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Net H^+ Influx in *Nitella clavata*

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Summary. Net H^+ influx measurements on the giant internodal cells of the alga, *Nitella clavata*, have been made by transferring the cells to solution of low pH, 4.5 to 4.7, and measuring the pH changes or the amount of acid addition required to maintain the low pH. The latter results are in very good agreement with those of Kitasato (1968) and thus support his hypothesis that the *Nitella* membrane is very permeable to H^+. Other results indicate or suggest that the internal pH is changed only slightly under these conditions and that the membrane may have a rather large capacity to adsorb H^+ on the external surface.

Although Kitasato's (1968) evidence for a very large passive H^+ influx in *Nitella* came as something of a surprise, it appeared to be quite consistent with membrane resistance values commonly obtained for internodal cells of the *Characeae* (e.g., *see* Williams, Johnston & Dainty, 1964). The detection of acidic and basic regions occurring in alternating bands along the lengths of *Nitella* cells (Spear, Barr & Barr, 1969) served to confirm Kitasato's conclusions and offer detailed evidence for his main hypothesis; this consisted of an active H^+ extrusion balanced almost entirely by the passive H^+ influx.

Quantitatively these results had extremely interesting implications in that the poising of the resting potential could be essentially accounted for by the H^+ fluxes; the contributions of K^+ and Cl^- (and presumably Na^+) to the membrane conductance were found to be quite small in these mature cells (Kitasato, 1968). Further progress toward the understanding of the regulation of the resting potential and its relation to internal pH control thus seemed assured, because of the simplicity of the system.

It is nevertheless true, that the predominance of H^+ in the ionic relations of the *Characeae* has to some extent remained open to question since unidirectional H^+ flux measurements are not possible, and the detection

of acid and base is not in itself proof of charge transfer. The results of Walker and Hope (1969) and Lannoye, Tarr and Dainty (1970) at least allow for the possibility that K^+ or Na^+, respectively, might be more important than H^+, although neither of these studies offers unequivocal evidence either in favor of these ions or against H^+. The careful resistance measurements of Spanswick (1970) have led him to suggest that perhaps one-half of the membrane conductance is attributable to K^+, Na^+ and Cl^-, the other half to H^+.

The present study is an attempt to test the Kitasato hypothesis by measuring net H^+ influxes in *Nitella clavata* cells immersed in solution of relatively low pH, 4.5 to 4.7. This was done in two ways: (a) by following the pH changes in the external solution, and (b) by adding acid at frequent intervals in order to maintain the external pH at 4.7. Information was also obtained on the rate of depolarization of the membrane potential as compared to the net H^+ influx. Membrane resistance measurements and a preliminary result on intracellular pH changes are also presented. The results are discussed in relation to the Kitasato hypothesis and some of the assumptions underlying it.

Materials and Methods

Nitella clavata, a green alga having giant internodal cells, was grown in open cultures containing the following nutrients (concentrations expressed in mmoles/liter): 3.0 $CaCl_2$, 1.0 $MgSO_4$, 0.2 KCl, 0.2 KNO_3, 0.004 KH_2PO_4, 2.0 $NaHCO_3$, 4.0 Tris-(hydroxymethyl)-aminomethane neutralized with HCl to pH 7.0, and micronutrients. One ml of stock micronutrient solution was used per liter of culture solution; the former was made by boiling 50 g of Brockport brown soil in 1 liter of 6.0 mM Na_3 EDTA for one-half hr and filtering, pH 8.0. Lighting consisted of 1,000 lx total illumination from equal numbers of Sylvania *Gro-lux* and cool white fluorescent tubes for 16 hr per day, alternating with 8 hr of darkness. Internodal cells were harvested from intact plants and conditioned in Kb or K solution (*see below*) under 500 lx cool white fluorescent illumination at 22 °C.

