AN ENZYMATIC ION EXCHANGE MODEL FOR ACTIVE SODIUM TRANSPORT*

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ABSTRACT

An enzymatic ion exchange model for active sodium transport is described. Kinetic equations relating net flux to time, and to concentration difference across the actively transporting membrane are derived. The second of these equations is tested, using the isolated frog skin in the "short-circuit" apparatus of Ussing. Reasonable linearity, as predicted by this equation, is observed.

The passive permeability coefficient for Na^+, is calculated as $5.3 \times 10^{-4} \pm 5.3 \times 10^{-4}$ cm./hr. If cholinesterase is assumed to be the enzyme responsible for transport, the activity required to account for the observations reported here is 17.7×10^{-4} mmoles/cm.^{2}/hr.

In a previous paper (8) the localization and characterization of frog skin cholinesterase were described. It was found that the enzyme was largely localized in the *tela subcutanea* and that it was of the butyrylcholinesterase (pseudocholinesterase) type. It seemed interesting to speculate on the possible role of this enzyme in the active sodium transport mechanism. Thomas (10) has recently proposed a model for ion transport involving a soluble enzymatic carrier. This model requires that the enzyme-substrate complex have ion-binding characteristics different from those of the free enzyme. This might occur if the pK_a of groups in the neutral pH range is shifted, increasing (or decreasing) the net charge of the enzyme. Such pK shifts have been observed in cholinesterase by Dixon (3). Application of this scheme to the frog skin active transport mechanism presents the difficulty that the enzyme is apparently tightly bound to some component of the *tela subcutanea*. Homogenization in frog-Ringer's destroys some 50 per cent of the activity; the remainder was found associated with the particulate fraction.

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That cholinesterase is involved in active sodium transport in frog skin is indicated by Kirschner's (7) investigations on the effects of cholinesterase inhibitors in this tissue. These inhibitors block the transport mechanism reversibly or irreversibly depending on the reversibility properties of their

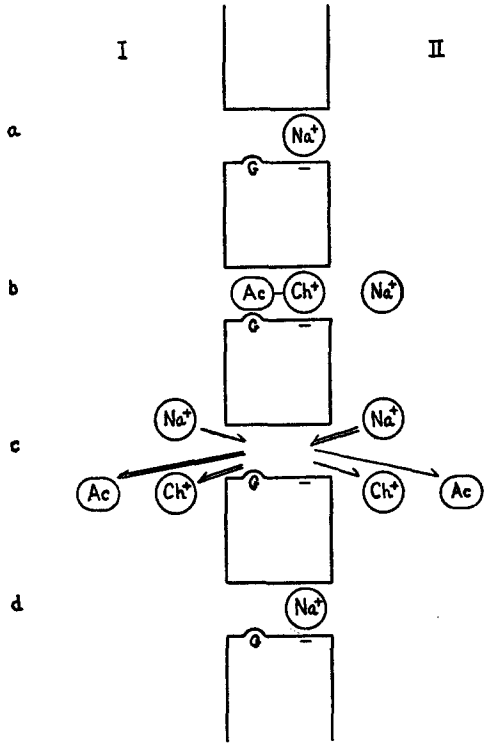

FIG. 1. Sequence of reactions leading to transport of a sodium ion from compartment I to compartment II. In *c*, displacement of hydrolysis products from pore into compartment II is indicated by single arrows. Displacement in the opposite direction is indicated by double arrows. (See text.) The parallel path of passive sodium permeation is not shown.

actions on the enzyme. Cholinesterase has also been implicated in active sodium transport in muscle (Van der Kloot (13)) and erythrocyte (Holland and Klein (4)).

Proceeding from these observations, a model in which the "carrier" enzyme is a structural component of an actively transporting membrane was developed. This model has the following properties. The membrane separates two compartments of equal size and bears pores having a diameter approximately equal to that of the hydrated sodium ion. The walls of some of these

pores incorporate the active surface of the enzyme cholinesterase. This active surface has been shown by Bergmann (1) to bear anionic sites.

A substrate is fed into one of the two compartments at a rate sufficient to keep the concentration constant. The sequence of events which moves sodium from the membrane into the second compartment is shown in Fig. 1.

In (*a*) the pore is shown charged with its sodium counterion. *G* represents the esteratic site of the cholinesterase active surface. In (*b*), the substrate has diffused from I into the pore, combined with the enzyme, and displaced the sodium ion unidirectionally, because of the approximate equality of the diameters of the pore and the hydrated sodium ion, into compartment II. Hydrolysis of the substrate regenerates the anionic site (*c*), which may be "recharged" with a sodium counterion from either I or II, as shown in (*d*).

