

# The Permeability of Endplate Channels to Monovalent and Divalent Metal Cations

DAVID J. ADAMS, TERRY M. DWYER, and BERTIL HILLE

From the Department of Physiology and Biophysics, SJ-40, University of Washington, Seattle, Washington 98195. Dr. Dwyer's present address is Department of Physiology and Biophysics, University of Mississippi, Jackson, Mississippi 39216.

**ABSTRACT** The relative permeability of endplate channels to monovalent and divalent metal ions was determined from reversal potentials. Thallium is the most permeant ion with a permeability ratio relative to  $\text{Na}^+$  of 2.5. The selectivity among alkali metals is weak with a sequence,  $\text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$ , and permeability ratios of 1.4, 1.3, 1.1, 1.0, and 0.9. The selectivity among divalent ions is also weak, with a sequence for alkaline earths of  $\text{Mg}^{++} > \text{Ca}^{++} > \text{Ba}^{++} > \text{Sr}^{++}$ . The transition metal ions  $\text{Mn}^{++}$ ,  $\text{Co}^{++}$ ,  $\text{Ni}^{++}$ ,  $\text{Zn}^{++}$ , and  $\text{Cd}^{++}$  are also permeant. Permeability ratios for divalent ions decreased as the concentration of divalent ion was increased in a manner consistent with the negative surface potential theory of Lewis (1979 *J. Physiol. (Lond.)*, **286**: 417–445). With 20 mM  $\text{XCl}_2$  and 85.5 mM glucosamine·HCl in the external solution, the apparent permeability ratios for the alkaline earth cations ( $X^{++}$ ) are in the range 0.18–0.25. Alkali metal ions see the endplate channel as a water-filled, neutral pore without high-field-strength sites inside. Their permeability sequence is the same as their aqueous mobility sequence. Divalent ions, however, have a permeability sequence almost opposite from their mobility sequence and must experience some interaction with groups in the channel. In addition, the concentrations of monovalent and divalent ions are increased near the channel mouth by a weak negative surface potential.

## INTRODUCTION

Endplate channels are permeable to many inorganic cations. Fatt and Katz (1951) found that the normal electrochemical driving force at the endplate reverses sign near 0 mV, rather than near the equilibrium potential of one of the major physiological ions, and they proposed that acetylcholine causes the endplate membrane to “break down” and become permeable to all ions. Since that time the physiologically important permeant ions have been shown to be  $\text{Na}^+$  and  $\text{K}^+$ , and all alkali metal ions and several divalent cations have been found to be permeant. In this paper we further characterize the permeation mechanism of endplate channels by measuring the relative permeabilities for the alkali metal cations,  $\text{Tl}^+$  ions, alkaline earth cations, five divalent transition metal ions, and six anions. The selectivity among the cations is extremely weak, and reversal potentials with mixtures of monovalent metal salts are well

described by the Goldman-Hodgkin-Katz voltage equation (Goldman, 1943; Hodgkin and Katz, 1949). Anions are negligibly permeant. The results are consistent with a pore of large diameter with neighboring negative charges of low field strength. A preliminary report of this work has been given (Dwyer et al., 1979).

## METHODS

### *Cation Selectivity Experiments*

The voltage clamp techniques are the same as in the preceding article (Dwyer et al., 1980): ionic currents are recorded from endplate regions of frog muscle fibers in response to iontophoretic "puffs" of acetylcholine (ACh). In most experiments the ends of the fiber were cut in an "internal" solution containing 115 mM NaF and 10 mM L-histidine (pH = 7.6). The control extracellular solution contained 114 mM NaCl, 1.0 mM CaCl<sub>2</sub>, and 10 mM histidine (pH = 7.6), and the test solutions for monovalent test ions were similar except that all of the NaCl was replaced by an osmotically equivalent amount of the test salt. The following salts of monovalent cations were used: LiCl, KCl, RbCl, CsCl, and TlNO<sub>3</sub>. In the experiments with thallous ions, the control and test solutions and the agar bridge to the extracellular reference electrode were made with nitrate salts. The test solutions for divalent ions contained only 80 or 83.5 mM divalent chloride and 10 mM histidine or 20-21 mM divalent chloride, 85.5 mM of the relatively impermeant glucosamine·HCl, and 10 mM histidine. The following salts of divalent cations were studied: MgCl<sub>2</sub>, CaCl<sub>2</sub>, SrCl<sub>2</sub>, BaCl<sub>2</sub>, MnCl<sub>2</sub>, CoCl<sub>2</sub>, NiCl<sub>2</sub>, ZnCl<sub>2</sub>, and CdCl<sub>2</sub>. For one series of experiments with divalent ions the "internal" solution contained no significantly permeant monovalent ion and consisted of 60 mM magnesium citrate (an acidic 1:1 salt) and 120 mM histidine, pH = 5.8. The external control solution in these experiments contained 60 mM MgCl<sub>2</sub>, 28.5 mM glucosamine·HCl, and 10 mM histidine.

Relative permeabilities for monovalent cations in endplate channels were calculated from the mean change of reversal potential upon switching from the control solution to the test solution, using Eq. 1 of the preceding article (Dwyer et al., 1980). All reversal potential measurements were corrected for junction potentials from the test solutions to the external bath electrode. For divalent ions and mixtures of divalent ions with monovalent ions, the following general implicit function relating reversal potential  $E_r$  and permeabilities  $P_j$  for the  $j$  relevant ions was solved by the Newton-Raphson technique,

$$0 = \sum_j P_j z_j \frac{E_r F^2}{RT} \frac{[j]_o - [j]_i \exp(z_j F E_r / RT)}{1 - \exp(z_j F E_r / RT)}, \quad (1)$$

where  $R$ ,  $T$ , and  $F$  are the usual thermodynamic quantities with  $RT/F = 24.66$  mV at 12°C,  $z_j$  is the valence and  $[j]_o$  and  $[j]_i$  are the external and internal ion concentrations. This method simply solves for the zero-current potential of the appropriate Goldman-Hodgkin-Katz current expressions (Goldman, 1943; Hodgkin and Katz, 1949). The calculations given in the tables are apparent permeability ratios not taking into account activity coefficients or surface potential effects. Activity coefficients are taken into account in the Discussion, and there the single ion activity coefficient of a divalent ion  $X^{++}$  in a pure solution of a salt  $XCl_2$  is considered to be the square of the mean activity coefficient of the salt (Butler, 1968), and the activity coefficient in mixtures follows Table I of Butler (1968).

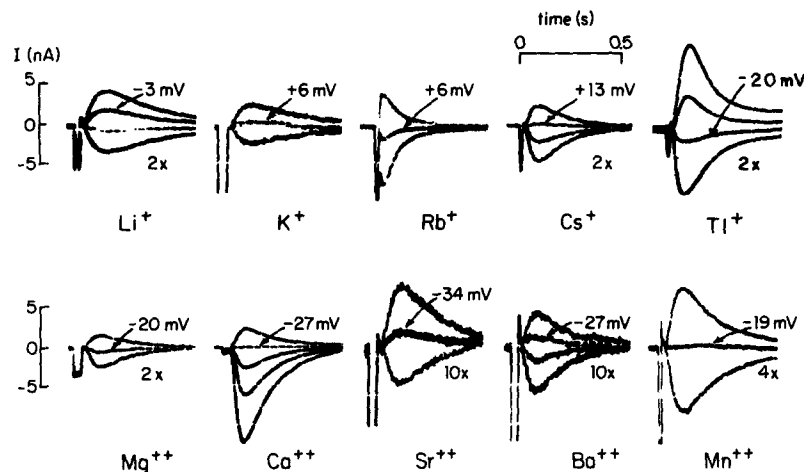
*Anion Selectivity Experiments*

Reversal potentials were measured in external solutions containing D-glucuronate, D,L-aspartate, acetate, nitrate, chloride, or fluoride as the only anion. The glucuronate-containing solution served as the reference solution that was applied between each test solution. All external solutions contained 115 mM L-arginine, 5 mM Na<sup>+</sup>, and no divalent ions or additional buffers. Several of the solutions were initially acidic and a small additional quantity of arginine free base was added to bring the final pH values to 6.5–7.1. The cut ends of the muscle fibers were bathed in 120 mM arginine-aspartate at pH 6.0. The reversal potential changes measured in the different solutions were corrected both for junction potential differences and for the Na<sup>+</sup> activity differences of the external solutions measured with a Corning No. 476210 Na<sup>+</sup>-sensitive electrode (NAS 11-18 glass, Corning Glass Works, Corning, N.Y.).

