fluid called sap, which is constantly circulating up and down the cell due to protoplasmic streaming, or cyclosis.

were grown in non-sterile conditions under constant illumination in 3 to 10 liter covered jars. They satisfied the usual criteria of health, such as turgor, solid green color, and constant cyclosis. Cell diameter was measured to  $\pm 1$  per cent under a microscope with a calibrated micrometer lens, and length was measured with a millimeter ruler to  $\pm 2$  per cent. Cell area and volume were then computed by the formulae for a right circular cylinder, to  $\pm 3$  per cent and  $\pm 5$  per cent respectively. Before the start of an experiment, single internodal cells were cut free of their neighbors and incubated for a day in the absence of  $K^{42}$  under conditions otherwise identical to experimental conditions. Cells being studied were manipulated only with a forceps using the shreds of adjacent cells as "handles"; the cell body itself was never touched. At the end of an experiment each cell was examined under the microscope and occasionally discarded because cyclosis was sluggish or had stopped.

#### 2. Equipment.—

Experiments were carried out in cylindrical glass boats 10 cm. long and 3 cm. in diameter (see Solomon (8) for details). Boats rested in a water bath which was heated

electrically and thermostated at  $27.0 \pm 0.2^\circ\text{C}$ ., with constant illumination from overhead. Through an opening on top of the boat, closed by a ground glass stopper when not in use, cells could be inserted and samples removed. Each boat contained two or more *Nitella* cells in 30 ml. of solution, and was mounted on a shaker to effect continuous stirring. Since the long axis of the boat was set perpendicular to the direction of motion, mechanical injury to the cells was prevented.

### 3. Experimental Solution.—

The solution consisted of  $6 \times 10^{-5}$  M KCl,  $1 \times 10^{-4}$  M NaCl,  $1 \times 10^{-5}$  M CaCl<sub>2</sub>, and  $1 \times 10^{-5}$  M MgCl<sub>2</sub>, buffered at pH  $7.0 \pm 0.1$  with  $1 \times 10^{-4}$  M imidazole. When K<sup>42</sup> was added, stable K was removed, if necessary, to keep the K concentration at  $6 \times 10^{-5}$  M. Periodic sampling of the solution in control boats without cells showed that there was no leaching of K off the glass of the boat, within the accuracy of photometric measurement ( $\pm 3$  per cent). Evaporation did not concentrate the solution by more than 1 per cent during an experiment, as seen from measuring the initial and final volume of solution. Since cells have been kept alive in this standard solution for 6 months and experiments are never run longer than 4 days, there is a wide margin of safety.

### 4. Procedure with Whole Cells.—

The kinetics of potassium exchange between whole *Nitella* cells and their environment could be studied in cells previously incubated in K<sup>42</sup>, by observing the loss of K<sup>42</sup> from the cells into a non-radioactive solution (referred to as an "efflux" experiment). Samples of the medium were taken with 100  $\lambda$  Misco pipettes (replicability  $\pm 1.1$  per cent). Alternatively, cells were periodically removed from a radioactive solution, and their K<sup>42</sup> uptake was determined (referred to as an "influx" experiment). Such cells were lightly blotted, dipped for 2 or 3 seconds into a rinse of  $10^{-4}$  M imidazole buffer to remove clinging drops of experimental solution, then blotted again. Each cell was pushed down to the bottom of a 5 ml. volumetric flask containing 0.5 ml. of 1 N HNO<sub>3</sub>, and the salts were extracted by boiling the flask until the cell debris was colorless. This procedure extracted more than 99.9 per cent of the radioactivity from cells that had been incubated 1 day with K<sup>42</sup>. The flasks were then made up to volume, and centrifuged aliquots were analyzed for K<sup>42</sup> and total K.

### 5. Cell Fractionation.—

The isolated cell wall was prepared by cutting off both ends of a cell and pushing out the vacuole, cytoplasm, and chloroplasts. When examined under a microscope, the resulting wall was a collapsed cylinder without apparent color or structure. Influx experiments on the wall were carried out in the same manner as on the whole cell, except that the 2 to 3 second rinse was omitted. Since a wall could be blotted and pressed dry much more firmly than an intact cell, the rinse was no longer essential for removing surface water.

For experiments on the vacuole, one end of a cell was cut off, and a drop of pure vacuolar sap was allowed to run onto a glass slide. This drop was immediately picked up in a calibrated 5  $\lambda$  pipette (controls showed that drops of liquid of this order of magnitude could be deposited and quickly recovered with an error less than 2 per

cent). In order to rule out the possible contribution of loose chloroplasts to the radioactivity in the sap, the measured drop was examined under the microscope. If care was taken not to squeeze the cut cell, the sap was uncontaminated by chloroplasts. After dilution the drop could be analyzed for  $K^{42}$  and total K.