In most of the experimental work the main solution employed was the K solution, or a slight modification of it, the Kb solution. The K solution contained the following concentrations of salts (mmoles/liter): 1.0 KCl, 0.1 NaCl, 0.1 $CaCl_2$, and 0.1 $MgCl_2$, with the pH adjusted according to experimental requirements. The Kb solution had the same composition as above except that 0.1 mM $KHCO_3$ was added to stabilize the pH at 7.0. Other solutions used occasionally are described under Results. Lighting during experiments consisted either of 500 lx cool white fluorescent illumination or 1,000 lx cool white plus 1,000 lx incandescent, as specified for the various results.

Membrane potential measurements on an internodal cell were done in a narrow plexiglass trough through which solution flowed at a rate of about 2 ml/min. Ag/AgCl microcapillary electrodes with 5 to 10 μm tips were inserted into the vacuole and measurements were made with either a General Radio 1230A electrometer or a Keithley 604 differential electrometer. The reference electrode consisted of a Colemen fiber type pH

reference reservoir into which a chlorided silver wire was inserted; this was located downstream in the measuring trough in order to eliminate possible effects from the salts escaping through the wick. The filling solution for both the microelectrode and the reference electrode consisted of 80 mM KCl, 30 mM NaCl and 5 mM $CaCl_2$, a solution simulating the vacuolar sap composition of *Nitella*. To obtain a measurement of the electrical potential contributed by the electrodes themselves, the microelectrode was immersed in a container holding the solution above, the reference electrode was immersed in a container of K or Kb solution, and the two solutions were connected by a filter paper bridge; thus each electrode was in the appropriate solution; i.e., corresponding to the situation for membrane potential measurements.

Membrane resistances were measured according to the method of Hogg, Williams and Johnston (1969) so that corrections for the leaky cable properties of *Nitella* cells would not be necessary. The membrane potentials measured here were done by a modified method; i.e., a microelectrode was used as the reference electrode and placed as close as possible to the insertion of the intracellular microelectrode so that the potential drop across the ambient solution would be insignificant. It was found that electrical artifacts were minimized by using the differential inputs of the Keithley 604 electrometer and grounding the flowing solution to earth via the Coleman reference electrode (*see above*). Small current densities of 10^{-7} to 10^{-6} amp cm^{-2} (cell surface) of 5- to 10-sec duration were used to cause displacement of the resting potential of 2 to 10 mV in each direction; if rectification occurred, the two were averaged to obtain the results presented. The currents were generated with an appropriate number of 9-V batteries in series with 100 MΩ resistor and monitored on a Simpson taut band microammeter. The current was delivered via Ag/AgCl microelectrode filled with 3 M KCl and returned through a silver wire running parallel to the length of the cell.

Net H^+ fluxes were determined in two ways: (a) by observing the time course of the pH increase caused by a number of cells in a small volume of K solution at an initial pH of 4.5 to 4.7, and (b) by holding the external pH constant at 4.7 through manual addition of small amounts of 5 mM H_2SO_4 at 10-min intervals via a home-made microburet; 20 cells in 3 ml volume were used, with the final volume after titration being less than 3.2 ml. In both cases the cells were preconditioned in K solution, pH 6.0, rather than Kb solution in order to eliminate the deposition of $CaCO_3$ or $MgCO_3$ in the cell walls (incrustations). Special precautions were taken to ensure that KCl from the reference electrode of the pH meter was not a factor in the results; at the end of the experiments the chloride concentration was checked with an Orion chloride electrode and found not to have changed in either of these two types of measurements. Potassium determinations were carried out on a Perkin-Elmer atomic absorption instrument.

The detection of external acid and base formation in alternating bands along the cell length was carried out as previously described (Spear *et al.*, 1969), i.e., with 0.1 mM potassium salt of phenol red in Kb solution at pH 7.0.

Definitions:

E_m: The electrical potential across the cell wall, cell membrane, and vacuolar membrane in series (microelectrode inserted into large central vacuole), but for convenience is referred to as the membrane potential.

