

Ammonium Ion Currents in the Squid Giant Axon

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ABSTRACT Voltage-clamp studies on intact and internally perfused squid giant axons demonstrate that ammonium can substitute partially for either sodium or potassium. Ammonium carries the early transient current with 0.3 times the permeability of sodium and it carries the delayed current with 0.3 times the potassium permeability. The conductance changes observed in voltage clamp show approximately the same time course in ammonium solutions as in the normal physiological solutions. These ammonium ion permeabilities account for the known effects of ammonium on nerve excitability. Experiments with the drugs tetrodotoxin (TTX) and tetraethyl ammonium chloride (TEA) demonstrate that these molecules block the early and late components of the current selectively, even when both components are carried by the same ion, ammonium.

INTRODUCTION

Recent voltage-clamp experiments on squid giant axons, in which either internal axoplasm was replaced by other ionic solutions (Chandler and Meves, 1965 *a*; Chandler, Hodgkin, and Meves, 1965) or the external bathing medium was systematically varied (Chandler and Meves, 1965 *b*; Meves, 1966; Meves and Chandler, 1965; Moore et al., 1966), have established a sequence of ionic permeabilities of the axon membrane to the alkali cations. There is evidence from these experiments, and from studies of the effect of the drugs tetrodotoxin (TTX) (Nakamura, Nakajima, and Grundfest, 1965; Hille, 1968), and tetraethylammonium chloride (TEA) (Armstrong and Binstock, 1965; Hille, 1967), suggesting that each of the two seemingly independent conducting processes first described by Hodgkin and Huxley (1952 *a*)—the early transient Na pathway and the delayed K pathway—can be characterized by its own sequence of ionic selectivities and its own specific blocking agents.

We were interested in the NH_4 ion because NH_4 is known to have some of the properties of both Na and K, and hence, in terms of the foregoing picture, might be highly permeable in both channels. NH_4 is known to restore excita-

bility to nerves in Na-free media and it is also a strong depolarizing agent. These properties were first established for frog A fibers by Lorente de N6 et al. (1957) and have been demonstrated for squid giant axon by Tasaki et al. (1965). Excitation and depolarization are usually assigned respectively to Na-like and K-like ions. Voltage-clamp experiments on the frog node in NH_4 solutions have been reported by Dodge (1963) and suggest that the Na pathway is permeable to NH_4 . The relative permeability of NH_4 in the K pathway has been difficult to study on intact axons (Dodge, 1963).

We studied the effects of replacing the normal physiological solutions by NH_4 solutions in intact and internally perfused squid giant axons. By varying the ionic composition inside or outside the axon we were able to explain the effects of NH_4 on nerve function by the ability of NH_4 to carry current with a considerable permeability in both channels.

METHODS

The preparation of the giant axons of the squid, *Loligo pealei*, was described in detail by Cole and Moore (1960). Tied off lengths of axon, about 5 cm long, were used. Axon diameters varied from 400 to 500 μ . The axons which were not perfused were fine dissected, but the internally perfused axons were left surrounded by a 50–75 μ layer of fibrous tissue. The axons were cleaned only enough so that we could easily see the internal electrodes and perfusion pipettes.

Voltage-clamp apparatus and technique have been described elsewhere (Cole and Moore, 1960). Modifications designed to speed the response of the voltage-clamp system were discussed by Armstrong and Binstock (1964). The main feature of their system was the replacement of the voltage-measuring microelectrode by a low impedance arrangement, consisting of a pipette voltage-measuring electrode surrounded by a current-carrying sleeve. Details of the construction of our electrode system were given in a previous paper (Lecar et al., 1967). The anomalous decay of the steady current noted in our earlier paper was eliminated in these experiments.

The internal perfusion technique was essentially the same as the Tasaki method (1963). Modifications were made to allow the use of the voltage-clamping technique, which required insertion of the coaxial electrode into the axon without stopping the perfusion flow. A glass capillary in the suction pipette with its end projecting about a millimeter, served as a guide for inserting the pipette into the axon. Once the suction pipette was inside the axon, the capillary was removed and the suction pipette was pushed further into the axon. The infusion cannula was inserted from the other end of the axon, and pushed through the suction cannula. As soon as the axoplasm was flushed out of the suction or outlet cannula and a good perfusion flow obtained, the two cannulae were separated, leaving a perfused zone 15 mm long. The perfusion fluid flowed at a rate of 5–15 $\mu\text{l}/\text{min}$. With a typical perfused volume of 3 μl , a complete solution replacement takes place in 24–30 sec and we always waited at least 5 min after a switch of perfusion fluids.

In these experiments the internal perfusion fluid was either 500 mM KF or 500 mM NH_4F buffered with 5 mM Tris and adjusted to pH 7.4. The external solution for the

control runs was artificial seawater (NaSW: 430 mM Na, 10 mM K, 10 mM Ca, 50 mM Mg, 560 mM Cl, and 5 mM Tris). Test external solutions had 430 mM NH_4 or 430 mM mixtures of NH_4 and choline in place of the 430 mM Na, with the other constituents unchanged.

TABLE I
RESTING MEMBRANE POTENTIALS FOR PERFUSED AXONS

(a) External substitution					
Test solutions: internal, 500 mM KF; external, NH_4SW					
Control solutions: internal, 500 mM KF; external, NaSW					
Axon	Temperature	$-V_{rest}, mV$		Test	Depolarization, mV ΔV
		Control	Control		
	$^{\circ}\text{C}$	<i>before</i>	<i>after</i>		
65-35	12	49	48	33	15
66-29	5.5	49	40	28	21
66-31	5	64	57	47	17
66-34	4	49	41	33	16
66-38	5	52	52	42	10
67-04	9.5	51	51	39	12
67-07	8	61	55	46	15
67-10	9	58	56	43	15
67-12	9.5	63	60	50	13
Average values		55 ± 6	51 ± 7	40 ± 7	15 ± 3
Average values corrected for liquid junction potentials		64	60	46	18
(b) Internal substitution					
Test solutions: internal, 500 mM NH_4F ; external, NaSW					
Control solutions: internal, 500 mM KF; external, NaSW					
Axon	Temperature	$-V_{rest}, mV$		Test	Depolarization, mV ΔV
		Control	Control		
	$^{\circ}\text{C}$	<i>before</i>	<i>after</i>		
66-29	5.5	58	53	35	23
66-33	6.5	57	48	17	40
66-41	6	57	56	16	41
67-37	8.5	47	50	15	32
Average values		55 ± 5	52 ± 4	21 ± 10	34 ± 8
Average values corrected for liquid junction potentials		64	61	30	34

RESULTS

Resting Potentials and Action Potentials

Resting potentials were measured between the internal voltage control electrode and an external reference pipette. The measured potentials were corrected for the liquid junction potentials at the solution-electrode interfaces, which were calculated from the Planck formula (MacInnes, 1961). Observed resting potentials and the corrected values are given in Table I.

