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Effects of Internal and External Sodium on the Sodium Current-Voltage Relationship in the *Squid* Giant Axon

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Summary. The early transient current-voltage relationship was measured in internally perfused voltage clamped squid giant axons with various concentrations of sodium on the two sides of the membrane. In the absence of sodium on either side there is an outward transient current which is blocked by tetrodotoxin and varies with internal potassium concentration. The current increases linearly with voltage for positive potentials. Adding sodium ions internally increases the slope of the current-voltage relationship. Adding sodium ions externally also increases the slope between +10 and +80 mV. Adding sodium to both sides produces the sum of the two effects.

The current-voltage relationships were fit by straight lines between +10 and +80 mV. Plotting the extrapolated intercepts with the current axis against the differences in sodium concentrations gave a straight line, $I_o = -P(c_o - c_i)F$. P, the Fickian permeability, is about 10^{-4} cm/sec. Plotting the slopes in three dimensions against the two sodium concentrations gave a plane $g = g_o + (a\mathrm{Na}_o + b\mathrm{Na}_i)F$. a is about 10^{-6} cm/mV-sec and b about 3×10^{-6} cm/mV-sec. Thus the current-voltage relationship for the sodium current is well described by $I = -P(c_o - c_i)F + (ac_o + bc_i)FV$ for positive potentials. This is the linear sum of Fick's Law and Ohm's Law. $P/(a+b) = 25 \pm 1$ mV ($N = 6$) and did not vary with the absolute magnitude of the currents. Within experimental error this is equal to kT/e or RT/F.

Increasing temperature increased P, a and b proportionately. Adding external calcium, lithium, or Tris selectively decreased P and a without changing b. In the absence of sodium, altering internal and external potassium while observing the early transient currents suggests this channel is more asymmetric in its response to potassium than to sodium.

Electrical nerve impulses are generated by a current of sodium ions passing through the nerve cell membrane. The dramatic transient increase in sodium permeability of the membrane which permits this flow of ions down their concentration gradient is itself turned on by a change in the transmembrane electrical potential, and so, in life, the process is self-regenerative and propagates along the nerve cell surface (Hodgkin & Huxley, 1952*b*).

In order to better understand the nature of this ionic current through the membrane we have measured the sodium current as a function of the internal and external sodium concentrations and the electrical potential difference between the two solutions using the techniques of voltage clamping and internal perfusion of squid giant axons (Hodgkin, Huxley & Katz, 1952; Tasaki, Watanabe & Takenaka, 1962; Chandler & Meves, 1965; Cole, 1972). We have found that a simple linear law will describe the current-voltage-concentration function. Preliminary reports have appeared (Landowne & Scruggs, 1978*a*, *b*).

Materials and Methods

Isolated segments of the hindmost axon of *Loligo pealii* were mounted horizontally in a chamber consisting of a 3 by 3 by 12 mm channel extending through three blocks of silver separated by about 0.2 mm of Teflon and epoxy; these formed the two guard electrodes and one central current-measuring electrode. The inner surfaces of the channel were platinized. The chamber was mounted on a peltier cooling device, and temperature was measured by an indwelling thermistor just below the center of the axon. Artificial seawater was continuously pumped through the chamber at about 0.5 ml/min.

A 200 μm diameter inlet cannula for internal perfusion was inserted into the axon about 5 mm beyond the chamber edge and was advanced through the axon until the cannula extended through a slit in the axon at the other end of the chamber. The internal electrode assembly was partially inserted into the cannula after starting flow through the cannula with fluid containing 0.5 mg/ml

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pronase. The entire assembly was moved back through the axon until the electrode was in position and the inlet cannula was near its original point of insertion. The perfusion fluid was then switched to a pronase-free solution. The electrode assembly was similar to that of Chandler and Meves (1965). It consisted of a 100-μm diameter platinized platinum wire glued to the outside of a 100-μm diameter glass or polyethylene tube containing 0.6 M KCl and a 20 or 50-μm diameter electrically floating Pt wire. With the plastic tube, the end was sealed and a side hole made electrical contact with the internal solution.

With the artificial seawater containing (in mM) 460 Na, 10 K, 10 Ca, 55 Mg, 5 Na N-2-hydroxyethylpiperazine-N′-2-ethanesulfonate (Na HEPES), pH 7.6, and the internal perfusion fluid containing (in mM) 260 KF, 30 K HEPES, pH 7.6, and 500 mannitol, the resting potential measured between the internal KCl bridge and a similar bridge just outside the axon was -51 ± 3 mV(21) mean ± SE (number of axons). The feedback loop was then closed and the voltage was clamped to a holding potenial of -60 mV (inside negative) (Hodgkin et al., 1952). Current through the membrane was measured as the current necessary to keep the central Pt-Ag chamber electrode at ground. The membrane area was calculated from three diameter measurements of the axon at the center and ends of the 3.4-mm long central electrode. "Compensation" was included for resistance in series with the membrane (Hodgkin et al., 1952). Standard families of current records were recorded for voltage pulses to values between -40 and $+180$ mV. A 20-msec prepulse to -80 mV to remove resting inactivation preceded each pulse (Hodgkin & Huxley, 1952*b*). The peak of the early transient current was measured with reference to the current just before the pulse and plotted against the potential. Leak conductance was determined from the current during a step to -160 mV. In freshly mounted axons the maximum early transient current was 3.0 ± 0.3 mA/cm^2(21) and the leak conductance was $1.3 \pm 0.2 \times 10^{-4}$ Ω^{-1} cm^{-2}(7).

The experiments consisted of recording a family of current traces for various external//internal solution combinations. Generally each fifth solution and occasionally every other solution was a repeat of the reference pair to control for axon deterioration. Deterioration was manifested as a reduction of the early transient current and was generally less than 10% or the experiment was disregarded.

The external solutions were as described above with various equimolar substitutions of choline and tetramethylammonium (TMA) ions for sodium. The internal solutions were either as above with equiosmotic substitution of NaCl for mannitol or, later in the majority of the experiments, the internal perfusion fluid contained (in mM) 160 KF, 30 K HEPES, pH 7.6, 255 mannitol, xKCl, yNaCl and zTMA Cl with $x+y+z=200$. Solution pairs will be represented as Na concentration outside//Na concentration inside, the double solidus representing the membrane. When not otherwise indicated the internal perfusion fluid contained 275 mM K. Most experiments were performed at 9–11 °C.

Results

The basic experiment was to study the early transient (nominally sodium) current-voltage relationship as a function of internal and external sodium concentrations. The results are presented firstly by varying internal sodium with no sodium outside, secondly by varying external sodium with no sodium inside, and finally by having sodium on both sides.

