The Influence of H^+ on the Membrane Potential and Ion Fluxes of *Nitella*

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ABSTRACT The resting membrane potential of the *Nitella* cell is relatively insensitive to $[K]_o$, but behaves like a hydrogen electrode. K^+ and Cl^- effluxes from the cell were measured continuously, while the membrane potential was changed either by means of a negative feedback circuit or by external pH changes. The experiments indicate that P_K and P_{Cl} are independent of pH but are a function of membrane potential. Slope ion conductances, G_K, G_{Cl}, and G_{Na} were calculated from efflux measurements, and their sum was found to be negligible compared to membrane conductance. The possibility that a boundary potential change might be responsible for the membrane potential change was considered but was ruled out by the fact that the peak of the action potential remained at a constant level regardless of pH changes in the external solution. The conductance for H^+ was estimated by measuring the membrane current change during an external pH change while the membrane potential was clamped at K^+ equilibrium potential. In the range of external pH 5 to 6, H^+ chord conductance was substantially equal to the membrane conductance. However, the $[H]_i$ measured by various methods was not such as would be predicted from the $[H]_o$ and the membrane potential using the Nernst equation. In artificial pond water containing DNP, the resting membrane potential decreased; this suggested that some energy-consuming mechanism maintains the membrane potential at the resting level. It is probable that there is a H^+ extrusion mechanism in the *Nitella* cell, because the potential difference between the resting potential and the H^+ equilibrium potential is always maintained notwithstanding a continuous H^+ inward current which should result from the potential difference.

INTRODUCTION

The electrical properties of the fresh water alga, *Nitella*, have been widely studied because of the cell's ability to generate an action potential. Of additional interest is the finding that the resting membrane potential is markedly depolarized by decreasing the pH of the external solution bathing the *Nitella* cell (Kishimoto, 1959). In the experiments to be reported here, the effect of pH on the membrane potential in *Nitella* cells was investigated.

The resting membrane potential of frog muscle fiber is affected by a decrease in external pH, and it has been shown that the decrease in Cl permeability is responsible for the membrane potential change observed (Hutter and Warner, 1967 *a*, *b*). Similarly, the effect of pH on the membrane potential of the esophageal cell of *Ascaris* has been tested (Castillo and Morales, 1967). The relation between permeability and pH in the red blood cell has also been examined (LaCelle and Rothstein, 1966). However, the membrane potential change observed in *Neurospora* when the external pH is changed is quite similar to that observed in *Nitella* (Slayman, 1965 *a*). It therefore seems attractive to investigate the kind of mechanism that might contribute to the change in membrane potential induced by a change in pH.

There are four possible factors which might contribute to a membrane potential shift due to change in pH. The first possibility is that the permeability for K^+ is influenced by changing pH, since it might be expected that an increase in H^+ concentration results in a decrease in the fixed negative charge density on the membrane. The second possibility is that there is a change in the phase boundary potential at the outer surface of the plasma membrane. Since the ionic strength of the external solution is very low, one might expect that the change in fixed negative charge density would bring about a large boundary potential change.

Other possibilities might be considered in the situation in which the membrane potential is maintained by an active electrogenic pump of some ion. If permeability to one ion is large compared with permeability to other ions, the membrane potential should behave as if it were a concentration cell with regard to the ion; on the other hand, if the ion is not actively transported, insofar as it penetrates the membrane it should be distributed so that its inside concentration is in equilibrium with the existing membrane potential and the outside concentration of the ion, regardless of its ease of penetration. Thus the membrane is not always permeable principally to the ion which has its equilibrium potential close to the resting potential. It is probable that ions with an equilibrium potential different from the resting potential are transported actively. The difference between the equilibrium potential of the ion and the resting potential is determined by the membrane permeability to the ion and the active transport rate of that ion. When the membrane potential is in the steady state, the sum of the passive currents carried by all the mobile ions is equal but opposite in sign to the sum of the active currents because in this state the total ionic current is zero. Under this condition the membrane potential, E_m, should be expressed by the following equation

$$E_m = (E_m)_o + \frac{\sum I_{j\pm}}{g_m} = (E_m)_o - \frac{\sum (I_{j\pm})_{\text{active}}}{g_m} \qquad (1)$$

Here, g_m is the chord conductance of the membrane. $I_{j\pm}$ is the current car-

ried by passive ionic movements, and $(I_{j\pm})_{active}$ is the current carried by actively transported ions. $(E_m)_o$ is the assumed membrane potential when the sum of passive currents is zero. $(E_m)_o$ is given approximately by the well-known Goldman equation

$$(E_m)_o = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o + P_H[H]_o + P_{Cl}[Cl]_i}{P_K[K]_i + P_{Na}[Na]_i + P_H[H]_i + P_{Cl}[Cl]_o} \quad (2)$$

A third possibility that might account for the membrane potential shift induced by external pH change is that the active transport rate of some ions may be affected by a pH change in the external solution. In this case the change in the value of the second term of equation (1) results in a change in the membrane potential.

A fourth possibility is that most of the passive membrane current is carried by H^+ which easily penetrates the plasma membrane, while the active transport rate of H^+ from inside to outside remains constant. In this case the increase in $[H]_o$ results in an increase in the passive inward membrane current. A part of this increased inward current flows into the capacitance and as a result the membrane is depolarized. In the same manner, when $[H]_o$ is decreased, the membrane is hyperpolarized. Now, if g_H is much larger than the sum of g_K, g_{Na}, and g_{Cl}, the membrane current is mainly carried by H^+, or $g_m \cong g_H$. This means that $(E_m)_o$ is nearly equal to H^+ equilibrium potential, E_H. Under this circumstance equation (1) can be approximated as

$$E_m \cong E_H - \frac{F\phi_H}{g_m} \quad (3)$$

Here, ϕ_H is the number of moles of H ions transported actively per unit time, and F is the Faraday constant. In this case the membrane potential should change as if it were a H^+ electrode, and should always be displaced from E_H to the extent of $-F\phi_H/g_m$, if the rate of active transport of H^+ remains constant throughout the whole experiment.

In order to determine which factors might contribute to the resting membrane potential shift induced by pH change, the following experiments were designed.

METHODS

Experimental Chamber Internodal cells of *Nitella clavata* (X033) were used throughout the experiments to be reported. The temperature at which all experiments were performed was 23°C. A *Nitella* cell was mounted in a small chamber, as shown in Fig. 1. This chamber was separated into three compartments by septa, each consisting of two thin plastic plates with silicone grease between. Both end compartments of the chamber were used to supply current to control the membrane potential of that part of the cell located in the central compartment. The membrane potential was

recorded from the length of the cell in the central compartment; the distance between the two septa was 2 cm. This value was a compromise between the desire for a very short distance to minimize nonlinearity in the electrotonic potential during voltage clamp, and to maximize the surface area involved when ionic flux measurements were carried out with radioisotopes. In each of the septa there was a narrow groove slightly larger than the diameter of the *Nitella* cell and the cell was laid in these grooves. After using filter paper to remove water from the cell surface at the septal interface, both end compartments were covered by glass plates spread with silicone

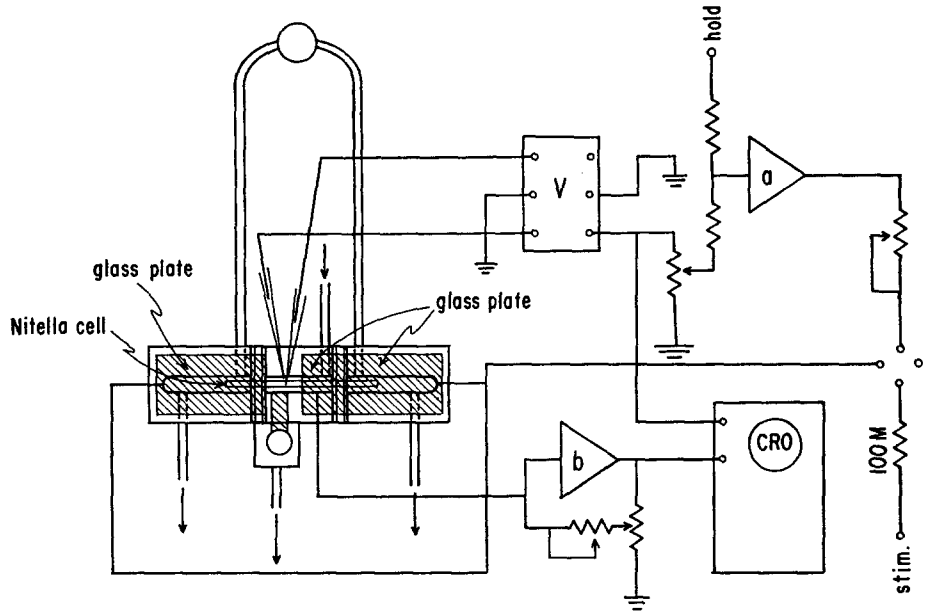

FIGURE 1. A chamber and the block diagram of the electric circuit used for this experiment. The *Nitella* cell was laid in the small plastic chamber shown on the left side. Both end compartments are covered by glass plates spread with silicone grease. Membrane potential is recorded from that part of the cell located in the central compartment. *v* indicates a vacuum tube voltmeter amplifier: *a* and *b* are operational amplifiers. *CRO* is a cathode ray oscilloscope.

grease and then the central compartment was partly covered by a piece of glass to make it easier to observe protoplasmic streaming in the cell. Both end compartments were attached to the same reservoir by a thin plastic tube and were perfused with artificial pond water (APW). The central compartment was connected through a specially designed eightway stopcock tube to reservoirs of experimental fluid; test solution came from one of these reservoirs. The solution which flowed into the central compartment flowed out via a filter paper wick into a small pot from which the solution was removed by vacuum. This method was effective in eliminating the electrical noise that is observed when fluid is aspirated directly from the central compartment. The perfusion flow rate was 2 ml per min and since the volume of the central com-

partment was 0.2 ml, the whole solution in the central compartment could be changed several times within a minute.

