J. Physiol. (1965), **180**, *pp.* 821–836
With 7 text-figures
Printed in Great Britain

THE EFFECT OF CHANGING THE INTERNAL SOLUTION ON SODIUM INACTIVATION AND RELATED PHENOMENA IN GIANT AXONS

BY W. K. CHANDLER, A. L. HODGKIN AND H. MEVES

From the Laboratory of the Marine Biological Association, Plymouth, and the Physiological Laboratory, University of Cambridge

(*Received* 23 *February* 1965)

This paper is concerned with the inactivation of the sodium system in perfused giant axons. Earlier experiments on perfused axons showed that when the artificial axoplasm was diluted with an isotonic sugar solution, action potentials could be obtained in the presence of little or no resting potential (Tasaki & Shimamura, 1962; Baker, Hodgkin & Shaw, 1962; Narahashi, 1963; Baker, Hodgkin & Meves, 1964). This is due to the fact that dilution of the internal potassium solution with a non-electrolyte causes both the inactivation curve and the relation between sodium conductance and membrane potential to shift in the direction of a more positive internal potential (Narahashi, 1963; Baker *et al.* 1964; Moore, Narahashi & Ulbricht, 1964). Baker *et al.* (1964) obtained some evidence that the shift in the inactivation, and probably the conductance, was due to the decrease in ionic strength rather than to the decrease in potassium concentration. The voltage clamp experiments presented in this paper support this conclusion. They show that the shift in the inactivation curve which occurs when the KCl is diluted with isotonic sucrose is absent when the KCl is diluted with NaCl, choline Cl or RbCl. This effect of ionic strength can be explained quantitatively by assuming a layer of fixed negative charges on the inside of the membrane. A preliminary report of some of these experiments has been given by Chandler & Meves (1964).

METHOD

The apparatus and experimental procedure are fully described in the preceding paper (Chandler & Meves, 1965).

Steady-state sodium inactivation was measured with a depolarizing test pulse applied 40–70 msec after the onset of a conditioning prepulse. The test pulse was chosen to give nearly maximum sodium inward current.

The rate of development of inactivation under a cathode and the rate of its removal under an anode were determined by changing the duration of the conditioning prepulse.

RESULTS

Steady-state inactivation

The results of the experiments on steady-state inactivation have been fitted to the empirical equation

$$h_\infty = \frac{1}{1+\exp\left(\frac{V-V_h}{k}\right)} \tag{1}$$

given by Hodgkin & Huxley (1952*a*). An expression which approximates closely to this for $h_\infty > 0{\cdot}02$ is obtained from the relation $h_\infty = \alpha_h/(\alpha_h+\beta_h)$ where the rate constants α_h and β_h are given by eqns. (23) and (24) of Hodgkin & Huxley (1952*b*) or eqns. (5) and (6) of this paper. Experimentally h_∞ is given by the peak sodium current expressed as the fraction of that obtained following large anodal polarizations. V is the internal potential during the conditioning prepulse, V_h is the value of membrane potential for which $h_\infty = 0{\cdot}5$, k is an empirical constant which determines the steepness of the function.

With 300 mM-KCl + sucrose as the internal solution, mean values of V_h and k were -59 mV and 7 mV, respectively (Table 1). From this it follows that $h_\infty = 0{\cdot}64$ at the average resting potential of -63 mV. These values are consistent with observations on intact axons where $h_\infty = 0{\cdot}6$ at the resting potential and $k = 7$ mV (Hodgkin & Huxley, 1952*a*).

Figure 1 illustrates an experiment designed to find out whether the shift in the inactivation curve which occurs when the internal KCl is diluted with sucrose is due to a decrease in ionic strength or a decrease in potassium. When the KCl was diluted from 300 to 50 mM with isotonic sucrose there was a shift in V_h of 21 mV. However, there was almost no shift when 300 mM-KCl was replaced with 50 mM-KCl + 250 mM-NaCl. Similarly, there was no significant change in V_h when the internal K was completely replaced by Rb as shown in Fig. 2.

The results of these and other experiments are tabulated in Table 1. In two axons reducing the internal KCl to 50 mM caused an average shift in V_h of 22 mV; in another axon a reduction to 24 mM caused a shift of 32 mV. These changes were accompanied by a slight broadening of the inactivation curve indicated by the increase in k. Similar changes were not observed when the KCl was replaced by NaCl, choline Cl or RbCl although at least in the case of choline there was a reversible increase in k. These experiments show that the position of the inactivation curve is affected by the internal ionic strength rather than by the potassium concentration.

Shifts in critical potential and sodium conductance relation

In two experiments the effect of dilution on the threshold was determined by applying brief pulses of current to the long internal electrode. Replacing 300 mM-KCl with 24 mM-KCl + sucrose shifted the critical potential by about 30 mV (28 and 32 mV) which is close to the shift in V_h of 32 mV found in axon 4 (Table 1). It is also in good agreement with the shift found by Baker *et al.* (1964).