To obtain the cytoplasm and chloroplasts, cells were cut at one end and their vacuoles drained, then a bent glass rod was used to push the cytoplasm and virtually all the chloroplasts out of the cell wall into a dish. This procedure removes the major fraction of the vacuolar sap from the cytoplasm-chloroplast fraction.

For study of the  $K^{42}$  exchange of isolated chloroplasts, the chloroplasts from twenty cells were suspended in 2 ml. of cell sap (obtained by centrifuging a blotted cut plant). A drop of stock  $K^{42}$  was added, and the suspension was pipetted into a small glass tube with a ground glass stopper and shaken under the usual conditions in the water bath. At intervals 100  $\lambda$  samples of the suspension were centrifuged at 7800  $g$  to pack down the chloroplasts, and the supernatant from the sample was discarded. The centrifuge tube was rinsed with distilled water. The pellet, a thin layer on the bottom of the centrifuge tube, was not stirred to avoid loss of  $K^{42}$ , and the tube with its pellet was transferred to a counter for  $K^{42}$  assay. Since extruded chloroplasts can fix radioactive carbon in the light (Tolbert and Zill (9)) and appear structurally normal, this procedure may give an approximation to their *in vivo* K exchange. Further more, cell sap and cytoplasm are similar in ionic composition (Bennett and Rideal (10)); hence the chloroplasts are in an environment resembling their natural one.

#### 6. Analytical Methods.—

Gamma radiation from  $K^{42}$  was measured in a Wood well-type scintillation counter (Type SC-2L) and expressed as counts per minute per  $cm.^2$  of cell surface. Total K was measured on a modified Beckman flame spectrophotometer (Solomon and Caton (11)) and expressed as moles per  $cm.^3$  of cell volume. Since  $10^{-4}$  M imidazole was used regularly as a buffer in the experimental solution, all flame photometric standards and extracted samples were made up to contain  $10^{-4}$  M imidazole before analysis to cancel any error due to interference in the flame. All other ions significantly present in samples were found to have a negligible effect on the K signal at the concentrations and measurement settings employed, except for phosphate. In a few experiments  $7 \times 10^{-5}$  M phosphate was added to the experimental solution, and it proved necessary to multiply the K readings of these samples by 1.06. The standard deviation from replicate determinations on eight cells was  $\pm 11.8$  per cent for  $K^{42}$ /cell area, and  $\pm 11.1$  per cent for total K/cell volume.

### RESULTS AND DISCUSSION

#### 1. Steady-State Conditions.—

Considerable effort was spent in devising experimental conditions that would simulate the environment of a fresh water pond and keep the cell in a steady state with respect to intracellular K concentration. Jacques and Osterhout (12) had demonstrated that another species of *Nitella*, *N. flexilis*, lost K into distilled water but gained K from solutions whose KCl concentration was above  $10^{-4}$  M. Hence presumably plant and solution were in equilibrium at

some intermediate level. Consequently, cells were incubated in solutions with KCl concentrations in the range 0 to  $10^{-4}$  M, and aliquots of solution were periodically analyzed for K. It was found that the K concentration in the medium remained relatively constant if set initially at  $6 \times 10^{-5}$  M, while cells withdrew K from a more concentrated solution and lost K into a more dilute solution. Since the K content of two average cells is roughly equal to the K content of the medium in the boat, changes in external K concentration reflect accurately changes in cell K. Fig. 2 shows the time course of extracellular K concentration when two cells are placed in 30 ml. of this standard solution. It appears that a steady state with respect to K is maintained for 7 days. Only after this interval do the cells slowly begin to lose K, and 70 per cent of the K in *Nitella* remains even after 37 days.

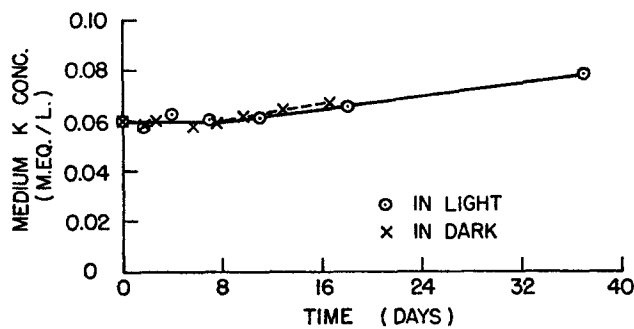


FIG. 2. Time course of changes in the K concentration of the medium. Each boat contained two cells in 30 ml. of the standard medium.

