THEORETICAL POTASSIUM LOSS FROM SQUID AXONS AS A FUNCTION OF TEMPERATURE

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ABSTRACT Theoretical net ionic movements have been calculated for the propagated impulse of the squid axon from the Hodgkin-Huxley equations. The computed potassium movements agree approximately with the experimental data of Shanes, but vary too much with temperature ($Q_{10} = 1/2.75$ from computation, 1/1.91 from experiment). Theoretical corrections providing higher ionic conductances increasing with temperature (according to J. W. Moore's experiments) give a Q_{10} of 1/2.24, but the incorporation of the higher values of the maximum conductances, as observed under improved environmental conditions, leads to potassium movements that are considerably higher than Shanes's values.

Theoretical net ionic movements per impulse were calculated by Hodgkin and Huxley (1952) from their model of the squid giant axon membrane for two different temperatures. Comparisons of such calculations with experimental data for the case of the propagated impulse provide a test of the validity of their model, which is based on data obtained under the electrically different case of voltage clamp. They showed that their calculations agree approximately with experimental measurements by Keynes and Lewis (1951). Shanes (1954) has measured experimentally the K loss per impulse with only an approximate agreement with Hodgkin and Huxley's theoretical values. Moore (1958) has found that the maximum values of the Na and K currents both increase with temperature instead of remaining constant as in the equations (see below). If the Hodgkin-Huxley (H-H) model is modified according to Moore's results, new theoretical values of ionic movement result, which, in the case of K, are markedly above Shanes's values. This can probably be explained by the improved condition of Moore's axons. This paper will present new theoretical figures for ionic movement for the modified model for comparison with experiment.

Hodgkin and Huxley made theoretical calculations of Na and K movements (q_{Na} and q_K) for 6.3° and 18.5°C, for the case of the non-propagated "membrane action potential" under space clamp. They also calculated ionic movements for the propagated action potential at 18.5°C only, and found slightly greater values than for the membrane action potential (*ca.* 8 per cent larger for Na and 5 per cent larger for K). Their propagated action potential for 18.5°C has been recomputed and checked by FitzHugh and Antosiewicz (1959) with a digital computer, and a new computation has been made for 6.3°C. From these have been calculated theoretical net movements of Na, K, and L (leakage ions, Table I). Since in the resting state none of the three ionic currents is zero, the net movement q in question is the integral, from $t = -\infty$ to $+\infty$, of each ionic current minus its resting value. Computation was stopped as soon as the solution of the equations entered a neighborhood of the resting singular point within which the equations could be linearized with sufficient accuracy (for mathematical details see FitzHugh and Antosiewicz, 1959). The computed currents were integrated numerically over this finite time interval. The tails of the integrals, for t tending toward $\pm\infty$, were found by integrating the linearized differential equations with an analog computer. The contributions of the tails were less than 0.2 per cent of the total for sodium and potassium, but as high as 5.5 per cent for leakage. Since the membrane potential V approaches zero as t tends to both plus and minus infinity, the total net charge moved across the membrane capacity by all ions during the impulse is theoretically zero. This provides a check on the accuracy of the calculation, and the sum of the q's shown in Table I differs from zero by no more than was expected from all errors of computation. Positive quantities in Table I indicate net inward transfer.

TABLE I

THEORETICAL NET IONIC MOVEMENTS FOR PROPAGATED IMPULSES IN THE HODGKIN-HUXLEY MODEL

(inward movements are positive)

Flux	6.3°C		18.5°C	
	ncoul/cm²‡	*pmole/cm²‡*	*ncoul/cm²*	*pmole/cm²*
q_{Na}	1436.9	14.890	420.46	4.3572 (4.33)*
q_K	−1409.5	−14.606	−413.57	−4.2857 (−4.26)*
q_L	−27.3	−0.283	−6.86	−0.0711
Total q	+0.1	+0.001	+0.03	+0.0004

*These values are from Hodgkin and Huxley (1952).

‡n = nano = 10^{-9}, p = pico = 10^{-12}.

The two figures with asterisks in Table I are taken from Hodgkin and Huxley (1952). Their figures are in satisfactory agreement with the recalculated values given immediately above them. They carried out the integrations only from $t = 0$ to the third zero in V after the action potential, instead of for $t \rightarrow \pm\infty$.