Electrical Measurements The membrane potential of the cells was measured by using two glass micropipettes filled with a solution of 100 mM KCl and 30 mM NaCl. Tip diameter of the electrodes was about 2 μ. One electrode was inserted into the cell and the other placed just outside. The potential difference between these two electrodes was led to the input of a vacuum tube electrometer amplifier (Keithley 603) and the output voltage of the amplifier, attenuated by a potentiometer, was led through a resistor of 100 kohms to the input of an operational amplifier (*a*) (Hewlett Packard). The output of this operational amplifier was connected through a variable resistor of 1 Mohm to Ag/AgCl wire electrodes placed in both end compartments. When constant currents were applied to the cell membrane, the connection between the operational amplifier and the electrodes in the end compartments was cut off and the electrodes were connected through a resistor of 100 Mohms to the output of an electronic stimulator (Tektronix 161). The current flowing across the cell membrane in the center compartment was measured by connecting two Ag/AgCl wires placed along the whole length of the central compartment to the input of the operational amplifier (*b*) (Hewlett Packard). The sensitivity was controlled by adjusting the value of the feedback resistor. Membrane potential and current were recorded simultaneously on an inkwriting recorder (Texas Instruments, Houston, Tex.). The output of the voltmeter and operational amplifier (*b*) were connected to the input of an oscilloscope (Tektronix 502) while the outputs of the oscilloscope were connected to the inkwriting recorder. Membrane potential was measured by inserting one of the two microelectrodes into the cell after ascertaining that there was no drift in the potential difference between the two electrodes when they were soaked in the perfusion solution in the central compartment for 30 min. After the experiment the microelectrode which had been inserted into the cell was withdrawn and the potential difference between the electrodes was checked again in the perfusion solution.

The Electronic Potential along the Cell Axis in the Central Compartment When current is flowing longitudinally through a cylindrical cell, from the end compartments to the central compartment, the displacement, V, of the resting membrane potential from the resting level in the longitudinal direction can be expressed as a function of the distance x from the septum separating the central compartment from the left end compartment. If the membrane resistance per unit length of the cell is r_m and the internal resistance per unit length of the cell is r_i, the well-known relation between the potential displacement and distance is

$$V = \frac{r_m}{r_i} \frac{d^2V}{dx^2} \tag{4}$$

The solution of the differential equation (4) generally can be written as follows:

$$V = Ae^{-x/\lambda} + Be^{x/\lambda} \tag{5 a}$$

This equation can be rewritten as follows:

$$V = V_1 \cosh \frac{x_1 - x}{\lambda} + V_2 \sinh \frac{x_1 - x}{\lambda} \qquad (5\,b)$$

λ is $\left(\frac{r_m}{r_i}\right)$ and is known as the length constant. x_1 is an arbitrary constant. V_1 is the potential displacement at $x = x_1$. The relation between V_1 and V_2 is as follows:

$$2A = (V_1 + V_2)e^{x_1/\lambda}; \qquad 2B = (V_1 - V_2)e^{-x_1/\lambda} \qquad (6)$$

The current flowing longitudinally, I_i, in the cell is the product of $-dV/dx$ and the reciprocal of the internal resistance per unit length of the cell, assuming that the resistance of the external solution per unit length can be negelected. I_i is expressed by the following equation:

$$I_i = -\frac{1}{r_i}\frac{dV}{dx} \qquad (7)$$

The derivative of potential displacement with respect to x can be obtained from equation (5 *b*) as follows:

$$\frac{dV}{dx} = \frac{V_1}{\lambda}\left[\sinh \frac{x_1 - x}{\lambda} - \frac{V_2}{V_1}\cosh \frac{x_1 - x}{\lambda}\right] \qquad (8)$$

From equations (7) and (8) the longitudinal current is given by the following:

$$I_i = -\frac{V_1}{\lambda r_i}\left[\sinh \frac{x_1 - x}{\lambda} - \frac{V_2}{V_1}\cosh \frac{x_1 - x}{\lambda}\right] \qquad (9)$$

There is no longitudinal current at the midpoint of the center compartment because the current controlling the membrane potential is supplied from both sides of the cell. If the value of x_1 is chosen as the distance from the septum on the left side to the midpoint of the center compartment, from equation (9) the value of V_2 can be determened ($V_2 = 0$). Then the potential displacement as a function of x is expressed by the following:

$$V = V_1 \cosh \frac{x_1 - x}{\lambda} \qquad (10)$$

The potential displacement at $x = 0$, V_0, is obtained from equation (10):

$$V_0 = V_1 \cosh \frac{x_1}{\lambda} \qquad (11)$$

For a *Nitella* cell, the resistance of the unit area of the membrane is 25 kohms cm^2, and the specific resistance of the cell sap may be about 100 ohms cm. From these

values one obtains the length constant as 1.9 cm. Since the distance between the septa is 2 cm, then $x_1 = 1$. When these values are used, the potential displacement along the cell axis in the central compartment can be expressed by the following equation:

$$V = 0.875\ V_o \cosh \frac{1 - x}{1.9} \qquad (12)$$

The solid curve in Fig. 2 is drawn from equation (12), while the broken curve shows the electrotonic potential along the cell axis when controlling current is supplied from only one side of the cell. As these curves show, when controlling current is supplied from both sides of the cell, the membrane potential displacement in the central compartment can be considered almost uniform (assuming that potential displacement does not change r_m).

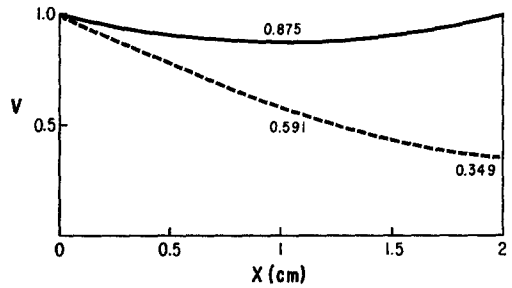

FIGURE 2. The electrotonic potential along the cell axis in the central compartment. The solid curve is drawn from equation (12); the broken curve is the electrotonic potential when controlling current is supplied only from the left side of the cell. Abscissa is v/v_o, and ordinate is the distance, x, from the left septum separating the central compartment from the left end compartment.

Solutions The *Nitella* cell was perfused continuously with a variety of test solutions. Artificial pond water (APW) had a composition of 2 mM NaCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.05 mM K_2SO_4, and 0.2 mM Tris (hydroxymethyl) aminomethane. The pH was adjusted to the required values by adding 0.1 N HCl or 0.1 N H_2SO_4. The composition of Cl-free APW was 1 mM Na_2SO_4, 1 mM $MgSO_4$, 1 mM $CaSO_4$, 0.025 mM K_2SO_4, and 0.2 mM Tris (hydroxymethyl) aminomethane. In this case, pH was adjusted by adding 0.1 N H_2SO_4. High K APW was prepared by adding K_2SO_4 to the normal APW and high Ca APW was prepared by adding $CaCl_2$ to normal APW. Ca-free APW was prepared by removing $CaCl_2$ from normal APW.

Flux Measurements To measure K^+ efflux from the *Nitella* cell, the cell was loaded with ^{42}K in APW containing radioactive K^+ for a period of 6 hr. The cell was then placed in the chamber described earlier and perfusion through this chamber was maintained continuously. 2 hr after the insertion of a glass micropipette (used for the measurement of membrane potential during the experiment), the solution perfusing the central compartment of the chamber was collected at 2 min intervals and these

collected samples were dried in stainless steel planchets and their radioactivity measured using a low-background automatic counting system with anticoincidence guard (Beckman Wide Beta II).