No Sodium Outside

When external sodium ions were replaced with choline or TMA, the inward early transient currents were replaced by outward transient currents with about the same time course. Fig. 1, left, shows these currents with 100 mM sodium inside (0//100). In the center panel, the currents with no sodium on either side (0//0) are also outward with about the same time course but about one-sixth the magnitude. These outward currents in the absence of sodium are carried by potassium ions as are the outward currents seen in the presence of external sodium (Chandler & Meves, 1965). Tetrodotoxin blocked all of the early transient currents carried by either ion and left the classical delayed rectifier potassium currents (Fig. 1, right) (*cf.* Narahashi, 1974).

In Fig. 2 the *I-V* relationships for these records are shown. After a transition region below about $+10$ mV the current increased approximately linearly with voltage. The conductance, or slope of the *I-V*

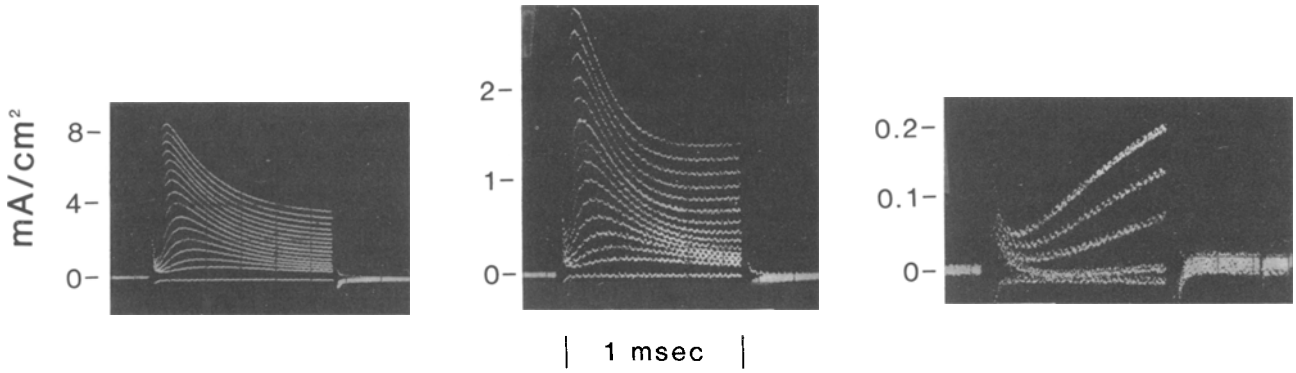

Fig. 1. Early transient currents in the absence of external sodium. In the left frame the internal perfusion fluid contained 100 mM sodium. Fifteen traces between 0 and $+140$ mV and one trace at -160 mV are shown. In the center, at higher gain, are the currents with no sodium on either side. At a still higher gain on the right are the currents remaining with 200 nM tetrodotoxin externally. The five traces in TTX are for $+30$, $+60$, $+90$, $+120$ and -160 mV. In this family the sodium concentrations were 460//0 which probably accounts for the very small inward current at $+30$ mV. Axon 23*b*, 17°, 493 μm

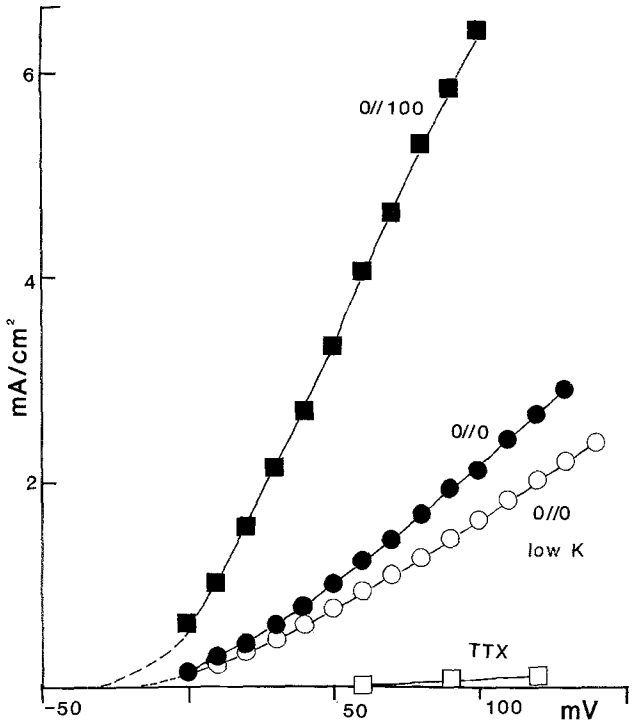

Fig. 2. The early transient current-voltage relationship in the absence of external sodium. The peaks of the transients shown in Fig. 1 are plotted against the potential. The filled squares correspond to the left frame with 0//100 sodium concentrations. The filled circles are the central 0//0 data, and the open squares represent the current in the presence of TTX measured at the time of the early transients. The current represented by the open circles was measured with the internal potassium concentration reduced from 275 to 175 mM. Axon 23*b*, 17°, 493 μm

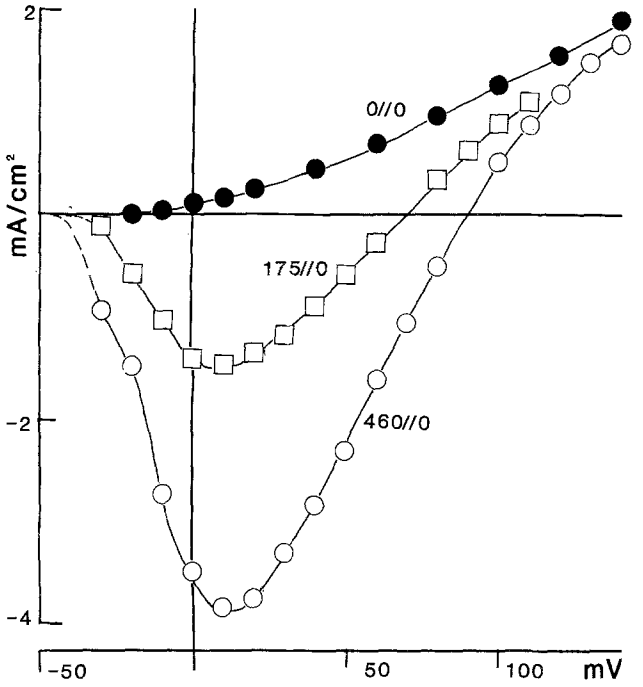

Fig. 3. The early transient current-voltage relationship in the absence of internal sodium. The filled circles represent current in the absence of sodium (0//0); the squares with 175//0, and the open circles with 460//0 sodium concentrations. Axon 40*a*, 11°, 466 μm

relationship in the linear region, clearly increased as the internal sodium concentration was increased from 0 to 100 mM.