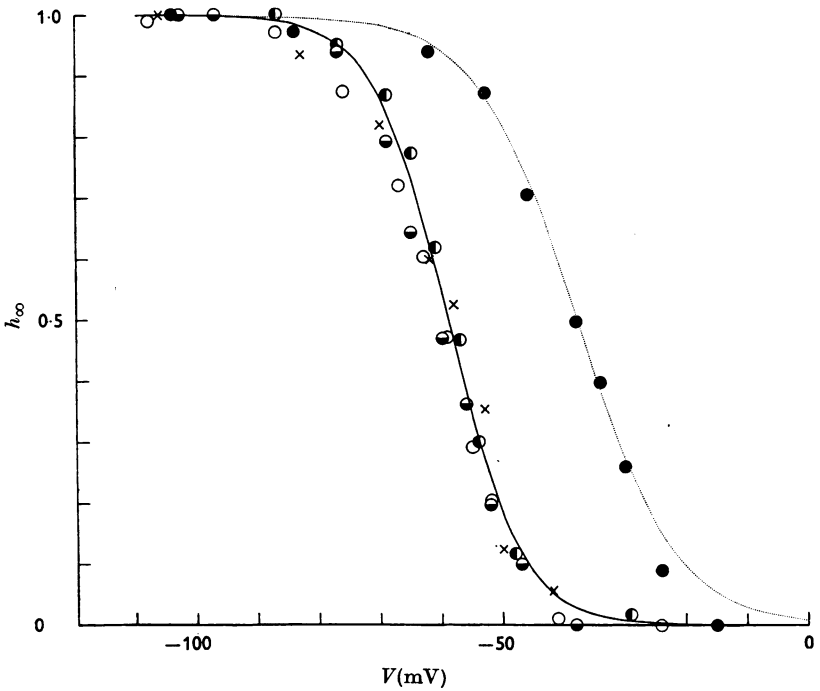

Fig. 1. Steady-state relation between h_∞ and membrane potential. Ordinate: sodium current associated with a test depolarization following a 70 msec prepulse, expressed in units of maximum current. Abscissa: prepulse potential V. Internal solutions: ◐, 300 mM-KCl; ●, 50 mM-KCl; ○, 300 mM-KCl; ×, 50 mM-KCl + 250 mM-NaCl; ◒, 300 mM-KCl. Isotonicity maintained with sucrose. External solution: K-free artificial sea water. The curves were drawn from eqn. (1) with the values $V_h = -59$ mV, $k = 6$ mV (solid) and $V_h = -38$ mV, $k = 8$ mV (dotted). Axon 18, diameter 651 μ. Temperature 2° C.

Voltage clamp records showed that the shift in the $g_{Na} - V$ curve between 300 and 50 mM-KCl was about 15–18 mV as against 22 and 21 mV for the change in V_h (inactivation) in the same axons. On the other hand when 300 mM-KCl was replaced with 50 mM-KCl + 250 mM-NaCl there was relatively little change in the position of the curve (+6 and −5 mV for g_{Na}, +1 and +2 mV for V_h).

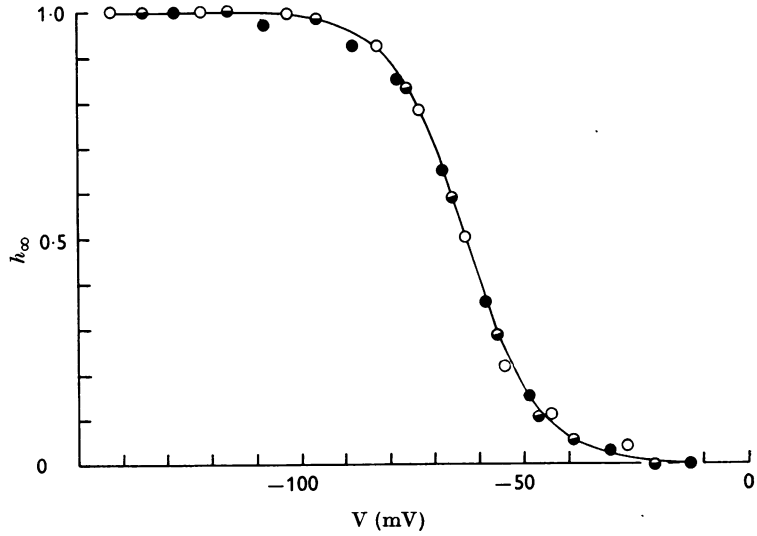

Fig. 2. Steady-state relation between h_∞ and membrane potential for axon perfused with ○, 300 mM-KCl; ●, 300 mM-RbCl; ◒, 300 mM-KCl. Isotonicity maintained with sucrose. External solution: K-free artificial sea water. Test depolarizations to about 0 mV. The curve was drawn from eqn. (1) with $V_h = -63$ mV and $k = 8{\cdot}0$ mV. Axon 14, diameter 673 μ. Temperature 0° C.

Kinetics of the inactivation process

As in intact axons, inactivation developed or was removed in an approximately exponential manner with a time constant which varied with membrane potential and had a maximum near the resting potential. In Fig. 3 the time constant τ_h of the inactivation process in five axons perfused with 300 mM-KCl is plotted against the internal potential. In Fig. 4 the rate constants, α_h and β_h, determined from τ_h and h_∞, are shown. These are obtained from the expressions

$$\alpha_h = h_\infty/\tau_h, \tag{2}$$

$$\beta_h = (1-h_\infty)/\tau_h \tag{3}$$

as given by Hodgkin & Huxley (1952*b*). The curves in the figures are drawn from the equations

$$\tau_h = 1/(\alpha_h+\beta_h), \tag{4}$$

$$\alpha_h = 0{\cdot}5 \times 0{\cdot}07 \exp\left(-\frac{V+62}{20}\right), \tag{5}$$

$$\beta_h = 0{\cdot}5 \times \frac{1}{\exp\left(-\frac{V+32}{10}\right)+1}. \tag{6}$$

TABLE 1. Experimental values for V_h and k

Axon	Internal solution	Resting potential (mV)	V_h (mV)	Change in V_h (mV)	k (mV)	Temperature (° C)
12	300 mM-KCl	−67	−60·5		8·5	−1·0°
13	300 mM-KCl	−61	−59		7·4	0°
	300 mM-RbCl	−47	−61·5	−3	7·4	0·4°
	300 mM-KCl	−57	−58·5		7·1	1·0°
14	300 mM-KCl	−65	−63		7·9	0°
	300 mM-RbCl	−49	−63	0	8·2	0·4°
	300 mM-KCl	−57	−63·5		7·8	0·8°
	150 mM-KCl + 150 mM-NaCl	−48·5	−64·5	−3	8·6	1·0°
	300 mM-KCl	−53	−60·5		8·1	1·0°
16	300 mM-KCl	−59	−57		7·2	0°
	50 mM-KCl + 250 mM-NaCl	−38	−62	−4	9·4	0°
	300 mM-KCl	−49	−59		8·6	0·5°
18	300 mM-KCl	−65	−58		5·6	2°
	50 mM-KCl	−62	−38·5	21	7·8	2°
	300 mM-KCl	−59	−60·5		6·5	2°
	50 mM-KCl + 250 mM-NaCl	−41	−59·5	1	6·2	2°
	300 mM-KCl	−56	−60·5		6·0	2°
19	300 mM-KCl	−59	−59·5		5·1	−0·5°
	50 mM-KCl + 250 mM-NaCl	−44	−58·5	2	5·6	−0·5°
	300 mM-KCl	−55	−60·5		5·9	−0·5°
	50 mM-KCl	−42·5	−40	22	10·8	−0·5°
	300 mM-KCl	−61	−64		7·6	0°
	50 mM-KCl + 250 mM Choline Cl	−29	−61	3	11·9	0°
	300 mM-KCl	−60	−63		6·7	0·2°
4	300 mM-KCl	−67	−55		7·1	0·5°
	300 mM-KCl	−61	−56·5		7·3	1·0°
	24 mM-KCl	−43	−27	32	8·6	0·5°
	300 mM-KCl	−58	−60·5		8·5	0·5°
Mean for 300 mM-KCl and S.E.		−63 ± 1	−59 ± 1		7·0 ± 0·5	