Both internal and external NH_4 substitutions depolarize the axon. For axons internally perfused with 500 mM KF, the average depolarization is 18 mv (corrected for junction potential) when NH_4 is substituted for Na externally. When the perfusion fluid was replaced by 500 mM NH_4F the depolarizations in NaSW ranged from 23 to 41 mv. The large spread in values for the internal

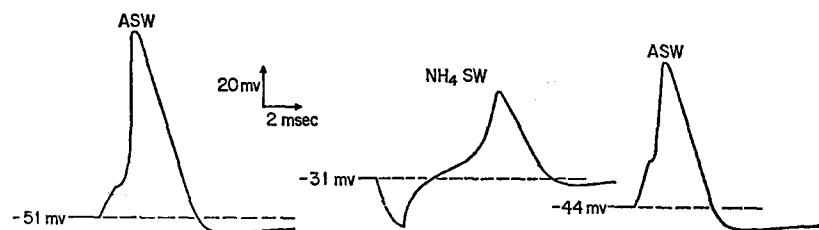


FIGURE 1. Action potentials of an unperfused axon in a typical experimental sequence in which the external solution was first NaSW, then NH_4SW , then returned to NaSW. The axon was space-clamped and stimulated by means of the internal current electrode. Axon 65-29a, temperature 9°C , stimulus duration for NH_4SW anodal break 9 msec.

substitutions may be attributable to incomplete exchange of the solutions in some of the perfusion experiments.

Axons immersed in NH_4SW give action potentials upon anodal break excitation, as shown in Fig. 1. The NH_4SW action potentials were blocked by externally applied tetrodotoxin and prolonged by injected tetraethylammonium chloride in the same way as normal NaSW action potentials. The crest of the NH_4 action potentials varied from 36 to 50 mv positive to the resting potential; the magnitude of the underswing varied from 0 to 6 mv. Internal perfusion with 500 mM NH_4F generally inhibited excitability.

Voltage-Clamp Currents

Fig. 2 shows the voltage-clamp currents in response to a series of depolarizing step voltages. In this sequence of experiments, the axon was internally perfused with 500 mM KF and immersed in NaSW for the runs shown in Figs. 2 a and 2 c. In Fig. 2 b the internal solution was replaced by 500 mM NH_4F with the external solution unchanged. Fig. 2 e shows the currents at early times for an

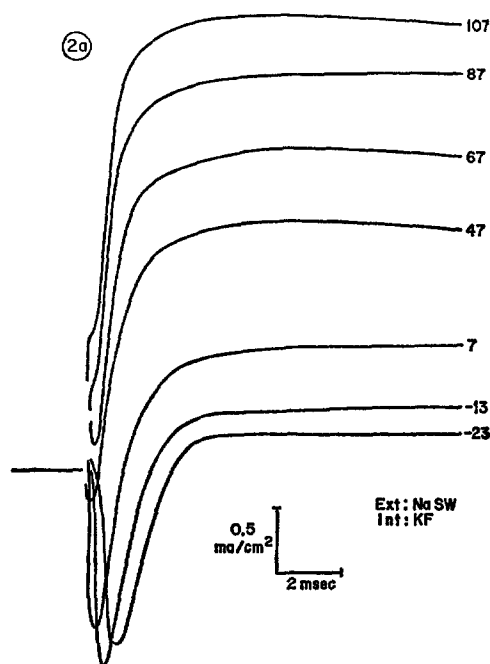


FIGURE 2 a

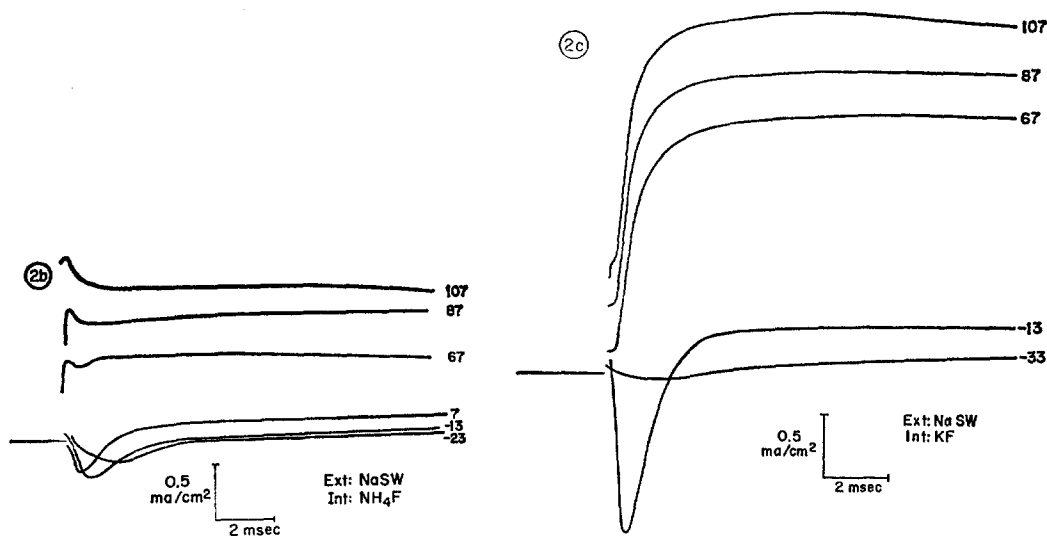


FIGURE 2 b

FIGURE 2 c

FIGURE 2. Voltage-clamp currents, following step depolarization to the potential given (millivolts). (a), (b), and (c) Sequence showing internal perfusion solution change from 500 mM KF to 500 mM NH_4F . Axon 65-35, temperature 12°C , holding potential -53 mv. (d), (e), and (f) Early currents for an intact axon which exhibited an NH_4 action potential. Sequence shows effect of external solution change from NaSW to NH_4SW . Axon 65-29a, temperature 9°C , holding potential -56 mv, hyperpolarizing prepulse -50 mv, 25 msec.