It is of interest that this equality of influx and efflux is dependent upon the extracellular rather than the intracellular [K]. Cells with intracellular [K] between  $7 \times 10^{-2}$  and  $17 \times 10^{-2}$  M remained in the steady state if the extracellular [K] was set at  $6 \times 10^{-5}$  M. In respect to this flexibility, *Nitella* differs from many organisms, as vertebrates, which regulate much more closely the concentrations of their ions.

Besides KCl, the standard solution contained NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, and buffer. Thus the major cations of pond water and of the cell sap are present. The requirements for the medium are not rigorous in that Ca and Mg can be omitted, phosphate or bicarbonate added, and Na increased sixfold without causing the cells to lose or gain K. Since *Nitella* is often found in stagnant, poorly aerated ponds, no gas was passed through the solution, and air was renewed only by diffusion through a small open gas vent (2 to 3 mm. in diameter, 55 mm. long) on top of each boat. In fact, when a continuous stream of air, pure O<sub>2</sub>, or O<sub>2</sub> with 0.5 per cent CO<sub>2</sub>, was run through a boat, the cells lost K.

Fig. 2 also shows that the K steady state is maintained for 7 days in darkness as well as in light. Energy is not directly required to maintain the K concentration gradient in *Nitella*, since the inside of the cell is negative to the outside by a resting potential sufficient to balance the concentration gradient (Hill and Osterhout (13); Blinks (14); Gaffey and Mullins (15)). However, this potential gradient presumably must be maintained by an active process; hence the retention of K by *Nitella* indirectly implies the consumption of energy. Since darkness fails to allow photosynthesis and the solution contains no organic compound that can be metabolized, the maintenance of the K steady state during a week of darkness implies that energy to maintain the resting potential can be derived from food reserves. In contrast, the marine alga, *Ulva lactuca*, has lost most of its K after 3 days without light (Scott and Hayward (16)).

## 2. Exchange of $K^{42}$ in Intact Cells.—

The evidence from fifty-six influx and thirty efflux experiments on whole cells indicated that  $K^{42}$  exchange involves three simultaneous processes. For example, Fig. 3, which represents a 5 minute efflux experiment on cells previously incubated 5 minutes in  $K^{42}$ , reveals an extremely rapid phase of exchange. When the period of incubation was extended to 22 hours and efflux was followed for 10 hours, as shown in Fig. 4, an additional medium speed process became apparent. Characteristically, the kinetics of efflux from this compartment should result in an exponential accumulation of radioactivity in the medium. The break in the curve in Fig. 4 at about  $5\frac{1}{2}$  hours shows that the curve is not exponential and suggests that a third intracellular compartment may be involved. This has been confirmed in influx experiments lasting more than a day which showed that the greatest part of the  $K^{42}$  was accumulated in a very slowly exchanging compartment. Thus, the three compartments were easily distinguished because of the large differences in their rates of  $K^{42}$  exchange. The apparent times required to attain half of the specific activity of the medium were 23 seconds, 5 hours,<sup>1</sup> and 40 days, and the fraction of cell K involved in each process (compartment "size") was, respectively, 0.1 per cent, 1.6 per cent, and over 90 per cent.

In a further exploration of these three processes, a large number of radioactive cells (as many as seven, previously incubated in  $K^{42}$  for 22 hours) were put into one boat, and K efflux was followed for a period of 48 hours. Fig. 5 is representative of the seven experiments carried out under these conditions.

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<sup>1</sup>The medium speed compartment, as will later be shown, exchanges both with the fast and the slow compartment. Within the first 24 hours its specific activity builds up to a quasi-steady-state value which is 27 per cent of that of the medium. The apparent half-time is the time taken to reach half of this quasi-steady-state value.

The specific activity of the medium reaches a peak at about 15 to 16 hours and then slowly falls. Two separate steps are involved.

*First Compartment.*—This compartment, which will be shown to represent the wall, exchanges very rapidly. In the first few minutes its specific activity

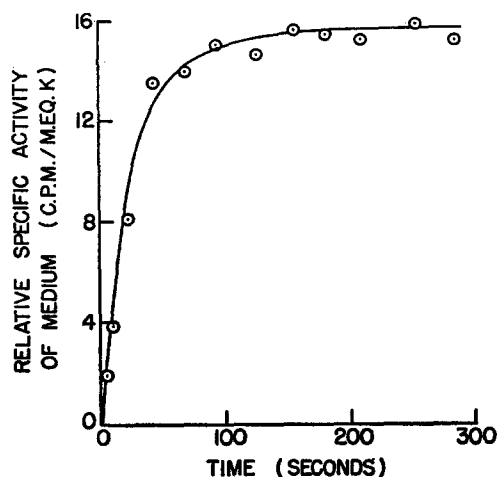


FIG. 3. Efflux of  $K^{42}$  into an initially non-radioactive solution from cells which had previously been incubated 5 minutes in  $K^{42}$ . The boat contained six cells in 30 ml. of the standard medium.