Reducing internal potassium reduced the outward transient currents (Fig. 2) as if potassium were a less favorable substitute for sodium as a current carrier. Thus $\Delta g/\Delta \mathrm{Na}_i = 4.2 \times 10^{-4}$ and $\Delta g/\Delta \mathrm{K}_i = 4.9 \times 10^{-5}$ $\Omega^{-1}\,\mathrm{cm}^{-2}\,\mathrm{mM}^{-1}$ or, dividing by F, Faraday's constant, 4.3×10^{-6} and 5.1×10^{-7} cm/mV-sec, respectively. In this experiment potassium had about one-ninth the permeability of sodium similar to the findings of Chandler and Meves (1965). This will be qualified below, after presenting results with high external potassium concentrations (p. 85).

The currents that occur in the presence of tetrodotoxin are quite small at the time of the normal early transient and arise from the leak conductance, the early turn-on of the classical potassium conductance (Hodgkin & Huxley, 1952*b*) and from the asymmetry or gating currents (Armstrong & Bezanilla, 1974; Keynes & Rojas, 1974; Meves, 1974). The data in this paper have not been corrected for these currents as they are relatively small and do not change much with the solution changes used in this study. Furthermore, most of the analysis is based on the comparison of two or more *I-V* relationships and any relatively unaltered currents drop out.

No Sodium Inside

Figure 3 shows the effect of increasing external sodium concentration with no sodium inside the axon. With sodium outside, three regions of the *I-V* relationship can be distinguished. There is a transition region of negative slope conductance below about +10 mV, a linear region of constant conductance from +20 to +80 followed by a bend towards the voltage axis and then another approximately linear region (Chandler & Meves, 1965). The transition region represents the turning on of the sodium currents (Hodgkin & Huxley, 1952*b*). It is the linear region between +20 and +80 which we will be describing. In the linear region the slope conductance is the same as Hodgkin and Huxley's chord conductance, g_{Na}.

As was the case with internal sodium, increasing external sodium increased the conductance. Thus the

slope between +20 and +80 mV increased from 0.012 to 0.030 to 0.055 Ω^{-1} cm^{-2} as the external sodium was increased from 0 to 175 to 460 mM, respectively. The extrapolated intercept of a straight line through the points between +20 and +80 mV with the 0//0 curve did not greatly change. This generates the bend at about +100 mV.

The change of conductance per unit change of external sodium concentration, $\Delta g/\Delta Na_o$, was 1.0 and 0.9×10^{-4} Ω^{-1} cm^{-2} mM^{-1} or, dividing by F, 1.1 and 0.9×10^{-6} cm/mV-sec. These numbers are smaller than those found with comparable changes in internal sodium concentration and this difference will be described with the accumulated results below.

The bend or rectification of the current which occurs near +100 mV becomes sharper as the difference between internal and external concentrations is made larger. Beyond this bend the magnitude of the current is not dependent on the external concentration.

Sodium on Both Sides

Increasing the sodium on either side of the membrane individually, increases the conductance without changing the intercept with the appropriate zero sodium current line. When sodium was increased on both sides these effects were found to sum (Fig. 4). The four concentration pairs and the associated conductances for the I-V relationships of Fig. 4 are 0//0, 0.034; 0//100, 0.073; 460//0, 0.099; and 460//100, 0.140 Ω^{-1} cm^{-2}. Increasing internal sodium by 100 mM increased the conductance by about 0.040 Ω^{-1} cm^{-2} in the presence or in the absence of external sodium. Similarly, increasing external sodium by 460 mM increased the conductance by about 0.066 Ω^{-1} cm^{-2} in the presence or absence of internal sodium. The conductance is more sensitive to a change in internal sodium. Thus $(1/F)\,\Delta g/\Delta Na_o = 1.4 \times 10^{-6}$ cm/mV-sec is about one third of $(1/F)\,\Delta g/\Delta Na_i = 4.5 \times 10^{-6}$ cm/mV-sec.

The I-V curves with external sodium bend toward the voltage axis and become asymptotic to the corresponding curve in the absence of external sodium at about the same potential for both internal sodium concentrations. The 460//100 curve is similar to the 460//0 curve but is shifted or rotated upward by the difference between the 0//100 and the 0//0 curve.

We were initially impressed by the relatively small effect on the current at 0 mV when 100 mM sodium was added to the inside of the axon with normal sodium outside. While the reversal potential where $I=0$ is reduced almost by half, the current at 0 mV is decreased by about one fifth. This is because increasing internal sodium had increased the membrane

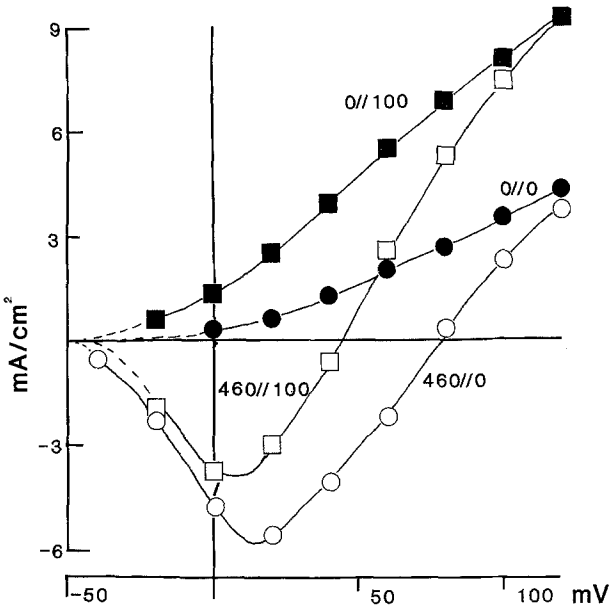

Fig. 4. The early transient current-voltage relationship of the squid axon membrane as sodium is varied independently on the two sides of the membrane. Filled circles, 0//0 sodium concentrations; filled squares, 0//100; open circles, 460//0; open squares, 460//100. Axon 6*b*, 3°, 670 μm

sodium conductance, g_{Na}, almost as much as it decreased the driving force $V\text{-}V_{Na} = V_{Na}$ at $V=0$, so their product, $g_{Na}V_{Na}$, is only slightly changed. The observed current at $V=0$ was reduced by a factor of 0.78. This is equal to the ratio of the sodium gradients, thus 360 mM/460 mM = 0.78.