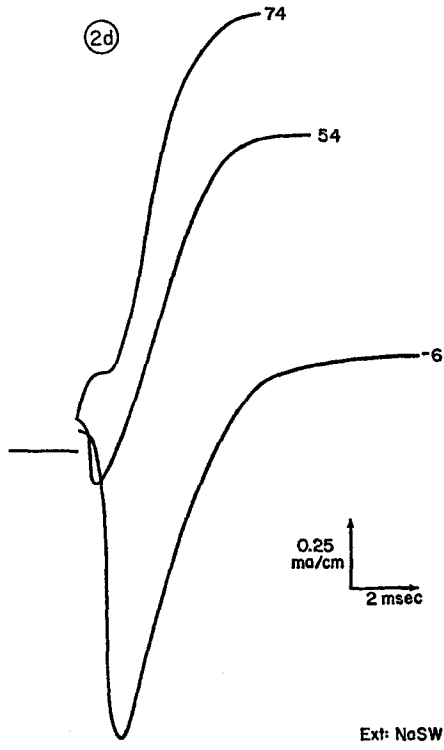


FIGURE 2 d

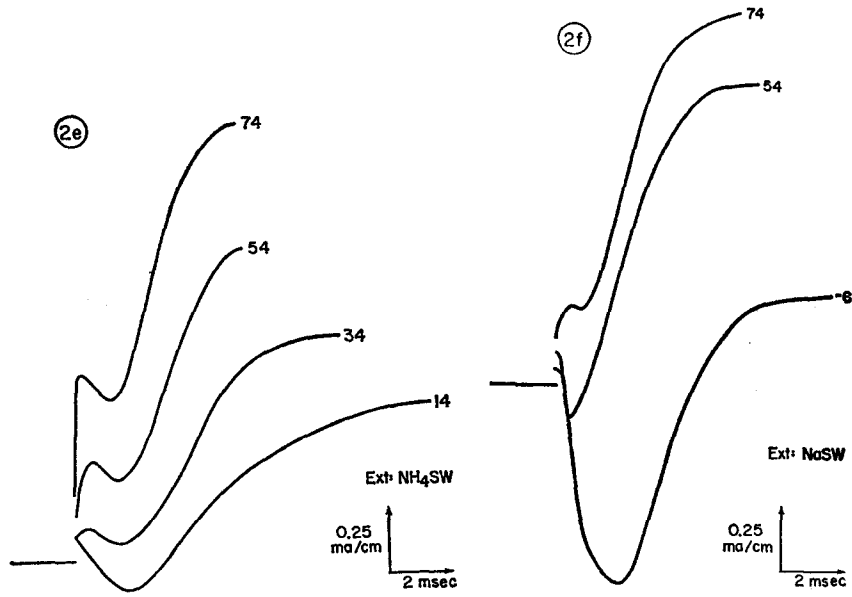


FIGURE 2 e

FIGURE 2 f

experiment in which the external solution was replaced by NH_4SW . In these experiments, the initial potential was held at the same value for all the solutions regardless of the true membrane resting potential. Each of the voltage-clamp sequences shows the same general features, a rapid surge of outward current, seen as a discontinuity in these records, followed by a transient peak which is inward for the lower voltages but reverses sign at some higher de-

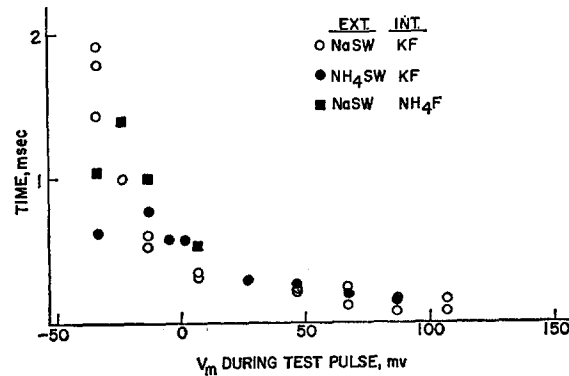
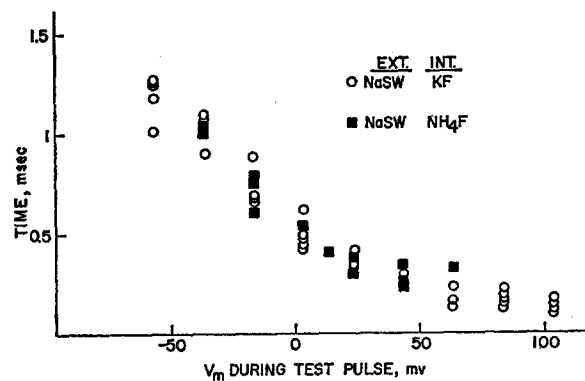
FIGURE 3 *a*FIGURE 3 *b*

FIGURE 3. Time to reach early peak current as a function of depolarizing voltage. (*a*) Axon 65-35, temperature 12°C , holding potential -53 mv. (*b*) Axon 66-41, temperature 6°C , holding potential -60 mv.

polarization, and a steady-state outward current. However, the relative magnitudes of the peak and steady-state components change for the NH_4 -substituted cases. For example, in Fig. 2 *b*, the responses to high depolarizations show a pronounced early peak of outward current. These early peaks seen with internal NH_4F are larger in magnitude than the early outward bumps observed with internal KF (Fig. 2 *a*).

The early outward bump has been shown to be a K current through the fast

channel (Chandler and Meves, 1965 *a*) having the same kinetics as the usual Na current. The larger outward peaks seen with internal NH_4 also exhibit the same "fast-channel" time course and hence it is clear that the outward peaks of Fig. 2 *b* represent NH_4 current through the fast channel. Also, it is clear that the channel must be more permeable to NH_4 than to K.

Similarly, Fig. 2 *e* shows the early current for an axon with external Na replaced by NH_4 . The early inward current and its reversal are clearly present,

TABLE II
APPARENT EQUILIBRIUM POTENTIALS

Axon	Test solutions		Test		Control		α	β
	External	Internal	V_e, mv		V_{Na}, mv			
			Meas-ured	Cor-rected*	Meas-ured	Cor-rected*		
64-36 (20°C)	NH_4SW	Axoplasm	10	4	43	34	0.30	—
65-25 (9°C)	215 mM NH_4SW 215 mM choline	Axoplasm	-10	—	39	—	0.28	—
65-29a (9°C)	NH_4SW	Axoplasm	29	23	64	55	0.28	—
65-31 (8°C)	NH_4SW	Axoplasm	17	11	58	49	0.22	—
65-35 (12°C)	NH_4SW	500 mM KF	33	27	66	57	0.26	0.09
65-35 (12°C)	NaSW	500 mM NH_4F	32	23	62	53	0.28	0.10
65-37 (11°C)	NaSW	500 mM NH_4F	37	28	63	54	0.24	0.10
66-41 (6°C)	NaSW	500 mM NH_4F	28	19	58	49	0.28	0.12
67-37 (8.5°C)	NaSW	500 mM NH_4F	24	15	58	49	0.33	0.12
Average values							0.27 ± 0.03	0.11 ± 0.01

* Corrected for liquid junction potentials at solution-electrode interfaces.

but the inward current is smaller in magnitude than the usual Na current and the reversal occurs at a lower potential.