Chloride efflux measurements were carried out using ^{36}Cl as a radioisotope, obtained as a 2.55 N HCl solution and neutralized with $NaHCO_3$ before use. Cells were loaded in a solution containing 10 mM NaCl, 0.05 K_2SO_4, 1 mM $CaCl_2$, and 1 mM $MgSO_4$. The loading time for this isotope was 1 wk. Sample collection during the experiment was at 10 min intervals because of the very low specific activity of ^{36}Cl (0.0117 mc/mole) compared to the specific activity of ^{42}K (11.13 mc/mole). The radioactivity of ^{36}Cl samples was measured as described above.

RESULTS

The Effect of pH on the Resting Potential and Membrane Resistance These experiments were originally begun in order to see whether changes in the fixed charge density at the outer surface of the cell membrane might affect the resting potential. In order to accomplish this the pH of the artificial pond water bathing the outside of the cell was changed over a range of 4 to 8 while the membrane potential was recorded continuously. As the pH of the external solution decreased, the internal potential became less negative; an example is shown in Fig. 3 A. The time course of the membrane potential change was almost exponential and the half-time of potential change, while differing from cell to cell, occurred mainly within 2–3 min. The relationship between membrane potential in the steady state and the external pH of the artificial pond water is shown in Fig. 3 B. For the pH range 4 to 6, the slope of the curve of membrane potential vs. pH was 56 mv per pH unit, while the response of the membrane potential to pH change in the range 6 to 8 had a slope that was less steep.

Membrane resistance was calculated by measuring the potential displacement in response to pulses of constant current. In the pH range of 5 to 6, the average membrane resistance in the resting state was $(2.5 \pm 0.7) \times 10^4$ ohm cm^2. This value is substantially equal to the value obtained by Findlay (1959) on a *Nitella* cell (unknown species), but somewhat lower than that described as a resistance of 40 Kohm-cm^2 for leakage current from voltage clamp experiments by Kishimoto (1964).

When external pH was changed from 5 to 6, the membrane resistance was increased 1.5 times; however, in some instances for an external pH change from 7 to 8, the membrane resistance decreased.

The Effect of K^+ on the Membrane Potential The K^+ concentration in the *Nitella* cell sap is about 100 mM while the K^+ concentration in the external solution is usually about 0.1 mM. The K^+ equilibrium potential calculated from these values is almost equal to the measured membrane potential at a pH of 6.5. It has been shown that the resting membrane potential of the *Nitella*

cell behaves as a H^+ electrode. This behavior might be the result of the influence of H^+ on the conductance for K^+ or it might be that H^+ itself is capable of carrying a considerable membrane current.

In order to have some quantitative information about P_K (the permeability coefficient for K^+), the membrane potential was measured at various K^+ concentrations as shown in Fig. 4. From these data it is clear that the membrane potential is not as highly sensitive to $[K]_o$ as would be expected from the Nernst relation. In particular, when $[K]_o$ was changed from 0.1 to 1 mM, no change in membrane potential occurred (actually a slight increase). In-

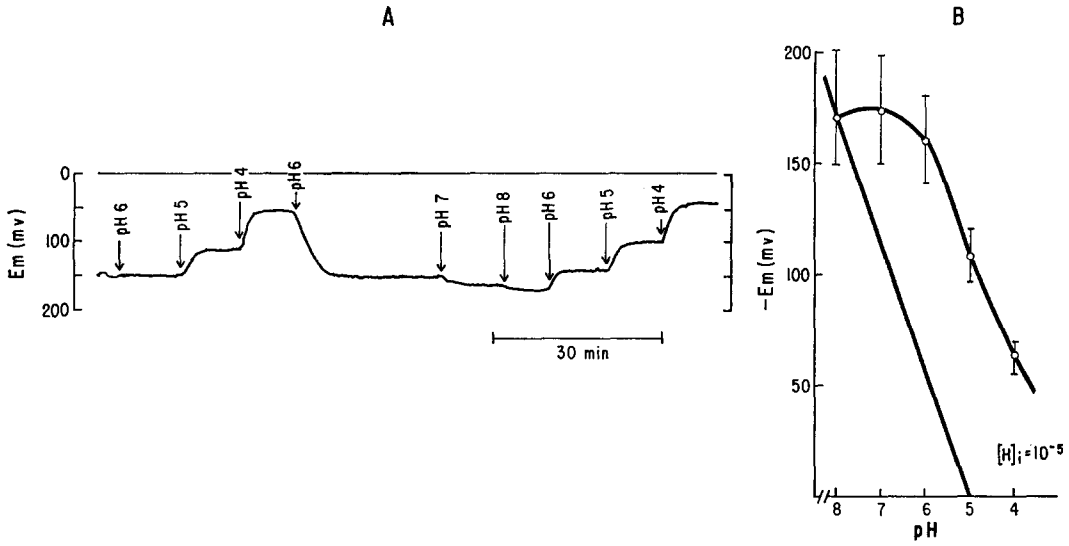

FIGURE 3. A, membrane potential change induced by the pH change in the solution bathing *Nitella*. Solution was changed at the arrows. B, the relation between external pH and membrane potential (solid curve), and between external pH and the H^+ equilibrium potential (straight line).

creasing $[K]_o$ from 1 to 10 and to 100 mM resulted in increases in the amount of depolarization with increased concentration, but this did not exceed 31 mv per decade of $[K]_o$. The results of measurements on several cells are given in Table I. Similar results were reported for the same species of *Nitella* (Barr, 1965) and for *Neurospora* (Slayman, 1965 *a*).

Some reservations must be made with respect to these findings. Following the excitation of a cell bathed in APW, the action potential returned smoothly to the base line level of resting potential, while if a cell was bathed in 10 mM K APW, it had a plateau in the falling phase of the action potential (Fig. 4 A). The level of this plateau was almost equal to the K^+ equilibrium potential calculated from $[K]_o$ and $[K]_i$. Similarly, if the external solution was 100 mM K APW and a depolarizing current pulse was supplied, the mem-

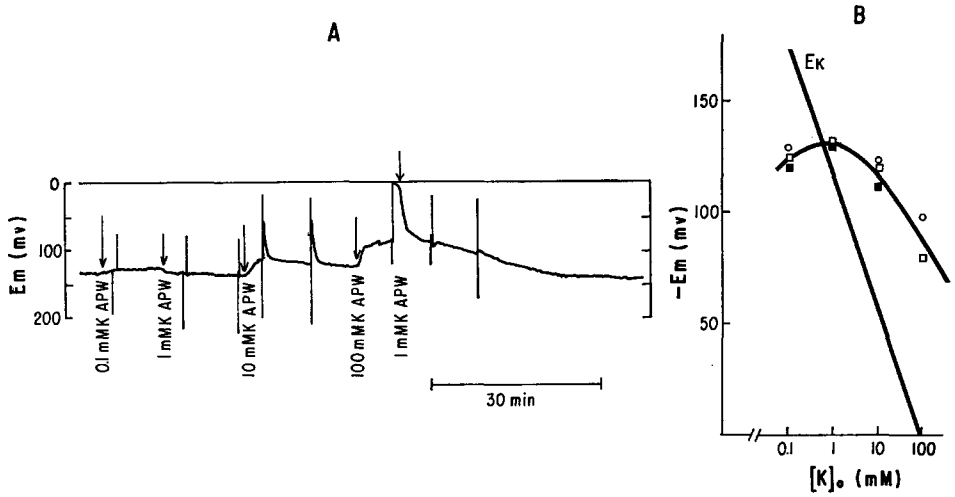

FIGURE 4. A, membrane potential change induced by the change in [K] in APW of pH 5.3. Solution was changed at the arrows. Sudden displacements of the membrane potential were caused by constant inward currents of 3.8 μamp/cm^2. B, the relation between $[K]_o$ and the membrane potential.

brane potential shifted suddenly from a resting value of about −80 mv to 0 and remained at this level. Again this value of 0 is close to the K^+ equilibrium potential. These findings suggest that during the falling phase of an action potential the membrane is highly permeable to K^+. Furthermore, if a cell was not bathed in a solution containing Ca^{++} (such as APW), but in one containing only KCl, the membrane behaved much more like a K^+ electrode than it did in the presence of Ca^{++}. This finding accords with the experiments on *Chara australis* in Ca-free APW (Hope and Walker, 1961).

TABLE I

MEMBRANE POTENTIAL OF *Nitella* CELL UNDER VARIOUS CONDITIONS

pH in the external solution	K^+ concentration in the external solution			
	0.1 mM	1 mM	10 mM	100 mM
	mv	*mv*	*mv*	*mv*
4	−64±7 (4)	−64±6 (7)		
4.5		−72±3 (2)		
5	−107±11 (4)	−109±9 (9)	−102±4 (2)	
5.3	−125±5 (3)	−130±2 (3)	−117±6 (3)	−87± (3)
6	−159±19 (4)	−160±20 (9)		
7	−170±24 (4)	−174±23 (9)		
8	−175±25 (4)	−170±27 (6)		

Variation expressed as standard error of the mean. Values in parentheses are numbers of experiments.

