

Potassium Channels in Myelinated Nerve

Selective permeability to small cations

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ABSTRACT The permeability of K channels to various cations is studied in myelinated nerve. Ionic currents under voltage clamp are measured in Ringer solution containing tetrodotoxin and a high concentration of the test ion. Reversal potentials for current in K channels are determined and used with the Goldman-Hodgkin-Katz equation to calculate relative permeabilities. The ratios $P_{\text{Tl}}:P_{\text{K}}:P_{\text{Rb}}:P_{\text{NH}_4}$ are 2.3:1.00:0.92:0.13. No other ions are found to be measurably permeant including Li^+ , Na^+ , Cs^+ , methylamine, guanidine, hydrazine, or hydroxylamine. The ratio $P_{\text{Na}}/P_{\text{K}}$ is less than 0.01. Potassium conductance is depressed at pH values below 5.0. Leakage conductance is higher in K, Rb, Cs, NH_4 , and Tl Ringer than in Na Ringer, but the selectivity sequence probably is not the same as for K channels. The hypothesis is offered that the narrowest part of the K channel is a circle of oxygen atoms about 3 Å in diameter with low electrostatic field strength.

INTRODUCTION

This paper reports measurements of the relative permeability of potassium channels to small cations. The study was undertaken because the ionic selectivity of potassium channels is virtually unknown yet essential to any attempt to formulate a molecular picture of the channel. Results are also given on the ionic dependence of leakage conductance and on the pH dependence of potassium currents. Some of this work has appeared in preliminary form (Hille, 1972 *a*).

METHODS

Nerve and Recording

The methods and some errors are described in detail elsewhere (Hille, 1971). Briefly, single myelinated nerve fibers from *Rana pipiens* are studied under voltage clamp conditions at 15°C. The currents shown in all figures are corrected for leakage assuming a pure ohmic leakage conductance. Capacity currents are not subtracted. The solution bathing the outside of the node of Ranvier under investigation can be changed in a

few seconds. The concentration of ions in the axoplasm can also be changed by cutting the internode at either end in solutions of different composition (Koppenhöfer and Vogel, 1969). The exchange of ions across the cut end takes at least 5 min and probably is rarely complete, so the internal ionic concentrations are not known with any precision. There is some evidence that the cut "heals" over and becomes relatively impermeable often in less than 10 min.

Rationale of Selectivity Measurement

Ionic selectivity of potassium channels is measured by the criterion used in previous papers on the selectivity of sodium channels (Hille, 1971, 1972 *b*). The reversal potential, E_r (zero current potential), for current in potassium channels is measured in a potassium-containing solution and then in a potassium-free solution containing the test cation X . The permeability ratio P_X/P_K is calculated from the change in E_r using the relation:

$$E_{r,X} - E_{r,K} = 2.303 \frac{RT}{F} \log_{10} \frac{P_X(X)}{P_K(K)},$$

where $2.303 RT/F$ is 57.2 mV at 15°C, and the parentheses indicate ionic activities. With the normal high internal potassium concentration, $E_{r,X}$ for poorly permeant test cations is quite negative. However, permeability ratios are best measured if both $E_{r,X}$ and $E_{r,K}$ are more positive than -40 mV, because at more negative potentials most of the potassium channels close. To make $E_{r,X}$ more positive in these experiments, the internal potassium concentration is lowered by cutting the ends of the fiber in 120 mM NH_4Cl or in isotonic mixtures of KCl with tetramethylammonium bromide (TMA·Br).

Solutions and Ionic Activities

The standard Ringer solution (called Na Ringer) contains 115 mM NaCl, 2 mM CaCl_2 , 55 nM tetrodotoxin, and 1 mM tris(hydroxymethyl)aminomethane buffer, pH = 7.4. By measurement the tetrodotoxin blocks more than 90% of the sodium channels. In the test solutions some or all of the NaCl is replaced with an osmotically equivalent amount of test salt. The following salts are tested: LiCl, KCl, RbCl, CsCl, TiNO_3 , NH_4Cl , methylamine·HCl, hydroxylamine·HCl, a mixture of hydrazine·2HCl and hydrazine, formamidine acetate, and guanidine·HCl. Isotonic solutions of these salts contain less than 150 μM sodium or potassium contamination. The pH of the hydroxylamine solution is lowered to 6.00 to ionize 50% of the test molecules. All other Ringer solutions have a final measured pH between 6.9 and 7.4. The TI Ringer is made entirely from nitrate salts and tested on the nerve in comparison with Na Ringer and K Ringer also made from nitrate salts. For convenience, the test solutions containing some sodium are designated simply as, e.g., $\frac{1}{8}$ K Ringer to mean a mixture of 1 part K Ringer and 7 parts Na Ringer. Only four ions are measurably permeant in potassium channels: K^+ , Rb^+ , NH_4^+ , and Ti^+ . At the concentrations used in these experiments the activity coefficients for these ions are assumed to be in the ratios 1.00:0.99:1.00:0.90 (Robinson and Stokes, 1965). Activity coefficient corrections are not used for the impermeant ions.

Another set of experiments measures the depression of steady-state potassium currents by external solutions of low pH. A 5 mM glycylglycine-piperazine buffer is used instead of Tris buffer. Otherwise the solutions are the same as the standard Na Ringer used in selectivity measurements.