One feature of the currents remains constant even when the NH_4 substitutions are made. Fig. 3 shows the time to reach peak current as a function of voltage for two axons. It can be seen that the time-to-peak data are similar for the different solutions. In terms of the usual Hodgkin-Huxley parameters, the invariance of the time-to-peak can be taken as a measure of the invariance of the early activation time, τ_m . From the Hodgkin-Huxley equations, one can

show that

$$t_{\text{peak}} \cong \tau_m \ln(1 + 3\tau_h/\tau_m), \quad (1)$$

where τ_h is the early channel inactivation time. Over the range of voltages studied here, τ_h varies from 5 to 10 times τ_m . Thus t_{peak} only changes from 2.7 to 3.4 times τ_m , and hence the measured value of t_{peak} is rather insensitive to variations of τ_h . Because the time course of the turn on of the early current is unchanged in the presence of the NH_4 solutions, we will regard the kinetics of the early conductance process as somehow reflecting a property of the membrane rather than of the particular ion carrying the current.

Next, we can determine the relative permeability of this early current pathway to the NH_4 ion. One quantity which depends on the relative permeability is V_e , the apparent equilibrium potential or "reversal potential" of the fast process. This is the voltage for which the peak current has a zero driving force. The apparent equilibrium potential can be determined from the voltage-clamp records as the depolarization value at which the early transient disappears. It can also be determined from the intersection of the leakage-corrected current-voltage curve with the zero-current axis. We assume V_e to have the form (Chandler and Meves, 1965 *a*; Goldman, 1943)

$$V_e = \frac{RT}{F} \ln \frac{[\text{Na}]_o + \alpha[\text{NH}_4]_o + \beta[\text{K}]_o}{[\text{Na}]_i + \alpha[\text{NH}_4]_i + \beta[\text{K}]_i}, \quad (2)$$

where the bracketed quantities are the activities for the various cations, and α and β are the permeabilities of NH_4 and K relative to Na. Here R , T , and F have their usual definitions as gas constant, absolute temperature, and Faraday's constant, respectively. Measured values of V_e for a variety of solution changes, both internal and external, are given in Table II. The average value of the permeability of NH_4 relative to Na from these measurements is $\alpha = 0.27$. Here β has also been determined independently from measurements of V_e in the control solutions. The value for the K permeability relative to Na is $\beta = 0.11$ in approximate agreement with previous measurements (Chandler and Meves, 1965 *a*; Moore et al., 1966). It is significant to note that the measured value of α is consistent for both internal and external NH_4 substitutions.

When a unique value of α has been determined, we ask whether this one quantity is sufficient to fit all the information about the peak currents. That is, can the peak currents be described completely by regarding NH_4 as behaving exactly like Na, but with 0.27 times the Na permeability? We know, for intact axons, that the current-voltage curves for various external Na concentrations can be reconstructed with reasonable accuracy from the curve in 430 mM external Na by the use of the independence principle (Hodgkin and Huxley, 1952 *a*). The independence principle relation for the ratio of the currents I and

I' measured at some voltage V for two sets of permeant ion concentrations, C_o, C_i and C_o', C_i' , is

$$\frac{I'}{\bar{I}} = \frac{C_o' - C_i' \exp \frac{FV}{RT}}{C_o - C_i \exp \frac{FV}{RT}} \quad (3)$$

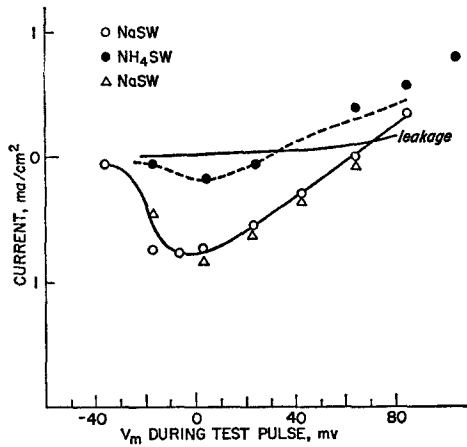


FIGURE 4 a. Peak current-voltage curves for an intact axon in NaSW and in NH₄SW. The dashed curve is the peak current-voltage curve obtained by the independence principle correction for the changed external solutions. Axon 65-29a, temperature 9°C, holding potential -56 mv, hyperpolarizing pre-pulse -50 mv, 25 msec.

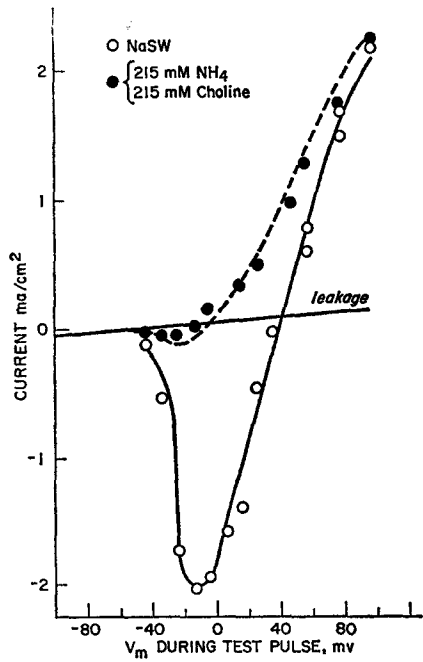


FIGURE 4 b. Peak current-voltage curves for an intact axon in NaSW and in a solution with 215 mM NH₄, 215 mM choline replacing the Na. The dashed curve is the peak current-voltage curve obtained by the independence principle correction for the changed solution. Axon 65-25, temperature 9°C, holding potential -63 mv. Corrected for leakage.