The Relation between Membrane Potential and K^+ Efflux The membrane potential of the *Nitella* cell could be clamped at any particular value using a negative feedback circuit (the voltage clamp). During 2 min step changes in membrane potential, K^+ efflux was measured simultaneously with the recording of membrane potential. The relationship between K^+ efflux and membrane potential is shown in Fig. 5 A. In these experiments the cell was perfused with 1 mM K APW of pH 5. Curve (*a*) shows an experimental membrane potential vs. K^+ efflux curve while curve (*b*) is a theoretical relationship calculated using the constant field assumption with P_K constant. K^+

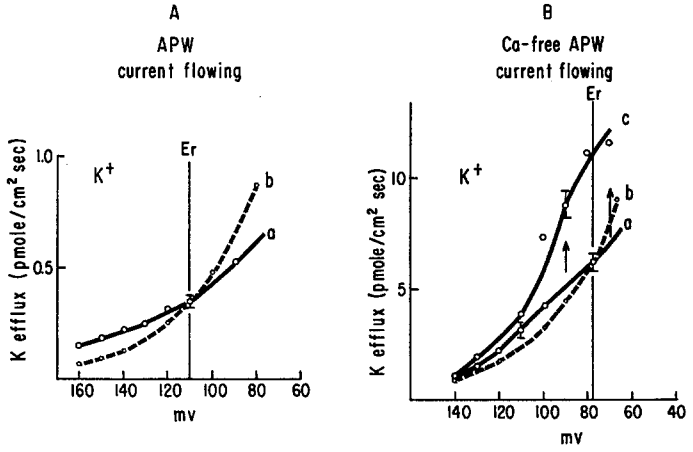

FIGURE 5. The relation between membrane potential and K^+ efflux from a cell perfused with 1 mM K APW of pH 5 (A), or from a cell perfused with Ca-free APW of pH 5 (B). Membrane potential was step-changed every 2 min by means of a negative feedback circuit. Curves (*a*) and (*c*) show the K^+ efflux vs. membrane potential relation obtained from the experiments. Curve (*b*) is a theoretical one calculated on the constant field assumption with constant P_K. In Ca-free APW the membrane potential vs. K^+ efflux curve shifted from (*a*) to (*c*) within the period of flux measurement.

efflux decreased with hyperpolarization of the membrane, but the slope of the membrane potential vs. K^+ efflux curve was not as steep as that of the theoretical curve, suggesting that in fact P_K increases with hyperpolarization.

For a *Nitella* cell in Ca-free APW, the membrane potential drifts gradually in a depolarizing direction. Fig. 5 B shows the relation between membrane potential and K^+ efflux for a cell in Ca-free APW at a pH of 5. The K^+ efflux increases about 10 times over that from control cells and the slope of the membrane potential vs. K^+ efflux curve is again close to the theoretical curve calculated for constant field conditions with constant P_K.

The Relation between K^+ Current and Membrane Current The relation between the K^+ efflux and the membrane current was examined during 2 min step changes in membrane potential. K^+ efflux was measured simultaneously

with the recording of membrane potential and membrane current. As described previously, K^+ efflux from a cell perfused with Ca-free APW is about 10 times larger than that from a cell perfused with normal APW. The relation between membrane current and I_K (the current carried by K^+) was obtained from this experiment as shown in Fig 6 A. In the case of no interaction between efflux and influx, the relation between the efflux and the influx is given by Ussing's equation (Ussing, 1949; Hodgkin and Huxley, 1952), and the net flux is given as the difference between the efflux and the influx. With these relations I_K was calculated as a product of the Faraday constant and net flux of K^+ from the values of K^+ efflux and membrane potential. Even in the Ca-free

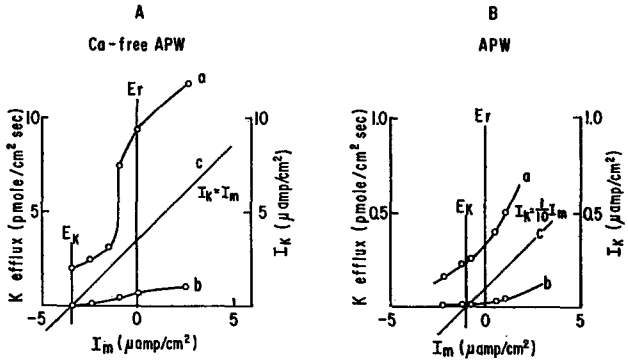

FIGURE 6. A, the relations between K^+ efflux, I_K, and the membrane current in a cell perfused with Ca-free APW. The membrane potential was step-changed by means of a negative feedback circuit. These relations were obtained from the same experiment which is shown in Fig. 5 B. Curve (*a*) shows the I_m vs. K^+ efflux relation. Curve (*b*) represents the relation I_m vs. I_K. Curve (*c*) indicates the I_m vs. I_K relation for the case in which the transport number of K^+ is 1. B, the relations between K^+ efflux, I_K, and membrane current of the cell perfused with Ca^{++} containing APW. These data were obtained from the experiment shown in Fig. 5 A. Curves (*a*) and (*b*) show the I_m vs. K^+ efflux and I_m vs. I_K relation, while curve (*c*) shows the I_m vs. I_K relation for the case in which the transport number of K^+ is 0.1.

solution, in which K^+ efflux is about 10 times the usual value in normal APW, only 20% of the passive ionic current is carried by K^+.

In normal APW the current carried by K^+ was much less than that in Ca-free APW. The relation between the membrane current and I_K in normal APW is illustrated in Fig. 6 B. As seen in this graph, I_K is about one-thirtieth of the total passive ionic current. This value is negligible compared to the total membrane current, and indicates that when Ca^{++} is removed from APW, the membrane conductance is increased about 20%. Experimentally a decrease in membrane resistance of about 20% was observed when Ca^{++} was removed from APW. When the external solution was replaced with a solution containing only KCl, the membrane resistance decreased further.

The Effect of pH on K^+ Efflux Since a change in pH of the external solution displaces the membrane potential of a *Nitella* cell, the relationship between membrane potential and K^+ efflux can be obtained by changing external pH without current flow, while the membrane potential vs. K^+ efflux relation is obtained from voltage clamp experiments under the conditions of membrane current flow.

Fig. 7 A shows the time course of membrane potential and K^+ efflux change. When the external solution was replaced by one of lower pH, the membrane potential was decreased and, simultaneously, K^+ efflux increased. The time course of K^+ efflux change followed the membrane potential change. By

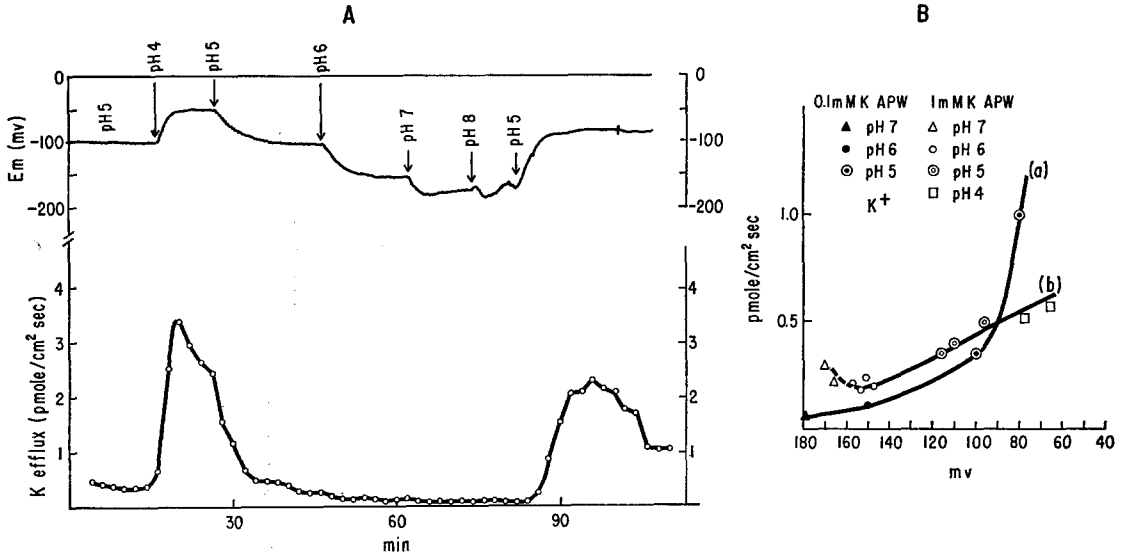

FIGURE 7. A, the simultaneous records of the time course of K^+ efflux and the membrane potential change induced by the change in the external solution. B, the relation between membrane potential in a steady state and K^+ efflux at various pH. Curve (*a*) is drawn from the experiment shown in A. Curve (*b*) is drawn from the experiment with 1 mM K^+ APW. The curve is very similar to that illustrated in Fig. 5 A.

plotting K^+ efflux with respect to the membrane potential in a steady state, a curve representing the relation between K^+ efflux and membrane potential was obtained. Two examples are shown in Fig 7 B. Curve (*a*) was drawn by using the data obtained from the experiment shown in Fig. 7 A. Curve (*b*) was drawn using data from the experiment with a solution containing 1 mM K^+, a concentration 10 times higher than that usually present in APW. The values of K^+ efflux at various values of membrane potential obtained by different methods are summarized in Table II. The membrane potential vs. K^+ efflux curve obtained from the experiments at various low pH's is very similar to that obtained from the voltage clamp experiments, while in high pH solution K^+ efflux shows a slight increase over that found with voltage clamp.