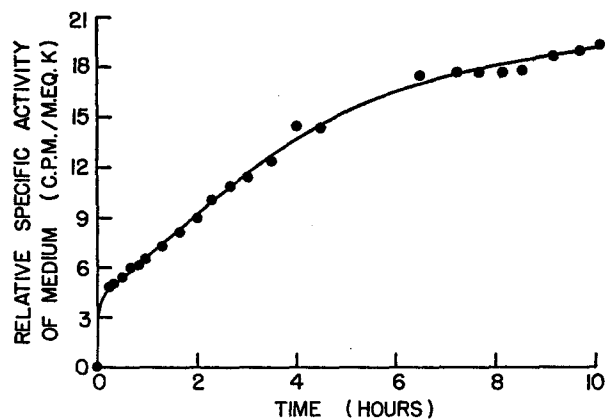


FIG. 4. Efflux of  $K^{42}$  into an initially non-radioactive solution from cells which had been incubated 22 hours in  $K^{42}$ . The boat contained three cells in 30 ml. of the standard medium.

has come to equality with that of the medium. After this time the first compartment remains in equilibrium with the medium and can no longer be distinguished from it.

*Second Compartment.*—In 22 hours of preincubation the specific activity of the second compartment has reached a high value. This compartment, which apparently comprises the chloroplast-cytoplasm layer, can lose radioactivity both into the vacuole and through the wall into the medium. At the close of the 22 hour preincubation its specific activity is higher than that of the vacuole, which fills very slowly. Upon transfer to a non-radioactive medium, the first compartment comes into equilibrium with the medium very quickly. Then the second compartment becomes the reservoir with the highest specific activity in the system. The continued supply of radioactivity to the medium from this reservoir serves to bring the specific activity of medium and of sec-

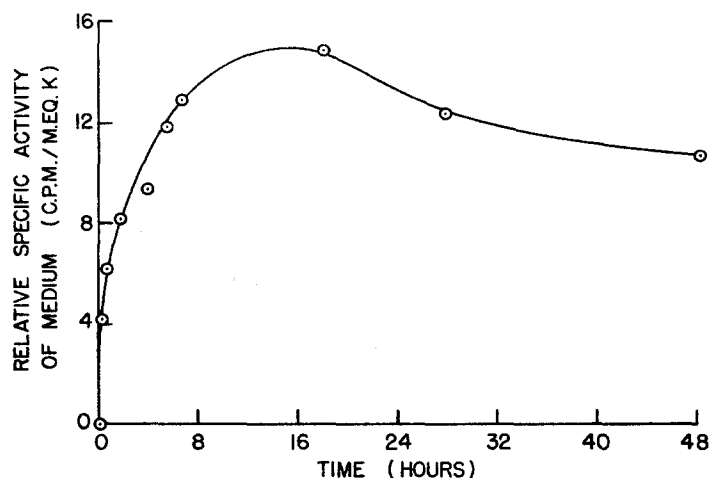


FIG. 5. Efflux of  $K^{42}$  into an initially non-radioactive solution from cells which had been incubated 22 hours in  $K^{42}$ . The boat contained six cells in 30 ml. of the standard medium.

ond compartment into equilibrium at about 15 to 16 hours. At this time the specific activity of medium, and of first and second compartments are all equal and above that of the vacuole. As a consequence, the vacuole begins to drain radioactivity from *all* the rest of the system, and thus causes the decrease in medium specific activity shown in Fig. 5.

The total system comprises (a) the medium, (b) the first compartment, (c) the second compartment, and (d) the vacuole. These four compartments, one extracellular and three intracellular, are all required in order to describe the kinetics of  $K^{42}$  distribution in *Nitella*. The hump in Fig. 5 is characteristic of a system in which there are two major intracellular compartments (in addition to the rapidly exchanging wall) and would not be observed if the intracellular radioactivity were contained only in the wall plus a single additional



compartment. In some experiments the medium has been prepared with an initial specific activity higher than the mean specific activity of the whole cell. Under these conditions too, the cell has lost radioactivity to the medium, confirming the presence of a compartment of high specific activity within the cell.

### 3. Identification of the Fastest Compartment.—

Influx experiments performed on the isolated cell wall showed that it exchanged  $K^{42}$  rapidly, with a half-time of less than 1 minute, which agrees well with the 23 second half-time previously obtained for the fastest compartment.