## RESULTS

*Thallos Ions and All Alkali Metal Ions Are Highly Permeant*

**PERMEABILITY RATIOS** The endplate channel discriminates poorly among small monovalent metal ions. The upper half of Fig. 1 shows records of



**FIGURE 1.** Reversal potential measurements with monovalent and divalent metal cations at the endplate. Currents in response to brief iontophoretic puffs of acetylcholine were recorded during step polarizations away from 0 mV. (Upper) Endplate currents in solutions containing 114 mM LiCl, KCl, RbCl, CsCl, or TlNO<sub>3</sub>, recorded at 7-mV intervals on either side of the reversal potential. (Lower) Endplate currents in solutions containing 20 mM of the chloride salt of a divalent metal and 85.5 mM glucosamine·HCl. Voltage steps for records in MgCl<sub>2</sub> and BaCl<sub>2</sub> were at 7-mV intervals, MnCl<sub>2</sub> at 14-mV intervals, and steps for CaCl<sub>2</sub> were to -13, -27, -41, -55, -71 mV and SrCl<sub>2</sub> to -20, -34, -41 mV. The notations "2X," "4X," and "10X" mean that a higher gain (2, 4, and 10X) was used than that corresponding to the calibration mark. Internal solution: 115 NaF. Temperature 12°C.

endplate currents in test solutions containing 114 mM monovalent test ions. Reversal potential changes,  $\Delta E_r$ , determined from such records using 114 mM  $\text{Na}^+$  as a reference are summarized in Table I. The permeability ratios calculated using Eq. 1 of the preceding article (Dwyer et al., 1980) are in the sequence  $\text{Tl}^+ > \text{Cs}^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+$ . With the alkali metals, the sequence of permeabilities is like that of the free solution mobility ratios of the ions, which range from 0.77 for  $\text{Li}^+$  to 1.54 for  $\text{Cs}^+$ , relative to  $\text{Na}^+$  (Robinson and Stokes, 1965). Thallous ions stand out as having a significantly higher relative permeability ( $P_{\text{Tl}}/P_{\text{Na}} = 2.51$ ) than aqueous mobility would predict ( $P_{\text{Tl}}/P_{\text{Na}} = 1.49$ ).

When compared in the same fiber and with the same iontophoretic current, the peak endplate conductance in different monovalent metal solutions correlated approximately with the permeability sequence of the ions. Conductance was two to three times larger with  $\text{Tl}^+$  than with  $\text{Na}^+$ , but puffs of ACh

TABLE I  
 $\Delta E_r$  AND PERMEABILITY RATIOS FOR MONOVALENT METAL IONS

<i>X</i>	$\Delta E_r \pm \text{SE}$ <i>mV</i>	<i>n</i>	$P_X/P_{\text{Na}}$
$\text{Tl}^+$	$22.7 \pm 1.0$	7	2.51
$\text{Cs}^+$	$8.7 \pm 0.7$	7	1.42
$\text{Rb}^+$	$6.6 \pm 0.7$	7	1.30
$\text{K}^+$	$2.5 \pm 0.4$	8	1.11
$\text{Na}^+$	0.0	—	1.00
$\text{Li}^+$	$-3.5 \pm 0.8$	9	0.87

Test and reference solutions: 114 mM  $X^+$ , 1 mM  $\text{Ca}^{++}$ , 116 mM  $\text{Cl}^-$  or  $\text{NO}_3^-$ , and 10 mM histidine.  $E_r = 1.8 \pm 0.6$  mV in NaCl reference solution. End-pool solution: 115 mM NaF, 10 mM histidine.

in  $\text{Tl}^+$  solutions always produced a progressive (use-dependent) block such that the fourth iontophoretic puff induced perhaps only 50% of the conductance induced by the first. With  $\text{K}^+$  and  $\text{Rb}^+$  (and  $\text{NH}_4^+$ ) solutions, the fiber quite obviously began to swell slowly, decreasing the distance to the iontophoretic electrode. The holding current became inward, the ACh-induced endplate currents became larger and faster, and the iontophoretic electrode soon penetrated the fiber if it was initially very close to the surface. Such slow swelling, which could occur with many membrane-permeant ions, was a reason for keeping the exposure to test solutions short (90–120 s) and for placing the electrode no closer than 20–25  $\mu\text{m}$  in all of the experiments of these two papers. Thallium solutions contained the less permeant anion nitrate rather than chloride and probably for that reason had less of a tendency to induce swelling.

**MIXTURES OF MONOVALENT CATIONS** Experiments were done to test if the permeability ratios for monovalent metals are influenced by the ionic composition of the external medium. In some permeability mechanisms where

more than one ion is in the channel at a time, the permeability ratios measured from reversal potentials in mixtures of ions can be quite different from those in pure solutions of the ions (Sandblom et al., 1977; Hille and Schwarz, 1978; Hladky et al., 1979). We have tested three kinds of mixtures of monovalent cations: NaCl with glucosamine·HCl, NaCl with KCl, and NaNO<sub>3</sub> with TiNO<sub>3</sub>. All solutions also contained 10 mM histidine and 1 mM CaCl<sub>2</sub> or Ca(NO<sub>3</sub>)<sub>2</sub>. The reversal potential changes with glucosamine mixtures are given in Table II, and the results of all three series of experiments are shown

TABLE II  
Δ*E<sub>r</sub>* FOR MIXTURES OF NaCl OR CaCl<sub>2</sub> WITH GLUCOSAMINE HCl

Sodium mixtures			Calcium mixtures			
[Na <sup>+</sup> ]*	Δ <i>E<sub>r</sub></i> ±SE	<i>n</i>	[Ca <sup>++</sup> ]‡	Δ <i>E<sub>r</sub></i> ±SE	<i>n</i>	<i>P<sub>Ca</sub></i> / <i>P<sub>Na</sub></i>
<i>mM</i>	<i>mV</i>		<i>mM</i>	<i>mV</i>		
114	0.0±0.0	—	80	-27.3±0.6	6	0.16
50	-19.4±0.3	8	40	-37.8±0.4	7	0.17
23	-36.3±0.8	8	20	-45.4±0.5	10	0.22
10	-53.9±1.1	8	10	-54.6±0.7	7	0.26
5	-67.5±1.0	7	5	-63.9±0.7	8	0.27
0	-78.8±1.6	10	2.5	-71.1±1.8	7	0.29
			1.0	-78.8±1.6	10	—

End-pool solution: 115 mM NaF, 10 mM histidine. *E<sub>r</sub>* = 1.8±0.6 mV in reference solution. *P<sub>Ca</sub>*/*P<sub>Na</sub>* calculation assumes *P<sub>Glucosamine</sub>*/*P<sub>Na</sub>* = 0.033.

\* Na<sup>+</sup> solutions contain 1 mM CaCl<sub>2</sub>, *X* mM NaCl, and (114-*X*) mM glucosamine HCl, 10 mM histidine.