Assuming that NH_4 , K , and Na ions all flow through the membrane independently, the formula can be generalized to a form applicable to our experiments. The ratio of the currents in the NH_4 -substituted case to that in the control solution is given by

$$\frac{I'}{I} = \frac{[\text{Na}]_o' + \alpha[\text{NH}_4]_o' + \beta[\text{K}]_o' - ([\text{Na}]_i' + \alpha[\text{NH}_4]_i' + \beta[\text{K}]_i') \exp \frac{FV}{RT}}{[\text{Na}]_o + \beta[\text{K}]_o - ([\text{Na}]_i + \beta[\text{K}]_i) \exp \frac{FV}{RT}} \quad (4)$$

Fig. 4 shows the peak current-voltage curves for axons bathed in 430 mM Na, 430 mM NH_4 , and 215 mM NH_4 . The dashed curves were calculated from

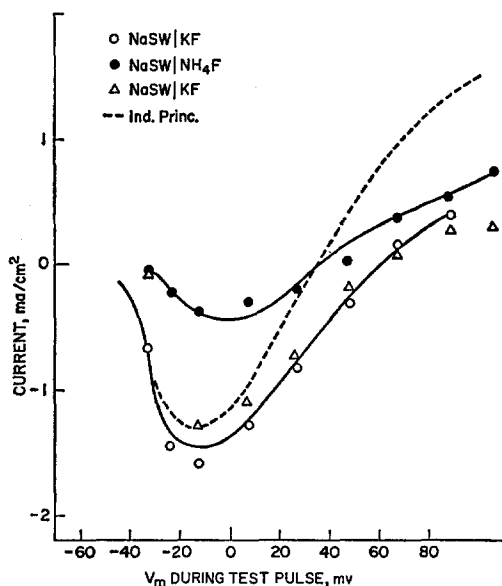


FIGURE 5. Peak current-voltage curves for a perfused axon in NaSW, with the internal perfusate changed from 500 mM KF to 500 mM NH_4F . The dashed curve is the peak current-voltage curve obtained by the independence principle correction for the changed internal solution. Axon 65-35, temperature 12°C , holding potential -53 mv. Corrected for leakage.

equation 4 with $\alpha = 0.3$ and $\beta = 0.1$. Fig. 5 shows the analogous curves for a perfused axon in NaSW when the internal solution is first 500 mM KF, then 500 mM NH_4F . In this case, the peak currents are considerably lower than the values predicted from equation 4. For axons perfused internally with NH_4F , the peak currents always ranged from one-half to one-third as large as expected from the independence principle.

The steady-state current-voltage curves for internal and external replacements are shown in Figs. 6 and 7. Fig. 6 shows that there is considerable outward steady-state current when NH_4 is the only internal cation. Thus the slow channel is certainly permeable to NH_4 . However, it is difficult to determine the exact value of this permeability from the outward currents because of uncertainties in the leakage current (see section on leakage).

In a preliminary report (Binstock and Lecar, 1967), we used the slope conductance of the steady-state current as a measure of the relative effectiveness of NH_4 for carrying outward current. These conductances and their ratios are given in Table III. Alternatively, we can estimate the relative permeability of the slow channel to NH_4 by fitting the steady-state current-voltage curves for internal NH_4 from those for internal K using the formula

$$\frac{I_{\text{NH}_4}}{I_{\text{K}}} = \frac{(P_{\text{NH}_4}/P_{\text{K}})_s [\text{NH}_4]_i - [\text{K}]_o \exp\left(-\frac{FV}{RT}\right)}{[\text{K}]_i - [\text{K}]_o \exp\left(-\frac{FV}{RT}\right)}. \quad (5)$$

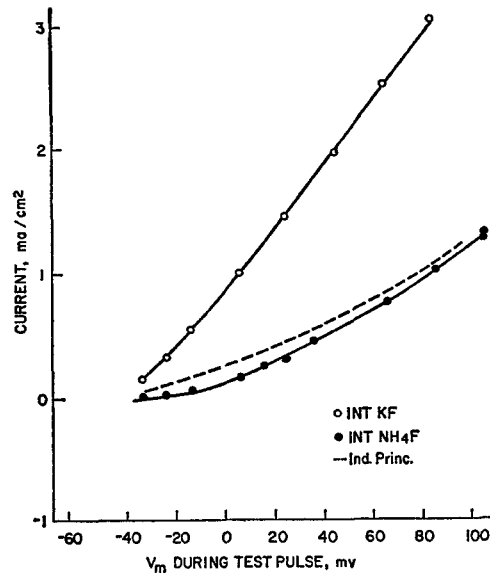


FIGURE 6. Steady-state current-voltage curves for an internally perfused axon in NaSW; the perfusate was first 500 mM KF, then 500 mM NH_4F . Axon 65-35, temperature 12°C , holding potential -53 mv. The dashed curve is the independence principle corrected curve for $(P_{\text{NH}_4}/P_{\text{K}}) = 0.35$.

Here and in the rest of this paper the subscripts s and f denote permeability in the "slow" and "fast" channels, respectively. In equation 5 we have assumed that the slow-channel Na permeability is negligible. First let us examine the currents for large depolarizations. In the limit of large depolarizing voltages, for our internal substitution experiments in which $[\text{NH}_4]_i = [\text{K}]_i$, equation 5 reduces simply to

$$\left(\frac{I_{\text{NH}_4}}{I_{\text{K}}}\right) \cong \left(\frac{P_{\text{NH}_4}}{P_{\text{K}}}\right)_s. \quad (6)$$

Table III shows the values of $(P_{\text{NH}_4}/P_{\text{K}})$ determined in this way.

As a check on the relative NH_4 permeabilities of Table III we calculated the NH_4 permeability from the slow-channel equilibrium potential which we

determined from the underswing of the action potential. According to the Hodgkin-Huxley picture, the hyperpolarizing underswing is caused by the opening of the slow channel during the last phase of the action potential, short-circuiting the other ionic pathways. If we assume that all other ions but K and NH_4 have negligible fluxes, then, for an external NH_4 substitution, we can fit the underswing, V_u , to the formula

$$V_u \cong \frac{RT}{F} \ln \left(\frac{P_{\text{NH}_4}}{P_{\text{K}}} \right)_s \frac{[\text{NH}_4]_o}{[\text{K}]_i}. \quad (7)$$

From the action potential shown in Fig. 1, we see that the underswing potential is -35 mv which, when corrected for liquid junction potentials, gives a value, $V_u = -41$ mv, from which we obtain $(P_{\text{NH}_4}/P_{\text{K}}) \cong 0.2$.

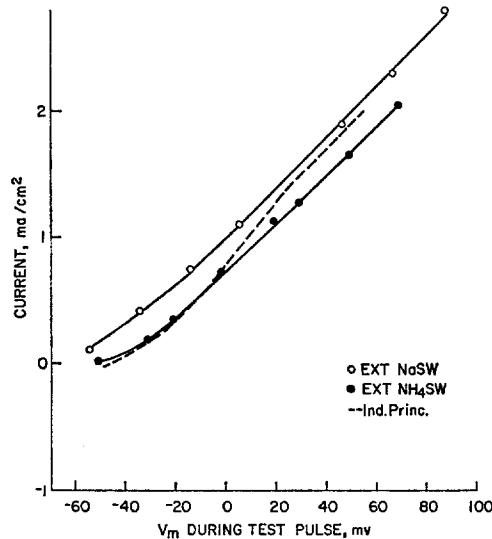


FIGURE 7. Steady-state current-voltage curves for a perfused axon in NaSW and in NH_4SW . Internal perfusion with 500 mM KF. The dashed curve shows the independence principle prediction for $(P_{\text{NH}_4}/P_{\text{K}}) = 0.35$ as in Fig. 6. Axon 65-35, temperature 12°C , holding potential -53 mv.