V_h and k were obtained by fitting experimental points such as those in Fig. 1 with eqn. (1). The duration of the conditioning prepulse was 40–50 msec for axons 12, 13, 14, 4 and 60–70 msec for axons 16, 18, 19. Measurements were made after passing at least 0·1 ml. solution through the axon. Means for 300 mM-KCl are based on values at the beginning of each experiment.

Equation (4) follows from (2) and (3). Equations (5) and (6) are similar to eqns. (23) and (24) of Hodgkin & Huxley (1952*b*) and can be made exactly equivalent by assuming (1) a Q_{10} of 3 for both α_h and β_h as found by Hodgkin & Huxley and (2) that the resting potential in their experiments, which was estimated as −60 to −65 mV, was −62 mV. The value of −62 mV was chosen so that $V_h = -59$ mV as observed in the experiments with 300 mM-KCl inside the axons. The agreement between the experimental results and the equations shows that the inactivation mechanism in axons perfused with 300 mM-KCl operates in a very similar manner to that in intact axons.

The effects of changing the composition of the internal solution on the kinetics of the inactivation process were investigated on four axons. The results given in Table 2 show that there was no significant change in τ_h when the standard perfusion fluid was replaced by 300 mM-RbCl. Partial replacement of K by Na increased the time constant about 50 %. Dilution of the internal KCl with sucrose appeared to shift the relation between τ_h and V in the positive direction and to increase the time constant expected at $V = V_h$.

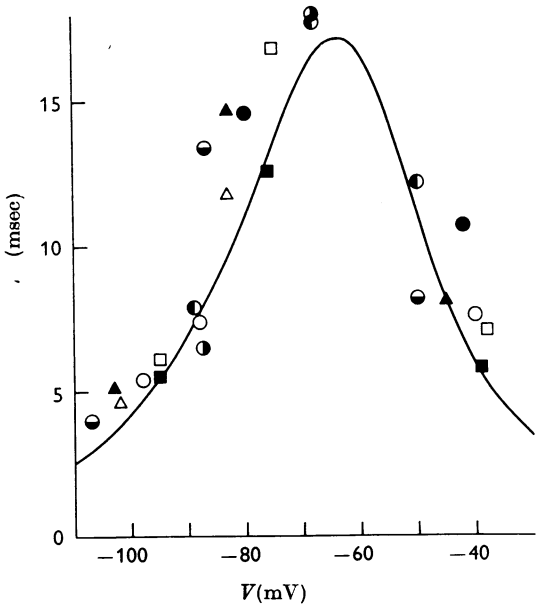

Fig. 3. Time constant of inactivation (τ_h) as a function of internal potential (V) for axons perfused with 300 mM-KCl at 0° C. ◒ axon 12; ■, □ axon 13; ▲, △ axon 14; ◐, ◑ axon 16; ●, ○ axon 4; the two different symbols for each axon (except 12) refer to measurements at the beginning and in the middle of the experiment. Experimental values (temperature range −1° C to 1° C) scaled to 0° C with a Q_{10} of 3. The curve was drawn according to eqn. (4).

Time course of the development of sodium inactivation

The equations used by Hodgkin & Huxley (1952*b*) imply that activation and inactivation proceed independently. An alternative is to suppose that inactivation affects only the active component (see Hoyt, 1963). On the first assumption inactivation should develop exponentially whereas on the second there should be an initial delay. An experiment designed to distinguish between these possibilities is shown in Fig. 5. The axon was perfused with 300 mM-RbCl so that there was very little delayed outward current (Chandler & Meves, 1965). Part *A* of the figure shows the inward current associated

with a depolarizing step from -60 to $+10$ mV. After about 4 msec the inward current decayed to zero with a time constant of 6·2 msec.

Part *B* shows the time course of inactivation determined with the pair of pulses illustrated in the inset. The interval between the two pulses was fixed and the experimental variable was the duration of the first pulse which determined the magnitude of the inward current associated with the second pulse. Curve *a*, which is considered to be a reasonable fit to the experimental points, was drawn from the equation