‡ Ca<sup>++</sup> solutions contain *Y* mM CaCl<sub>2</sub> and (80-*Y*) 114/80 mM glucosamine HCl, 10 mM histidine.

graphically in Figs. 2 A and 3. The solid curves are the predictions of the Goldman-Hodgkin-Katz voltage equation using fixed permeability ratios determined from the pure K<sup>+</sup>, Tl<sup>+</sup>, and glucosamine solutions, and the dashed curves show the expected potential values if these permeability ratios were changed by ±5% for Tl<sup>+</sup>, ±10% for K<sup>+</sup>, or ±20% for glucosamine. The observations fall within the limits of these curves, showing that Na<sup>+</sup> ions have little measurable effect on the permeability to K<sup>+</sup>, Tl<sup>+</sup>, and glucosamine in these experiments and that the Goldman-Hodgkin-Katz voltage equation gives a good description of reversal potentials at the endplate. To permit a closer comparison with similar experiments by Takeuchi and Takeuchi, (1960) and Takeuchi (1963 *a*) the "internal" solution for the experiments with external Na<sup>+</sup>-K<sup>+</sup> mixtures contained a high concentration of K<sup>+</sup> ions: 105 mM KF, 10 mM NaF, and 10 mM histidine.

#### *Nine Divalent Metal Ions Are Permeant*

REVERSAL POTENTIALS Endplate currents in solutions containing high concentrations of divalent ions were always small, as if these ions produced a "pharmacological block." Nevertheless, all divalent alkaline earths and tran-

sition metals tested were quite permeant by the reversal potential criterion. The four alkaline earths and  $Mn^{++}$  ion could be studied at full strength, 80–83.5 mM, and examples of currents in these solutions are shown in the lower half of Fig. 1. However, with the other transition metals,  $Co^{++}$ ,  $Ni^{++}$ ,  $Zn^{++}$ , and  $Cd^{++}$ , the currents were so small that the concentration had to be reduced to 20–21 mM by dilution with glucosamine to get unambiguous  $E_r$  measurements. Even at this dilution, a strongly use-dependent block developed with these four ions; particularly with  $Zn^{++}$ , all currents (inward and outward) vanished after only a few activations by puffs of ACh. Currents returned after a wash with  $Na^+$  reference solution, but they were always reduced in comparison with their previous size.

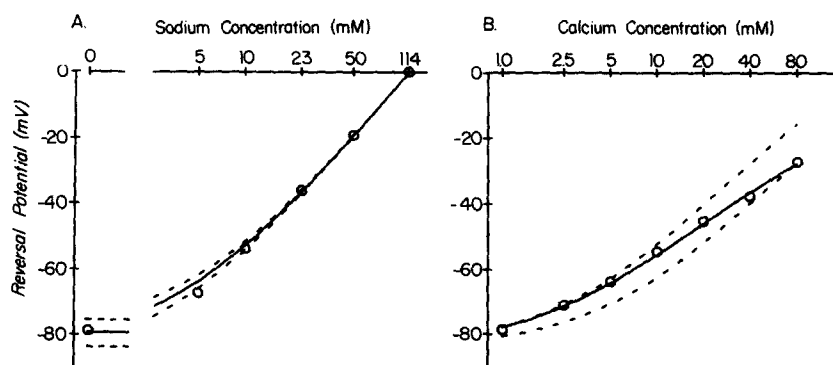


FIGURE 2. Concentration dependence of reversal potentials in mixtures of  $NaCl$  with glucosamine·HCl or  $CaCl_2$  with glucosamine·HCl. The symbols show the means of six measurements and are larger than the standard error of the mean. For both sets of measurements the reversal potential in the  $Na$  reference solution was used to define 0.0 mV. The theoretical lines were calculated assuming an internal  $Na$  concentration of 114 mM. (A) The solid line is the prediction of the Goldman-Hodgkin-Katz equation with  $P_{glucosamine}/P_{Na} = 0.033$ . The dashed lines are for permeability ratios of 80% and 120% of this value. (B) The dashed lines are from the Goldman-Hodgkin-Katz equation with  $P_{Ca}/P_{Na} = 0.155$  and 0.29. The solid line is from the surface potential theory of Lewis (1979) as described in text. Internal solution: 115 NaF.

Reversal potential changes with different divalent ion solutions are summarized in Table III. The first two columns of  $\Delta E_r$  values represent reversal potential changes with respect to the usual control solution containing 114 mM  $Na^+$  and with the ends of the fibers cut in 115 mM NaF. The reversal potentials with 80–83.5 mM divalent salts are 20–36 mV negative, and those with 20–21 mM divalent salt, 42–55 mV negative from the  $NaCl$  control solution. The third  $\Delta E_r$  column in Table III represents changes with respect to a solution containing 60 mM  $MgCl_2$ , 28.5 mM glucosamine·HCl, and 10 mM histidine after the ends of the fibers were cut in 60 mM magnesium citrate and 120 mM histidine. This latter set of experiments was done in an attempt to escape the theoretical complications of calculating selectivity ratios

among divalent ions from experiments with highly permeant monovalent ions inside the fiber. However, only after a series of experiments had been done did we realize that 60 mM magnesium citrate at pH 5.8 contains but 7 mM free  $Mg^{++}$  ion (Sillén and Martell, 1971). In addition, visual inspection and electrical criteria suggested that the cut fiber ends tended to seal up in the magnesium citrate solution; these fibers, therefore, were always cut a second time a few minutes before recording was begun. Nevertheless, the internal ionic composition of the fibers did change in magnesium citrate, since the absolute reversal potentials were  $\sim 25$  mV more positive than for fibers cut in NaF. The absolute reversal potential with 114 mM  $Na^+$  Ringer outside was  $30.3 \pm 0.8$  mV ( $n = 12$ ), and in one trial with 23 mM  $Na^+$  out, it was  $-1.6$  mV.

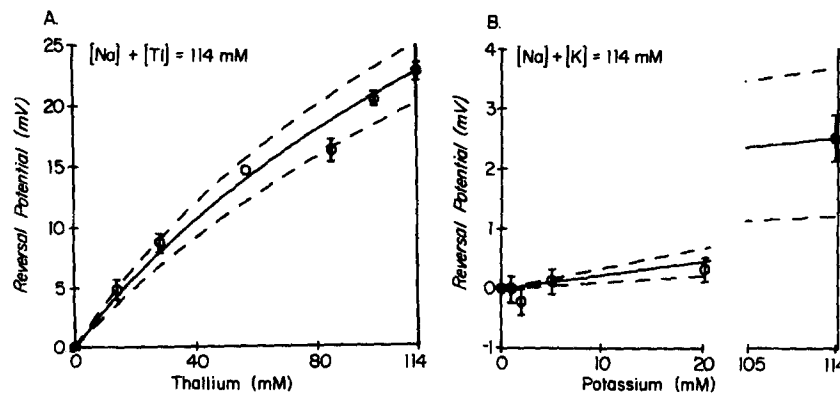


FIGURE 3. Mole-fraction dependence of the reversal potentials with  $Na^+K^+$  and  $Na^+Tl^+$  mixtures containing a constant total of 114 mM test salt. All predictions are from the Goldman-Hodgkin-Katz equation. (A) Symbols show the means of six measurements and bars indicate  $\pm$  standard error (SE). Internal solution: 115 mM NaF. Solid line is the prediction for  $P_{Tl}/P_{Na} = 2.51$ , and dashed lines are for permeability ratios of 90 and 110% of this value. (B) Symbols show the means of four to eight experiments and bars indicate  $\pm$ SE. Internal solution 105 mM KF and 10 mM NaF. Solid line is the prediction for  $P_K/P_{Na} = 1.1$ , and dashed lines are for permeability ratios of 95% and 105% of this value.

No inward or outward currents could be recorded in 21 mM  $NiCl_2$  with five fibers containing magnesium citrate, although the same solution gave clear currents the next day with fibers containing NaF. All the divalent metals studied occasionally gave biphasic currents at the voltage step closest to  $E_r$  similar to those seen with a few organic cations as reported in the preceding article (Dwyer et al., 1980).