Kinetic equations for such a model can be developed. Let us assume that the hydrolysis of each molecule of substrate hydrolyzed represents the movement of one sodium ion from the membrane to compartment II, and that the rate of "recharging" of regenerated anionic sites from each compartment is proportional to the concentration of sodium ion in that compartment. Assume further that at zero time the sodium concentrations in the two compartments are equal at N_o. If the volumes V_I and V_{II} are equal,

$$N_I + N_{II} = 2N_o$$

in which N_I and N_{II} are the Na^+ concentrations in compartments I and II, at any time t. Further, we must define the following variables and constants:

R = enzymatic hydrolysis rate in mmoles/cm.^{2} of membrane/hr.
k = passive sodium permeability constant in cm./hr.
N_o = original sodium concentration in I and II in mmoles/cc.
N_I = (Na^+) in compartment I at time t in mmoles/cc.
N_{II} = (Na^+) in compartment II at time t in mmoles/cc.
A = area of membrane in cm.^{2}
t = time in hours
V = volume of compartment I = volume of compartment II in cc.
$\frac{dN}{dt}$ = rate of net flux from I to II in mmoles/cm.^{2}/hr.
$\frac{dN_I}{dt}$ = rate of increase in N_I in mmoles/cc./hr.

We will assume that k is independent of concentration and that the P. D. across the membrane (should one develop) does not affect the rates of sodium movement in the two directions. (Experimental determination of net sodium flux in frog skin is usually accomplished by measuring the current required to hold the P. D. to zero; this is the short-circuit method of Ussing (12).)

The rate of increase of N_I, $(dN/dt)_I$, has two components

$$\left.\frac{dN_I}{dt}\right|_a = \text{active component, and } \left.\frac{dN_I}{d_t}\right|_p = \text{passive component.}$$

Since displacement of a sodium ion from the membrane into compartment II will be effective in lowering N_I only if the displaced ion comes into the membrane from compartment I, we will assume that at any time, the fraction of Na^+ which did so enter the membrane is $N_I/(N_I + N_{II}) = N_I/2N_o$. This is equivalent to the assumption that the relative rates of recharging of regenerated sites are proportional to the relative values of N_I and N_{II} mentioned above.

We may now write

$$\left.\frac{dN_I}{dt}\right|_p = \frac{A}{V} k(N_{II} - N_I)$$

and

$$\left.\frac{dN_I}{dt}\right|_a = -\frac{A}{V} R \frac{N_I}{2N_o} \S$$

Adding these,

$$\frac{dN_I}{dt} = -\frac{A}{V}\left[R\frac{N_I}{2N_o} - k(N_{II} - N_I)\right]$$

But $N_{II} = 2N_o - N_I$,

so

$$-\frac{dN_I}{dt} = \frac{A}{V}\left\{\left[\frac{R}{2N_o} + 2k\right] N_I - 2kN_o\right\} \tag{1}$$

The flux, dN/dt, is related to $(dN/dt)_I$ as follows:—

$$\frac{dN_I}{dt} = -\frac{A}{V} \cdot \frac{dN}{dt}$$

so that

$$\frac{dN}{dt} = \left[\frac{R}{2N_o} + 2k\right] N_I - 2kN_o \tag{1a}$$

§ The rate term for the reverse of this reaction (synthesis of the products in compartment II coupled with displacement of sodium ion from the membrane into compartment I) is ignored in this treatment. It is assumed that this term is of negligible magnitude.

Integration of (1) gives us the time course of N_I:

$$N_I = \frac{RN_o}{R + 4kN_0} \cdot e^{-\frac{A}{V}\left[\frac{R}{2N_o} + 2k\right]t} + \frac{4kN_o^2}{R + 4kN_o} \tag{2}$$

This immediately gives the equilibrium value of N_I since

$$\lim_{t \to \infty} N_I = \frac{4kN_o^2}{R + 4kN_o} \tag{3}$$

Substitution of (2) into (1 *a*) gives the useful results:

$$\frac{dN}{dt} = \frac{R}{2} \cdot e^{-\frac{A}{V}\left[\frac{R}{2N_o} + 2k\right]t} \tag{4}$$

and

$$\left.\frac{dN}{dt}\right|_{t=0} = \frac{R}{2} \tag{5}$$

Equation (4) can be solved for t, giving,

$$t = -\frac{V}{A}\left[\frac{2N_o}{R + 4kN_o}\right] \ln \left[\frac{2}{R} \cdot \frac{dN}{dt}\right] \tag{6}$$