$$0{\cdot}34 + 0{\cdot}66 \exp(-t/6{\cdot}2).$$

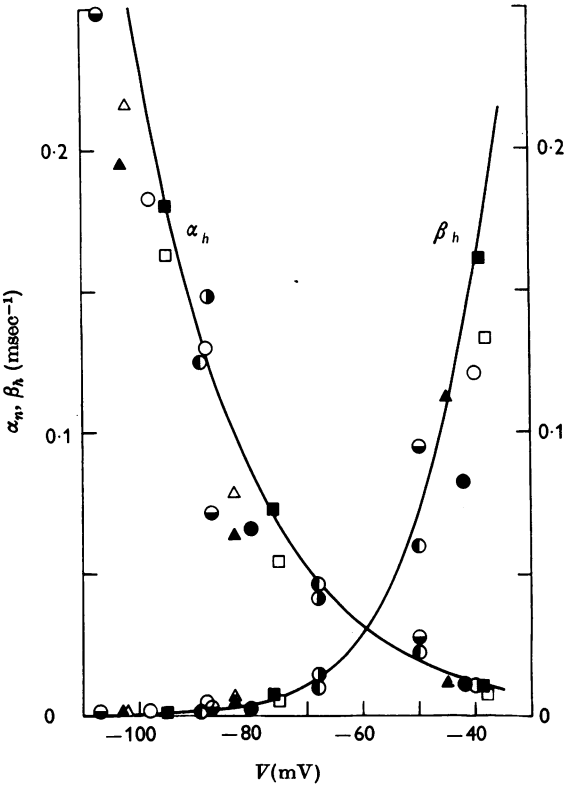

Fig. 4. Rate constants, α_h and β_h, as a function of internal potential, V, for axons perfused with 300 mM-KCl at 0° C. The experimental points were determined by applying eqns. (2) and (3) to the data in Fig. 3 and Table 1. The symbols have the same meaning as in Fig. 3. The curves were drawn from eqns. (5) and (6).

This can be derived from the Hodgkin–Huxley equations or more generally from the assumption that h decreases exponentially during the first pulse and increases exponentially during the interval between the two pulses. The time constant, τ_h, during the first pulse was taken as 6·2 msec and as

TABLE 2. Effect of changing the composition of the internal solution on the time constant τ_h of the removal of sodium inactivation

1 Axon	2 Internal solution	3 Membrane potential during prepulse (mV)	4 τ_h (msec)	5 Calculated τ_h, $V = V_h$	6 Ratio of τ_h, test: 300 mM-KCl
13	300 mM-KCl	−76	12·5	15·6	
	300 mM-RbCl	−76	17·2	19·6	1·08
	300 mM-KCl	−75	16·8	20·6	
	300 mM-KCl	−95	5·5	16·5	
	300 mM-RbCl	−95	8·1	21·5	1·23
	300 mM-KCl	−95	6·1	18·6	
14	300 mM-KCl	−83	14·6	20·6	
	300 mM-RbCl	−83	12·3	17·3	0·94
	300 mM-KCl	−83	11·8	16·3	
	300 mM-KCl	−103	5·1	18·8	
	300 mM-RbCl	−102	5·4	18·8	1·09
	300 mM-KCl	−102	4·6	15·7	
16	300 mM-KCl	−68	17·7	18·2	
	50 mM-KCl + 250 mM-NaCl	−66	28·0	26·7	1·49
	300 mM-KCl	−68	17·9	17·6	
	300 mM-KCl	−89	7·9	19·4	
	50 mM-KCl + 250 mM NaCl	−86·5	14·8	25·5	1·55
	300 mM-KCl	−87·5	6·5	13·5	
4	300 mM-KCl	−80	14·6	23·9	
	24 mM-KCl	−85	5·3	47·4	2·46
	300 mM-KCl	−88	7·4	14·6	

τ_h was measured at temperatures ranging from −1 to 1° C (see Table 1) and corrected to 0° C by using a Q_{10} of 3. For the calculation of τ_h in column 5 the theoretical curve in Fig. 3 was moved along the voltage axis so that the theoretical V_h coincided with the experimental one (Table 1). The theoretical ratio of τ_h at V_h to that at V was multiplied by the experimental value (column 4). In column 6 the calculated value of τ_h in the test solution is compared with that obtained in 300 mM-KCl.

18·5 msec during the interval. Allowance for removal of inactivation during the interval between the pulses leads to the constants 0·34 and 0·66. Curve *b* was drawn on the assumption that the rate of inactivation during the first pulse was proportional to sodium conductance. Owing to the time taken for the sodium conductance to increase following depolarization, the theoretical curve has a marked delay which is not shown by the experimental points. A curve based on the assumption that the rate of inactivation is proportional to the cube root of the sodium conductance was also inconsistent with the experiment. The conclusion is that inactivation follows an approximately exponential time course from the beginning of a depolarizing pulse and thus does not seem to depend on the sodium channel being turned on.