E_k: The potassium equilibrium potential, i.e., the electrical potential required for potassium in the vacuole to be in electrochemical equilibrium with potassium in the external solution. It does not necessarily mean that K is at equilibrium across the cell membrane although this is generally assumed.

E_H: Hydrogen ion equilibrium potential (*see* E_k).

Results

External pH Changes Caused by Nitella

Fig. 1 shows the time course of the pH rise of K solution, initially at a pH of 4.5 to 4.7, after *Nitella* cells are transferred to it. The net H^+ influxes presented in Fig. 2 have been derived from Fig. 1, based on the assumption that the pH changes are due solely to net H^+ uptake by the cells; these are plotted on a logarithmic scale. Cell walls were used as the control since there was the possibility of some buffering effect of the wall material; however, this proved not to be so.

The initial net H^+ fluxes of about 35×10^{-12} mol cm^{-2} sec^{-1} are 2 or 3 times greater than those obtained by Kitasato (1968) on the same species at pH 4.7 where the membrane potential was clamped at -110 mV. Our higher values can perhaps be attributed to the lower Ca concentration present, 0.1 mM as compared to 1.0 mM in Kitasato's solution. Although both results presumably consist of net H^+ influxes, an important difference is that in Kitasato's work the ionic current through the membrane is an overall net charge transfer, whereas in our work, the net H^+ influx appears

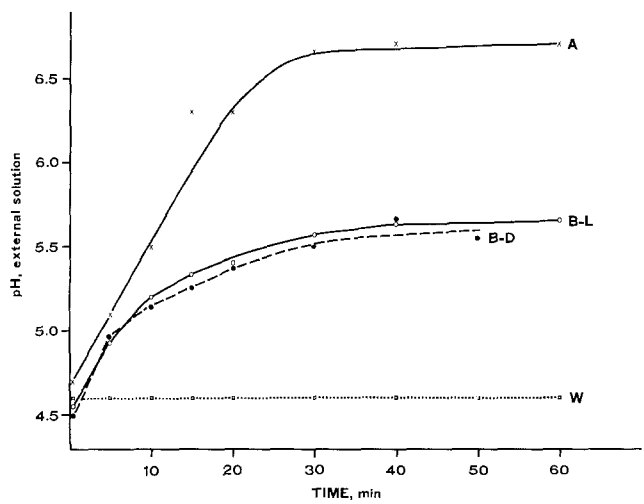

Fig. 1. Time course of pH changes caused by *Nitella* cells placed in 5 ml of K solution. *A* cells: 9 cells with total surface area of 11.5 cm^2 were preconditioned 14 days in K solution, pH 6.0; lighting was 500 lx cool white fluorescent throughout. *B* cells: 10 cells with total surface area of 8.9 cm^2 were preconditioned 17 days in Kb solution under same lighting as above; the cells were rinsed in pH 6.0 K solution before the experiment above which was conducted under 2,000 lx illumination (B–L) or in darkness (B–D). The solution pH eventually stabilized at about 7.6 in both cases. *W*: Walls from 18 cells which had dried out for several days; the surface area was 10.4 cm^2 and the conditions were the same as for those for B–L

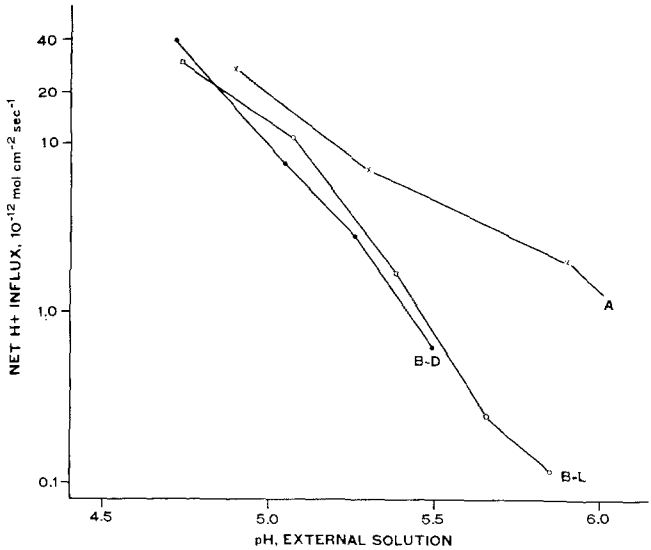

Fig. 2. Net H^+ influxes calculated from the disappearance of H^+ from the external solution, as obtained from suitable time intervals of Fig. 1