The temperature dependence of the H-H model is based on the Arrhenius equation (1). In general, the temperature dependence of a physical quantity X can be written: (Höber *et al.*, 1945, p. 388):

$$X(T) = A \exp\,[-a/(273.2 + T)] \tag{1}$$

where A and a are constants, T is the temperature in °C, and $273.2 + T$ is the absolute temperature in °K. Choose a reference temperature $T_0(= 6.3°\text{C}$ here); define

$$\phi(T) = X(T)/X(T_0) \quad \text{and} \quad Q_{10} = \phi(T_0 + 10).$$

$$\ln \phi(T) = -a[1/(273.2 + T) - 1/(273.2 + T_0)]$$

$$\text{Then} \qquad \ln \phi(T) = \frac{a(T - T_0)}{(273.2 + T)(273.2 + T_0)}$$

$$\ln Q_{10} = \frac{10a}{(273.2 + T_0 + 10)(273.2 + T_0)} \tag{2}$$

$$\frac{\ln \phi(T)}{\ln Q_{10}} = \frac{T - T_0}{10} \cdot \frac{273.2 + T_0 + 10}{273.2 + T} \doteq \frac{T - T_0}{10} \tag{3}$$

$$\phi(T) \doteq Q_{10}{}^{(T-T_0)/10} \tag{4}$$

The approximation in equations (3) and (4) is best for T near $T_0 + 10$. Hodgkin and Huxley assume a temperature dependence only for the time derivatives dn/dt, dm/dt, dh/dt of their conductance variables of state (n = K activation, m = Na activation, h = Na inactivation) which are proportional to $\phi(T)$ as given by formula (4), with $Q_{10} = 3$ (or, equivalently, the time constants τ_n, τ_m, τ_h have a Q_{10} of 1/3). Otherwise their model is independent of temperature; the only effect of a temperature increase on their theoretical ionic currents during voltage clamp is a uniform contraction of the curves along the time axis. In particular, the maximum peak value of the Na current and steady-state value of the K current (and the corresponding values of conductance) are independent of temperature.

For the case of a propagated impulse, the effect on the ionic movements cannot be simply predicted from a knowledge of $\phi(T)$, and the only way to determine them is to solve the differential equations for various temperatures. Shanes's average values of K movement (q_K) are -11.4 pmole/cm^2 at 6.1°C and -3.7 pmole/cm^2 at 24°C (1 pmole = 10^{-12} mole). Since Shanes's pair of temperatures is different from Hodgkin and Huxley's, these two pairs of values can be compared by assuming, for lack of any better information, that q_K also varies with temperature according to equation (1). This equation can of course be fitted to any data given at a pair of temperatures, but a value at a third temperature, at least, would be needed to test its accuracy.

Fig. 1 shows log $|q_K|$ plotted against the reciprocal of the absolute temperature. Equation (1) is plotted as a straight line segment in this plane, joining a pair of points. The two lower curves represent Shanes's experimental averages and the theoretical ones as calculated for the H-H equations. The latter are too high at the lower temper-

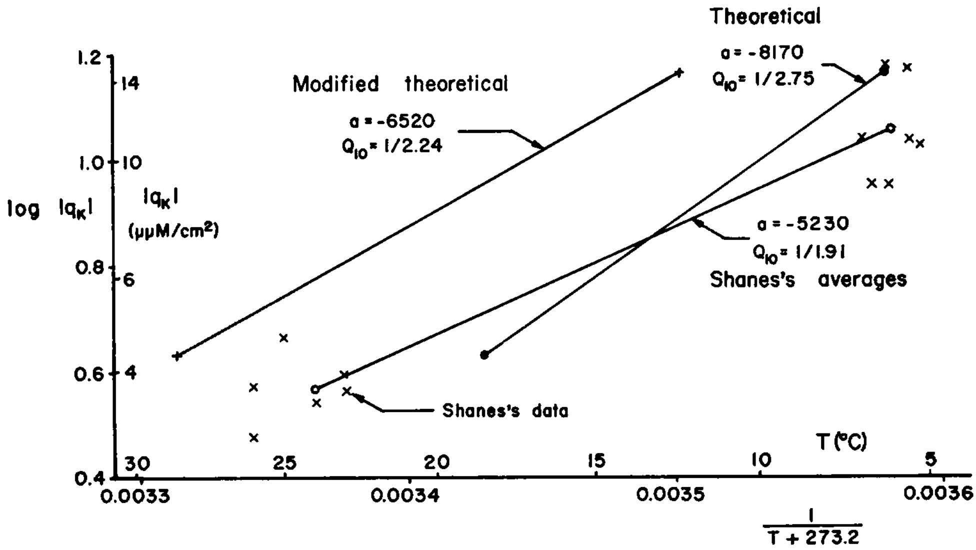

FIGURE 1 Effect of temperature T on net outward potassium movement q_K. Straight lines represent the Arrhenius equation (1) with $X = q_K$. Lines are shown for Shanes's experiments, for the H-H equations, and for the H-H equations modified as described in the text.