Leakage

The leakage currents were estimated from the initial discontinuous jump of outward current seen in the clamp records. This measurement is inaccurate because the very fast rise of the fast-channel conductance in the region of high depolarizations makes it difficult to separate the leakage current from the early transient. The value of leakage current obtained in this way is accurate to no more than 25%, and this inaccuracy can lead to a 5–10 mv variation in the value of V_e measured from the peak current-voltage curve. In order to minimize this difficulty, the values of α in Table II were calculated from the change in V_e upon solution change rather than from V_e itself, since the shifts in V_e are much less sensitive to inaccuracies in the leakage determination.

A more serious difficulty in correcting for leakage current is the leakage rectification (Adelman and Taylor, 1961). The usual way to correct for leakage is to measure the leakage current for hyperpolarizing voltages and then assume that the leakage current-voltage curve is symmetrical for the depolarizing voltages (Hodgkin and Huxley, 1952 *a*; Chandler and Meves, 1965 *a*). However, Adelman and Taylor (1961) have shown that leakage current increases nonlinearly in the region of high depolarizations. This apparent "turn-up" of the leakage current is evident in our clamp records (see Fig. 8 *a*) and has been seen for other preparations (Binstock and Goldman,

TABLE III
STEADY-STATE CURRENTS
Axons internally perfused with NH_4F .

Axon	Temperature °C	Steady-state conductance		Conductance ratio ($\text{NH}_4\text{F}/\text{KF}$)	($P_{\text{NH}_4}/P_{\text{K}}$) _s *
		Internal KF mmho/cm ²	Internal NH_4F mmho/cm ²		
65-30	12.5	34	14	0.4	0.26±0.03
65-35	12	20	12	0.6	0.22±0.09
65-37	11	23	13	0.6	0.40±0.03
66-33	6.5	41	12	0.3	0.24±0.10
66-36	1.5	30	15	0.5	0.24±0.07
67-37	8.5	—	—	0.3	0.26±0.15
66-41	6	—	—	0.3	0.17±0.15
					0.26±0.04

*Ratio of steady state currents

$$(P_{\text{NH}_4}/P_{\text{K}})_s \equiv \left(\frac{I_{(\text{NH}_4)} - I_{\text{Leak}}}{I_{(\text{KF})} - I_{\text{Leak}}} \right)_{V = 100 \text{ mv.}}$$

Experimental error estimated from probable error of leakage measurement.

private communication, *Myxicola* giant axon; Rojas, private communication, *Dosidicus gigas* giant axon). Our estimates for the steady-state NH_4 permeability can vary from ~0.2 to ~0.4 depending on whether we use a linear leakage extrapolated from the low voltage region or a point-for-point leakage correction.

Effects of TTX and TEA

The drugs, tetrodotoxin and tetraethylammonium chloride, exert very specific actions on axons in the normal physiological solutions. TTX in the external medium reduces the early inward Na current leading to a blocking of excitability but does not affect the delayed K currents (Nakamura et al., 1965). TEA reduces the delayed current, resulting in plateau action potentials, but

does not block the early currents (Armstrong and Binstock, 1965). Analogous results were obtained with NH_4 -substituted solutions. TTX in the external solution blocked the early NH_4 current, and for axons perfused internally with NH_4F , the addition of TEA to the perfusate reduced the steady-state current. These effects are illustrated in Fig. 8.

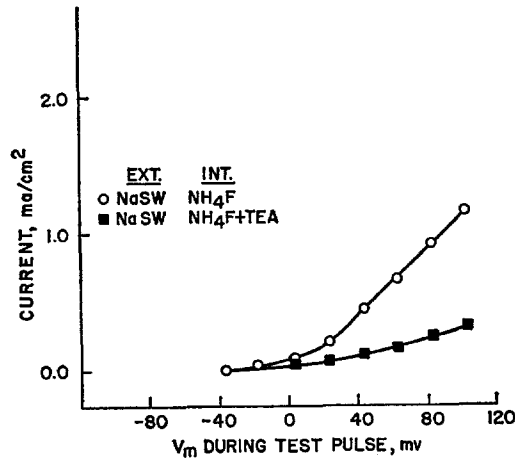


FIGURE 8a. Steady-state current-voltage curves for an internally perfused axon, showing the effect of TEA on the outward NH_4 current. Axon 65-37, temperature 11°C , holding potential -57 mv.

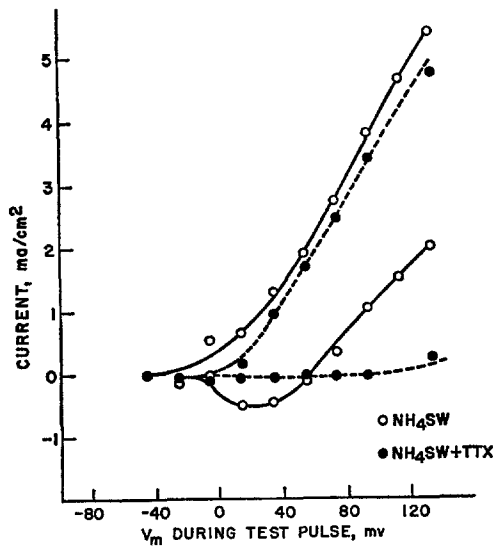


FIGURE 8b. Effect of TTX on the inward currents. Axon 66-14, temperature 7.5°C , holding potential -65 mv. Corrected for leakage.

Since many of the quaternary ammonium ions can cause excitation (Lorente de Nó et al., 1957; Tasaki et al., 1965), we also did some preliminary voltage-clamp experiments testing the effect of 430 mM TEA as an external current-carrying ion. External TEA does not affect the slow channel, but is sufficiently permeable in the fast channel to give measurable inward

currents and concomitant active responses. Furthermore the TEA inward currents and "action potentials" are blocked by TTX.

DISCUSSION

The voltage-clamp currents for NH_4 can be described by assuming that the two time-dependent conductance pathways are both permeable to NH_4 , and that the kinetics of the conductance changes are independent of the particular ion being transported.