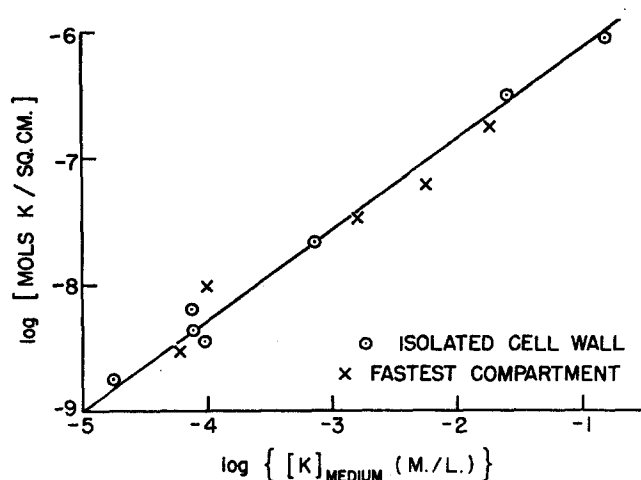


FIG. 6. Comparison of the amount of K in the wall and the fastest compartment, as external [K] is varied over a 10,000-fold concentration range. Each point is based upon six or more cells.

In equilibrium with a  $10^{-4}$  M KCl solution, the wall<sup>2</sup> contained  $7200 \pm 3300$  pmol K/cm.<sup>2</sup>. Under the same conditions the size of the fastest compartment was  $6500 \pm 3500$  pmol. K/cm.<sup>2</sup>; hence the two values agree within experimental error. Furthermore, this apparent identity was maintained when the K concentration in the solution was varied over a 10,000-fold range in fourteen experiments, each involving six or more cells. A straight line is obtained (as shown in Fig. 6) when the logarithm of the K content of the wall or in the fastest compartment is plotted against the logarithm of the external K concentration. This graph is characteristic of a Freundlich (or "classical") adsorption isotherm. It implies that the fastest uptake process involves passive adsorption; and that under normal circumstances (*i.e.*, in pond water or

<sup>2</sup> 1 pmol =  $10^{-12}$  mol. Errors are standard deviations.

the standard medium) few of the available binding sites are occupied by K. *Nitella* wall is largely cellulose (Amin (17); Hough *et al.* (18)), but among the other components present is a polymer of a uronic acid, probably D-glucuronic acid. If adsorption arises from directed binding of K by acidic groups, the amount of this polymer is sufficient to account for K adsorption under standard conditions but not in more concentrated solutions.<sup>3</sup>

#### 4. Identification of the Slowest Compartment.—

Since the vacuole contains almost all the cellular K, it seemed likely that it corresponded with the slowest compartment, which had been shown to contain more than 90 per cent of the exchangeable K. This question was therefore examined in seven experiments. The time course of K<sup>42</sup> uptake by

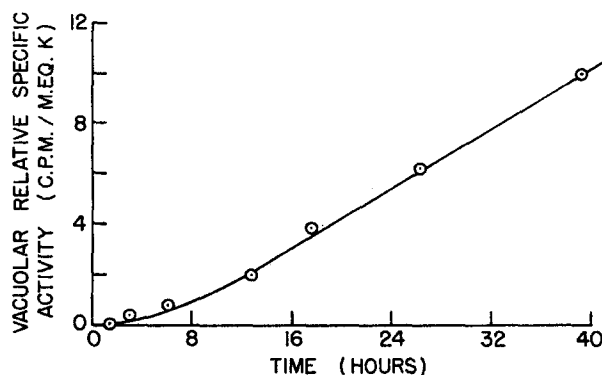


FIG. 7. K<sup>42</sup> relative specific activity in the vacuole, as determined by direct analysis of cell sap. Each point represents the average of samples from three vacuoles.

vacuolar sap under the standard conditions is presented in Fig. 7. After 12 hours, K<sup>42</sup> is entering the sap steadily at a rate corresponding to  $0.19 \pm 0.01$  pmols K/cm.<sup>2</sup>, sec.; at this time the specific activity of the vacuole is still so

<sup>3</sup> We are indebted to Dr. J. Dainty for pointing out the relevance of the following considerations. 7200 pmols of K/cm.<sup>2</sup> in a wall 2  $\mu$  thick corresponds to a fixed negative charge density of about 40 mM/liter in the wall. This must represent only a fraction of the total number of negative charge groups since the other ions in the medium, particularly Ca and Mg, are presumably also adsorbed. Furthermore the 23 second half-time is very long compared to the rate of KCl diffusion in solution. A layer of distilled water 2  $\mu$  thick placed in contact with a KCl solution would come to half diffusion equilibrium in about 0.5 millisecond. Thus the entrance of KCl into the fastest compartment is some 40,000 times slower than free diffusion. This discrepancy could be accounted for in terms of a 50  $\mu$  unstirred layer of solution at the outside of the cell wall.

low that efflux from the vacuole may be neglected. This rate of entrance of  $K^{42}$  into the separated vacuolar sap agrees well with the rate of entrance into the slowest compartment,  $0.22 \pm 0.04$  pmols/cm.<sup>2</sup>, sec., as obtained graphically from the time course of  $K^{42}$  uptake by whole cells after a day. It may be noted from Fig. 7 that the slope of vacuolar  $K^{42}$  uptake approaches zero at zero time. This behavior is characteristic of a compartment separated from the source of radioactivity (in this case, the medium) by another compartment which is initially free of radioactivity.