This finding would seem to indicate that P_K is not a function of the external pH but of membrane potential, if one could prove in any way that no boundary potential change is induced by pH changes. This point will be discussed later.

The K^+ efflux from a cell perfused with 1 mM K APW was 0.35 ± 0.035 pmole/cm² sec at a membrane potential of −110 mv. This value is in accord with that observed by Barr (1965) with the same species of *Nitella*. When membrane potential is equal to the K^+ equilibrium potential, the relation between K^+ efflux and the slope conductance for K^+ can be expressed as a simple equation (Hodgkin and Keynes, 1955)

$$G_K = \frac{F^2}{RT} \vec{f}_K \tag{13}$$

TABLE II

K^+ EFFLUX AT VARIOUS MEMBRANE POTENTIAL LEVELS OBTAINED BY DIFFERENT METHODS

	K^+ efflux, *pmoles/cm²sec*		
$-E_m$	Voltage clamp (in APW)	Changing external pH (in APW)	Voltage clamp (in Ca-free APW)
mv			
90	0.54±0.054	0.55±0.055	9.8±0.49
110	0.33±0.033	0.35±0.035	4.1±0.20
130	0.27±0.027	0.27±0.027	2.2±0.11
150	0.20±0.020	0.21±0.021	
170		0.28±0.028	

$[K]_o$ was 1 mM. Resting potential at pH 5 was −108 mv in APW. In Ca-free APW the resting potential at pH 5 was −78 mv. It was difficult to displace the membrane potential of the cell bathed in Ca-free APW by chainging external pH. The values of K^+ efflux at the indicated potential levels in the experiments with pH changes were interpolated from the experimental membrane potential vs. K^+ efflux relation.

Here F, R, and T have the usual meaning, and $\vec{f}_K$ is the K^+ efflux. Since the resting membrane potential at pH 5 (−109 ± 9 mv) is close to the K^+ equilibrium potential when the external K^+ concentration is 1 mM, the slope conductance for K^+ at the K^+ equilibrium potential might be considered as the K^+ conductance at resting potential. When the K^+ efflux is 0.3 pmole/cm² sec at the K^+ equilibrium potential, K^+ conductance is obtained as 1.1×10^{-6} mho/cm².

When the constant field assumption is used, K^+ efflux is expressed by the following equation

$$\vec{f}_K = -\frac{E_m F}{RT} P_K \frac{[K]_i}{e^{-E_m F/RT} - 1} \tag{14}$$

When the values of membrane potential, K^+ concentration, and K^+ efflux are inserted into this equation, the value of P_K can be calculated. When $[K]_i$

is 100 mM, the value of P_K is calculated as 4.05×10^{-8} cm/sec. This value is somewhat smaller than that obtained from flux measurements on the corticated *Chara* cell (Gaffey and Mullins, 1958).

The Effect of pH on the Action Potential Since the measured membrane potential is the sum of the boundary potential and the actual transmembrane potential within the membrane (presumably within the plasma membrane), the peak of the action potential should shift in parallel with the shift of the resting membrane potential, if the membrane potential change is due to the change in the boundary potential at the outer surface. For this reason, action potentials were elicited electrically at various resting potential levels that had resulted from changes in the external pH. As shown in Fig. 8, the peaks of action potentials were almost constant over the pH range 4.5 to 8. The threshold membrane potential also remained almost constant regardless of the

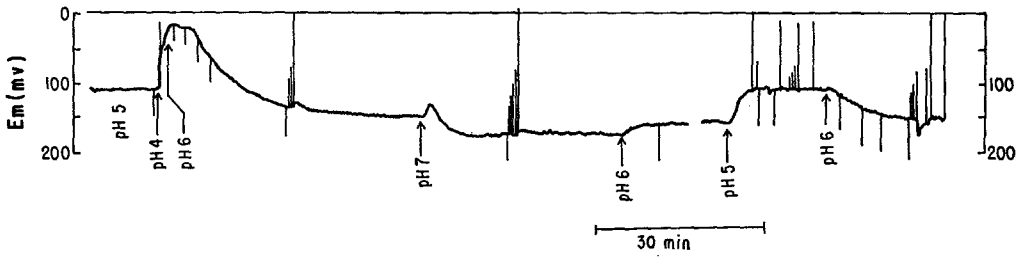

FIGURE 8. Action potential elicited from the various resting potential levels which resulted from the change in the external pH. Downward deflections of the membrane potential were caused by inward currents of constant amount of 2 μamp/cm^2. Solution was changed at the arrows.

change in the resting potential. At pH 4.5 the peak of the action potential shifted slightly to the hyperpolarized side. This may be accounted for by an increase in the inactivation of g_{Cl} induced by the long-lasting depolarization. From this finding it seems unlikely that the boundary potential changes in response to changes in pH.

Cl^- Efflux and Membrane Potential By changing the pH of the external solution the relation between Cl^- efflux and membrane potential was also examined under the condition of zero membrane current. As shown in Fig. 9 B, Cl^- efflux increased with increases in membrane potential. The Cl^- efflux at the resting potential of -110 mv was 0.5 ± 0.1 pmole/cm^2 sec, a value somewhat smaller than that obtained by others (Mullins, 1962; Hope and Findlay, 1964). When the membrane was depolarized by any means to a level less than about -80 mv, action potentials were generated and an increase in Cl^- efflux was observed.

It is impossible to displace the membrane potential to the level of the Cl^-

equilibrium potential without generating an action potential because the Cl^- equilibrium potential calculated from the Cl^- concentration in the external solution (4 mM) and that in cell sap (120 mM; Barr, 1965) is about +85 mv. For this reason slope conductance for Cl^- at E_{Cl} cannot be obtained directly from the Cl^- efflux measurement. However, Cl^- conductance at the resting potential level can be estimated from the slope of the I_{Cl} vs. E_m curve (where I_{Cl} is the current carried by Cl^-). Since the difference between resting potential and E_{Cl} is large, at the resting potential the passive Cl^- influx is almost negligible compared to Cl^- efflux. In this situation Cl^- efflux can be con-

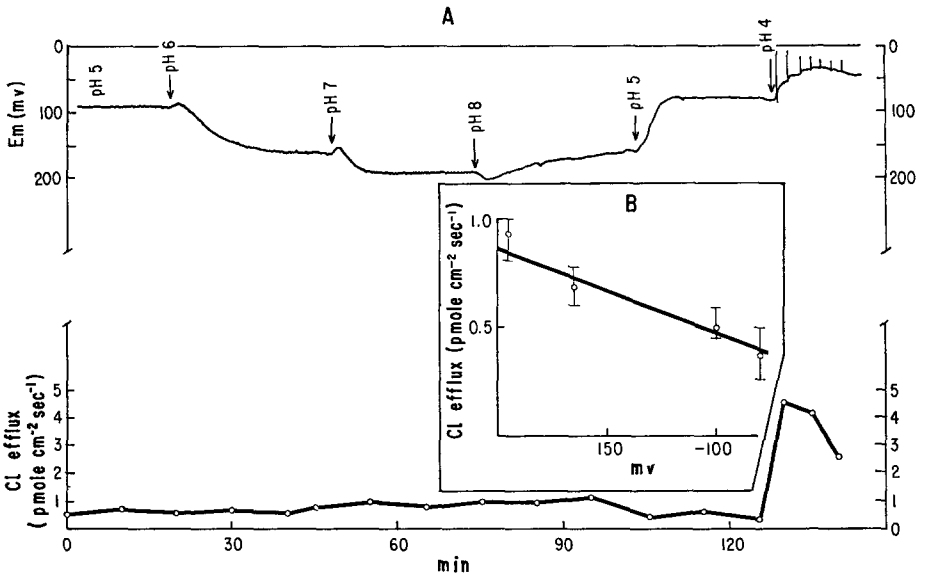

FIGURE 9. A, the simultaneous records of the membrane potential and Cl^- efflux from a cell perfused with 1 mM K APW of various pH. The solution was changed at the arrows. B, the relation between the membrane potential in the steady state and Cl^- efflux in APW of various pH.

sidered as an approximation of the passive net flux of Cl^-. As $-I_{Cl}$ is the product of the Faraday constant and net passive Cl^- flux, the membrane potential vs. Cl^- efflux curve would represent approximately the I_{Cl} vs. E_m relation. The slope conductance for Cl^-, G_{Cl}, is calculated from the slope of the curve as 3×10^{-7} mho/cm^2. The chord conductance, g_{Cl}, also was obtained from the following equation as 2.5×10^{-7} mho/cm^2

$$g_{Cl} = \frac{I_{Cl}}{E_m - E_{Cl}} \qquad (15)$$

These values of G_{Cl} and g_{Cl} are very similar.