ature and too low at the higher one. These discrepancies should be compared with the spread of Shanes's individual experiments, plotted as crosses.

The slope of such a line is $-a$, from which Q_{10} can be calculated as follows.[1] In equation (2), let $T_0 = 6.3°C$. Then

$$\log Q_{10} = 0.4343 \ln Q_{10} = 0.00005367a. \tag{5}$$

Although the ionic conductances in the H-H model are independent of temperature, Hodgkin, Huxley, and Katz (1952, p. 442) state that they in fact do vary with a Q_{10} of 1.0 to 1.5. More accurate measurements were made by Moore (1958), who found that squid axons in improved physiological condition have, under voltage clamp, maximum Na and K conductances which (*a*) are larger than those in the H-H equations and (*b*) show a linear increase with temperature not provided for by the equations. A representative axon was chosen from his data (Moore, personal communication), having maximum K conductances ($\bar{g}_K$) equal to 53 and 89 mmho/cm² for $T = 5°$ and 20°C, respectively, as compared with 36 mmho/cm² in the H-H model (for all temperatures). Fitting these values with a straight line gives the following dependence of $\bar{g}_K$ on temperature:

$$\bar{g}_K = 36\eta, \quad \eta(T) = 1.1389 + 0.06667T \tag{6}$$

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[1] Since the ionic movements decrease with temperature, their Q_{10} values are less than 1. Since we are accustomed to thinking in terms of a Q_{10} which is a ratio of a larger to a smaller number, we write all values of Q_{10} which are less than 1 as a fraction with numerator 1.

Assume for simplicity that all three ionic conductances ($\bar{g}_K$, $\bar{g}_{Na}$, g_L) have the same temperature dependence as in equation (6); *i.e.*, the H-H values are multiplied by the function $\eta(T)$. It is then possible, using a dimensional method similar to that described by Huxley (1959), to find a new pair of temperatures at which the computed net movements will apply under the new assumption of temperature dependence. The details of this procedure are given in the Mathematical Appendix.

The upper straight line in Fig. 1 passes through a pair of points computed by this method. Its Q_{10} is 1/2.24, which is intermediate between those of the two lower lines. That is, making the conductances increase with temperature decreases the discrepancy in Q_{10} between the theory and experiment, but does not eliminate it. Moreover, the curve lies considerably higher than that of Shanes and therefore is not directly comparable with it, presumably because of the better condition of Moore's axons. An attempt to carry out the dimensional procedure for one of Moore's axons which had lower ion movements comparable with those of Shanes unfortunately failed. It was impossible to find an equivalent temperature for 6.3°C by this method; mathematically, the two curves representing the left and right sides of equation (13) in the Appendix did not intersect.

One other possible effect of temperature would be to change the membrane capacitance *C*. Taylor and Chandler (1962) reported that the real part of the complex constant phase-angle capacitance of the squid axon membrane, measured at a frequency of 30 kc, varied with temperature *T* (in °C) by a factor $(1 + 0.0073T)$. Equation (7) of the Appendix shows that a change of *C* could be accounted for by multiplying *C* by a factor $1/\eta(T)$. Since $\eta(T)$ in equation (6) varies proportionally to $(1 + 0.0585T)$, the effect of changing *C* according to Taylor and Chandler's measurements would be only one-eighth of that obtained by changing the conductances according to Moore's measurements, and in the opposite direction. This effect would therefore make the fit of Q_{10} to experiment slightly worse. In any case, the frequencies important in the action potential are those near 1 kc, and dependable measurements of the effect of temperature change on capacitance do not seem to be available for this frequency.