Brooks (3) and Mullins (7) also found that radioactive ions entered the vacuole more slowly than the rest of the cell; so slowly indeed, that Brooks (4) suggested that no activity at all reached the vacuole except erratically, by contamination from detached fragments of the cytoplasm-chloroplast layer. The present experiments do not support Brooks's suggestion, since the rate of entrance of  $K^{42}$  into the vacuole was regular and no chloroplasts were observed in the sap if cells were cut carefully. In *Nitellopsis obtusa*, MacRobbie and Dainty (19) have found that K enters the vacuole at rates comparable to the present rates for *Nitella*.

##### 5. Identification of the Medium Speed Compartment.—

Cells were prepared with practically all their radioactivity in the medium speed compartment by incubating them in  $K^{42}$  for 1 hour, then rinsing them for 5 minutes. The rinse washed  $K^{42}$  out of the fast wall compartment, while the short time of incubation prevented appreciable amounts of radioactivity from reaching the slowest compartment. On the basis of previous experiments, the K flux into the medium speed compartment should have totalled 1700 pmols K/cm.<sup>2</sup> in 1 hour. When the cytoplasm-chloroplast layer was isolated from these cells,  $K^{42}$  equivalent to 1200 pmols K/cm.<sup>2</sup> was found to have entered this layer within 1 hour. This total cannot be ascribed to contamination by sap as a result of incomplete drainage of the vacuole, since  $K^{42}$  corresponding to only 150 pmols K/cm.<sup>2</sup> had entered the vacuole at the end of 1 hour. Some cytoplasm sticks to the cell wall and may account for the missing 500 pmols/cm.<sup>2</sup> of K. With this combination of incubating and rinsing, the radioactivity should be concentrated in the medium speed compartment; the fact that so much of it is found in the cytoplasm-chloroplast layer suggests that this layer represents the medium speed compartment.

Since *Nitella* chloroplasts have differentially permeable membranes, it at first seemed surprising that data on the intact cell gave no indication of the chloroplasts constituting a compartment distinct from the cytoplasm. Hence four influx experiments were carried out on preparations of isolated chloroplasts, resuspended in cell sap. Table I shows that chloroplasts contain as much  $K^{42}$  after 6 minutes, when the earliest sample was taken, as after several hours or even a day. Such a finding is compatible with the data of Mercer

*et al.* (20), who reported on the basis of conductivity measurements that the exchange of  $K^{42}$  in the chloroplasts must be virtually complete within 3 minutes. If this conclusion holds *in vivo*,  $K^{42}$  that has crossed the external cytoplasmic membrane must be distributed within the chloroplasts almost as readily as through the rest of the cytoplasm. Hence the chloroplast-cytoplasm layer, though structurally inhomogeneous, appears as a single compartment.

TABLE I  
*Influx of  $K^{42}$  into Preparation of Isolated Chloroplasts*

Time	$K^{42}$ /chloroplast
min.	C.P.M.
6	780
21	450
32	660
53	700
75	560
206	840
1308	570

TABLE II  
*Cellular K Concentration after 4 Day Exposure to Cyanide*

[CN] in medium*	[K] in cell
mols/liter	mols/liter
0	0.11
$1 \times 10^{-6}$	0.11
$5 \times 10^{-5}$	0.11
$5 \times 10^{-4}$	0.11

\* All media contained  $5 \times 10^{-4}$  M Na; K, Ca, Mg, imidazole as in the standard medium.

#### 6. The Effect of Cyanide.—

Since cyanide is a potent inhibitor of salt accumulation in higher plants, it was of interest to determine whether it exerted a similar effect on K accumulation by *Nitella*. Hoagland *et al.* (21) showed indirectly that *Nitella* is permeable to cyanide. Table II shows that the K concentration of cells, immersed for 4 days in cyanide solution, is independent of cyanide concentration up to  $5 \times 10^{-4}$  M CN. In two additional experiments *Nitella* retained its original K concentration in the presence of  $10^{-3}$  M CN. The rate of inward movement of K across the external cytoplasmic membrane after 4 days of exposure to  $5 \times 10^{-5}$  M CN was 0.36 pmols/cm.<sup>2</sup>, sec. (as measured by  $K^{42}$ ),

which does not differ significantly from the control value for this series of  $0.30 \text{ pmols/cm}^2, \text{ sec}$ . The exposed cells showed vigorous cyclosis and the usual turgor.