**APPARENT PERMEABILITY RATIOS** For the experiments with monovalent test ions, the Goldman-Hodgkin-Katz voltage equation gave self-consistent, permeability ratio values that were independent of concentration in mixtures of permeant ions. When the divalent ion concentration is varied, however, the calculated permeability ratios are no longer constant (Lewis, 1979). The right

side of Table II illustrates this effect with mixtures of  $\text{CaCl}_2$  and glucosamine-HCl. Dilution of  $\text{Ca}^{++}$  makes the reversal potential more negative, as expected; however, the less  $\text{Ca}^{++}$  there is in the solution, the higher is the calculated  $P_{\text{Ca}}/P_{\text{Na}}$  value using Eq. 1. The lack of fit with constant values of  $P_{\text{Ca}}/P_{\text{Na}}$  and  $P_{\text{glucosamine}}/P_{\text{Na}}$  is shown again in Fig. 2 B by the deviation of the dashed lines calculated from the Goldman-Hodgkin-Katz equation for  $P_{\text{Ca}}/P_{\text{Na}} = 0.29$  and  $0.16$ . The lines go through some of the points, but with these or any other choices of  $P_{\text{Ca}}/P_{\text{Na}}$  the predicted slopes are too steep. The possible cause of this deviation is considered in the Discussion. Here we note the discrepancy but continue to use Eq. 1 as a definition of apparent permeability ratios in discussing the different divalent metals. The solid line in Fig. 2 B comes from the theory of Lewis (1979) to be considered in the Discussion.

TABLE III  
 $\Delta E_r$  FOR SOLUTIONS OF DIVALENT CATIONS

X	Isotonic divalent salt			Divalent diluted with glucosamine				
	(X)	115 mM NaF inside*		(X)	115 mM NaF inside*		60 mM Mg citrate inside‡	
		<i>mM</i>	$E_r \pm \text{SE}$ <i>mV</i>		<i>n</i>	<i>mM</i>	$E_r \pm \text{SE}$ <i>mV</i>	<i>n</i>
$\text{Mg}^{++}$	80	$-24.8 \pm 0.8$	6	20	$-43.1 \pm 0.8$	7	$-15.7 \pm 0.7$	11
$\text{Ca}^{++}$	80	$-27.3 \pm 0.6$	6	20	$-45.4 \pm 0.5$	10	$-18.4 \pm 0.9$	9
$\text{Sr}^{++}$	80	$-35.7 \pm 1.0$	6	20	$-49.3 \pm 0.7$	8	$-21.4 \pm 0.9$	12
$\text{Ba}^{++}$	80	$-30.6 \pm 0.8$	7	20	$-46.7 \pm 0.7$	9	$-18.9 \pm 0.6$	12
$\text{Mn}^{++}$	83.5	$-20.4 \pm 0.9$	6	21	$-42.7 \pm 1.0$	7	$-13.3 \pm 0.8$	12
$\text{Co}^{++}$	—	—	—	21	$-43.6 \pm 1.4$	7	$-25.5 \pm 0.4$	7
$\text{Ni}^{++}$	—	—	—	21	$-42.0 \pm 2.6$	6	—	—
$\text{Zn}^{++}$	—	—	—	21	$-41.7 \pm 1.8$	6	$-24.0 \pm 0.8$	8
$\text{Cd}^{++}$	—	—	—	20	$-55.1 \pm 2.5$	7	$-32.0 \pm 1.6$	7

\* Reference solution: 114 mM NaCl, 1 mM  $\text{CaCl}_2$ , 10 mM histidine.  $E = 1.8 \pm 0.6$  mV in reference solution.

‡ Reference solution: 60 mM  $\text{MgCl}_2$ , 28.5 mM glucosamine HCl, 10 mM histidine.  $E = -2.4 \pm 1.8$  mV (16) in reference solution.

The results of applying Eq. 1 to the reversal potential measurements of Table III are listed in Table IV. For fibers cut in 115 mM NaF, the alkaline earths have apparent permeability ratios  $P_X/P_{\text{Na}}$  of 0.10–0.18 at 80 mM concentration and of 0.18–0.25 at 20 mM, assuming that the permeability ratio for glucosamine is 0.033 (only a small correction). Each divalent ion tested has a higher permeability ratio in 20–21 mM solutions than in 80–83.5 mM solutions. The third column of numbers gives the permeability ratios with respect to calcium,  $P_X/P_{\text{Ca}}$ , calculated from the ratios in column two. Three features stand out. (a) The alkaline earth permeabilities are nearly all the same, with a very weak selectivity  $\text{Mg}^{++} > \text{Ca}^{++} > \text{Ba}^{++} > \text{Sr}^{++}$ . (b) Four of the transition metals,  $\text{Mn}^{++}$ ,  $\text{Co}^{++}$ ,  $\text{Ni}^{++}$ , and  $\text{Zn}^{++}$ , are similar to  $\text{Mg}^{++}$  and  $\text{Ca}^{++}$  in permeability. (c) The  $\text{Cd}^{++}$  ion seems to stand alone as an ion of low permeability. However, this result can be entirely explained by the formation of chloride complexes,  $[\text{CdCl}_n]^{+2-n}$ , which reduces the free  $\text{Cd}^{++}$  to



less than 5 mM in the solution used (Sillén and Martell, 1971). Recalculating the permeability ratio for only 5 mM free  $\text{Cd}^{++}$ , would make  $\text{Cd}^{++}$  by far the most permeant ion, an unlikely result that may indicate that the dominant ( $\approx 10$  mM) complex,  $\text{CdCl}^{+1}$ , also carries current. The unique chemistry of  $\text{Cd}^{++}$  is reflected also in the mean activity coefficient, which at 0.1 molal is only 0.228 for the chloride salt of cadmium, compared with 0.503–0.523 for the chloride salts of the other eight divalent ions (Robinson and Stokes, 1965).

The last column of Table IV is calculated from the experiments on fibers cut in magnesium citrate. The exact permeability ratios obtained with Eq. 1 depend in this case on the unknown monovalent and divalent ion concentrations inside the fiber, so these concentrations were estimated using Eq. 1 and the absolute reversal potentials for Na Ringer, 23 mM Ringer, and 20 mM

TABLE IV  
GOLDMAN-HODGKIN-KATZ PERMEABILITY RATIOS FOR  
DIVALENT CATIONS

X	$P_X/P_{\text{Na}}^*$		$P_X/P_{\text{Ca}}^\ddagger$	
	( $X^{++}$ )= 80–83.5 mM	20–21 mM	20–21 mM	20–21 mM
$\text{Mg}^{++}$	0.18	0.25	1.1	1.2
$\text{Ca}^{++}$	0.16	0.22	1.0	1.0
$\text{Sr}^{++}$	0.10	0.18	0.8	0.8
$\text{Ba}^{++}$	0.13	0.21	0.9	0.9
$\text{Mn}^{++}$	0.22	0.25	1.1	1.3
$\text{Co}^{++}$	—	0.23	1.0	0.6
$\text{Ni}^{++}$	—	0.25	1.1	—
$\text{Zn}^{++}$	—	0.26	1.1	0.7
$\text{Cd}^{++}$	—	0.13	0.6	0.4

Columns 1–3 are from fibers cut in NaF in Table III.

Column 4 is from fibers cut in Mg citrate in Table III.

\*  $P_X/P_{\text{Na}}$  values calculated with Eq. 1 and absolute reversal potentials.

‡  $P_X/P_{\text{Ca}} = (P_X/P_{\text{Na}})/(P_{\text{Ca}}/P_{\text{Na}})$ .

$\text{Mg}^{++}$ . Assuming that the permeability ratios for  $\text{Mg}^{++}$  and glucosamine were 0.25 and 0.033 gave reasonable internal concentrations of 4 mM  $\text{Mg}^{++}$  and 24.8 mM  $\text{Na}^+$  (or an equivalent quantity of some other permeant monovalent cation). The other permeability ratios  $P_X/P_{\text{Ca}}$  were calculated with these assumptions. They give the same, weak selectivity among alkaline earths, and a larger divergence among transition metals than before, but still, neglecting  $\text{Cd}^{++}$ , no more than a total factor of two spread in permeability.