A final set of experiments measures the effect of cations on the leakage conductance. Here all the NaCl is replaced by isotonic concentrations of the test salt. These solutions all contain 6 mM tetraethylammonium bromide to block potassium channels. The composition of these solutions is given in detail in Hille (1971, 1972 *b*).

Currents and Potentials

Outward membrane currents are called positive. Membrane potentials are given as inside potential minus outside with the nominal holding potential being -80 mV. As before (Hille, 1971) corrections for measured junction potentials in the "A" pool ranging from -0.4 to $+1.5$ mV and for the "attenuation artifact" are applied to all measurements. The attenuation correction is 8.0 % for the $260\text{ }\mu\text{m}$ B pool width used. Absolute potential levels are not known reliably and probably should not be compared from one fiber to the next. One source of variation in these experiments is the variety of solutions used at the cut ends of the fiber. These introduce uncontrolled liquid junction potentials in the seals and electrodes as well as a junction potential with axoplasm that probably depends on whether a new membrane has formed over the cut end.

The reversal potential measurements involve a voltage clamp in two steps. First a depolarizing prepulse to near -30 mV opens most of the potassium channels. Then test pulses to different voltages measure the current-voltage relations of potassium channels. This two step procedure is used because some of the reversal potentials are close to the holding potential (-90 mV) where few potassium channels would be open in the steady state. The depolarizing prepulse also inactivates sodium channels not already blocked by tetrodotoxin.

An experimental artifact complicates the measurement of reversal potential. If a large ionic current is driven through potassium channels, local accumulation or depletion of permeant ions can develop. Armstrong and Hille (1972) argue that the inward tail of current sometimes seen upon repolarization of a node from a large and long depolarizing pulse is due to accumulation of K^+ ions just outside the node. The tail, called I_p by Frankenhaeuser (1963), decays with a time constant on the order of 1 ms, appreciably shorter than the time constant for closing potassium channels at rest. Presumably the concentration of accumulated ions falls with a time constant of 1 ms. Because of the inward I_p , current-voltage measurements made during this first millisecond in Na Ringer could give the false impression that potassium channels are appreciably permeable to sodium ions. Similar errors are well-known for potassium accumulation in the Frankenhaeuser-Hodgkin (1957) space of squid giant axons, except that the time constant of clearing that space is 30–100 ms.

Three procedures in my experiments reduce the error caused by external accumulation. (*a*) The prepulse is kept as small as possible consonant with opening enough potassium channels. (*b*) Most of the potassium in the axoplasm is replaced by other less permeant ions, to decrease the outward current during the prepulse. (*c*) All current-voltage relations used to obtain reversal potentials are measured both early

(300 μ s) and late (8–18 ms) in the test pulse. Any difference in the reversal potential between these times would suggest relaxation of an undesirable ionic accumulation. With the precautions described, the reversal potentials measured at these two times generally agree within 3 mV and rarely suggest a measurable systematic error. Finally the node is kept most of the time in Na Ringer to minimize resting ionic fluxes which might slowly change the concentration of internal cations.

RESULTS

K⁺, Rb⁺, Tl⁺, and NH₄⁺ are Permeant in Potassium Channels

Voltage clamp measurements in NH₄⁺ Ringer illustrate the two step method for obtaining current-voltage relations. The current records in Fig. 1 are from a node bathed in NH₄⁺ Ringer. Both internodes are cut in a solution containing 6 mM KCl and 114 mM TMA·Br. About 50% of the potassium channels are opened by a 40 ms prepulse to -31.4 mV, giving the small outward current indicated at the left in the figure. The rest of the figure is taken up by the variable test pulse and the return to the holding potential. The test pulse to -47.6 mV is just a few millivolts below the zero current potential. At more negative test potentials, currents flow inward and decay as potassium channels close. Inward currents are carried by NH₄⁺ ions. At the most positive test

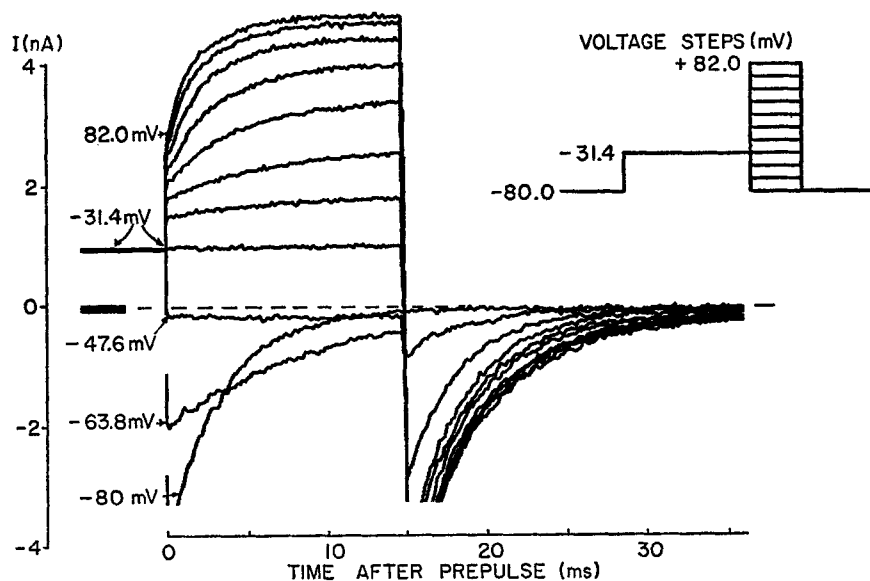


FIGURE 1. Voltage clamp currents for a node bathed in NH₄⁺ Ringer. A 40 ms prepulse to -31.4 mV has opened slightly more than half the potassium channels. The membrane potential is then clamped to 11 different test potentials for 15 ms and then returned to the holding potential, as in the inset. The largest "tails" of inward NH₄⁺ current reach -7 nA but are cut off in the picture. Ends of the fiber are cut in 6 mM KCl + 114 mM TMA·Br. Node number 3 (Table I).

potentials, currents flow outward and grow as more potassium channels open. Outward currents are carried by K^+ ions alone, since tetramethylammonium ions are impermeant. At the reversal potential, the influx of NH_4^+ and the efflux of K^+ are equal.