to be very nearly balanced electrically by some other ionic current; the basis for this conclusion is presented in detail below.

Fig. 1 also shows that for the "*B*" cells, the pH changes are essentially the same in light and darkness. This is not entirely unexpected since it is known that light promotes both H^+ extrusion (Spear *et al.*, 1969) and an enhanced membrane conductance (Hope, 1965; Nishizaki, 1968). The tentative conclusion here is that H^+ extrusion and passive H^+ influx are promoted to about the same extent by light, and thus the net influx is about the same in light and darkness. The greater net H^+ influx for "*A*" cells is consistent with the fact that they were not selected for high rates of acid and base formation as detected by phenol red (*see* Materials and Methods), whereas only active cells were used for the "*B*" experiment. The greater H^+ influx for "*A*" cells is then attributed to a lower rate of H^+ extrusion.

H^+ Uptake at pH 4.7

Fig. 3 shows the amount of H^+ which was added to K solution in which 20 cells were immersed in order to maintain the pH at 4.7 over a 4-hr period. The rate of H^+ uptake decreases approximately linearly with time, extrapolating to a zero rate at about 5 hr. The initial net H^+ influx of about 8×10^{-12} mol cm^{-2} sec^{-1} is in good agreement with Kitasato's

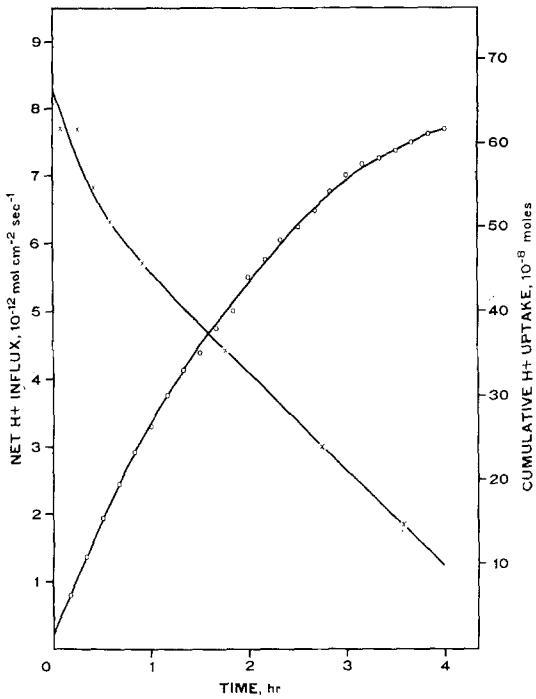

Fig. 3. Ascending curve: cumulative amount of H^+ added to 3 ml of K solution in order to maintain the pH at 4.7 after 20 cells were placed in it. Descending curve: net H^+ influx computed from suitable intervals of the above curve. The 20 cells, having a total surface area of 10.0 cm^2, were preconditioned 13 days in Kb solution under 500 lx cool white illumination. The conditions immediately preceding the experiment and the experimental conditions are the same as those given under Fig. 4. The mean E_m for 36 cells in Kb solution (pH 7.0) was -136 mV and -59 mV for 19 cells at pH 4.3. The interpolated value for pH 4.7 is about -80 mV. *See* Materials and Methods for further details

(1968) values but considerably lower than our own values derived from pH changes (Fig. 2).