In higher plants the CN sensitivity of salt uptake and of the increased respiration associated with salt uptake has led Lundegårdh (22–24) to propose that anions are transported into cells in chemical combination with the cytochromes. Cations then follow passively by electrostatic attraction. In *Nitella*, CN insensitivity makes it unlikely that such a direct link exists between CN-sensitive cytochromes and the potential difference which maintains the K concentration gradient. Furthermore, the K transport mechanism itself is presumably not directly linked with CN-sensitive cytochromes.

#### 7. Compartment Sizes and Fluxes.—

Since the apparent half-times for exchange of each of the three compartments differ by more than two orders of magnitude, the size and flux rates for each compartment can be computed graphically from a logarithmic plot of  $\text{K}^{42}$  concentration against time on the assumption that other compartments either have attained equilibrium or are exchanging at a negligible rate. A suitable choice of time interval makes it possible to neglect the effect of back fluxes. The error involved in making these approximations is many times less than the error of the experimental measurements in this study. Thus, the flux rate across the plasma membrane into the cytoplasm may be found from the rate of influx of  $\text{K}^{42}$  into cells between 10 minutes and 2 hours. During this period the vacuole acquires practically no  $\text{K}^{42}$ , while the wall has the specific activity of the solution, hence both make negligible contributions to the flux of  $\text{K}^{42}$  into the cell. The gain and loss of  $\text{K}^{42}$  by cells before 10 seconds and after a day are practically linear, and can be used to calculate the flux rates into the wall and into the vacuole, respectively. In the latter case, it is necessary to correct for the cytoplasm specific activity, which after a day reaches a quasi-steady-state level, 27 per cent of that of the medium. As previously discussed, the vacuolar flux rate was also measured directly, and found to agree with the rate calculated graphically. The amount of  $\text{K}^{42}$  in the wall compartment, measured indirectly as total cell  $\text{K}^{42}$  after 3 minutes of influx or as total  $\text{K}^{42}$  lost after 3 minutes of efflux, has also been shown to agree with measurements on the isolated wall (Fig. 6).

The combined data from direct and indirect analysis of each compartment fit the assumption that the four compartments are arranged in series, as depicted in Fig. 8. Table III gives the standard error of the mean for the fluxes. Comparable data have been obtained in *Nitellopsis obtusa* by MacRobbie and Dainty (19) who report values of 4 and  $0.25 \text{ pmols/cm}^2, \text{ sec}$ . for flux from wall to cytoplasm and cytoplasm to vacuole respectively.

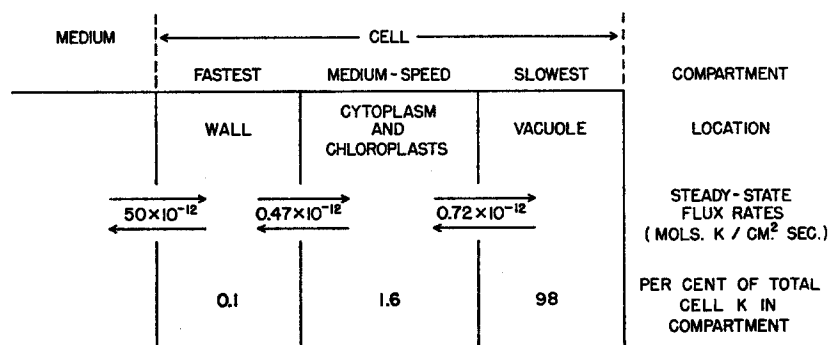


FIG. 8. Arrangement of *Nitella* compartments, combining data from direct and indirect analysis of each compartment.

TABLE III

*Steady-State Flux of K in Nitella*

	Flux	Standard error of mean	No. of experiments
	<i>pmols/cm.<sup>2</sup>, sec.</i>		
From medium to wall . . . . .	50	4	13
From wall to cytoplasm . . . . .	0.47	0.02	24
From cytoplasm to vacuole . . . . .	0.72	0.05	7

#### DISCUSSION

It is necessary to examine in further detail the correspondence between the structural model and the compartments which have been observed in the  $K^{42}$  uptake studies. It seems unlikely that multiple compartments have arisen as artifacts from diffusion gradients in the relatively huge vacuole, because the contents of the vacuole are kept in constant motion by cyclosis. The two oppositely directed streams of cyclosis produce turbulence where they pass each other; hence the vacuole must approximate closely a well stirred compartment. Since the location and properties of the intracellular structures postulated as compartments have been checked by direct analysis, the structural model appears to account in a satisfactory fashion for the gross features of  $K^{42}$  exchange by *Nitella*. Undoubtedly further subcellular structures escape detection as independent compartments in the whole cell because they contain only a small fraction of the total cell K or because they are in relatively rapid equilibrium with their intracellular surroundings. The chloroplasts provide an example of such a subcellular compartment which is not reflected in the over-all uptake of  $K^{42}$  by the whole cell.