As a rough measure of this independence, we have shown the invariance of the time-to-peak. We have also derived curves of the Hodgkin-Huxley conductance parameters, $m_\infty(V)$ and $n_\infty(V)$, and their voltage-dependent time constants, $\tau_m(V)$ and $\tau_n(V)$, by fitting our data to the currents predicted from the Hodgkin-Huxley equations with parameters modified to agree with NH_4 permeabilities. Within experimental accuracy, about 20%, these functions are unchanged by the ionic substitutions. One uncertainty in these data is a possible shift of the conductance curves along the voltage axis. The possibility that $n_\infty(V)$ can shift along the V axis as the external K concentration is varied was discussed by Ehrenstein and Gilbert (1968), and our analysis is beset by the same difficulties as theirs.

The invariance of the kinetics to various ionic substitutions is in agreement with a number of other observations. Chandler and Meves (1965 *a, b*) and Rojas and Atwater (1967) have shown that the time course of K in the fast channel is the same as that of Na, and data extracted from the voltage-clamp curves of Moore et al. (1966) show the fast process occurring with identical time-to-peak for all the alkali cations substituted externally. These results are all consistent with the experiments (Armstrong and Binstock, 1965; Nakamura et al., 1965; Hille, 1967, 1968) which have shown that when TEA and TTX are used to eliminate one component of the current, the other remains and follows its time course undisturbed. In addition, our result showing that TTX blocks the NH_4 current in the fast channel is similar to the finding of Rojas and Atwater (1967) that TTX blocks the K current through the fast channel.

One parameter we have not studied is the early-channel inactivation process, h . Adelman and Senft (1968) have shown that the inactivation process is altered when the delayed currents are blocked by internal Cs solutions. Thus, there may be some interaction between the delayed and early currents. In so far as the early and delayed pathways exhibit functional independence, our measurements determine the relative permeabilities of each channel to NH_4 .

For the fast channel, we found $(P_{\text{NH}_4}/P_{\text{Na}}) = 0.27$ from the shift in V_e upon NH_4 substitution. The shift for external NH_4 substitution is a direct measure of $(P_{\text{NH}_4}/P_{\text{Na}})$, whereas the shift for internal substitution really determines the ratio $(P_{\text{NH}_4}/P_{\text{K}})$ which must then be multiplied by $(P_{\text{K}}/P_{\text{Na}})$ to

get $(P_{\text{NH}_4}/P_{\text{Na}})$. But $(P_{\text{K}}/P_{\text{Na}})$ can be determined from V_e , hence the two independently measured values of $(P_{\text{NH}_4}/P_{\text{Na}})$ constitute an experimental check of the relation

$$\left(\frac{P_{\text{NH}_4}}{P_{\text{Na}}}\right) = \left(\frac{P_{\text{NH}_4}}{P_{\text{K}}}\right) \left(\frac{P_{\text{K}}}{P_{\text{Na}}}\right) \quad (8)$$

which would be expected to hold if the permeabilities were unique numbers for each ion.

Another test of the consistency of the permeability ratios is the fit of the NH_4 peak current-voltage curves to the curves predicted from the independence principle. The independence principle (equation 3) is not a general formula for change in current as a function of ion concentration, but is merely an expression obtained from a number of simplified models of ion transport (Hodgkin and Huxley, 1952 *a*). However, it is in agreement with experimental evidence on the effect of changes in external Na concentration on the peak current for intact squid giant axons (Hodgkin and Huxley, 1952 *a*; Adelman and Taylor, 1964). From Figs. 4 and 5, we see that the agreement is quite good for the external NH_4 substitutions but that currents for internal NH_4 are only one-third as large as predicted. This result is surprising because one would assume that the peak inward current is carried almost entirely by Na regardless of the internal cation. This is similar to the findings of Chandler and Meves (1965 *a, b*) for axons perfused with mixtures of NaCl and KCl. The peak currents were consistently lower than the values predicted by the independence principle. Adelman and Senft (1966) have proposed that the permeable ions often have a twofold effect—they carry current and they can block current-carrying pathways. For example Cs is slightly permeable in the fast channel, but internal Cs blocks the outward slow K current. The analogous interpretation of our results is that external and internal NH_4 are permeable in the fast channel with a unique permeability, but NH_4 inside tends to block the inward flow of Na. Since the shift in V_e is exactly the amount expected from the permeability ratios, the lowering of the current would seem to be caused by some lowering of the conductance for the early current rather than by some change in the ionic selectivity. Experiments to test this hypothesis would involve internal perfusion with Na- NH_4 and NH_4 -K mixtures, which we did not do. The decrease in peak current was not caused by a shift in the Na activation curve, $m_\infty(V)$, since merely shifting $m_\infty(V)$ along the voltage axis would not fit the data for the larger depolarizations.

The steady-state currents can be fit by assuming that $(P_{\text{NH}_4}/P_{\text{K}}) = 0.26$. The dashed curves in Figs. 6 and 7 were calculated from equation 5 using this value of the permeability ratio. The underswing of the NH_4 action potential leads to the value $(P_{\text{NH}_4}/P_{\text{K}}) \cong 0.2$.

Finally we might ask whether the K-like permeation observed for NH_4 is consistent with the data on resting depolarizations (Table I). We have attempted to fit the resting depolarizations to an expression of the constant-field type and found that the only way to make our internal and external substitution data consistent was to assume values for the anion permeability for internal substitutions different from those for external substitutions. This difficulty is not unique to our experiment. Hodgkin and Katz (1949) used the value $P_{\text{Cl}} = 0.2$ to fit data on external ionic changes, whereas Baker et al. (1964) have found that $P_{\text{Cl}} = 0.02$ for internal solution changes. When we use these particular values for our two substitutions, we find that the depolarization data are fit by the value $(P_{\text{NH}_4}/P_{\text{K}}) = 0.2$.

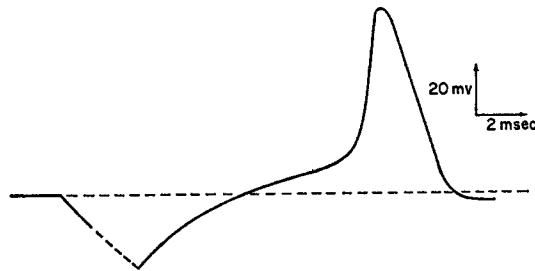


FIGURE 9. An NH_4 action potential computed from the Hodgkin-Huxley equations under current clamp. The changed parameters for conductances and reversal potentials in the presence of external NH_4SW were as follows:—

$$\begin{aligned} \bar{g}'_{\text{Na}} &= 36 \text{ mmho/cm}^2, & V'_{\text{Na}} &= 87 \text{ mv}, \\ \bar{g}'_{\text{K}} &= 12 \text{ mmho/cm}^2, & V'_{\text{K}} &= 6 \text{ mv}. \end{aligned}$$

Temperature 6.3°C , stimulus duration 9 msec. In this convention, potentials are taken relative to rest.