Sodium Permeability and Sodium Conductance

When the axon is voltage clamped to 0 mV there is no electrical force driving ions from one side to the other. In this situation, in the simplest case, Fick's Law would apply; that is, the flux of an ion is linearly proportional to the difference in concentrations on the two sides of the membrane. Expressed in an equation in terms of electrical current $I = -P(c_o - c_i)F$. This is consistent with the finding in the previous paragraph. In order to test this proposition we performed the same type of experiment as Fig. 4 with 12 different pairs of solutions. We made a least squares fit to the data between +20 and +80 mV and extrapolated back to the voltage axis. This represents the current that would be present at 0 mV if the transition were complete and all the channels were opened. Similar results were obtained by clamping the potential to +40 and then measuring the "instantaneous" current-voltage relationship at the time of the peak sodium current.

In Fig. 5 the extrapolated current at zero voltage is plotted against the difference in sodium concentra-

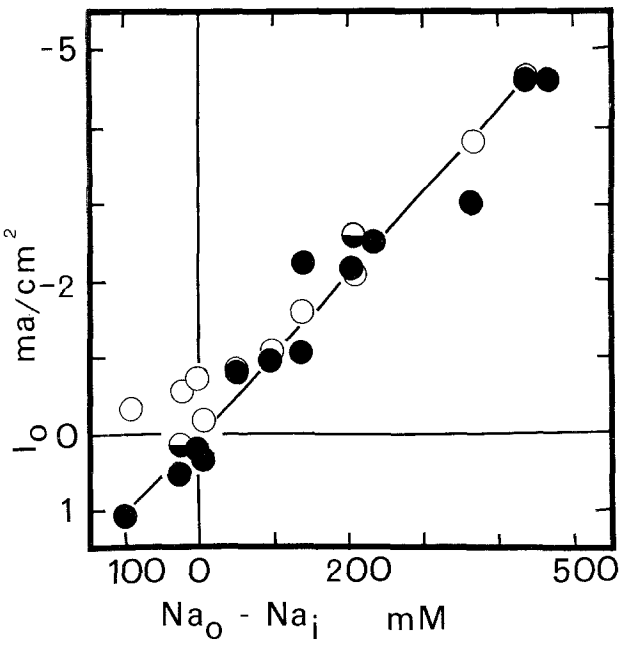

Fig. 5. The sodium permeability of squid axon membrane. *Ordinate:* the early transient current flowing through the membrane with 0 mV potential difference (extrapolated, *see* text); *abcissa:* the difference in sodium concentration of the two media bathing the membrane. Filled symbols: axon 132, choline replaced sodium extracellularly. Open symbols: axon 141, Tris replaced sodium externally. Half filled symbols: axon 141 with choline replacement. The current magnitude of axon 141 has been multipled by the ratio of the sodium conductances for the two axons, 1.53, to facilitate comparison

tions on the two sides. The straight line through the filled points with choline substituting for sodium has the equation $I_o = -P(Na_o - Na_i)F$ with $PF = 9.8 \times 10^{-3}$ mA cm^{-2} mM^{-1} or $P = 10^{-4}$ cm/sec. P is the Fickian permeability. The deviation from the line by the unfilled circles represents an artifact of high Tris concentrations and will be discussed below (p. 85). The value of P varies from axon to axon (Table 1), but in any individual fiber it is constant and independent of sodium concentration. We do not know whether this variability arises from differences between the squid or from mechanical manipulation of the nerves. The maximum inward current measured at the beginning of our experiments in 460//0 was 3.0 ± 0.3 mA/cm^2 (21) corresponding, with extrapolation, to a permeability of about 10^{-4} cm/sec (excluding axons with less than 1 mA/cm^2). The largest inward current we have seen was 10.5 mA/cm^2 with 460//30 sodium concentrations corresponding to a permeability of about 3×10^{-4} cm/sec.

The conductance data for 12 pairs of sodium concentrations are shown in Fig. 6. The conductance is plotted vertically against the two horizontal concentration axes. The internal sodium axis projects back into the plane of the paper. The data fall on a tilted plane with sides whose slopes are aF and bF. a and b correspond to the electrical admittance and measure the willingness of the membrane to accept sodium from outside and inside the axon, respectively. The

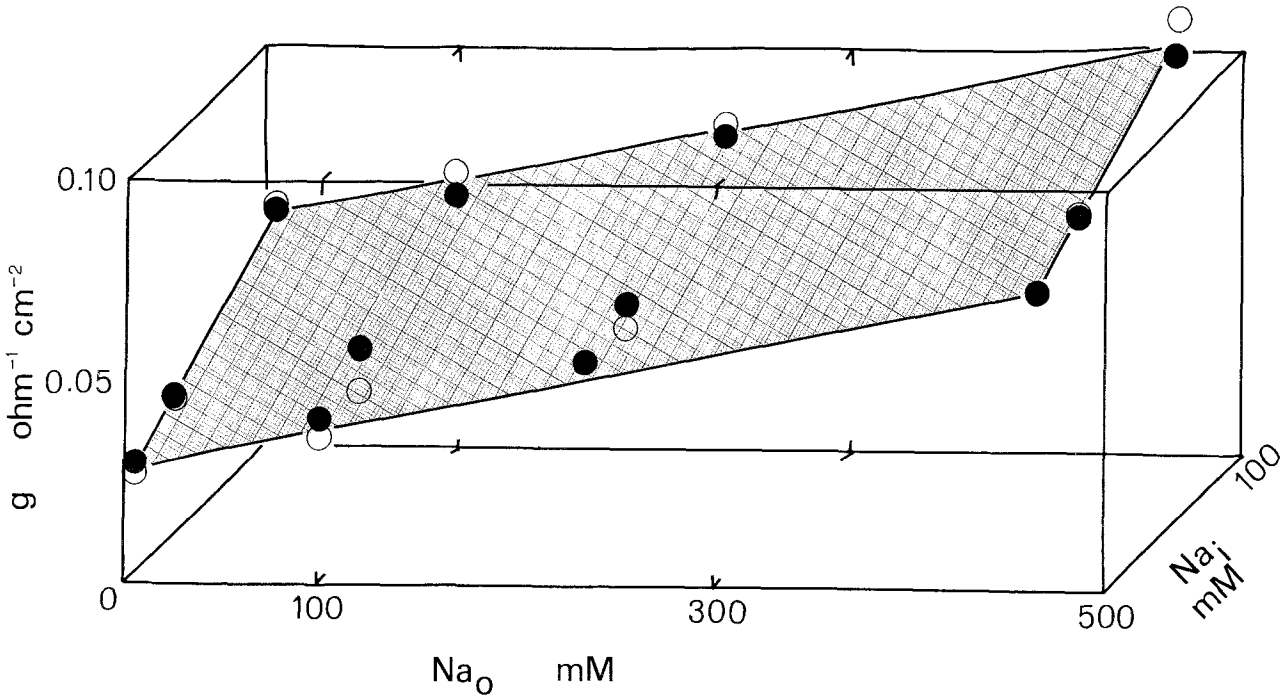

Fig. 6. The sodium conductance of squid axon membrane. On the vertical axis the membrane conductance during the early transient phase of current is plotted against the two sodium concentrations of the solutions bathing the two sides of the membrane. The internal sodium concentration axis projects behind the plane of the paper. Same symbols as Fig. 5