When the constant field assumption is used, Cl^- efflux is expressed by the following equation

$$\overrightarrow{f_{Cl}} = -\frac{E_m F}{RT} P_{Cl} \frac{[Cl]_i e^{-E_m F/RT}}{e^{-E_m F/RT} - 1}$$

By inserting the values of Cl^- efflux, membrane potential, and internal Cl^- concentration, P_{Cl} (the permeability coefficient for Cl^-) is obtained as 1.04×10^{-9} cm/sec.

The Effect of Na^+ on the Membrane Potential The membrane potential was measured at various Na^+ concentrations over the range of 1 mM to 100 mM. The perfusion solution was prepared by adding Na_2SO_4 to APW of pH 6. As shown in Fig. 10 there is almost no change in resting potential with increase in $[Na]_o$. This finding suggests that the conductance for Na^+ is low compared to that for other ions. According to Barr's experiment (1965), the observed Na^+ efflux from a cell bathed in 2 mM Na APW is 0.033 pmole/cm² sec and the

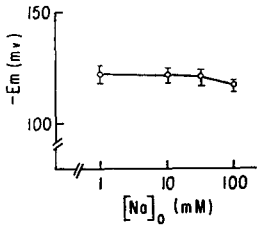

FIGURE 10. The effect of Na^+ on the resting membrane potential.

vacuolar Na^+ concentration is 22 ± 1 mM. From these values the chord conductance for Na^+, g_{Na}, is calculated as 5.6×10^{-8} mho/cm² on the assumption that there is no active inward movement, and no interaction between passive influx and efflux of Na^+. The permeability coefficient for Na^+, P_{Na}, also is calculated as 2.06×10^{-9} cm/sec using the constant field assumption. This value is very small compared to P_K.

Membrane Current under Voltage Clamp Conditions The H^+ conductance can be obtained by measuring the change in membrane current when the membrane potential is clamped at a given potential while the external pH is changed and other components are left unchanged. For convenience, the membrane potential was clamped at the K^+ equilibrium potential when $[K]_o$ was 1 mM, since at this membrane potential I_K could be eliminated and currents carried by Cl^- and Na^+ were expected to be negligible. In this situation the change in the membrane current should depend only on the change in $[H]_o$ (unless the active transport of H^+ is not affected by a change in the external pH), because the boundary potential is not influenced by the change in the external pH.

Fig. 11 shows the membrane current change induced by pH changes under the voltage clamp condition with simultaneous records of K^+ efflux. When the external solution was replaced with a solution of lower pH, the membrane current flowed inward and when it was replaced with solution of higher pH, membrane current flowed outward, while K^+ efflux remained almost constant. This finding indicates that the actual transmembrane potential was effectively clamped at a constant level. The time course of the membrane current change was nearly exponential.

When an external solution of pH 5 is replaced with a solution of pH 6, the change in the membrane current should be expressed by the following equa-

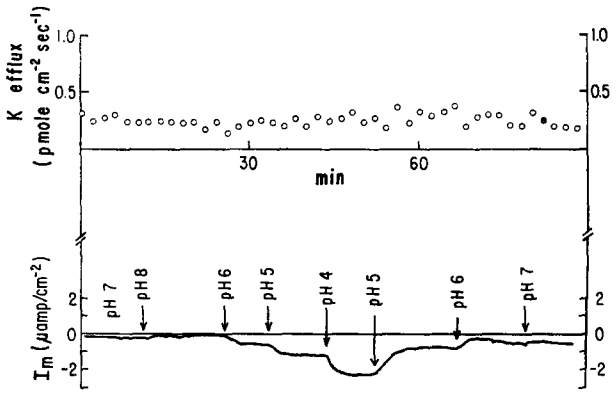

FIGURE 11. Simultaneous records of K^+ efflux and the membrane current. The membrane potential was clamped by means of a negative feedback circuit at -110 mv, while the external pH was changed at the moment indicated by the arrows. Outward current has a positive sign. Inward current has a negative sign.

tion

$$\Delta I_m = \Delta I_H = (g_H)_{pH\ 6}[E_m - (E_H)_{pH\ 6}] - (g_H)_{pH\ 5}[E_m - (E_H)_{pH\ 5}] \quad (17\,a)$$

$$= [(g_H)_{pH\ 6} - (g_H)_{pH\ 5}][E_m - (E_H)_{pH\ 5}] + 0.058(g_H)_{pH\ 6} \quad (17\,b)$$

Since the ionic conductance is a function of the ionic concentration of the solutions on both sides of the membrane, $(g_H)_{pH\ 6}$ is not always equal to $(g_H)_{pH\ 5}$. But as a rough approximation, by assuming that $(g_H)_{pH\ 6}$ is equal to $(g_H)_{pH\ 5}$, a simple equation representing the relation between the magnitude of the membrane current change and the H^+ conductance can be obtained from equation (17)

$$(g_H)_{pH\ 6} = \frac{\Delta I_m}{0.058} \quad (18)$$

Since on the average ΔI_m is 2.2 μamp/cm^2, when the external solution of pH 5 was replaced with a solution of pH 6, H^+ conductance was obtained as 3.8×10^{-5} mho/cm^2. This value is substantially equal to the average membrane conductance of a *Nitella* cell at pH 5 to 6 (4.0×10^{-5} mho/cm^2). This indicates that the membrane conductance of the cell bathed in a solution of pH 5 to 6 is mainly represented by a H^+ conductance.

The Effect of DNP on the Membrane Potential When the membrane conductance is represented by a H^+ conductance in the steady state and a strong

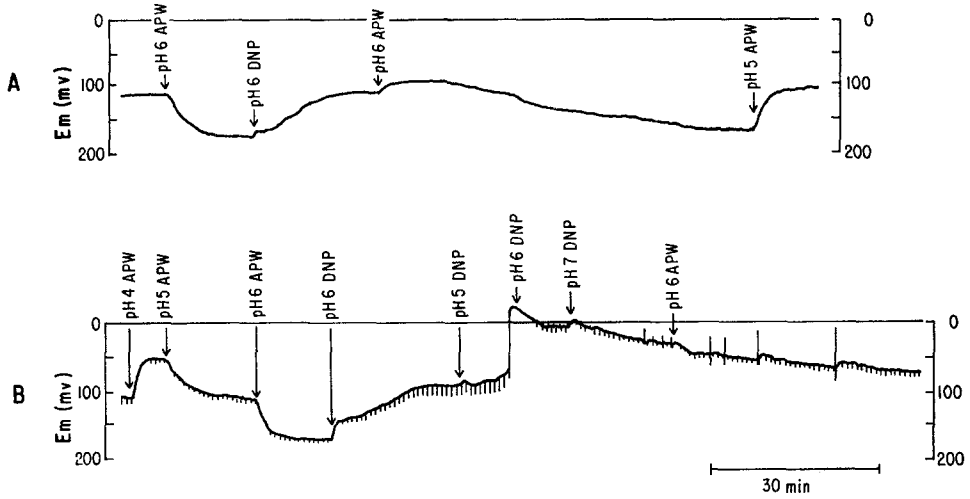

FIGURE 12. A, the effect of DNP on the membrane potential. By replacing 1 mM K APW of pH 6 with pH 6 DNP solution the membrane potential was depolarized gradually. When perfusion solution was returned to pH 6 1 mM K APW, the membrane potential slowly recovered to the normal value, after transient depolarization. Solution was changed at the arrows. All the external solutions contained 1 mM K^+. B, the change in the membrane resistance induced by DNP. Downward deflections of the membrane potential were caused by pulses of the constant inward current of 0.3 μamp/cm^2. Experiments A and B were carried out on different cells.

H^+ pump exists, the resting membrane potential should be expressed by equation (3). If some metabolic inhibitor depresses the active transport of H^+ completely, the membrane potential should depolarize to the extent of $F\phi_H/g_m$. Stated in another way, the membrane potential should reach the H^+ equilibrium potential.