So-called isochronal current-voltage relations are used to measure the reversal potentials. Fig. 2 (left) shows current-voltage relations obtained at 0.3, 2, 4, and 18 ms after the beginning of the test pulse in a fiber in NH_4 Ringer. The essential point is that the reversal potential, the intersection of the curves with the voltage axis, does not change over 18 ms. Although the

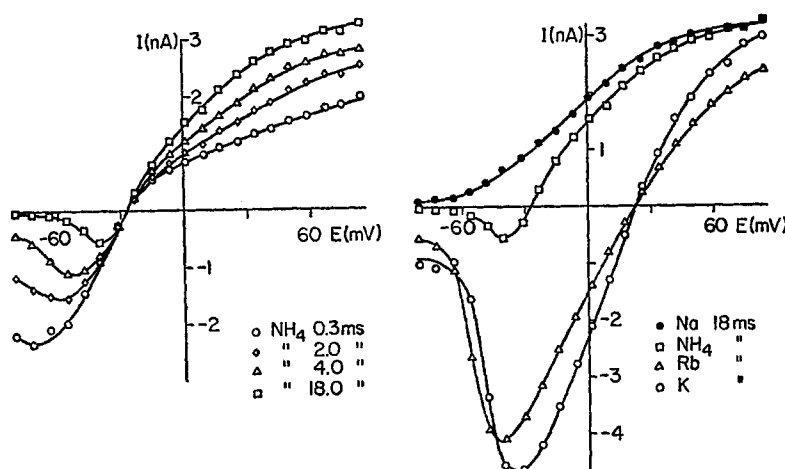


FIGURE 2. Isochronal current-voltage relations for a node bathed in Na, NH_4 , Rb, and K Ringer. The node is depolarized by a 50 ms prepulse to -31.4 mV to open potassium channels and then stepped to various test potentials as in Fig. 1. The points show the size of current in potassium channels at the indicated time after the start of the test pulse. Ends of the fiber are cut in 6 mM KCl + 114 mM TMA·Br. Node number 2 (Table I).

node and reversal potentials are different, the original current traces for this figure are quite similar to those in Fig. 1. Fig. 2 (right) shows the current-voltage relations at 18 ms for the same fiber bathed in Na Ringer, NH_4 Ringer, Rb Ringer, and K Ringer. In these experiments, a more positive reversal potential means a more permeant ion, so by inspection of the figure, the sequence of permeability is $K^+ \approx Rb^+ > NH_4^+ > Na^+$. Indeed the absence of any inward currents in Na Ringer means that Na^+ ions are not measurably permeant in this experiment.

The reversal potential in K Ringer is high ($+23.7$ mV) as expected with only 6 mM KCl at the cut ends. Taken literally, this potential indicates that the internal K^+ concentration has dropped to 44 mM. The reversal potentials of the node in Fig. 1 indicate about 90 mM K^+ inside, a difference perhaps due to little K^+ exchange at the cut end and to the uncertainty in the position of

true zero millivolts. In both nodes the steady-state outward I_K at high potentials is smaller than in fibers cut in 120 mM KCl. The currents at +82 mV are only 3 and 4.5 nA, while after cutting in 120 mM KCl in other experiments the current averages 17 nA. The large drop in I_K after cutting in the low-potassium solution is due partly to the reduction in axoplasmic K^+ concentration and partly to a voltage-dependent block of potassium channels by internal tetramethylammonium ions.

All measurements of reversal potentials with permeant ions are summarized in Table I together with the relative permeability P_X/P_K for each ion calcu-

TABLE I
PERMEABILITY RATIOS FOR PERMEANT CATIONS IN THE
POTASSIUM CHANNEL

Node number	[K ⁺] at cut end	External reference solution				
		K	½ K	K	½ K	½ K
		External test solution				
		Rb	½ Rb	NH ₄	NH ₄	½ Tl
Change of reversal potential*						
	<i>mM</i>			<i>mV</i>		
1	12	—	—	-54.3‡	—	—
2	6	1.0	—	-48.5	—	—
3	6	—	—	—	3.1	—
4	6	—	—	—	3.8	—
5	120	-6.3	—	-47.7	—	—
6	60	—	-1.3‡	—	-1.7‡	—
7	60	—	—	—	—	20.3
8	60	—	—	—	—	15.4
9	60	—	—	—	—	19.4‡
Mean change, <i>mV</i>		-2.7	-1.3	-50.2	1.7	18.4
<i>P_X/P_K</i>		0.90	0.95	0.13	0.13	2.3

* Reversal potential in test solution minus reversal potential in reference solution.