#### *Anions Are Not Measurably Permeant*

Previous work with anions studied reversal potential changes when  $\text{Cl}^-$  was replaced by glutamate, propionate,  $\text{NO}_3^-$ , and  $\text{SO}_4^{--}$ , and the reversal potential was estimated by extrapolation (Oomura and Tomita, 1960; Takeuchi and Takeuchi, 1960; Takeuchi, 1963 *a*) or by actual reversal of the ACh response (Ritchie and Fambrough, 1975; Steinbach, 1975; Lassignal and Martin, 1977; Linder and Quastel, 1978). No corrections were made for junction potentials

or  $\text{Na}^+$  activity changes, but all authors concluded that there was no significant change of  $E_r$ . With depolarized, denervated muscle Jenkinson and Nicholls (1961) reported small, ACh-induced fluxes of  $^{36}\text{Cl}$  that were, however, significantly smaller than the fluxes of  $^{24}\text{Na}$  and  $^{42}\text{K}$ .

In this paper and the preceding one (Dwyer et al., 1980), the equations used assume that anions are not measurably permeant. This important assumption was tested again by measuring reversal potential changes when the external anion was changed and the only cations were 115 mM arginine and 5 mM  $\text{Na}^+$ . If anions are impermeant, then the measured  $\Delta E_r$  values should deviate from 0 mV only because of junction potentials and small activity differences of  $\text{Na}^+$  ions among the solutions. Therefore, we corrected the  $\Delta E_r$  for measured junction potential and  $\text{Na}^+$  activity differences. In six experiments with glucuronate as the reference anion, the corrected  $\Delta E_r$  values in order of increasing crystal sizes were:  $\text{F}^-$   $1.6 \pm 0.3$  mV,  $\text{Cl}^-$   $0.1 \pm 0.4$  mV,  $\text{NO}_3^-$   $1.4 \pm 0.2$  mV, acetate  $0.4 \pm 0.5$  mV, and aspartate  $0.2 \pm 0.2$  mV. The absolute  $E_r$  was  $-33.4 \pm 2.9$  mV in the reference solution. Glucuronate was chosen as the reference anion because, with a mean diameter of  $7.1 \text{ \AA}$  and a molecular weight of 193, it is too large to fit through the  $6.5 \times 6.5 \text{ \AA}$  pore proposed for the endplate channel in the previous paper (Dwyer et al., 1980). We thus assume it is impermeant. If any of the other smaller anions were permeant, they should have given negative values of  $\Delta E_r$ ; for example, if  $P_{\text{Cl}}/P_{\text{Na}}$  were 0.01,  $\Delta E_r$  should have been  $-1.3$  mV. Our results rule out a permeability ratio as high as 0.01 for any of the anions, and we attribute the slightly positive  $\Delta E_r$  values found to residual systematic errors or to a small effect of anions on the cation selectivity.

## DISCUSSION

### *Comparison with Previous Work*

Many studies report that alkali metal ions and some divalent metal ions are permeant at the endplate. Early work established the existence of permeability to  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{++}$  and, at most, a small permeability to anions by electrophysiological criteria (Oomura and Tomita, 1960; Takeuchi and Takeuchi, 1960; Takeuchi, 1963 *a,b*), by tracer fluxes in denervated muscle (Jenkinson and Nicholls, 1961), and by the detection of local contractures at the site of ACh stimulation in Ca-containing solutions. Early observations also show that  $\text{Li}^+$  ions are permeant, since in isotonic lithium solutions there are normal neuromuscular transmission (Overton, 1902), endplate depolarization with applied ACh (Fatt, 1950), and normal miniature endplate potentials (Onodera and Yamakawa, 1966). More recently, there have been several quantitative studies of reversal potential changes with alkali metals. Unfortunately many of these are hampered by the simultaneous presence of several permeant ions in the external solution and insufficient attention to proper correction for junction potentials when measuring tiny changes. With mammalian tissue, Ritchie and Fambrough (1975) found the sequence  $\text{Na}^+ > \text{K}^+$  with permeability ratios (1:0.6), and Linder and Quastel (1978) found  $\text{Li}^+ > \text{Na}^+ > \text{K}^+$  (1.1: 1:0.8); with amphibian endplates, Lewis (1979) found  $\text{K}^+$

> Na<sup>+</sup> (1.1:1) and Gage and Van Helden (1979) found Cs<sup>+</sup> > K<sup>+</sup> > Na<sup>+</sup> > Li<sup>+</sup> (1.25:1.1:1.0:0.9); and with *Electrophorus* electric organ, Lassignal and Martin (1977) found K<sup>+</sup> > Na<sup>+</sup> (1.1:1). In a tracer flux study on chick myotubes, Huang et al. (1978) found the flux sequence Cs<sup>+</sup> > Rb<sup>+</sup> ≈ K<sup>+</sup> > Na<sup>+</sup> (1.9:1.5:1.5:1). Our work is in good qualitative agreement with the four studies that are not on mammalian muscle.

Divalent ions have also been studied recently. Katz and Miledi (1969) found endplate potentials with reversal potentials of -35 and -39 mV in frog muscle bathed in solutions containing 83 mM Ca<sup>++</sup>, 2 mM K<sup>+</sup>, and 5 mM tetraethylammonium ion. They also observed miniature endplate potentials in isotonic Ca-Ringer's and in isotonic Mg-Ringer's. Inward endplate currents have now been reported with isotonic solutions of MgCl<sub>2</sub>, CaCl<sub>2</sub>, SrCl<sub>2</sub>, MnCl<sub>2</sub>, and CoCl<sub>2</sub> (Bregestovsky et al., 1979). Evans (1974) demonstrated transmitter-induced uptake of <sup>45</sup>Ca at the endplate of innervated mouse diaphragm. From the ratio of <sup>45</sup>Ca fluxes to <sup>24</sup>Na fluxes in K-depolarized chick myotubes, Huang et al. (1978) calculated a calcium permeability ratio of 0.22, in close agreement with our results. From the reversal potential obtained in a solution containing 32 mM K<sup>+</sup>, 20 mM Ca<sup>++</sup>, and 105 mM glucosamine, Lassignal and Martin (1977) estimated a permeability ratio  $P_{Ca}/P_{Na}$  of 0.8 in the electric organ. On the other hand, Lewis (1979) demonstrated that a constant  $P_{Ca}/P_{Na}$  ratio does not adequately describe reversal potentials in the frog endplate. She calculated a value of 1.0 in solutions of low ionic strength containing only 2 mM Ca<sup>++</sup>, 1.2 mM Na<sup>+</sup>, and 2.5 mM K<sup>+</sup> as cations (with sucrose to bring up the osmolarity), a value significantly larger than would fit at higher concentrations of divalent ion. The reversal potential changes,  $\Delta E_r$ , she found on going from Ringer's to 80 mM CaCl<sub>2</sub> or 78 mM MgCl<sub>2</sub> + 2 mM CaCl<sub>2</sub> are -26.0 and -20.7 mV, which with Eq. 1 are equivalent to  $P_{Ca}/P_{Na} = 0.17$  and  $P_{Mg}/P_{Na} = 0.22$ . These values are close to ours (Tables I and III), with a sequence Mg<sup>++</sup> > Ca<sup>++</sup>, and they show the same trend, a decreasing permeability ratio as the divalent ion concentration is raised. Lewis proposed that the apparent variability of the divalent ion permeability ratio can be explained by taking into account surface potentials, a theory which we consider in the next section.

#### *Description of Reversal Potentials*

**PARALLEL-CONDUCTANCE THEORY** In their classic analysis of the ionic basis of endplate currents, Takeuchi and Takeuchi (1960) and Takeuchi (1963 *a*) reached the conclusion that reversal potentials and conductance changes in mixtures of NaCl, KCl, and sucrose or dextrose could not be described by the Goldman-Hodgkin-Katz formalism used in this paper. The deviations seemed particularly striking when the K<sup>+</sup> concentration was changed in the range 0.2-7.0 mM and the Na<sup>+</sup> concentration was nearly normal. The Goldman-Hodgkin-Katz equation would predict virtually no change of  $E_r$  for these conditions, but experimentally,  $E_r$  was said to vary from -42 to -3 mV. To account for these observations, Takeuchi and Takeuchi (1960) described endplate currents as the sum of currents in two parallel, ohmic pathways,

which they called Na channels and K channels. Within this empirical theory, endplate currents are characterized by independent conductance changes,  $\Delta g_{\text{Na}}$  and  $\Delta g_{\text{K}}$ , and by electromotive forces,  $E_{\text{Na}}$  and  $E_{\text{K}}$ .