If we define the half-time, $t_{1/2}$, as the time required for the flux to fall to one-half its value at $t = 0$, we have, from (5) and (6),

$$t_{1/2} = 0.69 \frac{V}{A}\left[\frac{2N_o}{R + 4kN_o}\right]. \tag{7}$$

The chambers used in this laboratory for short-circuit experiments are so designed that $V/A \cong 2$ cm. Chemical estimation of R (8) indicates a maximum value of about 2×10^{-3} mmoles/cm.^{2}/hr. These enzymatic essays, it should be noted, are performed at a substrate concentration which is presumably more nearly optimum than that which obtains in frog skin. Taking $N_o = 0.050$ mmoles/cc.,

$$t_{1/2} = \frac{0.138}{0.002 + 0.2k}$$

For $k = 0$, (membrane completely impermeable to Na^+)

$$t_{1/2} = 69 \text{ hours.}$$

For $k = 10^{-2}$ (a "very high" value)

$$t_{1/2} = 34.5 \text{ hours.}$$

Using frog skin equation (7) cannot, therefore, be easily validated experimentally. We can, however, derive an equation which relates dN/dt to the

concentration difference across the skin, $N_{II} - N_I$. This can be most simply accomplished by making use of the relation

$$N_I = N_o - \frac{N_{II} - N_I}{2}.$$

Substitution into equation (1 *a*), and rearrangement, yields

$$\frac{dN}{dt} = \frac{R}{2}\left[1 - \frac{R + 4kN_o}{2RN_o}(N_{II} - N_I)\right]. \tag{8}$$

In the short-circuit apparatus each microampere represents a transport rate of 3.73×10^{-5} mmoles/hr., hence,

$$I = 13.4 \times 10^3 AR\left[1 - \frac{R + 4kN_o}{2RN_o}(N_{II} - N_I)\right] \tag{9}$$

in which I is the short-circuit current in microamperes.

When $N_{II} = N_I$ (equivalent to $t = 0$)

$$I_{t=0} = 13.4 \times 10^3 AR. \tag{10}$$

This provides an estimate of R.

When $I = 0$ (equivalent to $t \to \infty$; equilibrium),

$$N_{II} - N_I = \frac{2RN_o}{R + 4kN_o}, \tag{11}$$

providing an estimate of k.

It is possible to test (9) by manipulating N_{II} and N_I under the condition $N_I + N_{II} = 2N_o$, and observing the changes in I.

Methods

The experimental system used was equivalent to that of Ussing and Zerahn (12). The unpigmented ventral skin of female *Rana pipiens* was used throughout. The medium was 50 mM NaCl, 5 mM KCl. After an initial equilibration period, the epidermal solution was diluted with 5 mM KCl and the dermal solution with 1000 mM NaCl, 5 mM KCl. These dilutions were performed as stepwise replacements so that $(Na^+)_{epid.} + (Na^+)_{derm.} \cong 100$ mM. The sequence of concentration changes was (epidermal concentration given first); 50, 50; 46, 53.8; 39, 61.7; 33, 66.4; 25, 74; 19.2, 81.5; 14.7, 85.4; 0, 100. After each step the current was read every 2 minutes until two consecutive equal readings occurred. This generally happened by the fourth reading. R was estimated from equation (10) and k from equation (11).

RESULTS

Fig. 2 shows the relation of I/I_o to $N_{II} - N_I$ for thirteen skins. Fairly convincing linearity is obvious.

Table I gives the values of R and k calculated from the data on individual

skins. Four skins (11-13, 12-21, 12-24, and 12-27) showed linearity when $N_{II} - N_I$ was less than 50 mM. For these the linear part of the curve was extrapolated to $N_{II} - N_I = 100$ and k' calculated from this extrapolation. It is interesting to note that k' is relatively high in these skins.