In view of the shortcomings of the dimensional method, further digital computations incorporating various assumptions regarding the temperature dependence of the conductances are needed to make a better test of the H-H model. More than two points per curve should be obtained in order to check the form of the temperature dependence of the ion movements. It may also be advisable to change the temperature coefficients of the Hodgkin-Huxley time "constants" τ_m, τ_h, τ_n, in the squid. A Q_{10} of 1/3, as originally given by Hodgkin, Huxley, and Katz (1952), for all three time constants has proven to be an excellent approximation for many purposes, but it is worth noting that Moore and Frankenhaeuser (1962) reported Q_{10} values from *Xenopus* node near 1/2 for τ_m and near 1/3 for τ_h and τ_n. Since further computations would be rather time-consuming, the results of this partial analysis are being published to make them available now.

Table II shows conduction velocities calculated (as described in the Appendix) for various temperatures and assumptions. In column 3 are the velocities for the H-H equations, both in their original form and as modified by equation (6). These were calculated using Hodgkin and Huxley's assumption that R_2, the axoplasmic specific resistance (equation 11), is independent of temperature. Schmitt (1955) measured axoplasmic resistance in kilohms per centimeter of a 0.53 mm diameter fiber. The corresponding values of specific axoplasmic resistance are given in column 4. Schmitt's measurements of R_2 match Hodgkin and Huxley's assumed value at 20°C. Introducing the temperature dependence of R_2 serves to increase the temperature dependence of conduction velocity (column 5).

TABLE II

THEORETICAL CONDUCTION VELOCITIES θ

Case	Temp. °C	θ for constant R_2 ($R_2 = 35.4\ \Omega$ cm)	R_2	θ
		m/sec.	(Ω cm)	m/sec.
H-H equations	6.3	12.32	49.5	10.42
	18.5	18.74	36.7	18.40
Modified H-H equations	12.4	17.27	42.2	15.82
	28.6	32.71	30.3	35.36

If the values in Table II were compared with experimental results, it would provide a check of the H-H equations and of the assumption regarding the temperature dependence of the ionic conductances and of axoplasmic resistance.

Because of the great variation of $\bar{g}_K$ with the physiological condition of the axon, comparison between experiment and theory must be made with caution. Until it is sure that the axons in which potassium loss is measured are in the same condition as those on which the equations are based, one cannot expect an accurate comparison.

MATHEMATICAL APPENDIX

The following method of correcting the computed relation between ion movements and temperature is similar to the "dimensional" method used by Huxley (1959), for the relation between conduction velocity and temperature.

For simplicity, assume that all the ionic conductances have the same temperature dependence; *i.e.*, that $\bar{g}_K$, $\bar{g}_{Na}$, and g_L are multiplied by the factor $\eta(T)$ defined in equation (6). The H-H equations for the propagated action potential (Hodgkin and Huxley, 1952, equations (31, 7, 15, 16) then take the form:

$$d^2V/dt^2 = K\left\{dV/dt + \frac{\eta(T)}{C}\left[\bar{g}_K n^4(V - V_K) + \bar{g}_{Na} m^3 h(V - V_{Na}) + g_L(V - V_L)\right]\right\} \qquad (7)$$

$$dn/dt = [(1 - n)\alpha_n(V) - n\beta_n(V)]\phi(T) \tag{8}$$

$$dm/dt = [(1 - m)\alpha_m(V) - m\beta_m(V)]\phi(T) \tag{9}$$

$$dh/dt = [(1 - h)\alpha_h(V) - h\beta_h(V)]\phi(T) \tag{10}$$

where T is the temperature in °C and $\phi(T) = 3^{(T-6.3)/10}$. At this stage, $\eta(T)$ can be any arbitrary function of T and is the factor by which the conductances are being multiplied in order to provide for their variation with temperature. (When $\eta(T)$ is identically equal to 1, the original H-H equations result.) For each temperature, one must find that value of K for which $V(t) \rightarrow 0$ as $t \rightarrow +\infty$. The procedure for searching for this value of K is described by Hodgkin and Huxley (1952) and FitzHugh and Antosiewicz (1959). The conduction velocity θ is found from K by the following equation:

$$\theta = \left(\frac{\mathrm{K}r}{2R_2C}\right)^{1/2} \tag{11}$$

where r is the axon radius, R_2 is the specific resistance of the axoplasm, and C the membrane capacitance per unit area.