Table 1. The sodium permeability and the sodium conductance of squid axon membranes[a]

Axon	$P\times10^4$ (cm/sec)	r^2	$a\times10^6$ (cm/mV sec)	$b\times10^6$ (cm/mV-sec)	b/a	$P/(a+b)$ (mV)	Temp. (°C)	Na substitute
132	1.02	0.987	0.99	2.42	2.4	29.9	8	Choline
141	0.66	0.997	0.70	2.31	3.3	21.9	9	Tris[b]
6	1.62	0.996	1.41	4.49	3.2	27.5	3	Choline
7	0.40	0.990	0.30	1.27	4.2	25.5	6	Choline
7	0.58	0.992	0.41	2.23	5.4	22.0	18	Choline
18	0.23	0.993	0.24	0.65	2.7	25.8	4	TMA
Mean ± SE					3.5 ± 0.5	25.4 ± 1.3		

[a] The sodium conductance is a bilinear function of sodium concentration given by $g=(a\ Na_o+b\ Na_i)F$.
[b] Excluding measurements made with complete (460 mM) substitution of Tris for sodium. r^2 is the correlation coefficient for the data expressed as $I_o=-P(c_o-c_i)F$.

plane intercepts the conductance axis at $0.032\ \Omega^{-1}\ cm^{-2}$. This intercept represents the early transient conductance in the absence of internal and external sodium. For the filled circles (choline substituted) $a=10^{-6}$ and $b=2.4\times10^{-6}$ cm/mV-sec. The fit to the plane is quite good in Fig. 6 with the variance of a or b and the covariance (a, b) less than 0.1%. The data from an axon with Tris substituting for sodium has been scaled by a factor of 1.53 and falls on a similar plane $g=g_o+aFNa_o+bFNa_i$ with slightly different constants. The values of a and b for several axons are accumulated in Table 1 where it can be seen that the ratio b/a is about three with some variability between axons.

There is a definite relationship between permeability and conductance. Both concepts relate to how easily ions move through the membrane with either a concentration gradient or a voltage gradient as the driving force. Einstein has shown the relationship between the diffusion coefficient D and the electrical mobility μ is $D/\mu=kT/e$ where k is Boltzmann's constant; T, the absolute temperature and e, the charge. Correspondingly, we find that $P/(a+b)=25\pm1$ mV or kT/e within experimental error (Table 1). This ratio $P/(a+b)$ did not vary systematically with the magnitude of the current, the temperature, or the sodium substitute used. The experimental errors are too large to see the relatively small increase expected for a 5% change in absolute temperature.

The ratio b/a is a measure of the membrane asymmetry in its willingness to accept sodium ions from the two solutions. It has a value of about 3.5 but is somewhat variable about this mean, more variable than would be expected from errors in measurement. The asymmetry is not correlated with the magnitudes of the currents or any experimental manipulation of which we are aware. Its variability may represent innate axonal differences or perhaps trauma associated with dissection and internal perfusion. The asymmetry was altered by external calcium ions as described below.

Temperature

In axon 7 of Table 1 it can be seen that increasing the temperature increased P, a and b with less effect on $P/(a+b)$. Figure 7 shows that in the absence of external sodium, raising the temperature increased the outward current at all potentials. In the inset (circles and left-hand axis) the conductance, measured between +20 and +80 mV, is seen to increase linearly with temperature. Thus for this axon $g=0.0134+0.00188\ C$ where C is the temperature in °C. A linear dependence of this sort has been reported by Moore (1958) in intact axons. Our data are better fit by a straight line ($r^2=0.999$) than by $A\exp(-B/T)$ where T is the absolute temperature ($r^2=0.958$).

The current at zero voltage and hence the permeability also increased with temperature and at about the same rate (inset, squares, and right-hand axis). The values of I_o are small, and this is not the best way to estimate permeability so we are not disturbed by the departure from linearity at the highest temperature. A 2-mV error in the potential could account for this deviation.

When the temperature was increased with sodium on the outside of an axon, there was a similar linear increase in the conductance with $g=0.0243+0.00123\ C$ (Fig. 8). In comparison with the axon of Fig. 7, this would suggest that a is less temperature dependent than b. This is also consistent with axon 7 (Table 1) where a 12° increase in temperature increased a by 36% and b by 76%. However, in the axon of Fig. 8, I_o and therefore the permeability increased slightly less with temperature than the conductance which suggests b has less temperature dependence than a since $P=(a+b)=kT/e$. Because of this and the