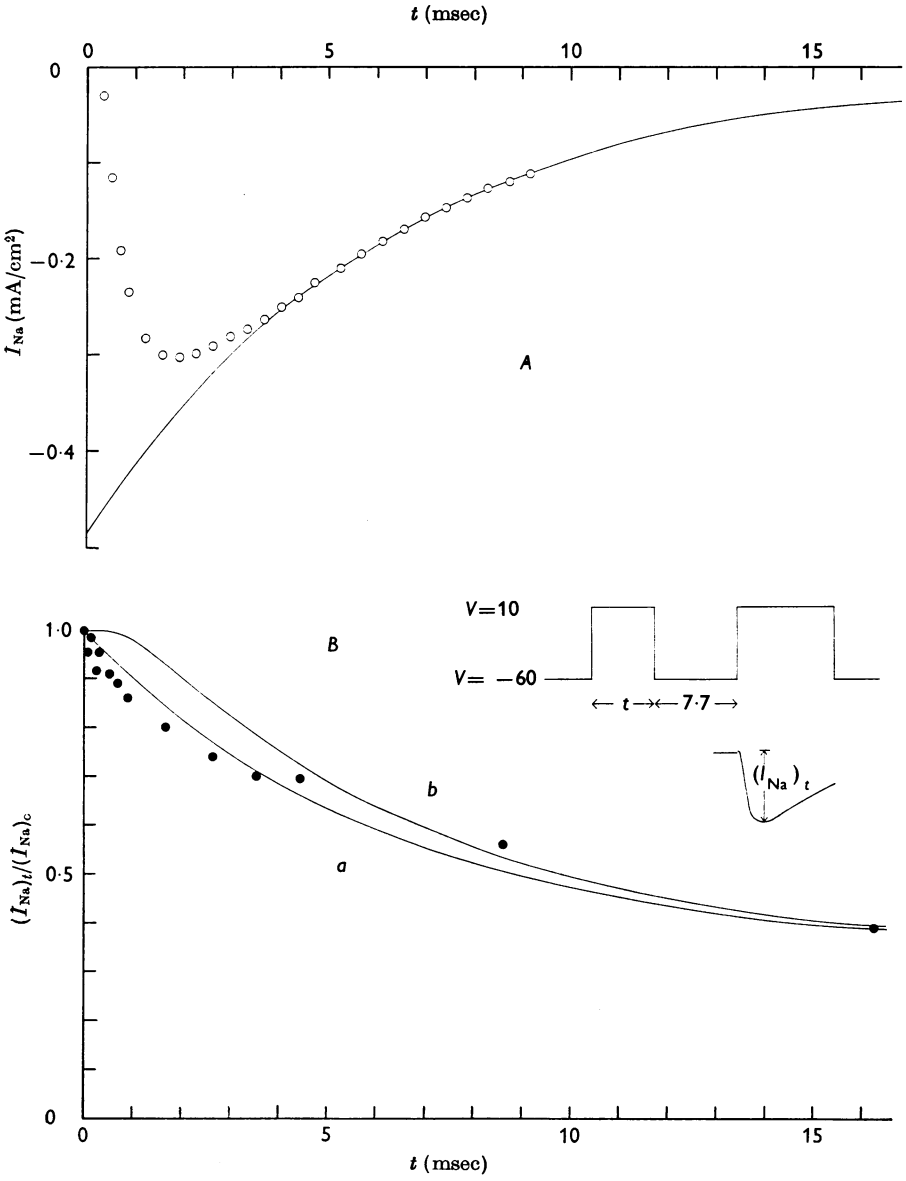

Fig. 5. Time course of the development of sodium inactivation. Axon perfused with 300 mM-RbCl + sucrose. Resting potential −40 mV; holding potential −60 mV. Part *A*: Inward current (○) vs time from onset of depolarizing pulse to $V = 10$ mV. Smooth curve given by $-0{\cdot}485 \exp(-t/6{\cdot}2)$ mA/cm². Part B: inset shows pulse arrangement. The experimental points (●) are determined from the peak currents $(I_{Na})_t$ developed during the second pulse. They are expressed in units of the current obtained without a first pulse. See text for details of curves *a* and *b*. External solution: K-free artificial sea water. Axon 28, diameter 738 μ. Temperature 0° C.

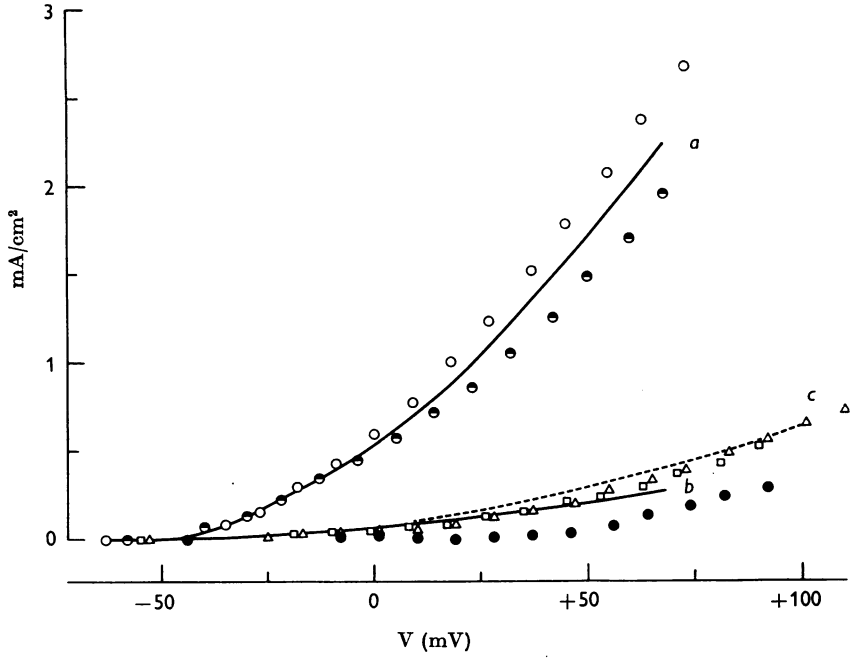

Fig. 6. Effect of low ionic strength on delayed currents. Ordinate: currents measured at 35 msec after the beginning of the cathodal pulse. The experimental results are not corrected for the effect of the small leakage conductance measured with an anodal pulse. Abscissa: internal potential. Internal solutions: ○, 300 mM-KCl (leakage resistance 33,000 ohm cm²); □, 24 mM-KCl (leakage 81,000 ohm cm²); ●, 12 mM-KCl + 12 mM-NaCl (leakage 33,000 ohm cm²) △, 24 mM-KCl (leakage 14,000 ohm cm²); ◓, 300 mM-KCl (leakage 4000 ohm cm²); isotonicity maintained with sucrose. External solution: K-free artificial sea water. Curve *a* was drawn through the experimental results with 300 mM; curve *b*, based on the independence principle, is given by multiplying curve *a* by 0·12, the ratio of potassium activities in 24 and 300 mM; curve *c* combines a shift of 16 mV in an assumed internal double-layer potential between 300 and 24 mM-KCl with the independence principle applied to the concentration and voltage at the inner edge of the membrane; internal concentration at the membrane was calculated by eqn. (9·0). Axon 27, diameter 715 μ. Temperature 0° C.

Effect of low ionic strength on delayed currents

The experiment in Fig. 6 shows the effect on the delayed currents produced by first diluting the KCl from 300 to 24 mM and then replacing half the K with Na. The effect of varying K is clearly consistent with the idea that this ion carries the delayed outward current.

Curve *a* was drawn through the results obtained with 300 mM-KCl and curve *b* gives the current expected with 24 mM on the independence principle; this was obtained by multiplying curve *a* by the ratio of

potassium activities, 0·12. The agreement with the experimental values might be taken as evidence that ionic strength does not affect the position of the potassium conductance on the voltage axis. However, an equally good fit can be obtained by assuming that negative charges at the inside of the membrane produce a potential drop in the internal solution which shifts the curve and also concentrates cations near the membrane. Curve *c* which was calculated by the methods outlined in the discussion was drawn for a shift of 16 mv between 300 and 24 mM-KCl. This value was chosen so that the curve would match the experimental points at the apparent sodium equilibrium potential, $V_e = 100$ mV, where any residual conductance through the sodium channel would not alter the experimental results.