In order to eliminate active H^+ movement, the *Nitella* cell was perfused with 1 mM K APW containing 0.2 mM 2,4-dinitrophenol (DNP solution). When 1 mM K APW of pH 6 was replaced with DNP solution of the same pH, the membrane potential depolarized progressively and as shown in Fig. 12 reached a steady membrane potential of about −80 mv 20 min after the

beginning of perfusion with the DNP solution. After reaching the steady potential level, the DNP solution was replaced with 1 mM K APW. The membrane potential then recovered gradually after transient depolarization (Fig. 12 A). It took 2 hr or more to recover completely. After replacing DNP solution with 1 mM K APW the most depolarized potential level was −60 mv. This value is in quite good agreement with the H^+ equilibrium potential expected from intracellular pH as measured by a micro pH glass electrode (Hirakawa and Yoshimura, 1964).

This finding seems to provide good evidence for the support of the speculation that the membrane potential of the *Nitella* cell is maintained by the H^+ pump. However, some problems remain concerning the fact that the internal potential of the cell during perfusion with the DNP solution of pH 6 was not exactly equal to the H^+ equilibrium potential but was more negative than the H^+ equilibrium potential, while the membrane potential of the cell perfused with 1 mM K APW of the same pH after administration of DNP was much closer to the H^+ equilibrium potential. If DNP depresses the permeability for H^+ without affecting P_K in addition to its effect on the metabolic process, the internal potential of the cell treated with DNP should be more negative than the H^+ equilibrium potential, because at pH 6 the K^+ equilibrium potential is larger than the H^+ equilibrium potential by about 50 mv. If the membrane conductance is mainly H^+ conductance, the decrease in H^+ conductance should result in an appreciable increase in membrane resistance.

Membrane resistance was estimated by measuring membrane potential displacement in response to pulses of constant inward current. One of the experiments is shown in Fig. 12 B. Membrane resistance increased gradually with time from the beginning of the perfusion with DNP solution, and then reached a steady value. The time course of membrane resistance change was almost in parallel with the alteration in the membrane potential change. The steady value of the membrane resistance during the perfusion with the DNP solution of pH 6 was 5 to 10 times larger than that of a normal cell at pH 6. This finding is quite similar to the resistance change in *Neurospora* observed during the administration of DNP (Slayman, 1965 *b*).

When a DNP solution of pH 6 was replaced by one of pH 5, the internal potential shifted in a depolarizing direction very slowly. After some time when the progressive depolarization had reached the threshold potential level for an action potential, the internal potential shifted suddenly to the level of +15 mv, a potential level more positive than the peak of the usual action potential. When the membrane potential reached this level, the membrane resistance decreased suddenly. When the pH 5 DNP solution was replaced with one of pH 6, the membrane potential shifted from +15 mv to −10 mv, a level much lower than that found during the initial perfusion with pH 6 DNP (with no prior pH 5 DNP perfusion). In addition, the membrane resistance was lower

than its previous value at pH 6. When the pH 6 DNP solution was replaced with one of pH 7, the internal potential shifted to the more negative side, but to an extent much less than 58 mv. Upon replacement of the DNP solution by 1 mM K APW, the level of the membrane resistance gradually returned to the initial value. Complete recovery of the membrane resistance took 4 or more hr.

The decrease in membrane resistance observed when internal potential shifted beyond threshold potential to a more positive level with the administration of pH 5 DNP solution might be interpreted as an increase in conductances for ions related to excitation (such as Cl^- and K^+), although the increase in membrane resistance by the perfusion with pH 6 DNP solution might be a result of a decrease in H^+ conductance.

TABLE III

CONSTANTS CONCERNING THE PERMEABILITY OF *Nitella* CELL IN APW (PH 5–6)

Ion species	Slope conductance G	Chord conductance g	Permeability coefficient P
	mho/cm²	*mho/cm²*	*cm/sec*
K^+	1.2×10^{-6}	1.2×10^{-6}	4.05×10^{-8}
Na^+	(5.6×10^{-8})	5.6×10^{-8}	2.06×10^{-9}
Cl^-	3×10^{-7}	2.5×10^{-7}	1.04×10^{-9}

The values of the permeability coefficient were calculated from the values of effluxes on the constant field assumption without considering any interaction between influx and efflux. The constants for Na^+ were calculated from the value of efflux (0.033 pmole/cm²sec; Barr, 1965) which would include passive efflux and active flux. Slope conductance for Na^+ shown in parentheses was assumed from the value of the chord conductance in analogy to other ion conductances.

DISCUSSION

The *Nitella* cell has a resting membrane conductance of the order of 40 μmho/cm² while the fluxes for the ions Na^+, K^+, and Cl^- yield a calculated membrane conductance of less than 2 μmho/cm², as summarized in Table III. This discrepancy between electrical and flux measurements is so large that it is difficult to ascribe it to the differing experimental conditions under which fluxes and electrical measurements were made. Indeed, it seems more likely that the discrepancy is a reflection of the fact that ions other than Na^+, K^+, and Cl^- carry a major fraction of the membrane current under the conditions in which conductance measurements are made. There are some instances in which the resting membrane conductances based on tracer experiments and those based on electrical measurements are different. Usually the resting K^+ conductance determined from flux measurement is smaller than the value measured electrically. For example the discrepancy is observed for the muscle fiber (Keynes, 1954; Hodgkin and Horowicz, 1959 *a*, *b*), Purkinje fiber

(Weidmann, 1952; Carmeliet, 1961), and frog auricle (Haas and Glitsch, 1962). For frog auricle muscle exchange diffusion (Haas, 1964) has been used to explain this discrepancy. For the *Nitella* cell, the discrepancy between K^+ conductance calculated from tracer experiment and the membrane conductance at rest is much larger than that observed in other cells.

The experiments reported in this paper were designed to determine the ion species which carried the major fraction of membrane current. The fact that in the range of pH 4 to 6 the internal potential shifts toward zero at a rate of 56 mv per 1 pH unit decrease, could not simply be explained by the evidence that the plasma membrane of *Nitella* is predominantly permeable to H^+. For example, the measured membrane potential could change if either some potential change occurred at the boundary between the cell membrane and the external solution, or if the active transport rate of some electrogenic ionic pump were changed. To reach the conclusion that H^+ carries the major part of the membrane current, it is necessary to eliminate the other possibilities that must be considered.

Hodgkin and Chandler (1965) suggested that the shift of the inactivation curve of the squid giant axon when it is perfused intracellularly with low ionic strength solution, such as 4 mM KCl plus sucrose, could be due to a change in the phase boundary potential at the inner surface of the membrane. Since the ionic strength of the solution normally bathing a *Nitella* cell is low (1 – 2 mM), a considerable potential difference must be expected at the boundary between the external solution and the membrane. It is possible that changes in pH could affect the ionization of specific groupings so as to alter the phase boundary potential without affecting the actual transmembrane potential within the membrane. However, this possibility seems unlikely because of the finding that the membrane potential vs. K^+ efflux relation obtained with the external pH changes was quite similar to the relationship between membrane potential and K^+ efflux from voltage clamp experiments. This finding suggests that changes in the external pH might result in the change in the actual transmembrane potential itself. Probably, more decisive evidence might be found in experiments investigating pH effect on action potential. If there were some changes in phase boundary potential resulting from changes in pH, one would expect either shifts in the resting potential without change in the amplitude of the action potential, or parallel shifts in the threshold potential level for generating the action potential; in fact the peaks of action potentials were almost constant over the pH range 4.5 to 8, and the threshold membrane potential level also remained almost constant (Fig. 8). From these findings the possibility of the change in phase boundary potential might be eliminated from the factors responsible for membrane potential change.

There should be some discussion about the effect of Tris buffer (which was used in this experiment) on the membrane potential. Van Steveninck (1965,

1966) reported that, in the presence of Tris buffer at a pH greater than 6, freshly sliced beetroot tissue accumulated K^+ from KCl solution at a greatly accelerated rate from the beginning of replacement with a solution containing Tris buffer, and yet Cl^- accumulation did not take place until many hours later when the tissue slices had aged. This pronounced effect of Tris buffer on cation fluxes was thought to be related to the active transport of such ions. However, the accumulation of cations also could be expected in the *Nitella* cell when the external solution was changed to a solution of higher pH, because the hyperpolarization resulting from the replacement with the higher pH solution should result in the net entry of cation into the cell. One might argue that the Tris cation might carry an appreciable fraction of membrane current. But this possibility could be eliminated since the change in membrane current observed under the voltage clamp increases with $[H]_o$ while the fraction of Tris in the ionic form ($pK_a = 8.3$) is almost unity in the range of pH lower than 6. At pH 5 to 6 the H^+ chord conductance estimated by the membrane current change under the voltage clamp condition was substantially equal to the membrane conductance. Kishimoto (1964) calculated from his voltage clamp experiments that the membrane resistance, assuming no leakage current, is 250 kohms cm^2 at rest (which would accord with the resistance for K^+), while the ordinary membrane resistance is within the range of 10-70 kohms cm^2. By comparing these values with the present experimental results, one can conclude that the so-called leakage current obtained by the usual voltage clamp experiment on the *Nitella* cell might correspond to the H^+ current.