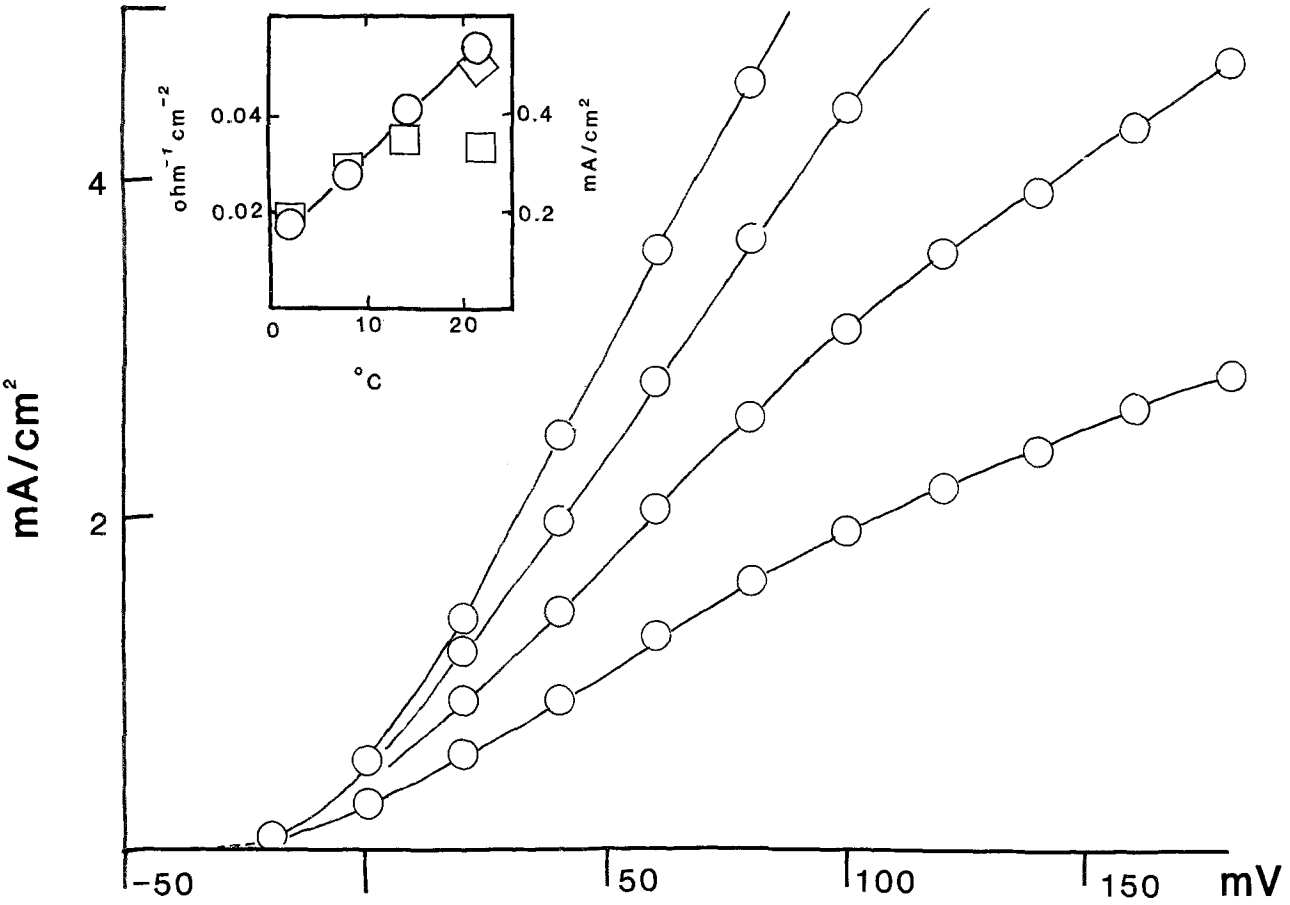

Fig. 7. The effect of temperature on the early transient current-voltage relationship with no sodium outside. Starting with the lowest curve, the measurements were made at 2.1, 8.1, 14.2, and 21.5 °C. A final curve (not shown) at 1.8° fell just below the data at 2.1°. In the inset the conductance (circles, left ordinate) and the current at 0 mV (squares, right ordinate) are plotted against the temperature. The diamond represents the current at +2 mV. Axon 44*b*, sodium concentrations 0//30, 486 μm

unexplained variability of *b*/*a* we have been unable to precisely determine their relative temperature dependence but conclude that they increase by 3–6% per degree of their value at 10°.

Other External Ions

Tris. Tris (hydroxymethyl) aminomethane is a common impermeable sodium substitute used in voltage-clamp experiments (Armstrong & Bezanilla, 1974; Keynes & Rojas, 1974; Landowne, 1977). It was somewhat surprising that its behavior differed from choline as seen in Fig. 5. At high Tris concentrations the sodium permeability is reduced but the sodium conductance (Fig. 6) is not changed. If the *I-V* curves for 0//30 sodium concentrations with Tris and choline sodium replacements are compared (not shown) the outward current curve in Tris is shifted about 20 mV towards more positive potentials. That is, Tris reduced the intercept with the current axis ($V=0$) without changing the slope or reduced P without changing *b*. This can be explained as an action of Tris on the membrane in which *a* is selectively reduced. The reduction in *a* in 460 mM Tris does not alter the conductance because $Na_o=0$ and the conductance is determined by *b* alone for large voltage pulses.

Replacing sodium with Tris also reduces the extra efflux of radioactive sodium associated with voltage-clamp pulses (Landowne, 1977). This was initially associated with the absence of sodium because three other substitutes had the same effect. Presently it seems more likely that the reduction of efflux reflects the action of Tris to reduce the sodium permeability. When choline was used as a sodium substitute in more recent experiments on radioactive sodium efflux from internally perfused axons, no change in extra efflux was seen whereas Tris still produced the reduction (Scruggs & Landowne, 1977).

Potassium. Internal potassium ions can carry outward early transient current through a tetrodotoxin-sensitive mechanism. We looked for inward potassium cur-