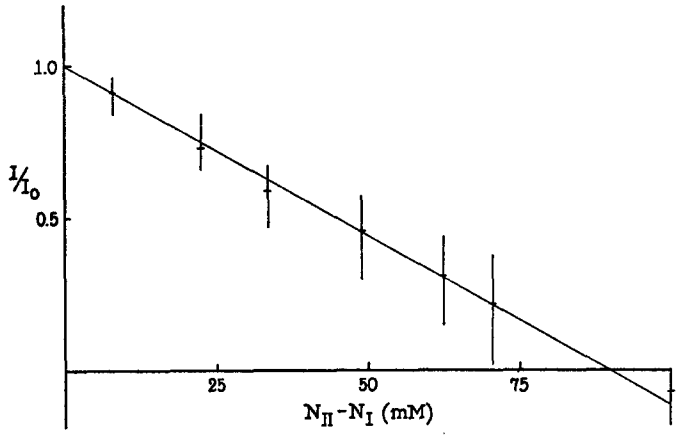

FIG. 2. Test of linearity predicted by equation (10). The relative short-circuit current, I/I_o, is plotted against the difference in sodium concentration across the skin. The horizontal lines give the average, and the vertical lines the range, for each value of $N_{II} - N_I$. Thirteen skins (see Table I).

TABLE I

Hydrolysis Rates (R) and Passive Permeability Coefficients (k) for Thirteen Skins

Skin	R	k	k'
	mmoles/cm.²/hr.	*cm./hr.*	*cm./hr.*
11-12	0.0017	0.0004	
11-13	0.0022	0.0003	0.0027
12-3	0.0030	0.0008	
12-21	0.0013	0.0003	0.0024
12-24	0.0012	0.0001	0.0017
12-26	0.0009	0.0002	
12-27	0.0008	0.0001	0.0024
1-3	0.0014	0.0006	
1-4	0.0015	0.0005	
1030-2	0.0012	0.0008	
115-1	0.0025	0.0020	
116-1	0.0028	0.0009	
1016-1	0.0025	0.0001	
Average	0.00177 ± 0.00074	0.00053 ± 0.00053	0.0023

The average values of R and k are of interest. $R_{ave} = 1.8 \times 10^{-3}$, not much less than can be calculated from the assays reported previously (8), which give an average of about 2×10^{-3}. This would indicate that the substrate concentration in the vicinity of the membrane must be relatively high. The value of k is about the same as that reported by Kirschner (6). It is remarkable that a fairly high equilibrium concentration difference can be maintained across a structure so permeable to Na^+ as frog skin seems to be.

DISCUSSION

The linearity predicted by equation (9) and observed in these experiments cannot be taken as a validation of the model for frog skin, as it is not known what other set of assumptions might lead to the same sort of linear relation. It should be noted that this formulation does not take into account the exchange between each compartment and the membrane-bound sodium. Such exchange may lead to outflux rates higher than those calculated from passive permeability alone when $N_{II} > N_I$. Under these circumstances passage of Na from II to I occurs through the mechanism, as well as parallel to it by the passive permeation pathway. Kirschner (6) has derived equations which relate influx and outflux to the outside sodium concentration. His equations explain the experimental observation that outflux increases with increasing outside concentration. If account is taken of an outflux pathway through the charged pores, this effect can be derived from the formulation presented here. On the other hand, if we neglect the term for carrier participation in outflux, Kirschner's equation for net flux, though formally similar to the equation given here, predicts a quite different result. His equation describes the variations in net flux which occur when N_{II} is held constant and N_I is varied from 0 to N_{II}. Neglecting the last term, the equation is

$$M_{net} = \frac{M_{c(max)} N_I}{K_s + N_I} + k(N_I - N_{II})$$

in which M_{net} is the net flux, $M_{c(max)}$ the maximum possible carrier-mediated influx, K_s is the dissociation constant for the carrier-sodium complex, and k, N_{II}, and N_I have their usual significance.

The equivalent form of equation (1 *a*) is

$$\frac{dN}{dt} = \frac{RN_I}{N_{II} + N_I} + k(N_I - N_{II}) \tag{12}$$

The striking difference between these two equations is that K_s is a constant independent of N_{II}. The experimental results given in Fig. 2 of Kirschner's paper seem to be a good fit to his equation. The only difference in procedure from the experiments presented here (other than the fact that N_{II} is held constant) is that Kirschner maintained the total osmotic pressure

in the outside solution constant by making sodium replacements in a choline-Ringer's solution. This procedure is justified by the statement that choline does not penetrate through the skin. We have found that choline does in fact so penetrate, its passive permeability coefficient being about 6×10^{-4} cm./hr. when the concentration difference across the skin varies from 10 to 25 mM. (Chemical measurements; choline chloride + 5 mM KCl on both sides of the skin.)