‡ Mean of two measurements.

lated from the Goldman-Hodgkin-Katz equation. To test for errors due to ionic accumulations or other factors, the experimental conditions are varied. The ends of the fibers are cut in solutions of different potassium concentration and the external reference and test solutions contain different concentrations of permeant ion. These variations, however, reveal no appreciable systematic errors. In summary the relative permeabilities to Tl⁺, K⁺, Rb⁺, and NH₄⁺ are in the ratios 2.3:1.00:0.92:0.13. For each solution the range in measured reversal potential change is about ±3 mV, corresponding to ±13% error in the permeability ratios.

The "independence principle" is not obeyed in my experiments. For example, axons bathed in NH₄ Ringer and in ½ K Ringer have the same

reversal potential and according to independence should also have the same current-voltage relations. Nevertheless the ratio of inward current "tails" for nodes repolarized to -80 mV from large depolarizations in NH_4 Ringer and $\frac{1}{8}$ K Ringer is 2.3 ± 0.2 (mean \pm SD), i.e., the inward NH_4^+ currents are larger than expected. By contrast, Rb^+ currents are smaller than expected by 25–35%. Even outward K^+ currents are smaller in Rb Ringer than in K Ringer (Fig. 2). Thus $P_{\text{NH}_4}/P_{\text{K}}$ would be overestimated and $P_{\text{Rb}}/P_{\text{K}}$, underestimated by applying independence to current amplitudes.

Three Alkali Cations and Many Small Organic Cations are Relatively Impermeant

It is not possible to determine reversal potentials for ions giving no detectable inward currents. Here E_r must be more negative than the most negative potential at which a net outward current can be observed in potassium channels. The limits of measurement are then determined by how small an outward current can be resolved and still be proven to pass through potassium channels. In these experiments current differences of 50 pA are resolved and a test Ringer with 6 mM tetraethylammonium ion plus the appropriate test ion is included as a control to check that the observed currents are flowing in potassium channels. These controls show that current in potassium channels defined as current blocked by tetraethylammonium ion agrees within 50 pA with the current obtained by subtraction of calculated leakage current from total measured current. In addition the ends of the fibers are cut in 120 mM NH_4Cl . Internal NH_4^+ ion has two advantages over K^+ diluted with other ions. Ammonium gives large outward currents without block, and yet, even after large depolarizations, the inward tail of current I_p is small. Presumably some NH_4^+ ions accumulate outside the node during a large depolarization, but being only 13% as permeant as K^+ ions, the accumulated ions have much less influence on the electromotive force in potassium channels.

Current-voltage relations for a node in Na Ringer and in NH_4 Ringer are compared in Fig. 3 (left). In NH_4 Ringer the reversal potential for current in potassium channels is close to 0 mV. Taken literally, this means that almost all the internal K^+ ion has exchanged for NH_4^+ ion across the cut ends. In Na Ringer a clear outward current carried by NH_4^+ ions is seen at all potentials above -60 mV. The outward current at 0 mV in Na Ringer is several times larger than the independence principle predicts for an NH_4 -filled node. The same current-voltage relations are expanded on the right in Fig. 3 to show the small currents around -60 mV better. Two arrows marked 1% and 2% show the value of reversal potential corresponding to permeability ratios $P_{\text{Na}}/P_{\text{K}}$ of 0.01 and 0.02. The positions of the arrows are calculated from the reversal potential in NH_4 Ringer assuming that P_{K} is 7.5 times P_{NH_4} as was found in other experiments (Table I). Since in several experiments outward currents are seen down to the 1% arrow, I conclude that $P_{\text{Na}}/P_{\text{K}}$ is

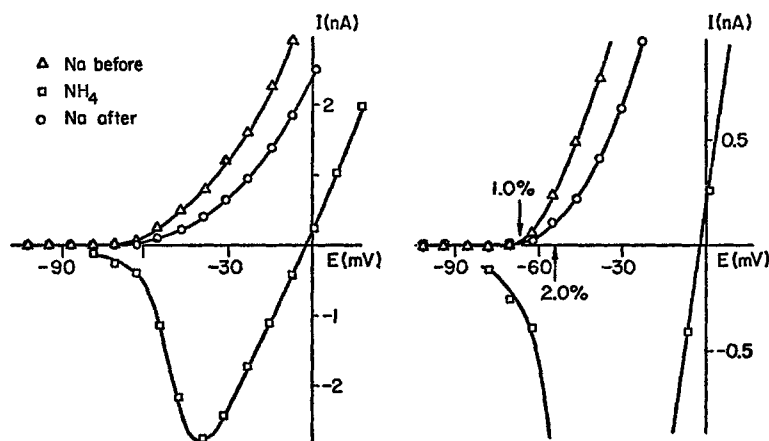


FIGURE 3. Isochronal current-voltage relations for a node in Na and NH_4 Ringer. The node is depolarized 15 ms by a prepulse to -31.4 mV and then stepped to various test potentials as in Fig. 1. The points (left) show the current in potassium channels 13 ms after the start of the test pulse. The right hand side shows the same currents on an expanded scale. In this experiment the currents measured 1.3 ms after the start of the test pulse were within 50 pA of the currents after 13 ms. Controls with 6 mM tetraethylammonium to block potassium channels verified that the horizontal line is within 50 pA of the base line. The arrows show the predicted reversal potentials in Na Ringer if $P_{\text{Na}}/P_{\text{K}}$ were 0.02 and 0.01. Ends of the fiber are cut in 120 mM NH_4Cl .

not larger than 0.01. How much smaller the sodium permeability ratio is cannot be determined from these experiments.