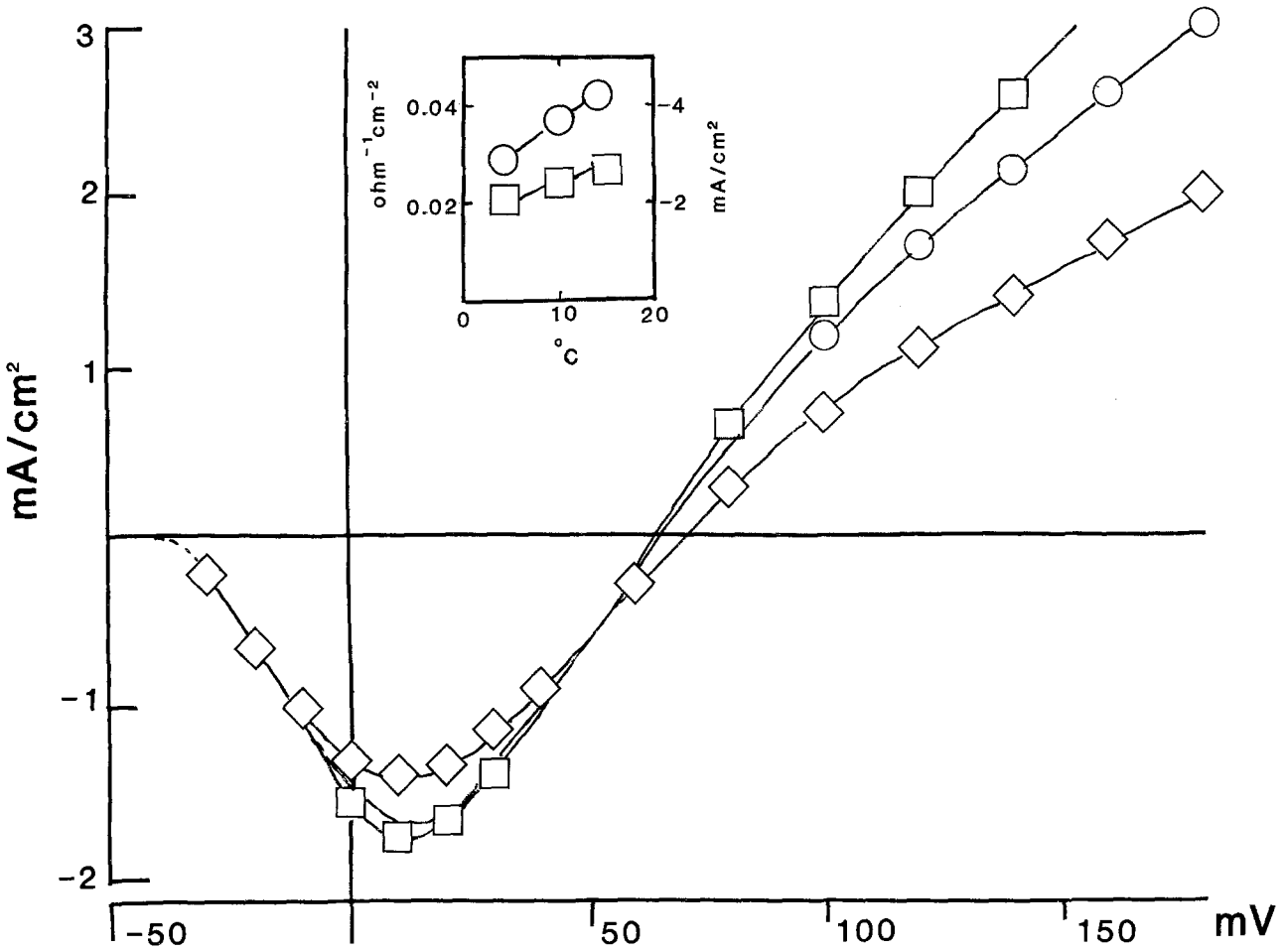

Fig. 8. The effect of temperature on the early transient current-voltage relationship with no sodium inside. Main figure: diamonds 4.0°, circles, 10.1°, squares, 14.0°. Inset: as in Fig. 7, circles and left ordinate are the conductance; squares and right ordinate, the current at 0 mV. Axon 43*a*, sodium concentrations 175//0, 492 μm

rent with K replacing Na in the external solution. With 470 mM K outside and 175 K inside no inward early transient current was seen at any potential. This confirms previous observations (Moore et al., 1966, Ebert & Goldman, 1976). Qualitatively, the outward early transient current was not altered when K replaced Na outside, but subtle changes may have been obscured by the change in the delayed potassium current.

This absence of inward potassium current and presence of outward potassium current suggests that both the permeability and the asymmetry may be different for different ions in the same membrane. It appears as if $a_K \ll b_K$ so that replacing TMA with K outside does not produce inward early transient currents whereas inside, raising K increases the outward early transient currents (Fig. 2).

Calcium. Raising external calcium concentration decreased the sodium permeability in a manner similar to the decrease seen with Tris. In the absence of external sodium the outward Na *I-V* curve was shifted towards more positive potentials when external calcium was increased. The slope was unchanged and the intercept with the current axis was reduced, suggesting that, like Tris, external calcium reduces the permeability by a selective action on *a*. This was supported by confirming the finding of Frankenhaeuser and Hodgkin (1957) that raising external calcium in the presence of external sodium decreases the inward Na currents with little effect on the outward Na currents.

Lithium. Externally, lithium is able to substitute for sodium in producing the action potential and the associated transient inward currents. When the Na and Li *I-V* curves are compared (not shown), the lithium curve is similar to the sodium curve with elevated external Ca. That is, in Li there is a shift of the transition region towards positive potentials, a reduction in the slope and zero voltage intercept, a crossing

of the two curves below voltage axis, and a less prominent bend near +100 mV. The shift of the transition is relatively small, about 10 mV for 460 mM Li replacing Na. Lithium also reduces the extra radioactive sodium efflux associated with voltage-clamp pulses (Landowne, 1977), again consistent with a Ca-like action of reducing P. Thus lithium is permeable, reduces the sodium permeability, and perhaps reduces its own permeability by a Ca-like effect.

Discussion

We have measured the relationship between the magnitude of the sodium current, the membrane potential, and the sodium concentrations on the two sides of the membrane. When we plot the current-voltage relationships we find that the intercept with the current axis (the short-circuit current) is linearly proportional to the difference between the two sodium concentrations (Fig. 5). This is a clear parallel to Fick's Law. For positive potentials the slope of the linear portion of the current-voltage relationship (the conductance) increases linearly with sodium concentration and not symmetrically from the two sides of the membrane (Fig. 6). Thus the current-voltage-concentration relationship is well described by

$$I = -P(c_o - c_i)F + (ac_o + bc_i)FV$$

I is the magnitude of the sodium current, c_o and c_i are the sodium concentrations on the outside and inside of the axon, respectively, F is Faraday's constant, and V is the membrane potential. P, a and b are experimentally determined membrane constants. This empirical equation could be seen as the simple sum of Fick's Law and Ohm's Law.

We also find, within experimental error (Table 1), that $P/(a+b) = kT/e$ where k is Boltzmann's constant; T, the absolute temperature and e, the unit electronic charge. That is, the membrane permeability, P, is related to the membrane conductivity in a way which is similar to the relationship between the diffusion constant of an ion in free solution and the conductivity associated with that ion.

The value in this finding lies in its simplicity, its accuracy in describing the currents, and its ability to describe membrane asymmetry. It is a first approximation to describing the current-voltage relationship; it does not describe gating or the rectification near +100 mV. This rectification occurs at the intersection of the current described by this equation in the presence and absence of external sodium. This equation is part of a piecewise-linear approximation of the current-voltage relationship. The segment beyond +100 mV is reasonably well described by the same equation with c_o set equal to 0. We have not analyzed the curvature of the bend. This curvature becomes important when considering the Nernst equilibrium potential. This will be discussed below after considering other published data and formal descriptions.

There have been very few systematic studies of the concentration dependence of the currents, none in which concentrations on both sides of the same membrane have been varied. Hodgkin and Huxley (1952*a*) present three experiments, each with two different external solutions. Qualitatively, increasing external sodium increases the conductance and also makes the short-circuit current (estimated at −60 mV on their scale) more inward. If P and a are estimated from their data the ratio of P/a is the same as reported here, within experimental error. Chandler and Meves (1965) reported an experiment in which they internally replaced 300 mM K with 150 mM K and 150 mM Na. There is qualitative agreement with data presented here; because they changed both Na and K, it is difficult to compare quantitatively.

The current-voltage relationship in frog node has the same general form as the squid. In the node the maximum inward current is at about −30 mV. There is a linear region from about −20 to +40 mV and then a bend toward the voltage axis. Hille (1975) presents current-voltage curves for four external sodium concentrations. Both the short-circuit current and the conductance in the linear region are linear functions of the external sodium concentration with r^2 of 0.99 and 0.97, respectively. The ratio of P/a in the frog appears to be 30 mV less than the squid and, correspondingly, the bend appears at a lower potential.