DISCUSSION

The experiments confirm the observation of Narahashi (1963), Baker *et al.* (1964) and Moore *et al.* (1964) that dilution of the internal potassium solution with a non-electrolyte causes the inactivation curve to shift in the direction of a more positive internal potential. They show that the shift is caused by a decrease of ionic strength rather than by a decrease of potassium concentration. Similar conclusions apply to the threshold and to the relation between sodium conductance and membrane potential.

A tentative explanation can be given along the lines mentioned by Baker *et al.* (1964). Suppose that there are phosphate or other negatively charged groups on both sides of the membrane and that those on the outside are neutralized by calcium and magnesium. (The second assumption is not essential but it is simpler to consider only one set of charges.) Since ions can pass through the membrane, the fixed charges do not make any direct contribution to the resting potential, and, if the latter were zero, the potential distribution should be like that in Fig. 7*A*. The combined effect of the fixed charges and of a resting potential of -63 mV is shown by the full curve in *B*. The magnitude of the potential difference (ψ_0) between the internal solution at the membrane and at some distant point depends on the ionic strength of the internal solution, and on the density of the fixed charges. If the concentration of KCl were reduced from 300 to 6 mM-KCl, the space constant of the ion atmosphere would increase from 5·5 to 39 Å, and, with a constant charge density of $1{\cdot}4 \times 10^{13}$ electronic charges/cm^2, ψ_0 would alter from -17 mV to -80 mV (curve *C*). From this it is clear that the p.d. across the membrane, as opposed to the total p.d. between internal and external solutions, would be the same in *C* (6 mM-KCl, no resting potential) as it is in *B* (300 mM-KCl, resting potential of -63 mV). This provides a possible explanation of the shift in threshold and inactivation curve, since changes in sodium permeability are likely

to be determined by the p.d. across the membrane, rather than by the total p.d.

The method of calculating ψ_0 from the charge density is similar to that described by Verwey & Overbeek (1948) or Parsons (1954) in connexion with the theory of the diffuse double layer (Gouy, 1910; Chapman, 1913). It is assumed, first, that the charges are smeared

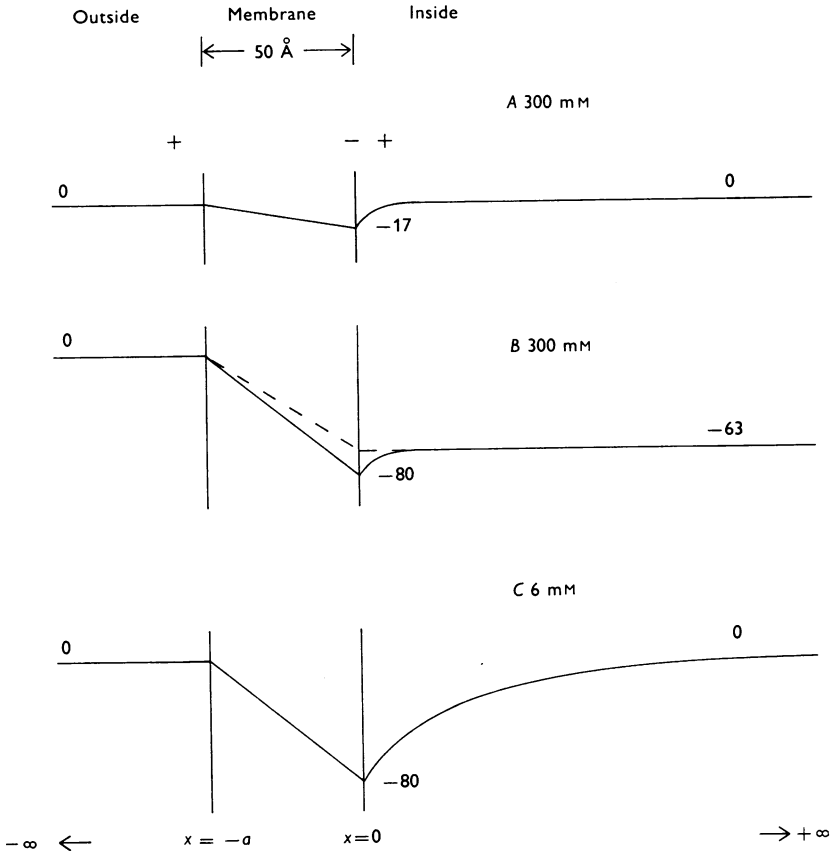

Fig. 7. Theoretical effect of fixed charges at the inside of the membrane on potential distribution (20° C).

A: no resting potential; fixed charge density, $-2{\cdot}23$ μcoulomb/cm^2 ($1{\cdot}39 \times 10^{13}$ electronic charges/cm^2); internal concentration 300 mM;

B: interrupted curve; resting potential, -63 mV; no fixed charges, 300 mM inside;

B: full curve; resting potential, -63 mV; same fixed charge density as in *A*, 300 mM inside;

C: no resting potential; same fixed charge density as in *A*, 6 mM inside.