Table II summarizes the permeability ratios for all cations tested. Most values are upper limits, determined as for Na^+ ion without any measurable reversal potential. The ions are arranged in order of increasing size using a method of measurement described in the Discussion. The measurably permeant ions fall between 2.66 and 3.0 Å in diameter. Seven of the "impermeant" ions are certainly no more than 3% as permeant as K^+ ions. External cesium tends to block potassium channels particularly at the negative potentials where outward current must be resolved to establish a low limit on permeability. This block might explain why the measured permeability limit for cesium is not as low as for other ions.

Low External pH Reduces Potassium Currents

The next part of the results is a search for evidence of acidic or basic chemical groups associated with potassium channels. The pH of the bathing solution is varied and I_{K} at the end of a 20 ms depolarization is determined about 1 min after the pH change. Control measurements at pH 7.4 precede and follow each exposure to a different pH. While most of the effects of pH changes reverse on return to pH 7.4, the control values after exposure to pH 4.5 or 4.2 are often lower than the previous control.

The open circles of Fig. 4 show the pH dependence of the amplitude of I_K recorded at +82 mV. All points are normalized to set the current at a pH of 7.4 equal to 100%. The smooth curve is the prediction if the conductance of potassium channels were controlled by the degree of ionization of single acid group with a pK_a of 4.4. This theory is only a poor fit as it predicts a steeper pH-dependence than is observed.

The effect of low pH on I_K is more complicated than implied so far. As is known from previous work, at low pH the membrane must be depolarized

TABLE II
PERMEABILITY RATIOS FOR ALL CATIONS IN THE
POTASSIUM CHANNEL

P_X/P_K	X	Minimum pore diameter*
		\AA
<0.018†	Lithium	1.20
<0.010†	Sodium	1.90
1.00	Potassium	2.66
2.3	Thallium	2.80
0.91	Rubidium	2.96
0.13	Ammonium	3.0
<0.029†	Hydrazine	3.3
<0.025†	Hydroxylamine	3.3
<0.077†	Cesium	3.38
<0.021†	Methylamine	3.6
<0.020†	Formamidine	3.6
<0.013†	Guanidine	4.8

* Measured from atomic models with a pentagonal pore described in Discussion section.

† Permeabilities measured using NH_4 Ringer as a reference and calculated as $P_X/P_K = 0.13 \times P_X/P_{\text{NH}_4}$.

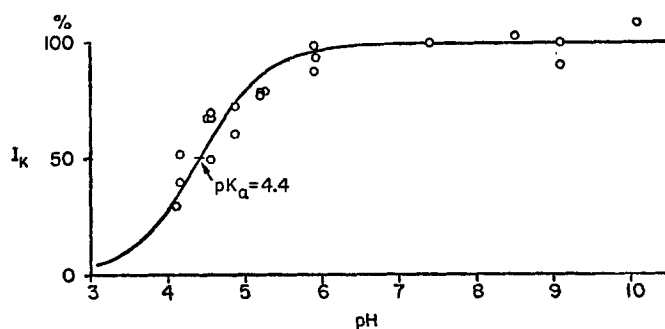


FIGURE 4. Block of potassium outward currents at low pH. The circles show the pH dependence of the amplitude of steady-state potassium currents at +82 mV relative to pH 7.4. Data from seven fibers all with ends cut in 120 mM KCl. The smooth curve is the prediction if each potassium channel were blocked by the protonation of an acid group with a pK_a of 4.4.

more to open potassium channels (Hille, 1968; Drouin and The, 1969; Mozhayeva and Naumov, 1970). This calcium-like shift of the opening of channels is also evident in my experiments. In addition, however, the block by protons at large depolarizations is voltage dependent. For example, in one experiment lowering the pH to 5.20 reduces I_K by 22% at +82 mV and by 30% at +18 mV. Because of this voltage dependence, the apparent pK_a of the "titration curve" depends on the voltage at which the current ratios are measured. The more positive the voltage, the lower the apparent pK_a .

Monovalent Cations Affect the Leakage Conductance

The final part of the Results deals with the ionic selectivity of the leakage conductance. Leakage conductance is measured from the step in membrane current during a step of membrane potential from -125 to -80 mV. In order to reduce the contribution of potassium channels to this current, the membrane is held at -125 mV for 40 ms before the measurement and all solutions contain 6 mM tetraethylammonium ion. These measurements were all made in conjunction with earlier published experiments on the ionic selectivity of sodium channels (Hille, 1971, 1972 *b*).

Table III summarizes the observations normalized to the leakage conductance in standard sodium Ringer with tetraethylammonium. The "depolarizing ions" K^+ , Cs^+ , NH_4^+ , Rb^+ , and Tl^+ all increase leakage conductance. The same ions also give steady inward leakage currents at -80 mV. The relative size of this steady current increases in the sequence $Na < Tl \approx \frac{1}{8} K < NH_4 < Cs \approx K$, the same as the sequence of leakage conductance. Neither the absolute size of this current nor the reversal potential for leakage channels was measured, so this information is insufficient to calculate permeability ratios from the Goldman-Hodgkin-Katz equation. All other sodium-free solutions including Li^+ and 15 organic cations decrease the leakage conductance by 5–10%. The organics include four guanidines, five hydroxy and methyl derivatives of ammonium, two amidines, imidazole, and choline (Hille, 1971).