The finding that the ratio $P/(a+b)$ is constant also means only two factors are required to determine the third. Any two could be chosen and, because the membrane is asymmetric in its response to sodium ions, two is the minimum number. Biological membranes are generally asymmetric in their lipid and protein make-up (Bergelson & Barsukov, 1977; Rothman & Lenard, 1977) and also in the amount of surface change at the two aqueous/membrane interfaces (McLaughlin & Harary, 1974). The two membrane constants could be a and b, the willingness of the membrane to accept sodium from its two sides, or P, the membrane permeability, and b/a, a measure of the membrane asymmetry. The membrane constants are specific both for the particular ion and the particular channel or conductance mechanism. Thus we have found that during the early transient current the membrane is more permeable to sodium than potassium, but the response to potassium is more asymmetric than the response to sodium. To a first approximation, raising external calcium, Tris, or Li

has a selective effect on a the membrane acceptance of sodium from the external sodium, although our experiments did not distinguish between calcium and ionic strength. Thus, in general, one might expect to find drugs or other treatments which selectively alter either or both the permeability and the asymmetry of a current pathway.

The empirical current-voltage-concentration relationship is a formal framework which well describes our experimental findings. If a potential energy barrier model is used to explain these data, it must be asymmetrical and contain a sufficient number of barriers to make the conductance ohmic. A rate theory analysis of the current-voltage characteristics of Danielli's (1943) potential energy barrier model shows that

$$I = P[-(c_o - c_i) + c_m 2n \sinh(eV/2nkT)]F,$$

$$P = [\sum \exp(e\phi_j/kT)]^{-1}$$

with ϕ_j being the height of the individual barriers and $c_m = \frac{1}{n}\sum c_j$ with c_j being the concentration in each of the n potential minima within the membrane. When $V \ll 2nkT/e$ this simplifies to

$$I = P[-(c_o - c_i) + c_m\, eV/kT]F$$

which can be compared with our experimental equation by defining

$$\bar{c}_m = (a c_o + b c_i)/(a+b),$$

the effective membrane concentration. Then, by substitution and using the experimentally found $P/(a+b) = kT/e$, the data may be described by

$$I = P[-(c_o - c_i) + \bar{c}_m\, eV/kT]F$$

as in the model. Thus a very general model for the membrane suggests the form of the current-voltage-concentration relationship we have obtained. A bulk diffusion model where the membrane is pictured as a medium with low sodium concentration and low mobility is also appropriate. The new findings presented here require that the two partition coefficients for the two aqueous/membrane interfaces be different by a factor of three. The effect of temperature to increase both the permeability and the conductance (Figs. 7 and 8) suggests that the ability to move through the membrane is being altered rather than the effective membrane concentration.

We would like to compare our linear equation with other formal descriptions of the relationship between current, voltage, and ionic concentrations. First we note that we have only studied one membrane and one species of ion, and clearly many more experiments will be required to test the generality of the equation. We expect some generality because our equation is similar to one proposed by Hodgkin and Huxley (1952*a*), namely $I = g_{Na}(V - E_{Na}) = -g_{Na}E_{Na} + g_{Na}V$. In the linear region of the I-V curve the conductance we measure is the same as Hodgkin and Huxley's. We have found this conductance to increase linearly with sodium concentration and not symmetrically from the two sides of the membrane. Hodgkin and Huxley do not explicitly give the concentration dependence of the conductance, although a nonlinear relationship can be derived from their discussion of predictions from the "independence principle." Our equation differs from Hodgkin and Huxley's more in the first term. We find the current at $V=0$ to be a direct measure of membrane permeability by a clear demonstration of Fick's law. In contrast, Hodgkin and Huxley predict a short-circuit current of $-g_{Na}E_{Na}$. Fick's Law does not depend on the concept of thermodynamic equilibrium, but describes what is essentially an irreversible process. With Fick's Law the current is well described when either concentration is zero.

Hodgkin and Huxley's equation worked very well in describing the effect of voltage changes on the current and permitted the mathematical reconstruction of the action potential. In order to describe changes in the current-voltage relationship when concentrations were changed they invoked the "independence principle" and an additional arbitrary scaling factor (Hodgkin & Huxley, 1952*a*; Chandler & Meves, 1965). Our equation retains the same form of voltage dependence as Hodgkin and Huxley's and in addition describes the effect of concentration changes quantitatively, without a scaling factor.

The major theoretical description of the relationship between current, voltage, and concentrations has been the constant field equation (Goldman, 1943; Hodgkin & Katz, 1949). This has recently been challenged by the results of Cahalan and Beginisich (1976) who find that when the reversal potential of the early transient current is analyzed using the constant field equation it appears as if the relative permeability of the membrane to Na and K varies with the K concentration. This is a violation of one of the assumptions made in deriving the equation and makes its use much less practical. In addition, our finding of an asymmetry in the dependence of the conductance on ionic concentrations suggests that the assumption of symmetrical partition coefficients made in deriving the constant field equation is inappropriate. One might use a modified constant field equation with two different permeability constants, one looking from the inside and one looking from the outside, but this

would clearly make the equation much more cumbersome.

A very interesting theoretical point is that our empirical equation does not reproduce the Nernst equation. We find at $I=0$, $V=P(c_o-c_i)/(ac_o+bc_i)$. By substituting $\Delta c=c_o-c_i$, $\bar{c}_m=(ac_o+bc_i)/(a+b)$ and $kT/e=P/(a+b)$ this becomes

$$V=\frac{kt}{e}\frac{\Delta c}{\bar{c}_m}$$

which is similar to but not exactly the same as the Nernst equation. Complete similarity would be achieved were the membrane symmetrical and of infinitesimal thickness. It then reduces to $dV=(kT/e)dc/c$.

Our equation predicts a voltage smaller than the Nernst potential. It is therefore not physically correct and only approximate. What is missing is a description of the curvature through the rectification. The data bends away from the equation towards more positive potentials. An exact description of the current-voltage-concentration relationship would include this curvature and have an equilibrium potential given by the Nernst equation. Caution should also be used when considering currents at potentials more negative than those we have examined. The peak currents decrease because of incomplete gating. The instantaneous current-voltage relationship is more linear but also bends towards the voltage axis at negative potentials (Chandler & Meves, 1965). There is uncertainty in making the "instantaneous" measurement at negative potentials because it is necessary to perform an extrapolation at a time when the current is rapidly changing. It is due to this uncertainty and that we actually wish to describe the peak currents that we have chosen to use peak rather than instantaneous current-voltage relationships. When we compared the peak curve with an instantaneous curve made at the time of maximum current at +40 mV, they superimposed, for potentials larger than +10, the region we are describing (*see also* Chandler & Meves, 1965).

The fact that our empirical equation is simple and parallels Fick's Law and Ohm's Law suggests that it may be theoretically simpler to consider the current through the membrane as two independent flows, one associated with the concentration gradient and the other associated with the voltage gradient. A simple superposition law seems adequate. It will be interesting to see if this can be verified with a wider range of experiments.

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