The curves were calculated from eqn. (7·8), with ψ_0 from (8·0), assuming a membrane capacity of 1 μF/cm^2 and 600 mM in the external solution. The numbers give the potential in mV with reference to the external solution ($x = -\infty$). The p.d. in the external solution ($V_{-a} - V_{-\infty}$) is too small to show on this scale; it amounts to $-0{\cdot}094$ mV in *A* and $-0{\cdot}44$ mV in *B* and *C*. The counterions in the external solution (shown at top) amount to 0·8 % of those inside in *A* and 3·8 % of those in *C*.

out over the inner edge of the membrane so that the problem is one-dimensional, and secondly, that the field within the membrane is constant. The small p.d. in the external fluid, which amounts to 0·55 % of the p.d. across the membrane, is neglected. From the Poisson and Boltzmann relations the Debye–Hückel equation for a uni-univalent electrolyte is obtained, i.e.

$$\frac{d^2\psi}{dx^2} = \frac{8\pi Fc}{\epsilon_w} \sinh \frac{F\psi}{RT}, \tag{7·0}$$

where R, T and F have their usual meaning, ψ is the potential (here defined with reference to the bulk of the internal solution), c is the bulk concentration in moles/cm^3 and ϵ_w is the dielectric constant of water. The boundary conditions are $\psi = 0$ at $x = \infty$, and, from electrostatics,

$$\epsilon_w \left(\frac{d\psi}{dx}\right)_{0_w} - \epsilon_m \left(\frac{d\psi}{dx}\right)_{0_m} = -4\pi\sigma, \tag{7·1}$$

where σ is the density of fixed charges at $x = 0$ and the suffix w or m denotes water or membrane, respectively. For $|\psi| < 25$ mV the approximate solution is

$$\psi = \psi_0 \exp(-x/l) \tag{7·2}$$

where

$$l = \sqrt{\left(\frac{\epsilon_w RT}{8\pi cF^2}\right)} \tag{7·3}$$

If the field in the membrane is constant eqn. (7·1) can be written

$$\frac{\epsilon_w}{4\pi}\left(\frac{d\psi}{dx}\right)_{0_w} - \frac{\epsilon_m}{4\pi a}(\psi_0 - \psi_{-a}) = -\sigma, \tag{7·4}$$

where a is the thickness of the membrane.
From the approximate solution, equation (7·2),

$$\frac{\epsilon_w}{4\pi l}\psi_0 + \frac{\epsilon_m}{4\pi a}(\psi_0 - \psi_{-a}) = \sigma. \tag{7·5}$$

Now $\epsilon_m/4\pi a$ is the electrical capacity of the membrane and may be taken as $1\mu F/cm^2$; $\epsilon_w/4\pi l$ depends on ionic strength and varies from 180 μF/cm^2 with 600 mM-KCl to 18 μF/cm^2 with 6 mM-KCl as the internal solution. Over most of the range little error is introduced by neglecting the second term in eqn. (7·5) so

$$\psi_0 = \frac{4\pi\sigma l}{\epsilon_w} = \frac{\sigma}{F}\sqrt{\left(\frac{2\pi RT}{\epsilon_w c}\right)}. \tag{7·6}$$

Equations (7·2) and (7·6) are accurate to within about 5 % up to $|\psi| = 25$ mV but for larger values it is best to avoid the approximation $\sinh u = u$. Integration of (7·0) with the boundary condition $d\psi/dx = 0$ at $x = \infty$ leads to

$$\frac{d\psi}{dx} = -\frac{2RT}{Fl} \sinh \frac{F\psi}{2RT} \tag{7·7}$$

and further integration to

$$\tanh \frac{F\psi}{4RT} = \left(\tanh \frac{F\psi_0}{4RT}\right) \text{e-p}(-x/l). \tag{7·8}$$

If the second term in (7·4) is neglected ψ_0 is given by

$$\sinh \frac{F\psi_0}{2RT} = \sigma\sqrt{\left(\frac{\pi}{2\epsilon_w cRT}\right)}. \tag{7·9}$$

A more accurate value can be obtained by including the second term of (7·4). In the absence of a resting potential

$$\psi_0\left[\frac{\epsilon_m}{4\pi a} + \frac{\epsilon_w}{4\pi l}\left(\frac{\sinh F\psi_0/2RT}{F\psi_0/2RT}\right)\right] = \sigma. \tag{8·0}$$

Experimental and theoretical values of the shift in voltage with concentration are compared in Table 3. The density of fixed charge chosen to fit these results, $1{\cdot}4 \times 10^{13}$ electronic charges/cm^2, does not seem unreasonable, since it corresponds to a linear separation of 27 Å between charges.

TABLE 3

1 Internal concentration (mM)	2 Temperature (°C)	3 Quantity	4 Observed shift from 300 mM (mV)	5 Calculated change in $-\psi_0$ from 300 mM (mV)	6 Absolute value of ψ_0(20° C) (mV)
300	20	—	(0)	(0)	−17
50	0	V_h	22	19	−38
24	0	V_h	32	31	—
24	0	V_c (threshold)	30	31	—
24	21	V_c (threshold)	38	34	−51
6	21	V_c (threshold)	65	63	−80

Values of V_c (threshold) at 21° C are from Baker *et al.* (1964). The value of V_c at 0° C and the values of V_h (inactivation) are from this paper. The last two columns were calculated from equation (8·0) with ϵ_w and T appropriate to the temperature, a membrane capacity of 1 μF/cm^2 and $\sigma = -2{\cdot}23$ μcoulomb/cm^2. Column 5, which is calculated for zero resting potential, should be within 1 mV of the observed shift in column 4. With 300 mM inside the mean values of V_h and V_c were −59 and −46 mV.

From the electrical effect of reducing the internal concentration of KCl on the resting potential, Baker *et al.* (1964) tentatively concluded that dilution changed the relative permeability of the membrane to cations and anions. With concentrations below 50 mM the results were fitted by permeability ratios of $P_K:P_{Na}:P_{Cl} = 1:0{\cdot}035:0{\cdot}02$. At concentrations of 100–600 mM a better fit was obtained with $P_K:P_{Na}:P_{Cl} = 1:0{\cdot}05:0{\cdot}1$. A change of this kind is expected from the hypothesis which has just been described. The fixed anions should concentrate cations and dilute anions by a factor ρ which is related to ψ_0 by

$$\rho = \exp(-F\psi_0/RT). \qquad 9{\cdot}0$$

On the constant field theory the total p.d. between internal and external fluids is therefore

$$V_i = \frac{RT}{F} \ln \frac{P_K[K]_e + P_{Na}[Na]_e + P_{Cl}[Cl]_i/\rho}{\rho P_K[K]_i + \rho P_{Na}[Na]_i + P_{Cl}[Cl]_e} + \frac{RT}{F} \ln \rho \qquad (9{\cdot}1)$$

or

$$V_i = \frac{RT}{F} \ln \frac{P_K[K]_e + P_{Na}[Na]_e + P_{Cl}[Cl]_i/\rho}{P_K[K]_i + P_{Na}[Na]_i + P_{Cl}[Cl]_e/\rho}, \qquad (9{\cdot}2)$$

where $[\ ]_e$ and $[\ ]_i$ are concentrations in the bulk of the external and internal solutions, respectively, and V_i is the total p.d. defined with reference to the external solution. The first term in eqn. (9·1) is the p.d. across the membrane, the second is the p.d. between the bulk of the internal solution and the inner edge of the membrane.