In equations (7–10) transform to a new time variable t^*:

$$t^* = t\eta(T) \tag{12}$$

For a given temperature, this is equivalent to a change in the units of time. After substituting from equation (12), make the substitutions from equations (13–14) to get equations (15–18):

$$\phi(T) = \eta(T)\phi^* \tag{13}$$

$$\mathrm{K} = \eta(T)\mathrm{K}^* \tag{14}$$

$$d^2V/dt^{*2} = \mathrm{K}^*\left\{dV/dt^* + \frac{1}{C}[\bar{g}_{\mathrm{K}}n^4(V - V_{\mathrm{K}}) + \bar{g}_{\mathrm{Na}}m^3h(V - V_{\mathrm{Na}}) + g_L(V - V_L)]\right\} \tag{15}$$

$$dn/dt^* = [(1 - n)\alpha_n(V) - n\beta_n(V)]\phi^* \tag{16}$$

$$dm/dt^* = [(1 - m)\alpha_m(V) - m\beta_m(V)]\phi^* \tag{17}$$

$$dh/dt^* = [(1 - h)\alpha_h(V) - h\beta_h(V)]\phi^* \tag{18}$$

The function $\eta(T)$ cancels out and equations (15–18) are the original H-H equations, with K, $\phi(T)$, and t replaced by K*, ϕ^*, and t^*. If T^* denotes the temperature at which the original equations have been solved, then $\phi^* = \phi(T^*)$. Two solutions for equations (15–18) have been computed, as described earlier; *i.e.*, for $T^* = 6.3°$ and 18.5°C. Knowing ϕ^*, one can solve for T graphically, by finding the intersection point of the curves of the left- and right-hand sides of equation (13), plotted against T. The values $T^* = 6.3°$ and 18.5° correspond to $T = 12.4°$ and 28.6°C, respectively. With K*, T, and therefore the value of $\eta(T)$ all known, K can be found from equation (14). To each solution of equations (15–18) corresponding to given values of ϕ^* and K*, there is a solution of equations (7–10) in which V, n, m, and h are functions of t, which are found by substituting for t^* by means of equation (12) in the solution of equations (15–18). Thus, equations (7–10) with an arbitrary $\eta(T)$

have been indirectly solved, though at a temperature which could not have been specified in advance.

It turns out that the ionic movements q for equations (7–10) are the same as the movements q^* already known for equations (15–18). This will be shown as follows for q_K, which is defined by the following equations:

$$q_K = \int_{-\infty}^{+\infty} (I_K - I_{KO})\, dt, \qquad I_K = \bar{g}_K n^4 (V - V_K)\eta(T). \tag{19}$$

I_K is the K current, a function of t, and the constant I_{KO} is its value in the resting state. The corresponding quantities for equations (15–18) are:

$$q^*_K = \int_{-\infty}^{+\infty} (I^*_K - I^*_{KO})\, dt^*, \qquad I^*_K = \bar{g}_K n^4 (V - V_K).$$

Thus at corresponding values of t and t^*,

$$I_K = I^*_K \eta(T). \tag{20}$$

Substitute from equations (12) and (20) into equation (19):

$$q_K = \int_{-\infty}^{+\infty} (I^*_K - I^*_{KO})\eta(T)\, dt^*/\eta(T) = q^*_K$$

Thus the value of q is not changed by making $\eta(T)$ different from 1, but the temperature to which that value corresponds is changed from T^* to T.

These calculations have actually been carried out with $\eta(T)$ given by equation (6).

The results are plotted as the upper left-hand line in Fig. 1. This line covers a range of temperatures which is different from that of the original theoretical pair, but still overlaps Shanes's pair sufficiently for comparison.

If it is assumed that R_2 in equation (11) is independent of temperature, then from equations (11) and (14) the conduction velocity for equations (7–10) is $[\eta(T)]^{1/2}$ times the corresponding velocity for equations (15–18), already calculated by Hodgkin and Huxley (1952) and FitzHugh and Antosiewicz (1959), with $R_2 = 35.4\ \Omega$ cm and $r = 0.238$ mm. The velocities computed in this way are given in Table II, column 3. Schmitt's (1955) measurements of axoplasmic resistance show, however, a temperature dependence. His Fig. 5 shows axoplasmic resistance in kilohms per centimeter of axon (ρ), plotted against temperature, where $\rho = R_2/\pi r^2$. From this figure and his assumed value of 0.265 mm for r, the values of R_2 in column 4 were calculated. The values of conduction velocity in column 5 were calculated from those in column 3, using the fact that θ varies as $R_2^{-1/2}$.

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