The only significant difference in the procedures of the two experiments was that a 1-hr exposure of the cells to pH 4.5 was used here, and this may have had a residual effect; recovery of the membrane potential in pH 6.0 solution appeared to require a full 2 hr (*see* Fig. 4). The ordinate intercept in Fig. 3 of 0.2×10^{-8} mole is in excellent agreement with the buffering capacity of the cell walls of *Chara australis* as reported by Dainty, Hope and Denby (1960); the calculated value of 0.18×10^{-8} mole is based on a cell wall carboxylate concentration of 0.6 mole/liter, an average pK of 2.2 and a wall thickness of 10 μm. This result serves to confirm the negligible buffering effect of the cell wall, which went entirely undetected in the Fig. 1 results.

Intracellular pH Changes

We looked for direct evidence for the net H^+ influx in a preliminary study consisting of pH measurements on the contents of 30 cells under essentially the same experimental conditions as above, i.e., 4 hr at pH 4.7 with the same pretreatment. Thirty cells in Kb solution under the same light conditions served as the control. The pH measurements were carefully made with very small electrodes on two cellular fractions: (a) "cell sap" consisting mostly of vacuolar sap along with some protoplasm, and (b) cell walls along with the remaining protoplasm to which was added 0.3 ml of K solution at pH 4.76 after blotting to remove the residual "cell sap"; the purpose of using the pH 4.76 solution was to obtain some indication of the buffering power present in the protoplasm clinging to the cell walls. As Table 1 shows, the cells exposed to the low pH had the same pH in the wall fraction as the control and only a slightly lower pH in the "cell sap". Thus, the only indication of net H^+ influx is the vacuolar pH decrease. It seems probable that the actual pH decrease in the vacuole of the experimental cells is greater than the above since the latter contained some protoplasm, which appears to have considerable buffering power. Buffering is attributed to the protoplasm on the basis of the results obtained on cell walls described above. The concentration of buffering substances in the cell would be, very roughly, on the order of 50 mM if confined to the protoplasm. The protoplasmic pH of at least 6.3 is in line with the generally accepted view among biochemists that a pH of 6 to 8 is essential for proper enzyme functioning. Vacuolar pH values reported elsewhere for *Nitella* average about 5.5 (Hirakawa & Yoshimura, 1964).

Table 1. Intracellular pH measurements

Cell fraction	pH	
	Low pH cells [a]	Control cells [b]
"Cell sap": Vacuolar sap and some protoplasm (*ca.* 0.2 ml)	5.88	6.03
Cell walls and some protoplasm plus 0.3 ml of pH 4.76 K solution	6.27	6.25

[a] 30 cells under same preconditions and experimental conditions as Fig. 4 except the pH of the K solution was 4.76. They were rinsed three times in pH 6.0 K solution before the pH determination.

[b] 30 cells from same batch as the low pH cells except they were kept in Kb solution, pH 7.0, under same lighting. They were rinsed three times in pH 4.76 solution, followed by three rinses in pH 6.0 K solution before the pH measurement. *See* text for further details.

H^+ Uptake at pH 4.7: Membrane Properties

Although it is predictable that *Nitella* cells cannot continue to take up H^+ indefinitely, the basis for the decreased rate with time (Fig. 3) was not evident. We investigated this by following the time course of the levels of the membrane potential and the membrane resistance under exactly the same preconditions and experimental conditions of Fig. 3, except that a flowing solution of pH 4.7 was used instead of a stagnant one. The results corresponding to Fig. 3 are presented in the center section of Fig. 4. As expected, a large depolarization of the membrane occurs at 4.7, but after 1 hr both the membrane potential and the membrane resistance assume quite constant levels while net H^+ influx continues to decrease for at least 3 hr. Thus, it appears that all of the driving forces (including the pH of the protoplasm) remain constant, but H^+ influx nevertheless decreases.