A few observations on switching from NaCl to NaBr or $NaNO_3$ reveal no

TABLE III
EFFECT OF MAJOR CATIONS ON LEAKAGE CONDUCTANCE

Relative leakage conductance (Mean \pm SD)	Cation
119 \pm 10	Potassium
119 \pm 8	Cesium
111 \pm 5	Ammonium
110 \pm 5	Rubidium
105 \pm 7	Thallium
100	Sodium
94 \pm 4	Lithium

systematic effect of anions at the 10% level. In 12 measurements the normalized leakage conductance in tetramethylammonium bromide Ringer is 94 ± 4 (mean \pm SD). Most of the measurements of leakage were made on fibers with ends cut in 120 mM KCl and some on fibers cut in 120 mM CsF. There was no systematic difference in results with these two procedures. (There is also no evidence to date on whether anions like Cl^- or F^- enter the axoplasm from a cut end.)

DISCUSSION

Comparison with Previous Work

SELECTIVITY My observations agree with the few permeability ratios reported in the literature. Previous reports fall into three categories: calculations from amplitudes of outward currents using expressions derived from the independence principle and calculations from reversal potentials and resting potentials using the Goldman-Hodgkin-Katz equation. Of these methods the resting potential is the least specific, because all ionic channels made a contribution to the resting potential, and the most common, because resting potentials are easiest to measure. Hagiwara et al. (1972) give the most extensive results with this method, reporting $P_{\text{Tl}}:P_{\text{K}}:P_{\text{Rb}}:P_{\text{NH}_4}:P_{\text{Cs}}$ as 1.8:1.0:0.71:0.25:0.18 in the squid giant axon. Except for the high value for P_{Cs} , these values are close to mine (Table I). The calculated P_{Cs} may be in part an artifact reflecting the block of resting P_{K} by added Cs^+ ions as well as a possible contribution of Cs to leakage conductance in squid. Hagiwara et al. summarize other resting potential measurements from the literature giving values for P_{Cs} and P_{Rb} similar to theirs. Binstock and Lecar (1969) calculate a value of 0.2 for $P_{\text{NH}_4}/P_{\text{K}}$ from the underswing of an action potential elicited in NH_4Cl artificial seawater. I have found no systematic investigation of reversal potentials under voltage clamp conditions. With squid axons in Rb seawater Moore et al. (1966) report a reversal potential sometimes (but not always) like that in K seawater, i.e., $P_{\text{Rb}}/P_{\text{K}}$ close to 1.0.

In systems obeying the independence principle, permeability ratios can be calculated from the ratio of current amplitudes with the test and reference solutions. Since reversal potentials predicted by independence are the same as those predicted by the Goldman-Hodgkin-Katz relation, the two methods give the same permeability ratios. In direct violation of the independence principle, however, outward potassium movements in potassium channels are reduced in the presence of internal Li^+ , Na^+ , Rb^+ , or Cs^+ (Chandler and Meves, 1965; Adelman and Senft, 1966; Bezanilla and Armstrong, 1972). Voltage-dependent block of the channels by these ions blurs the meaning of permeability ratios calculated from independence, but should not affect ratios calculated from reversal potentials. Bezanilla and Armstrong (1972) measured the amplitude of late outward currents in squid giant axons perfused with

pure 275 mM RbF or NaF. Application of the independence principle to the magnitude of the currents gives a value of 0.25 for $P_{\text{Rb}}/P_{\text{K}}$ and less than 0.06 for $P_{\text{Na}}/P_{\text{K}}$. The low value for $P_{\text{Rb}}/P_{\text{K}}$ probably reflects block of Rb^+ fluxes by Rb^+ ions.

The deviation from independence is different in the case of NH_4^+ . Lüttgau (1961) concluded from current clamp measurements on nodes of Ranvier that NH_4^+ ions (and Rb^+) can pass through potassium channels. Binstock and Lecar (1969) compared the late outward current in squid giant axons perfused internally with KF and NH_4F . Applying the independence principle gave a value of 0.26 for $P_{\text{NH}_4}/P_{\text{K}}$. Their calculation accords with my observation that both inward and outward ammonium currents are larger than is predicted from the value of $P_{\text{NH}_4}/P_{\text{K}}$ (0.13) obtained from reversal potential measurements.

In systems not obeying independence, the word "permeability" evidently acquires two different meanings depending on the criterion of measurement. This difference has hardly been explored in the theoretical literature, so these phenomena remain uninterpreted for the moment. In the meantime the existence of the difference must be recognized to decrease the confusion engendered by using one word for two concepts. Work is now in progress on a model system with several ion binding sites, single file flow, and saturation kinetics which can reproduce some of the phenomena seen. Discussion of this model is reserved for a later paper.

BLOCK AT LOW pH My observation on the depression of potassium currents by low pH confirms the work of Drouin and The (1969). They suggest that the current is controlled by the dissociation of a weak acid with a pK_a of 4.6. My experiments are fitted poorly by assuming a single group with a pK_a of 4.4. A better fit could be obtained by assuming that the degree of titration of several neighboring groups affects the conductance. These groups could be part of the "surface fixed charge" of the membrane. The scatter of the data however does not justify an extended analysis. The apparent voltage dependence of the block at high voltages in my experiments can also be detected in the current-voltage relations given by Drouin and The. A similar voltage dependence of the block of sodium channels at low pH suggests a Boltzmann distribution of hydrogen ions between the external medium and a binding site part way across the membrane within the sodium channel (Woodhull, 1973). Perhaps the effect in potassium channels has a similar explanation.