If there were actually two distinct conductance channels in the endplate the analysis given in our two papers would be wrong; however, several observations argue against the parallel-conductance theory. All of the pertinent results of Takeuchi and Takeuchi (1960), Takeuchi (1963 *a*), and of much work agreeing with theirs (see references reviewed by Rang, 1975) were reversal potentials estimated by linear extrapolation of the peak current-voltage relation from very negative potentials beyond the contraction threshold for the muscle. Linear extrapolation has since then been recognized to give incorrect results (Dionne and Stevens, 1975). Direct measurements of reversal potentials without extrapolation have now been repeated four times with solutions containing varying low concentrations of  $\text{K}^+$  and with high concentrations of  $\text{Na}^+$  (Ritchie and Fambrough, 1975; Steinbach, 1975; Linder and Quastel, 1978; this paper, Fig. 3 B). In each case the results were in full accord with the Goldman-Hodgkin-Katz voltage equation, showing only small changes of  $E_r$ , as in our Fig. 3 B, rather than the large changes originally supposed on the basis of extrapolation. We also find that mixtures of  $\text{Na}^+$  with  $\text{Tl}^+$  and  $\text{Na}^+$  with glucosamine give no appreciable deviations from the predictions of the voltage equation (Figs. 2 A and 3A and Table II). Thus, the endplate channel can be a single entity with fixed values of  $P_{\text{Na}}$ ,  $P_{\text{K}}$ , etc., defined from reversal potentials by conventional electrodiffusion theory. These observations are evidence against flux interactions among  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Tl}^+$  ions in the pore such as can be found in multi-ion, single-file pores (Hille and Schwarz, 1978). Our observations are not in contradiction to the suggestion that endplate channels are a single-ion pore with ion-binding and conductance saturation (Lewis, 1979; Lewis and Stevens, 1979). The channel could even be a multi-ion pore that does not have the combination of barrier heights needed to give flux interaction (Hille and Schwarz, 1978).

Using an entirely different approach, Dionne and Ruff (1977) have shown that Na and K currents in endplate channels are gated by the same gate at the molecular level. They found that the endplate current noise (fluctuations) reaches a minimum at the macroscopic reversal potential, as required by a one-channel theory, rather than having separate minima at  $E_{\text{Na}}$  and  $E_{\text{K}}$ , as required by a two-channel theory. Hence, we assume that endplate currents should no longer be discussed in terms of a separate  $\Delta g_{\text{Na}}$  and  $\Delta g_{\text{K}}$  or in terms of a two-channel hypothesis. Indeed, if we accept that the endplate has a channel with approximately the  $6.5 \times 6.5 \text{ \AA}$  size suggested in the preceding article (Dwyer et al., 1980), and with no strong preference for organic cations of a particular size or chemical structure, it would be difficult to imagine how that channel could distinguish strongly between  $\text{Na}^+$  and  $\text{K}^+$  ions.

**DIVALENT IONS AND SURFACE POTENTIALS** Although reversal potentials with mixtures of monovalent ions fit the Goldman-Hodgkin-Katz equation with constant permeability ratios, potentials with monovalent and divalent ions both present do not (Lewis, 1979; this paper, dashed lines, Fig. 2 B). The divalent ion permeability seems to fall as the divalent ion concentration is

increased. Lewis attributed this not to a structural effect on the pore but rather to an effect on the membrane surface potential and hence on the local ionic concentrations at the mouth of the pore. If the subsynaptic membrane has a negative surface potential like that postulated for other excitable membranes, then monovalent cations will be concentrated at the membrane surface by a factor  $\beta$ , and divalent cations by a factor  $\beta^2$ , where  $\beta$  is  $\exp(-\Psi_o F/RT)$  and  $\Psi_o$  is the surface potential. Qualitatively, divalent cations would then have an advantage,  $\beta$ , over monovalent ions in permeation; at low ionic strength,  $\Psi_o$  is very negative and the advantage,  $\beta$ , is large; while at higher ionic strength,  $\Psi_o$  is less negative and the advantage is smaller.

Several specific surface-potential theories can be fitted to our observations. The solid line in Fig. 2 B is calculated following exactly the method of Lewis (1979); surface potentials are calculated from the Grahame (1947) equation, activity coefficients from the data of Butler (1968), and membrane potentials from the Goldman-Hodgkin-Katz voltage equation modified for the effect of surface potentials by the method of Frankenhaeuser (1960). A very low negative surface charge density ( $-0.0014e/\text{\AA}$ ) was assumed, giving surface potentials changing from  $-26$  to  $-16$  mV as the calcium concentration is increased from 1.0 to 80 mM. With these parameters, the intrinsic permeability ratio  $P_{Ca}/P_{Na}$  of the channel was 0.26, in complete accord with the values given by Lewis (1979). Equally good agreement could be obtained with different assumptions. Assuming that the pore is physically at some distance from a region of high charge density, so that it sees, for example, 25% of the surface potential reported by the *m*-gating system of Na channels (charge density, ca.  $-0.01 e/\text{\AA}^2$ , Hille et al., 1975; Campbell and Hille, 1976) gives a good fit with  $P_{Ca}/P_{Na} = 0.3$ , not a surprising result, as the potentials assumed are about the same as those in the first model. Still another good fit to our measurements is obtained by assuming a constant, concentration-independent, surface potential of  $-45$  mV and  $P_{Ca}/P_{Na} = 0.15$ . This entirely *ad hoc* model works because a given surface concentration of calcium ions acts like the same surface concentration of monovalent ions with a voltage-dependent effective permeability,  $4P_{Ca} \{1 + \exp[(E - \Psi_o) F/RT]\}^{-1}$ , according to the Goldman-Hodgkin-Katz equation (e.g., see Lewis, 1979). At negative values of  $E - \Psi_o$ , the effective permeability is constant and equal to  $4P_{Ca}/P_{Na}$ , while at positive  $E - \Psi_o$ , the effective permeability is voltage-dependent and smaller.

There already is much evidence for a negative surface potential at the external face of electrically excitable membranes (see references in Hille et al., 1975). As we have seen, the variation of the permeability ratios for divalent ions at the endplate is consistent with the surface-potential theory of Lewis (1979). Also at the endplate, a speeding of the decay phase of the miniature endplate potential (Cohen and Van der Kloot, 1978) and an apparent increase of the affinity of curare for the ACh receptor (Jenkinson, 1960) as the ionic strength of the medium is lowered have been interpreted as surface-charge effects (Cohen and Van der Kloot, 1978; Van der Kloot and Cohen, 1979). In the next section we conclude that there are no negative charges of high field strength within the narrow part of the pore, so the surface negative charge could be acid groups on protein or lipid close to but not in the pore.

*Permeation at the Endplate Pore*

Any realistic model of permeation at the endplate will now need to reconcile a variety of observations: Fig. 4 is a grand summary of all of our permeability ratio measurements for monovalent organic cations and monovalent and divalent metal cations. The permeabilities are plotted vs. the ionic crystal diameter for metals (Pauling, 1960) or the geometric mean, van der Waals diameter for organics (Dwyer et al., 1980). Taken together, the monovalent ions show a permeability maximum in the diameter range 2.5–3.5 Å. The channel is large enough to pass organic molecules like triethanolamine and glucosamine, so the narrowest part must be at least  $6.5 \times 6.5$  Å (Dwyer et al., 1980). Small neutral molecules, monovalent metal and organic cations, and divalent metal and organic cations all have similar permeabilities (Huang et al., 1978; and Fig. 4), whereas anions have a much lower permeability, if any.