In terms of the Kitasato (1968) hypothesis, the only explanation remaining is that active H^+ extrusion is somehow stimulated by the low external pH. There is both positive and negative evidence bearing on this interpretation. Fig. 5 shows the hyperpolarizing effect induced by first exposing *Nitella* cells to low pH solution and then returning them to neutral solution. This has been observed in a large number of cells, with the hyperpolarized state persisting, sometimes indefinitely. Sometimes E_m reaches -200 mV or more negative (*cf.* the initial E_m in Fig. 4). The E_m for this state is consistently more negative than either E_K or E_H ($[K]_i$ is no greater than about

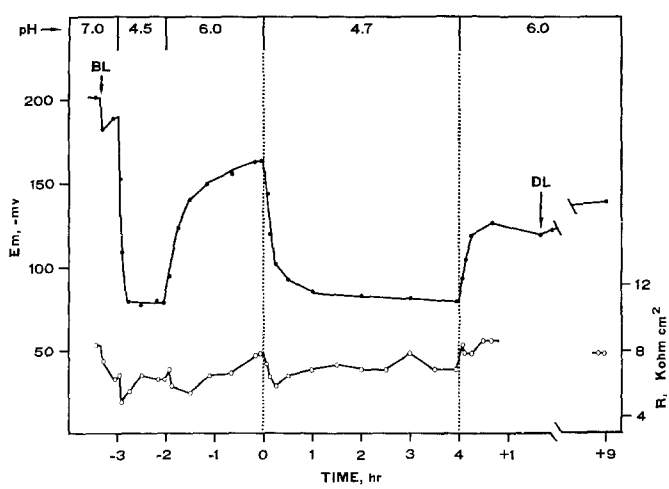

Fig. 4. Time course of membrane potential and resistance changes of a *Nitella* cell as related to pH changes of the K solution. The cell was preconditioned 17 days in the same way as the 20 cells of Fig. 3. BL refers to bright light, i.e., 2,000 lx, and DL refers to dim light of 500 lx

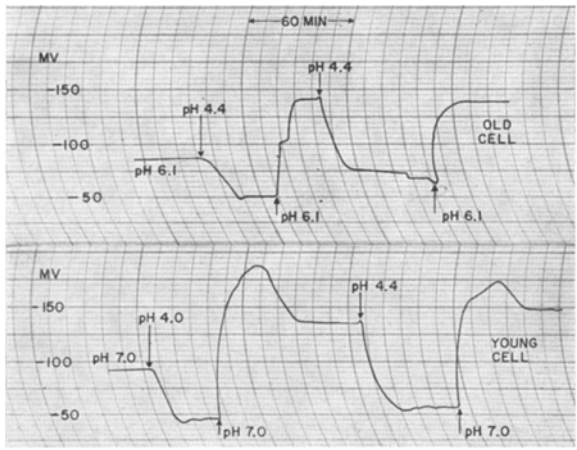

Fig. 5. Dependence of the level of the membrane potential on external pH of the K or Kb solution, and the hyperpolarizing after-effect of low pH treatment. The "humps" of the young cell are perhaps related to the presence of bicarbonate at pH 7.0 (*cf.* Hope, 1965)

100 mM [Barr, 1965]), and in view of the membrane conductance (Fig. 4), a stimulated H^+ extrusion seems to be the only explanation. Some cells apparently attain the hyperpolarized state by some means other than low pH treatment.

The evidence against an enhanced H^+ extrusion under low pH conditions comes directly from Fig. 4 and is therefore more decisive: subsequent to the pH 4.7 treatment, E_m returns to only -120 mV at pH 6.0. Although this potential is close to E_k, we do not have the information on the unidirectional K^+ fluxes which is necessary to ascertain if they are large enough to control the potential. Kitasato's (1968) results on the same species would seem to have eliminated this possibility, but this point requires verification since the experimental conditions were somewhat different. Our chemical analysis on the solution used in the Fig. 3 work was inconclusive: the mean net effluxes of both K^+ and Cl^- were 3×10^{-12} mol cm^{-2} sec^{-1} during the 4-hr exposure to pH 4.7.