Mozhayeva and Naumov (1970, 1972) also studied potassium conductances at low pH. Their measurements on nodes of Ranvier in isotonic K Ringer use extremely slow (2 mV/s) voltage ramps and include the effects of inactivation of potassium channels brought on by the many seconds of depolarization. Mozhayeva and Naumov find less reduction of potassium conductance than is

found with fast voltage clamping in Na Ringer. In their work the conductance at pH 4 is still more than 60% of the control value at pH 7, and the depression spreads over a much wider pH range than would be fitted by the dissociation of a single acid. Their schematic curves given for potassium conductance show no voltage dependence of the depression at higher voltages.

The pH measurements are included in this paper because they offer some evidence, albeit negative, on the "chemistry" of potassium channels. The "titration" curve (Fig. 4) indicates that no groups with pK_a 's between 5 and 10 lie near enough to the channels to alter potassium conductance. Thus there are no partially ionized groups titrating at physiological pH. Functionally this means that there can be no competition (ion exchange) between H^+ ions and K^+ ions in normal conditions. Chemically this implies that there are no imidazole, phosphate, or di- or polycarboxylic acid groups in the mouth of the channel. By all measurements, the apparent pK_a is 4.6 or less, significantly lower than the apparent pK_a of 5.2 for block of sodium channels (Hille, 1968; Drouin and The, 1969). Woodhull (1973) gives evidence that the titratable group governing sodium permeability is actually within the pore of the sodium channel. In the language of Eisenman (1962), potassium channels have a lower effective field strength than sodium channels.

LEAKAGE CONDUCTANCE Leakage is the current left over after the obviously time- and voltage-dependent sodium and potassium currents are subtracted from voltage clamp records. In the node the leak is clearly physiological. Since sodium channels and potassium channels are virtually entirely closed in resting nodes of Ranvier, the leak is essential to establish the normal resting potential. Dissection of single fibers does not seem to change the leak because the measured RC membrane time constant is like that estimated from strength-duration curves for external stimulation in nerve trunks and because the measured leak is about the value required in computer predictions of conduction velocity from voltage clamp parameters (Goldman and Albus, 1968).

The normal leakage potential of the node of Ranvier is probably between -70 and -80 mV (resting potential, Huxley and Stämpfli, 1951). Frankenhaeuser and Huxley (1964) used -70 mV and Dodge (1963), -75 mV. Dodge (1963) summarized arguments that this leakage is primarily a potassium conductance, citing flux measurements and experiments with changes of anions and external potassium. Frankenhaeuser (1962) saw no change in total outward current in a clamp step to E_{Na} when chloride was replaced by methylsulphate or isethionate. Koppenhöfer (1965) measured current-voltage relations under current clamp when chloride was replaced with nitrate or thiocyanate. In the hyperpolarizing direction the constancy of the slope and extrapolated intercept of the I/V relation indicate no change of leakage potential or conductance. I confirm this result with nitrate, bromide, and

iodide. Dodge (1963) showed that the leakage potential falls to 0 mV and the leakage conductance increases on bathing a node in K Ringer. Finally, in contrast to the squid, the major outward current during the action potential of the node is (by calculation) leakage current and nevertheless Asano and Hurlbut (1958) found a net loss of potassium matching the net gain of sodium in active sciatic nerves. All of these experiments argue for potassium and against anions in the leak.

My observations suggest that the leak accepts several metal cations and ammonium. Cations larger than cesium may be excluded. Lithium seems also to be excluded while sodium can at least increase the leakage conductance relative to lithium and large organic cations. The inability of either internal or external tetraethylammonium ion to block the leak and the sequence $\text{Cs} \approx \text{K} > \text{NH}_4 > \text{Tl}$ for leak imply that leakage is not simply a voltage-independent modification of K channels ($\text{Tl} > \text{K} > \text{NH}_4 > \text{Cs}$). Indeed no treatment except raising the divalent ion concentration (or irreversible breakage) seems to have much effect on the leak in nodes. Although the evidence is incomplete, leakage channels seem to be potassium-preferring cation channels with less ionic selectivity than potassium channels. A resting potential of -75 mV corresponds to a selectivity ratio $P_{\text{Na}}/P_{\text{K}}$ of 0.05.

Possible Explanation for Selectivity

In an earlier study of the sodium channel, seven organic cations and three metal cations were found to be permeant (Hille, 1971, 1972 *b*). Particularly with the organic ions the results offered enough geometrical and chemical information to suggest a specific hypothesis for the selectivity filter of the sodium channel. In the present study, only one organic cation and three metal cations are measurably permeant. This information is insufficient to elaborate detailed hypotheses and could presumably be explained in numerous ways.

This discussion assumes that the channel is a long aqueous pore. Evidence for a pore comes from the ratio of K^+ influx to K^+ efflux in the potassium channel of squid giant axons. The measured ratio differs from that expected from free diffusion in a manner explainable if the potassium channel were a long, narrow pore containing several K^+ ions crossing the membrane in single file (Hodgkin and Keynes, 1955). Further evidence for a pore comes from the channel-blocking effects of internally applied quaternary ammonium ions both in squid and in frog node (Armstrong, 1971; Armstrong and Hille, 1972). Because there may be several K^+ binding sites and numerous energy barriers for ions to cross in the channel, ionic selectivity may be a multi-stage process.