Thus the K-like permeation is consistent with the action of NH_4 as a depolarizing agent. NH_4 substitution for K is known for a number of permeability phenomena (Lüttgau, 1961). The Na-like permeation explains NH_4 excitability. The reason NH_4 action potentials are only obtained by anodal break is because NH_4 is also a depolarizing agent and hence a nerve with NH_4 in the external solution is always inactivated in the resting state. In Fig. 9 we show an NH_4 action potential computed from the Hodgkin-Huxley (1952 *b*) equations using the NH_4 permeability values determined from our voltage-clamp data. We note that the theoretical action potential agrees well with the observed NH_4 action potential of Fig. 1 without the necessity of modifying any of the time constants or normalized conductance functions in the Hodgkin-Huxley equations. The only parameters changed were the absolute values of the conductances and the reversal potentials (as indicated in the figure legend).

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REFERENCES

- ADELMAN, W. J., JR., and J. P. SENFT. 1966. Voltage clamp studies on the effect of internal cesium ion on sodium and potassium currents in the squid giant axon. *J. Gen. Physiol.* 50:279.
- ADELMAN, W. J., JR., and J. P. SENFT. 1968. Dynamic asymmetries in the squid axon membrane. *J. Gen. Physiol.* 51 (5, Pt 2):102.
- ADELMAN, W. J., JR., and R. E. TAYLOR. 1961. Leakage current rectification in the squid giant axon. *Nature.* 190:883.
- ADELMAN, W. J., JR., and R. E. TAYLOR. 1964. Effects of replacement of external sodium chloride with sucrose on membrane currents of the squid giant axon. *Biophys. J.* 4:451.
- ARMSTRONG, C. M., and L. BINSTOCK. 1965. Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. Gen. Physiol.* 48:859.
- ARMSTRONG, C. M., and L. BINSTOCK. 1964. The effects of several alcohols on the properties of the squid giant axon. *J. Gen. Physiol.* 48:265.
- BAKER, P. F., A. L. HODGKIN, and H. MEVES. 1964. The effect of diluting the internal solution on the electrical properties of a perfused giant axon. *J. Physiol. (London).* 170:541.
- BINSTOCK, L., and H. LECAR. 1967. Ammonium ion substitutions in the voltage clamped squid giant axon. Abstracts of the Biophysical Society 11th Annual Meeting. Houston, Texas. WC4. 19.
- CHANDLER, W. K., A. L. HODGKIN, and H. MEVES. 1965. The effect of changing the internal solution on sodium inactivation and related phenomena in giant axons. *J. Physiol. (London).* 180:821.
- CHANDLER, W. K., and H. MEVES. 1965 a. Voltage clamp experiments on internally perfused giant axons. *J. Physiol. (London).* 180:788.
- CHANDLER, W. K., and H. MEVES. 1965 b. Ionic selectivity in perfused giant axons. *J. Cell. Comp. Physiol.* 66: (3, Pt. II):65.
- COLE, K. S., and J. W. MOORE. 1960. Ionic current measurements in the squid giant axon membrane. *J. Gen. Physiol.* 44:128.
- DODGE, F. A., JR. 1963. A study of ionic permeability changes underlying excitation in myelinated nerve fibers of the frog. Doctoral Dissertation, unpublished. The Rockefeller University, New York.
- EHRENSTEIN, G., and D. L. GILBERT. 1968. Voltage shift of steady-state conductance curve of squid axon in high potassium. Abstracts of the Biophysical Society 12th Annual Meeting. Pittsburgh, Pennsylvania. T12. A-132.
- GOLDMAN, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* 27:37.
- HILLE, B. 1967. The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. *J. Gen. Physiol.* 50:1287.
- HILLE, B. 1968. Pharmacological modification of the sodium channels of frog nerve. *J. Gen. Physiol.* 51:199.
- HODGKIN, A. L., and A. F. HUXLEY. 1952 a. Currents carried by sodium and potassium ions through the membrane of the giant axon of *Loligo*. *J. Physiol. (London).* 116:449.
- HODGKIN, A. L., and A. F. HUXLEY. 1952 b. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* 117:500.
- 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. (London).* 108:37.
- LECAR, H., G. EHRENSTEIN, L. BINSTOCK, and R. E. TAYLOR. 1967. Removal of potassium negative resistance in perfused squid giant axons. *J. Gen. Physiol.* 50:1499.
- LORENTE DE NÓ, R., F. VIDAL, and L. M. H. LARRAMEDÍ. 1957. Restoration of sodium-deficient frog nerve fibers by onium ions. *Nature.* 179:737.

- LÜTTGAU, H. C. 1961. Weitere Untersuchungen über den passiven Ionentransport durch die erregbare Membran des Ranvierknotens. *Pfluegers Arch. Gesamte Physiol. Menschen Tiere.* **273**:302.
- MACINNES, D. A. 1961. *The Principles of Electrochemistry.* Dover Publications, Inc., New York.
- MEVES, H. 1966. Experiments on internally perfused squid giant axons. *Ann. N. Y. Acad. Sci.* **137**:807.
- MEVES, H., and W. K. CHANDLER. 1965. Ionic selectivity in perfused giant axons. *J. Gen. Physiol.* **48** (5, Pt. 2):31.
- MOORE, J. W., N. ANDERSON, M. BLAUSTEIN, M. TAKATA, J. Y. LETTVIN, W. F. PICKARD, T. BERNSTEIN, and J. POOLER. 1966. Alkali cation selectivity of squid axon membrane. *Ann. N. Y. Acad. Sci.* **137**:818.
- NAKAMURA, Y., S. NAKAJIMA, and H. GRUNDFEST. 1965. The action of tetrodotoxin on electrogenic components of squid giant axon. *J. Gen. Physiol.* **48**:985.
- ROJAS, E., and I. ATWATER. 1967. Effect of tetrodotoxin on the early outward currents in perfused giant axons. *Proc. Nat. Acad. Sci. U.S.A.* **57**:1350.
- TASAKI, I. 1963. Permeability of squid axon membrane to various ions. *J. Gen. Physiol.* **46**:755.
- TASAKI, I., I. SINGER, and A. WATANABE. 1965. Excitation of internally perfused squid giant axons in sodium-free media. *Proc. Nat. Acad. Sci. U.S.A.* **54**:763.