Rate of Depolarization at pH 4.7

Common with all of the results obtained by others on this point (Kitasato, 1968; Walker & Hope, 1969; Lannoye *et al.*, 1970) the depolarization caused by low pH treatment (Figs. 4 and 5) actually requires several minutes whereas the discharge of the membrane capacity should require only a

fraction of a second (membrance capacitance about 1 or 2 $\mu F/cm^2$). The net H^+ influx values obtained in our work thus do not represent overall net positive ionic currents into the cell, even during the initial phase of depolarization. The initial rates of depolarization at pH 4.5 or 4.7 correspond to net ionic currents of between 10^{-15} and 10^{-14} Equiv $cm^{-2}\,sec^{-1}$; the discrepancy between these and the net H^+ influxes is at least three order of magnitude. Net K^+ efflux cannot be the balancing current during the initial phase when E_m is -150 mV or more negative since E_k is about -120 mV or more positive. The same argument would hold for Na^+. The only other current which might serve to nearly balance H^+ influx would be Cl^- influx, but low pH appears to have no effect on this ionic movement in *Chara australis* (Lannoye *et al.*, 1970) and only a relatively small effect in *Chara corallina* (Smith, 1970). In the latter study the enhanced Cl^- influx occurring when the pH is lowered to 4.7 may be estimated by extrapolation as being no more than 1×10^{-12} mol $cm^{-2}\,sec^{-1}$. Until tracer studies on Cl^- fluxes are carried out, however, the possibility of a large Cl^- inward current cannot be rigorously eliminated.

Mailman and Mullins (1966) found that the cell wall of *Nitella* offered considerable resistance to Cl^- penetration, but, as they pointed out, this is most probably due to the high density of fixed carboxylate groups and referred to phenomena indicating a much faster movement of cations through the wall.

In summary, the rapid disappearance of H^+ from the external solution is extremely puzzling in view of the slow depolarization rates. It is true that it may take a minute or two for the flowing solution to actually deliver the required amount of H^+ to the membrane, but at that point the membrane should discharge at a rate much faster than that observed. There appears to be no simple explanation for it.

Discussion

Net H^+ Influxes

The main results of our study indicate that H^+ is readily removed by *Nitella* cells from solution when the pH is below 5. The rate of removal, on a cell surface basis, is of the proper magnitude (ca. 10^{-11} mol $cm^{-2}\,sec^{-1}$) to be consistent with Kitasato's (1968) hypothesis. According to this, the passive H^+ influx and the active H^+ extrusion are by far the largest ionic fluxes, amounting to about 40×10^{-12} mol $cm^{-2}\,sec^{-1}$ each, for cells in neutral or slightly alkaline solution. At low pH a net H^+ influx should

occur, but its magnitude cannot be accurately predicted since there is no way of measuring the unidirectional H^+ fluxes isotopically, and thus the effect of the external pH on H^+ extrusion cannot be evaluated.

It is possible to make a quasi-direct comparison of our results on net H^+ influx with those of Kitasato (1968). By interpolation of his data the inward positive current at pH 4.7 is estimated as 10^{-11} Equiv cm^{-2} sec^{-1}, with E_m clamped at -110 mV; in our work the net H^+ influx was 7 or 8×10^{-12} mol cm^{-2} sec^{-1} for the same average E_m (Figs. 3 and 4, first half hour at pH 4.7). The agreement between these values provides strong evidence in favor of H^+ as the predominant ion in membrane traffic, as elucidated in the Kitasato hypothesis.

Depolarizing Action of H^+

One of the paradoxes in the results is that the large net H^+ influx at low pH should cause an almost instantaneous depolarization of the membrane since the capacitative charge on the membrane is of the order of 10^{-12} Equiv cm^{-2}. Although one can argue that it may take a minute or so for H^+ to actually reach the membrane, the required time of a few minutes becomes extremely difficult to account for.