A tentative hypothesis can be offered which, like the model previously proposed for sodium channels (Hille, 1971, 1972 *b*), uses geometric, chemical, and energetic factors in distinguishing ions. In brief the potassium channel

is assumed to be a pore with some region as narrow as 3.0–3.3 Å. The narrow part includes several oxygen-containing groups. The mean “electrostatic field strength” of the channel is low. Bezanilla and Armstrong (1972) have proposed about the same model.

SIZE OF THE PORE Table II lists the cations tested in the order of a “minimum pore diameter” required to let them pass. The minimum pore diameter is defined in the following arbitrary way. Atomic models of the ions are constructed from Corey-Pauling-Koltun (CPK) models (Koltun, 1965). Five oxygen atoms representing the pore are arranged in a perfect pentagon, and the size of the pentagon is adjusted until the CPK model of the cation can just pass through the hole in the middle. The diameter of the pore is measured from the diameter of the circle which just touches the inner van der Waal’s surface of all oxygens. The centers of the oxygens lie 1.4 Å outside this circle. Since the test pore is made of oxygen atoms, the cation models are built with hydrogen-bonding hydrogens where appropriate.

The suggested pore diameter of 3.0–3.3 Å explains some of the ionic selectivity of potassium channels. Specifically the impermeability to all cations below ammonium on Table II is accounted for on geometric grounds. They are too large. The permeability to K^+ , Tl^+ , and Rb^+ is understandable on geometric grounds, and the permeability to NH_4^+ is understandable because hydrogen bonds to oxygens of the pore reduce the effective radius of the cation to a suitable size. At most three oxygens of the pore can form bonds to one NH_4^+ ion, so at least two of the five hypothetical atoms forming the pore need not be oxygen atoms.

FIELD STRENGTH Geometric factors alone do not account for the strong selectivity against the small Na^+ and Li^+ ions. Electrostatic theories have been used to explain equilibrium ion exchange selectivity favoring large cations (Eisenman, 1962). There the preference for larger cations means that more work is required to move small cations from water to the binding site than large cations. Because more work is always required to remove water from small cations than from large cations, selectivity favoring large cations must occur whenever the site does not provide a much stronger attraction for small cations than for large cations. As Eisenman and coworkers have analysed in great detail, such a site could be an atom or group of atoms with low surface charge density (large radius or charge diluted over several atoms) or it could be a weak dipole, group of weak dipoles, or dipoles of any strength at some distance away (Eisenman et al., 1971). The properties of the site are summarized by the phrase “low field strength.”

Permeability ratios in aqueous pores are more complicated than ion exchange equilibria. Bionic potentials depend both on equilibrium affinities for the “sites” in a pore and on the ability to move to and from the site. Bezanilla and Armstrong (1972) discuss this question with a one-site, two-

barrier model of potassium channels. Computer tests on a two-site, three-barrier model show that fluxes are quite complex functions of the barrier heights and equilibrium properties of the sites. This problem will be spelled out in detail elsewhere. Thus whether the selection against Na^+ and Li^+ is accomplished by low field strength barriers, low field strength sites, or both is not resolved by the observations given here. A plausible suggestion given by Bezanilla and Armstrong (1972) is that the narrow part of the channel is a barrier to sodium flow because the dipoles (carbonyl groups) of the wall are held rigidly at the diameter of a K^+ ion (2.66 Å) and cannot all approach the small Na^+ ion (diameter 1.90) as closely as the dipoles of water can in solution. This is equivalent to the statement that for Na^+ ions the channel presents a barrier of lower equivalent field strength than water.

Eisenman and Krasne (1973) make a different suggestion. They collated equilibrium thermochemical heats of transfer of ions between different solvents. The different solvent molecules have oxygen atoms of different dipole strength: hydroxyl, ether, amide, ester, etc. They show that the selectivity sequence of the potassium channel (Hille, 1972 *a*), particularly the special positions of Tl^+ and NH_4^+ is like that for transfer of ions from water to a solvent with sixfold coordination and oxygens "more ether-like than ester carbonyls . . . possibly amide carbonyls from the backbone of membrane proteins." This approach would be useful if the dipoles of the channel are free to move and can be pulled in by small ions and pushed back by large ones. Both such a flexible channel or the rigid channel of Bezanilla and Armstrong seem consistent with available information.

REMARKS ON THE PORE The narrowest part of the postulated potassium channel (3 Å) is smaller than the narrowest part (3×5 Å) of the sodium channel postulated before (Hille, 1971, 1972 *b*). This forces the permeating cations to abandon all but two water molecules in the potassium channel as against retaining three to four waters in the sodium channel.

In this hypothesis the selection against Na^+ is accomplished in a narrow (3 Å) part of the pore. Other evidence indicates that a short part of the inner end of the pore is wide enough to accommodate blocking quaternary ammonium ions (diameters ca. 8 Å) and that a longer part will accept impermeant sodium, cesium, and methylamine (Armstrong, 1971; Armstrong and Hille, 1972; Bezanilla and Armstrong, 1972; Hille, unpublished). Some of the K^+ sites needed for single file behavior could be in these parts of the channel. The K^+ ions would be kept from passing by each other by mutual repulsion despite the increased width of the channel. To keep one K^+ ion from repelling all other K^+ ions from the channel, there probably are several negative charges (ionized acids) in the length of the pore. According to the low effective pK_a for titration, these negative charges are not very close together.

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