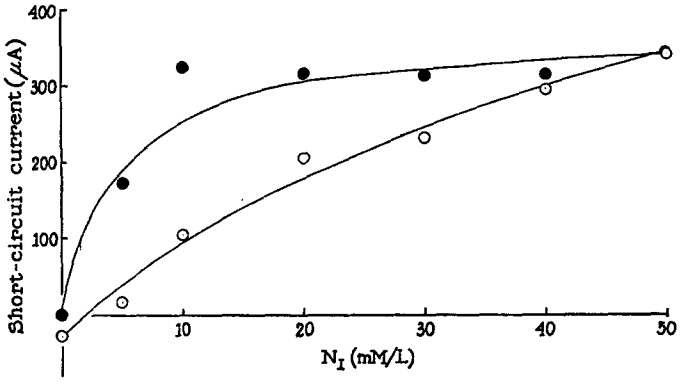

FIG. 3. The effect of choline on sodium transport. The filled circles represent the short-circuit current at varying epidermal sodium concentrations when the total cation concentration is maintained at 50 mM with choline chloride. The open circles represent the short-circuit current at the same concentrations when no choline is present. The upper curve is calculated from the simplified form of Kirschner's Equation 11 given in the text. $M_{c(max)}$ was estimated from the value of the short-circuit current when $N_I = N_{II}$. K_s was taken as 4.3 mM. The lower curve is calculated from Equation 12. R was estimated from the value of the short-circuit current when $N_I = N_{II}$. The passive permeability coefficient, k, was taken as 5.3×10^{-4} cm./hr. in both equations. The dermal solution was 50 mM NaCl, 5 mM KCl and replacements were made so that the epidermal solutions were 5 mM KCl throughout.

To see whether choline was, in fact, responsible for these differences in behavior, the following experiment was performed. An isolated piece of skin was equilibrated with 50 mM NaCl, 5 mM KCl on both sides. By partial replacement with 5 mM KCl, the concentration of sodium in the epidermal solution was reduced to 40, 30, 20, 10, 5, and 0 mM. The epidermal solution was replaced with fresh medium and replacements made through the same epidermal sodium concentrations using 50 mM choline chloride, 5 mM KCl. The results are shown in Fig. 3. The open circles represent the variation in short-circuit current when choline is omitted and the filled circles the variation when choline is used. The upper curve was calculated from the version of Kirschner's equation given above; K_s was taken as 4.3 mM and $M_{c(net)}$ was

calculated by solving the equation when $N_I = N_{II} = 50$ mM. The lower curve was calculated from equation (12), in which $N_{II} = 50$ and R was calculated from the short-circuit current when $N_I = 50$. The value of k used for both curves was 5.3×10^{-4} cm./hr.

In terms of the model, the fact that higher flux values are obtained when choline is present than when it is not is suggestive of competition between choline and a choline ester for storage sites. The stimulation of sodium transport by potassium might also be explained in these terms. Huf *et al.* (5) found that 5.12 mM was the optimum potassium concentration when N_I was 47.9 mM. Under these conditions the net sodium flux was 70 per cent higher than when potassium was lacking from the outside solution. At higher concentrations, net sodium flux fell; at the highest concentration tested, 17.2 mM, it was only 30 per cent higher than the base value. The latter observation throws some light on the ion exchange aspect of the transport process. It is known that synthetic cation exchangers bind K^+ more tightly than Na^+. Boyd *et al.* (2) give ΔF for the reaction

$$A^+ + HX \rightarrow AX + H^+$$

as -530 cal. when A^+ is K^+, and -320 cal. when A^+ is Na^+. (X represents a typical synthetic resin ion exchanger, amberlite IR-1.) This means that the fraction of sites occupied by K^+ increases as the K/Na ratio rises. Under such conditions R is not numerically equivalent to the rate of movement of sodium from the membrane into compartment II.

The question of why potassium is not transported when the K/Na ratio is higher than optimum for sodium transport remains to be answered. It might tentatively be suggested that replacement of K^+ by choline ester is not unidirectional due to the small radius of the hydrated potassium ion. That the pore diameter is somewhat greater than the diameter of the hydrated sodium ion is apparent from the fact that lithium ions are transported by frog skin (11). It may be the case that the pore can accommodate both choline and potassium but not choline and sodium.

Zerahn (14) has reported that the oxygen consumption of isolated skin is increased during sodium transport. This result would not be expected on the basis of the model. It is possible, however, that the mechanism is self-regulating, choline esterification being depressed in the absence of sodium.

Patlak (9) has proposed a "gate type non-carrier" mechanism which resembles, in some respects, the mechanism considered here. In Patlak's model, the point at which energy is made available for the transport process is not specified. Moreover, the passenger species acts as a trigger in the sequence of reactions which lead to its transfer across an energy barrier.

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