The pH of the cell sap measured by various methods is within 5 to 6 (Albaum et al., 1937; Baily and Zirkle, 1931; Hirakawa and Yoshimura, 1964). The measured internal potential is not equal to the H^+ equilibrium potential calculated from the H^+ concentrations inside and outside, but is about 100 $\pm$ 25 mv more negative than E_H. Two membranes exist between the external solution and the internal solution or cell sap (the plasma membrane and the tonoplast) and some potential difference between protoplasm and cell sap might be expected. In *Chara australis* the potential level in the cell sap is positive to the extent of about 10 mv with respect to that in protoplasm (Findlay and Hope, 1964). This potential difference is opposite in sign to that expected and too small to account for the discrepancy between the resting membrane potential and the H^+ equilibrium potential.

Since the cell membrane of the *Nitella* cell appears to be highly permeable to H^+, the discrepancy between resting membrane potential and E_H must result in considerable passive inward H^+ current. The quantity of H^+ moving passively across the cell membrane is calculated to be about 4×10^{-11} moles/cm^2 sec, or 0.72×10^{-11} mole/sec for unit length of the cell (of 0.06 cm diameter), while moles of H^+ in cell sap of unit length are 2.7×10^{-11} moles

for the case in which the internal pH is 5. From a comparison of the value of passive H^+ movement and the moles of H^+ in cell sap, it is clear that a H^+ extrusion mechanism is necessary if intracellular pH is to be held at its observed level of about 5. The transport rate of the H^+ pump should be much larger than the sum of transport rates of the Na^+ pump and the Cl^- pump. There are some circumstances in which the sum of ion fluxes actively transported might not be zero, as for example in the muscle fibers recovering from Na loading (Mullins and Noda, 1963; Mullins and Awad, 1965), and in the stretch receptor neuron of crayfish when it is recovering from repetitive firing (Nakajima and Takahashi, 1966). A similar situation was suspected in spinal motoneurons (Ito and Oshima, 1964).

When an ionic pump moves electric charges without coupling to any other ionic pump, electric current must flow across the cell membrane. When the membrane potential is in a steady state the sum of the currents resulting from active transport and from passive fluxes should be zero. Under these circumstances, the membrane potential is not given by the well-known Goldman equation, because the derivation of this equation requires that the sum of the passive currents be zero. In this situation passive currents could be expressed by the following:

$$\Sigma z_{j\pm} F f_{j\pm} = I_m = g_m[E_m - (E_m)_o] \qquad (19)$$

where $(E_m)_o$ is the assumed membrane potential when the sum of the passive currents is zero. From equation (19) one may obtain an equation which represents the membrane potential when the sum of the passive currents is not zero:

$$E_m = (E_m)_o + \frac{F\Sigma z_{j\pm} f_{j\pm}}{g_m} = (E_m)_o + \Delta E_m \qquad (20)$$

ΔE_m is the membrane potential displacement from $(E_m)_o$ resulting from electrogenic ionic pumps, and g_m is the chord membrane conductance.

The existence of an electrogenic H^+ pump cannot be directly demonstrated by radioisotope measurements and for this reason some features of the problem remain unclear. It is impossible to be certain that no boundary potential exists at the interphase between the external solution and the plasma membrane. While the evidence reported in this paper shows that the boundary potential does not change with pH, it is still possible to imagine boundary potentials that might be unaffected by pH changes. The experimental fact is that the $[K]_i$ is such as to suggest that K^+ is distributed passively across the cell membrane. Stated in another way, the H^+ pump is working to accumulate K^+ in the cell by keeping the membrane potential more negative than E_H. One could argue, however, that K^+ are not distributed passively, but are also pumped inward (possibly by a Na-K–coupled pump) (Barr,

1965), because this coupled pump has no effect on the second term of equation (20). It would also be expected that the membrane potential would be almost equal to the K^+ equilibrium potential in the case in which the transport rate by the Na-K–coupled pump is not so large as the rate by H^+ pump.

Perhaps the effect of DNP is the most conclusive evidence for a metabolic component of the observed membrane potential of the *Nitella* cell. Such a substance could not be expected to alter a phase boundary potential, but it could clearly be expected to uncouple phosphorylation in the cell and so decrease the energy stores available to an ionic pump.

The membrane potential when the sum of passive ionic currents is zero, $(E_m)_o$, can be expressed by the Goldman equation. If the potential displace-

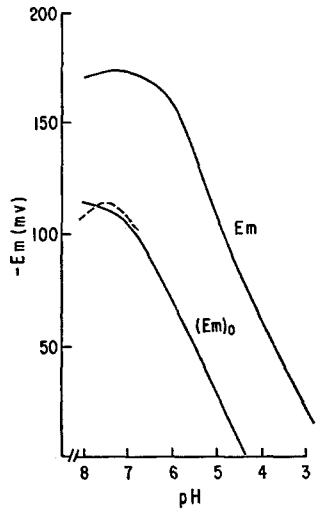

FIGURE 13. The relation between observed membrane potential, E_m, and the assumed membrane potential, $(E_m)_o$, at various pH. The solid curve of $(E_m)_o$ was drawn by assuming values of P_H, P_K, and P_{Cl} as 10^{-3}, 10^{-7}, and 10^{-9} cm/sec, respectively. The dashed curve indicates the pH vs. $(E_m)_o$ relation for the case in which P_K is increased twofold at a pH higher than 7 and P_{Cl} increased twofold at pH 8.

ment from $(E_m)_o$ resulting from electrogenic active transport is constant over the range of pH 4 to 8, the pH vs. membrane potential curve should be parallel with the pH vs. $(E_m)_o$ curve. The solid curve shown in Fig. 13 was drawn by using values of P_H, P_K, and P_{Cl} as 10^{-3}, 10^{-7}, and 10^{-9} cm/sec, respectively on the assumption that $[H]_i$, $[K]_i$, and $[Cl]_i$ are constant over the range of pH. The value of P_H was estimated from the value of g_H with constant field assumptions. The dashed curve in Fig. 13 indicates the relation for the case in which P_K is increased twofold at a pH higher than 7 and P_{Cl} is increased twofold at pH 8. This curve is almost parallel with the pH vs. membrane potential curve. However, there must be some discussion regarding whether the potential displacement from the $(E_m)_o$ would remain constant even at high pH, because it is presumed that the decrease in $[H]_i$ caused by the decrease in inward H^+ movement would result in a halt in H^+ pump activity. Experimentally the gradual decrease in membrane potential was observed when the

Nitella cell was bathed in a solution of pH 8 (Figs. 7–9). At high pH such as pH 9 the values of $P_H[H]_o$ and $P_H[H]_i$ are small compared with the values of $P_K[K]_o$ and $P_K[K]_i$, respectively. In this situation $(E_m)_o$ should be substantially equal to E_K, and passive membrane current caused by ΔE_m resulting from the remaining H^+ pump activity might not be carried by H^+ but mainly by K^+; i.e., this cell is in a position to gain K, while the internal pH should become higher. It seems that with the decrease in the H^+ active transport rate the membrane potential finally reaches E_K, whose value is larger than the value calculated from the usual value of $[K]_i$ at pH 5 to 6. The final value of E_K may not be estimated precisely, but it might be larger than the usual E_K and smaller than the usual $E_K + 100$ mv.

The permeability coefficient for H^+ estimated from the experimental data in this paper is extremely high compared to the usual ionic permeability coefficient. For example, the P_K of frog muscle fiber is of the order of 10^{-5} cm/sec (Adrian and Freygang, 1962), and similarly that of frog myelinated nerve at resting level is of the order of 10^{-5} cm/sec while at E_{Na} it is calculated to be of the order of 10^{-3} cm/sec from voltage clamp experiments (Frankenhaeuser, 1962). However, the permeability coefficient of the *Nitella* cell for the water molecule is calculated to be of the order of 10^{-2} cm/sec from the osmosis experiment of Kamiya and Tazawa (1956). A similar result has been obtained on the *Chara* cell by a more accurate method (Dainty and Hope, 1959; Dainty and Ginzburg, 1964 *a*), and the permeability coefficient for methanol is calculated to be about 3×10^{-4} cm/sec by different methods (Zwolinski, Eyring, and Reese, 1949; Dainty and Ginzburg, 1964 *b*). It is suggested that the value of the permeability coefficient for H^+ estimated from this experiment ranges between the values of the permeability coefficient for water and that of methanol (which has less capacity for hydrogen bonding than water).

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