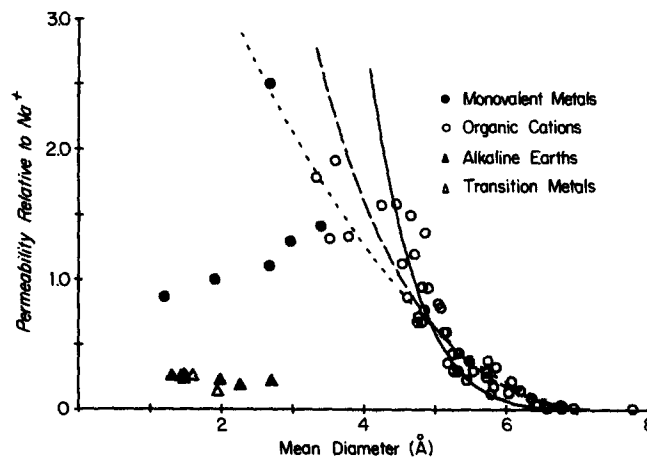


FIGURE 4. The relation between relative permeability in endplate channels and ionic diameter. Values for monovalent organic cations (○) are taken from the preceding article (Dwyer et al., 1980), and the lines represent predictions of three empirical pore theories, as in Fig. 8 of that paper. Permeability ratios for monovalent (●) and divalent (▲, △) metal cations are from this paper, and the ionic crystal radii are from Pauling (1960).

The selectivity among monovalent metals is very weak, and so is that among divalent metals (Fig. 4 and literature summarized at beginning of Discussion). The single-channel conductance is  $\approx 27$  pS in frog Ringer's solution (Neher and Stevens, 1977). There may be a negative surface potential near the external mouth of the channel, as well as saturation or binding of permeant ions within the channel (Lewis, 1979; Lewis and Stevens, 1979; and Fig. 2 B), and there is no evidence for flux interaction of the type found in multi-ion, single-file pores (Figs. 2 B, 3 A, 3 B).

The high conductance and low selectivity for metal ions are consistent with a water-filled pore of the large size deduced from studies with organic cations (Dwyer et al., 1980). A weak discrimination between monovalent and divalent

ions, between small metals of different crystal radius, and between organic molecules of different chemical properties all suggest that, within the narrow part of the pore, the walls lack negative charges of high field strength (Eisenman, 1962). The permeation of ions would then not depend on a strong interaction with special chemical groups in the pore, but might instead be limited by access to the pore, the energy of partial dehydration, and friction between the stationary walls and the moving ion and associated water molecules. As with aqueous mobility, small cations, which hydrate strongly, would be retarded in accordance with a larger hydrated size, while large cations, with weak hydration, would act in accordance with their crystal size. This would account for the correlation between permeability ratio and aqueous mobility seen with alkali metals; it would account for the approximate similarity between the permeability ratios for the smallest organic cations and those for the larger alkali metals (Fig. 4); and it would account for the falloff of permeability for ions of larger size. On the basis of their single-channel conductance measurements with alkali metal ions, Barry et al. (1979) have also presented a neutral-pore theory for the endplate channel, with a more extensive mathematical analysis. Their theory includes the idea of a long pore containing several ions and counterions simultaneously.

The picture so far does not offer a ready explanation for the permeability sequence,  $Mg^{++} > Ca^{++} > Ba^{++} > Sr^{++}$ , which is nearly the inverse of the aqueous mobility sequence,  $Ba^{++} > Sr^{++} > Ca^{++} > Mg^{++}$ , and is not even one of the Eisenman (1962; Diamond and Wright, 1969) sequences. The only suggestion we can make is that some additional interaction, e.g., polarization inducing a strong dipole, might give the permeation of divalents a different character than the permeation of most monovalents. Some polar interaction with the walls might also be needed to explain the higher-than-expected permeability of the more polarizable monovalent cations (e.g.,  $Tl^+$  and guanidinium derivatives, Dwyer et al., 1980). Still another kind of interaction with the pore is needed to explain blockage of the pore by external cations such as local anesthetic derivatives, decamethonium and related quaternary ammoniums, guanidinium derivatives, etc. (Adams, 1977; Adams and Sakmann, 1978; Neher and Steinbach, 1978; Adler et al., 1979; Farley et al., 1979). These blocking effects with fairly large, and often partly hydrophobic, compounds are often use-dependent and voltage dependent suggesting that opening of a pore creates a wide, perhaps hydrophobic, mouth that extends quite far into the electric field in the membrane.

The observed discrimination against anions and permeability to nonelectrolytes and monovalent and divalent cations could be due to the combination of two electrostatic factors. First, there would be the negative surface potential that would change the local concentrations of these four charge types by factors of  $1/\beta$ ,  $1$ ,  $\beta$ , and  $\beta^2$ , respectively. Second, there is the dielectric work of bringing charges into a region of lowered mean dielectric constant (the inside of the pore). This work, proportional to the square of the charge, is a dehydration energy in the sense that the ion leaves at least some of the more distant water molecules behind. If we call  $\alpha$  the value,  $<1.0$ , of the effective

partition coefficient attributable to the dielectric work, then the electrostatic and dielectric factors together would affect the four charge species by factors  $\alpha/\beta$ , 1,  $\alpha\beta$ , and  $\alpha^2\beta^2$ . Now, for the sake of argument, if  $\alpha$  were 0.1 and  $\beta$  were 10 then the net result would be 0.01, 1, 1, and 1. Hence anions would hardly permeate, while the other three species would pass with equal ease. Of course, the numbers were chosen arbitrarily and the theory lacks sophistication, but it gives one intuitive explanation for the results.

In summary, the endplate channel forms a large aqueous pore that is permeable to neutral and cationic molecules. The weak selectivity may be based less on specific chemical interactions with the permeant species than on size-dependent friction and hydration and on an interaction with a diffuse negative surface charge.

We thank Lea Miller for invaluable secretarial help and assistance in all phases of this work, Barry Hill for building the electronics, and Dr. Theodore H. Kehl and his staff for providing computer resources. We are grateful to Dr. Wolfgang Nonner and Dr. Wolfhard Almers for reading a draft of the manuscript.

This research was supported by grants NS 08174, NS 05082, and FR 00374 from the National Institutes of Health. Dr. Adams is a Fellow of the Muscular Dystrophy Association of America.

*Received for publication 1 November 1979.*

#### REFERENCES

- ADAMS, P. R. 1977. Voltage jump analysis of procaine action at frog end-plate. *J. Physiol. (Lond.)* **268**:291–318.
- ADAMS, P. R., and B. SAKMANN. 1978. Decamethonium both opens and blocks endplate channels. *Proc. Natl. Acad. Sci. USA* **75**:2994–2998.
- ADLER, M., A. C. OLIVEIRA, E. X. ALBUQUERQUE, N. A. MANSOUR, and A. T. ELDEFRAWI. 1979. Reaction of tetraethylammonium with the open and closed conformations of the acetylcholine receptor ionic channel complex. *J. Gen. Physiol.* **74**: 129–152.
- BARRY, P. H., P. W. GAGE, and D. F. VAN HELDEN. 1979. Cation permeation at the amphibian motor end-plate. *J. Membr. Biol.* **45**:245–276.
- BREGESTOVSKY, P. D., R. MILEDI, and I. PARKER. 1979. Calcium conductance of acetylcholine-induced endplate channels. *Nature (Lond.)* **279**:638–639.
- BUTLER, J. N. 1968. The thermodynamic activity of calcium ion in sodium chloride-calcium chloride electrolytes. *Biophys. J.* **8**:1426–1433.
- CAMPBELL, D. T., and B. HILLE. 1976. Kinetic and pharmacological properties of the sodium channel of frog skeletal muscle. *J. Gen. Physiol.* **67**:309–323.
- COHEN, I., and W. VAN DER KLOOT. 1978. Effects of  $[Ca^{2+}]$  and  $[Mg^{2+}]$  on the decay of miniature endplate currents. *Nature (Lond.)* **271**:77–79.
- DIAMOND, J. M., and E. M. WRIGHT. 1969. Biological membranes: the physical basis of ion and nonelectrolyte selectivity. *Annu. Rev. Physiol.* **31**:581–646.
- DIONNE, V. E., and R. L. RUFF. 1977. End-plate current fluctuations reveal only one channel type at frog neuromuscular junction. *Nature (Lond.)* **266**:263–265.
- DIONNE, V. E., and C. F. STEVENS. 1975. Voltage dependence of agonist effectiveness at the frog neuromuscular junction: resolution of a paradox. *J. Physiol. (Lond.)* **251**:245–270.