Neither the buffering capacity of the cell wall (Figs. 1 and 3) nor the calculated H^+ diffusion time through the wall appear to be large enough to explain the disappearance of H^+ from solution without a correspondingly rapid membrane depolarization. In looking for a balancing current to explain this result, net K^+ loss by the cell can be ruled out since during the initial phase of depolarization, E_m is sufficiently electronegative to cause the net flux to be inward or at least near zero.

If, then, the missing H^+ is not in the protoplast, nor in the solution, nor exchanged for cations of the cell wall, it must be present on the membrane itself. If, for example, it takes 2 or 3 min for $1{,}000 \times 10^{-12}$ mole of H^+ to diffuse to the membrane and adsorb there, the surface density of H^+ would be one H^+ per 15 $Å^2$. This figure corresponds to a perfectly flat membrane; most likely the membrane is considerably convoluted and with ectodesmata projecting into the wall. This would give a lower surface density, and perhaps a value of one H^+ per 30 $Å^2$ is more realistic. Although there is no direct proof for this, certain incidental information suggests that it is possible, viz, from Kitasato's voltage clamp experiment one can calculate that the H^+ permeability (as defined by the Goldman (1943) equation) decreased fivefold as the pH is lowered from 5 to 4. This indicates a profound effect of low pH on the physical state of the membrane and suggests

that the adsorption of H^+ or the conversion of carboxylate groups to the undissociated form might be responsible. Summing up, it is suggested that the membrane's outer surface can adsorb a considerable number of H^+ while allowing relatively few to penetrate, and this drastically lowers the H^+ permeability.

Once the E_m has been depolarized to about -100 mV, net K^+ movement out of the cell begins and can possibly account for the continued slow rate of depolarization. The gradualness of the depolarization curves in Fig. 5 indicates that during the *net* movement of H^+ into the cell there must be an oppositely moving ionic current which almost balances it. An obligate exchange process within the membrane is suggested, but this explanation carries with it the implication that H^+ extrusion and passive H^+ influx are interdependent processes which together control the rate of net charge transfer through the membrane under resting conditions. Further study is needed to evaluate this suggestion.

H^+ Uptake and Intracellular pH

Our results on the intracellular pH changes for cells exposed 4 hr to pH 4.7 solution are quite meager and serve mainly as a future guideline for more careful work. The detection of only a small lowering of the internal pH is, nevertheless, significant in that it is consistent with the common assumptions of a well-controlled internal pH and considerable buffering capacity. Also, the results suggest that excess acidity may be routed to the vacuole. Any hypothesis regarding the mechanism of transfer should take into account the probability that most of the buffering power is localized in the protoplasm.

One possibility is that a slight lowering of the protoplasmic pH occurs which, in turn, brings about the partial conversion of the anionic form of an organic acid to the undissociated form. The undissociated species might then pass through the vacuolar membrane readily; about 90% of it would be expected to be in the vacuole at equilibrium; i.e., because of the vacuole's large volume. Such a buffering system would be much more effective if the anionic form were also to be present in the vacuole; even a concentration of a few millimolar of both forms combined would suffice to be consistent with our results.

A second possibility is that the vacuolar membrane is capable of active H^+ extrusion; if inward H^+ extrusion is another means whereby the proto-

plasmic pH is kept constant, the situation would be quite complex and difficult to analyze.

A third explanation may be found in the ideas developed by MacRobbie (1970) as a result of her extensive studies; she concludes that the processes of influx into the cell and transfer to the vacuole are intimately linked, possibly through the formation and movement of small vesicles (Costerton & MacRobbie, 1970). One of our results, the decrease in net H^+ influx without an apparent change in electrochemical gradient (and negative evidence for an enhanced H^+ extrusion), at least suggests the possibility of this type of transport. The cell membrane and the vacuolar membrane are not necessarily limited to one or two types of transport mechanisms; they may be capable of a variety of responses in controlling ionic exchanges with the environment, and these may differ according to the conditions.

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