Since ρ increases as the salt concentration is reduced, dilution should decrease the permeability of the anions relative to that of the cations. Using the values of ψ_0 in Table 3, ρ increases fourfold between 300 and 24 mM, which is roughly consistent with the change in permeability inferred by Baker *et al.*

If the fixed charge hypothesis is taken in its most literal form, one would expect that dilution would shift the potassium conductance relation to the same extent as the curves determining sodium permeability. However, this prediction is somewhat unrealistic, since the charge density might be high near the sodium channels and low, or absent, near the potassium channels. The assumption of a uniformly charged layer was made for simplicity and is not a particularly good description if the charges are 27 Å apart. The experimental position is equally indeterminate. Moore *et al.*'s results (1964) suggest that the delayed currents may be shifted by dilution to about the same extent as the sodium currents. Our results (Fig. 6, 300–24 mM) indicate that the shift might be between zero, curve *b*, and 16 mV, curve *c*, the latter being less than the 31 mV expected from the shift in the sodium curves. However, both curves depend on the independence relation and if this is inapplicable no definite conclusion can be drawn from Fig. 6. A different type of experiment, possibly with high external potassium, is required to settle the point.

Besides illustrating the influence of a low internal salt concentration on the inactivation curve, the experiments showed that the inactivation mechanism in perfused axons was strikingly similar to that in intact axons (Hodgkin & Huxley, 1952*a*, *b*). Further experiments are needed to study the detailed effects of varying the internal ionic concentrations on the time course of inactivation. There was some evidence that partial replacement of K with Na increased τ_h but no successful experiments were carried out under conditions where very long action potentials are obtained (Narahashi, 1963; Baker *et al.* 1964).

Since replacing internal K with Rb prolongs the action potential (Baker *et al.* 1962) it might be supposed that the potassium channel is not open to Rb, or that Rb affects sodium inactivation, or that both mechanisms are affected. The first alternative now appears to be correct since the time constant of inactivation was little affected by substituting Rb for K (Table 2), whereas the late outward current was greatly reduced (Chandler & Meves, 1965).

SUMMARY

1. The effect of internal solutions on steady-state sodium inactivation was investigated on perfused axons using the voltage clamp method.

2. Dilution of the internal KCl solution with isotonic sucrose shifted the inactivation curve along the voltage axis in the positive direction. The shift did not occur when KCl was replaced by NaCl, choline chloride or RbCl at constant ionic strength.

3. It is concluded that the position of the inactivation curve is determined by the ionic strength of the internal solution rather than by the potassium concentration.

4. Additional experiments showed that the kinetics of inactivation in perfused axons were similar to the kinetics in intact axons.

5. Measurements of the time course of the inactivation indicated that the inactivation follows an approximately exponential time course from the beginning of a depolarizing pulse.

We wish to thank the Director and staff of the Laboratory of the Marine Biological Association at Plymouth for providing space and assistance. Financial support was provided by the National Institute at Neurological Diseases and Blindness (special fellowship) and the Deutsche Forschungsgemeinschaft.

REFERENCES

BAKER, P. F., HODGKIN, A. L. & MEVES, H. (1964). The effect of diluting the internal solution on the electrical properties of a perfused giant axon. *J. Physiol.* **170**, 541–560.

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1962). The effects of changes in internal ionic concentrations on the electrical properties of perfused giant axons. *J. Physiol.* **164**, 355–374.

CHANDLER, W. K. & MEVES, H. (1964). Sodium inactivation in internally perfused squid giant axons. *Pflüg. Arch. ges. Physiol.* **281**, 25–26.

CHANDLER, W. K. & MEVES, H. (1965). Voltage clamp experiments on internally perfused giant axons. *J. Physiol.* **180**, 788–820.

CHAPMAN, D. L. (1913). A contribution to the theory of electrocapillarity. *Phil. Mag.* **25**, 475–481.

GOUY, G. (1910). Sur la constitution de la charge électrique à la surface d'un électrolyte. *J. physique* (4), **9**, 457–468.

HODGKIN, A. L. & HUXLEY, A. F. (1952*a*). The dual effect of membrane potential on sodium conductance in the giant axon of *Loligo*. *J. Physiol.* **116**, 497–506.

HODGKIN, A. L. & HUXLEY, A. F. (1952*b*). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500–544.

HOYT, ROSALIE, C. (1963). The squid giant axon. Mathematical models. *Biophys. J.* **3**, 399–431.

MOORE, J. W., NARAHASHI, T. & ULBRICHT, W. (1964), Sodium conductance shift in an axon internally perfused with a sucrose and low-potassium solution. *J. Physiol.* **172**, 163–173.

NARAHASHI, T. (1963). Dependence of resting and action potentials on internal potassium in perfused squid giant axons. *J. Physiol.* **169**, 91–115.

PARSONS, R. (1954). Equilibrium properties of electrified interphases. In *Modern Aspects of Electrochemistry.*, ed. BOCKRIS, J. O'M. & CONWAY, B. E. London: Butterworths Scientific Publications.

TASAKI, I. & SHIMAMURA, M. (1962). Further observations on resting and action potential of intracellularly perfused squid axon. *Proc. Nat. Sci., Wash.*, **48**, 1571–1577.

VERWEY, E. J. W. & OVERBEEK, J. TH.G. (1948). *Theory of the Stability of Lyophobic Colloids.* Amsterdam–New York: Elsevier.