- DWYER, T. M., D. J. ADAMS, and B. HILLE. 1979. Ionic selectivity of end-plate channels. *Biophys. J.* **25**:67a. (Abstr.)
- DWYER, T. M., D. J. ADAMS, and B. HILLE. 1980. The permeability of endplate channels to organic cations in frog muscle. *J. Gen. Physiol.* **75**:469-492.
- EISENMAN, G. 1962. Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2** (2, Pt. 2):259-323.
- EVANS, R. H. 1974. The entry of labelled calcium into the innervated region of the mouse diaphragm muscle. *J. Physiol. (Lond.)*. **240**:517-533.
- FARLEY, J. M., S. WATANABE, J. Z. YEH, and T. NARAHASHI. 1979. Mechanism of end-plate channel block by *N*-alkyl guanidine derivatives. *Biophys. J.* **25**:16a. (Abstr.)
- FATT, P. 1950. The electromotive action of acetylcholine at the motor end-plate. *J. Physiol. (Lond.)*. **111**:408-422.
- FATT, P., and B. KATZ. 1951. An analysis of the end-plate potential recorded with an intracellular electrode. *J. Physiol. (Lond.)*. **115**:320-370.
- FRANKENHAEUSER, B. 1960. Sodium permeability in toad nerve and in squid nerve. *J. Physiol. (Lond.)*. **152**:159-166.
- GAGE, P. W., and D. F. VAN HELDEN. 1979. Effects of permeant monovalent cations on end-plate channels. *J. Physiol. (Lond.)*. **288**:509-528.
- GOLDMAN, D. E. 1943. Potential, impedance and rectification in membranes. *J. Gen. Physiol.* **27**:37-60.
- GRAHAME, D. C. 1947. The electrical double layer and the theory of electrocapillarity. *Chem. Rev.* **41**:441-501.
- HILLE, B., and W. SCHWARZ. 1978. Potassium channels as multi-ion single-file pores. *J. Gen. Physiol.* **72**:409-442.
- HILLE, B., A. M. WOODHULL, and B.I. SHAPIRO. 1975. Negative surface charge near sodium channels of nerve: divalent ions, monovalent ions, and pH. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **270**:301-318.
- HLADKY, S. B., B. W. URBAN, and D. A. HAYDON. 1979. Ion movements in pores formed by gramicidin A. In *Membrane Transport Processes*, Vol. 3. Stevens, C. F. and R. W. Tsien, editors. Raven Press, New York. 89-103.
- HODGKIN, A. L., and B. KATZ. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.)*. **108**:37-77.
- HUANG, L. M., W. A. CATTERALL, and G. EHRENSTEIN. 1978. Selectivity of cations and nonelectrolytes for acetylcholine-activated channels in cultured muscle cells. *J. Gen. Physiol.* **71**:397-410.
- JENKINSON, D. H. 1960. The antagonism between tubocurarine and substances which depolarize the motor end-plate. *J. Physiol. (Lond.)*. **152**:302-324.
- JENKINSON, D. H., and J. G. NICHOLLS. 1961. Contractures and permeability changes produced by acetylcholine in depolarized denervated muscle. *J. Physiol. (Lond.)*. **159**:111-127.
- KATZ, B., and R. MILEDI. 1969. Spontaneous and evoked activity of motor nerve endings in calcium ringer. *J. Physiol. (Lond.)*. **203**:689-706.
- LASSIGNAL, N. L., and A. R. MARTIN. 1977. Effect of acetylcholine on postjunctional membrane permeability in eel electroplaque. *J. Gen. Physiol.* **70**:23-36.
- LEWIS, C. A. 1979. Ion-concentration dependence of the reversal potential and the single channel conductance of ion channels at the frog neuromuscular junction. *J. Physiol. (Lond.)*. **286**:417-445.
- LEWIS, C. A., and C. F. STEVENS. 1979. Mechanism of ion permeation through channels in a postsynaptic membrane. In *Membrane Transport Processes*, Vol. 3. Stevens, C. F. and R. W. Tsien, editors. Raven Press, New York. 89-103.

- LINDER, T. M., and D. M. J. QUASTEL. 1978. A voltage-clamp study of the permeability change induced by quanta of transmitter at the mouse endplate. *J. Physiol. (Lond.)*. **281**:535-556.
- NEHER, E., and J. H. STEINBACH. 1978. Local anaesthetics transiently block currents through single acetylcholine-receptor channels. *J. Physiol. (Lond.)*. **277**:153-176.
- NEHER, E., and C. F. STEVENS. 1977. Conductance fluctuations and ionic pores in membranes. *Annu. Rev. Biophys. Bioeng.* **6**:345-381.
- ONODERA, K., and K. YAMAKAWA. 1966. The effects of lithium on the neuromuscular junction of the frog. *Jpn. J. Physiol.* **16**:541-550.
- OOMURA, Y., and T. TOMITA. 1960. Study on properties of neuromuscular junction. In *Electrical Activity of Single Cells*. Y. Katsuki, editor. Igako Shoin, Ltd., Tokyo. 181-205.
- OVERTON, E. 1902. Beiträge zur allgemeinen Muskel- und Nervenphysiologie. II. Über die Unentbehrlichkeit von Natrium- (oder Lithium-) Ionen für den Kontraktionsakt des Muskels. *Pflügers Archiv. Gesamte Physiol. Menschen Tiere.* **92**:346-386.
- PAULING, L. 1960. *Nature of the Chemical Bond*. 3rd edition. Cornell University Press, Ithaca, N.Y. 260, 514.
- RANG, H. P. 1975. Acetylcholine receptors. *Q. Rev. Biophys.* **7**:283-399.
- RITCHIE, A. K., and D. M. FAMBROUGH. 1975. Ionic properties of the acetylcholine receptor in cultured rat myotubes. *J. Gen. Physiol.* **65**:751-767.
- ROBINSON, R. A., and R. H. STOKES. 1965. *Electrolyte Solutions*. Second edition. Butterworth and Co., Ltd., London. 479-496.
- SANDBLOM, J., G. EISENMAN, and E. NEHER. 1977. Ionic selectivity, saturation, and block in gramicidin A channels. I. Theory for the electrical properties of ion selective channels having two pairs of binding sites and multiple conductance states. *J. Membr. Biol.* **31**:383-417.
- SILLÉN, L. G., and A. E. MARTELL. 1971. Stability constants of metal-ion complexes. Special Publication No. 25. The Chemical Society, London. 180-181, 412-413.
- STEINBACH, J. H. 1975. Acetylcholine responses on clonal myogenic cells *in vitro*. *J. Physiol. (Lond.)*. **247**:393-405.
- TAKEUCHI, A., and N. TAKEUCHI. 1960. On the permeability of end-plate membrane during the action of transmitter. *J. Physiol. (Lond.)*. **154**:52-67.
- TAKEUCHI, N. 1963 *a*. Some properties of conductance changes at the end-plate membrane during the action of acetylcholine. *J. Physiol. (Lond.)*. **167**:128-140.
- TAKEUCHI, N. 1963 *b*. Effects of calcium on the conductance change of the end-plate membrane during the action of transmitter. *J. Physiol. (Lond.)*. **167**:141-155.
- VAN DER KLOOT, W. G., and I. COHEN. 1979. Membrane surface potential changes may alter drug interactions: an example, acetylcholine and curare. *Science (Wash. D.C.)*. **203**:1351-1353.