The K flux between the cytoplasm and vacuole of 0.72 pmols/cm.<sup>2</sup>, sec. is

hardly greater than the flux of  $0.47 \text{ pmols/cm}^2, \text{ sec.}$ , between medium and cytoplasm, which are separated by a highly impermeable plasma membrane. If diffusion through the cytoplasm presented the only barrier to K entrance into the vacuole and the diffusion coefficient of K were the same in the cytoplasm as in water, the flux of K into the vacuole should be more than  $10^6$  times as rapid as the value observed, and the vacuole should not appear as a separate compartment. It would seem difficult to believe that diffusion through the cytoplasm is a million-fold slower than through water; and this possibility is rendered more unlikely by the high K permeability of the chloroplasts, which occupy much of the volume of the cytoplasmic layer. Furthermore, even in the gelatinous axoplasm of cuttlefish axon, Hodgkin and Keynes (25) found the diffusion coefficient of K to remain virtually unchanged from its value in water. Consequently, it seems preferable to conclude that the barrier to K diffusion lies in the membrane between the cytoplasm and vacuole recently observed with the electron microscope by Hodge (26). This is in agreement with the conclusions drawn by Osterhout (27, 28) on the basis of the electrical characteristics of *Nitella*. A similar explanation might hold for the marine alga, *Valonia*, whose vacuole has been shown by Mullins (29) to behave as an independent compartment. Since the cell vacuoles of higher plants are known to be surrounded by membranes of restricted permeability, it is not surprising that the vacuolar membranes in these algae are also semi-permeable. Thus, the metabolically active part of the cell—the cytoplasm—is surrounded on both the outside and the inside by membranes which exercise specific effects on the permeability of ions.

In the multicellular tissues of higher plants, the uptake of radioactive cations generally involves two distinct processes, one metabolically dependent and the other metabolically independent. The latter has been compared to a solid cation exchanger (Epstein (30)) and arises from a compartment termed the "apparent free space" (Briggs and Robertson (31)). It bears striking resemblances to the wall compartment of *Nitella* in three respects: (1) uptake by the "apparent free space" is an adsorption process and, in at least two higher plants (potato tubers and maize), it has been shown to follow an adsorption isotherm (Higinbotham and Hanson (32); Helder (33)); (2) this uptake is independent of respiratory poisons and does not require metabolic energy; (3) the "apparent free space" is a fast compartment, with a half-time of the order of 10 or 15 minutes. The 23 second apparent half-time of the wall of *Nitella* is even more rapid, as is to be expected, since the ions do not have to penetrate interstitial spaces as in multicellular tissues. In *Nitellopsis obtusa*, MacRobbie and Dainty (19) have also assigned a similar fast compartment to the "apparent free space". Analogous fast compartments have been described in the fresh water alga, *Chara contraria* (half-time, 10 minutes; Gaffey and Mullins (15)), in the marine alga, *Ulva lactuca* (half-time, 1 to 2

seconds; Scott *et al.* (34, 35)), and in the bacterium, *E. coli* (half-time around 1 minute; Cowie *et al.* (36)). All these organisms are characterized by the presence of cell walls. In contrast, no such rapid compartment has been reported for human erythrocytes, which lack a cell wall (Solomon *et al.* (37)). Thus, rapid adsorption on cell walls, as in *Nitella*, may be a characteristic phenomenon of all organisms which are protected by such walls.

The cellulose wall, which is about  $2 \mu$  thick, is essential since it allows *Nitella* to be hypertonic to its environment. An osmotic pressure difference of about 8 atmospheres exists between the interior of the cell and pond water, and this difference is maintained by the selective movement of ions into the cell. Because of the resulting hydrostatic pressure exerted by the cell sap on the wall, *Nitella* cells can maintain form and grow erect in stationary water while laying down very little structural material in their strong but thin walls. The potential difference, presumably resulting from the selective ion movement, is reflected in the unequal distribution of K. Consequently, K must be the most mobile ionic constituent of *Nitella*, and its rate of exchange, 1.2 per cent of the cell content per day, would be expected to be greater than that of other ions, as confirmed experimentally by Gaffey and Mullins (15) for the related genus, *Chara*. These remarkably slow rates of exchange, due to the relative impermeability of the cellular membranes, may be useful in enabling *Nitella* to retain its ions against their steep concentration gradients with a small expenditure of energy.

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