TEMPERATURE COEFFICIENTS OF THE SODIUM TRANSPORT SYSTEM OF ISOLATED FROG SKIN*

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Although the sodium transport system of the frog skin has been intensively studied, and found to be dependent upon energy derived from cellular metabolism[1,2], no systematic study of the temperature coefficients of the process has been reported. Effects of varying the temperature on the skin potentials were noted by a number of earlier investigators[3–7]. Both FRANCIS[6] and BARNES[7], who performed the most complete studies, found rather large temperature coefficients for the skin potentials, and BARNES[7] concluded that the potential was metabolically linked. More recently CAPRARO[8] and OIKAWA[9] have studied the influence of temperature on the water permeability of the skin. With the accumulation of knowledge about the sodium transport system of the frog skin, especially with respect to its energetics[10,11], and in view of the model mechanisms recently proposed[12,13], further study of the temperature coefficients seemed desirable.

In this paper are reported the results of an investigation of the effects of temperature on the sodium transport system of the isolated skin of *Rana pipiens*. The results are analyzed in terms of a model mechanism modified from that proposed by LINDERHOLM[12] and extended from that used by KIRSCHNER[13]. The temperature coefficients for the several parameters of the transport system are presented.

METHODS

The method of USSING AND ZERAHN[14] of estimating the net sodium flux across the skin by measuring the short-circuit current, particularly convenient and adaptable to studies of the temperature effects, has been used in this investigation.

The lucite chambers for the solutions bathing the inner and outer surfaces of the skin were jacketed to permit water from a thermo-regulated bath to be circulated for adequate temperature control. Chambers of two sizes were used. In the initial experiments the chambers had an effective skin surface area of 7.9 cm^2 and the volume on each side was 12 ml. Subsequently, to permit simultaneous duplicate experiments, smaller chambers were employed with an effective area of 2.9 cm^2 and a volume of 6 ml per side. In the duplicate experiments the abdominal skin of a single frog was divided into approximately symmetrical halves. The potential-measuring electrodes were saturated KCl, calomel half cells, with 3 M KCl, agar bridges that terminated in juxtaposition to the skin on each side. The skin potentials were measured with a Leeds and Northrup potentiometer to the nearest millivolt or better. The current-carrying electrodes were Ag, AgCl, and the shunting current was measured using either a Triplett 0–100 microammeter or a Minneapolis-Honeywell Brown recording potentiometer shunted with a standard resistance. The absence of significant interfering thermal E.M.F.'s in the potential-measuring circuit was assured by

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replacing the frog skin with a ring gasket and varying the temperature of the chamber solutions.

The sodium Ringer's solution used in these experiments had the following composition:

$NaCl$	115 mM/l
$KHCO_3$	2.5 mM/l
$CaCl_2$	1.0 mM/l
$Cu(NO_3)_2$*	10^{-3} mM/l

Reagent grade chemicals were used. Choline Ringer's solution was identical except that 115 mM/l choline chloride (Choline Chloride, Merck) replaced the NaCl. All solutions were equilibrated with air prior to use, and had a pH of 8.2–8.4. The sodium concentration was varied by making the appropriate mixtures of sodium Ringer's solution and choline Ringer's solution. The concentration in each case is referred to in terms of the percentage of sodium Ringer's solution in the mixture.

The fluid bathing the inside skin surface was invariably 100% sodium Ringer's solution. Whenever the concentration of sodium in the solution bathing the outer skin surface was changed, this chamber was first rinsed twice with the new solution. In general the solutions once placed in the chambers were not aerated, but instead were replaced at 10-minute intervals with fresh, aerated solutions. In those experiments in which the temperature of the solutions was allowed to change gradually over a longer period of time the solutions were aerated within the chambers.

The net sodium flux inward across the skin mounted between the chambers was determined by measuring the current flow with the skin potential difference, $\emptyset_{\text{inside}} - \emptyset_{\text{outside}} = \Delta\emptyset_m$, shunted to zero. The skin conductance, $\Lambda_m = -dI/d(\Delta\emptyset_m)$, was measured by the approximation $I_0/\Delta\emptyset_m^0$, where $\Delta\emptyset_m^0$ is the potential difference across the unshunted skin. This approximation is without serious error, since Λ_m was found to be nearly independent of $\Delta\emptyset_m$ from $\Delta\emptyset_m = 0$ to $\Delta\emptyset_m = \Delta\emptyset_m^0$.

RESULTS

Experiments were performed in which, with sodium Ringer's solution bathing both surfaces of the skin, the temperature of the solutions was allowed repeatedly to increase gradually from an initial low value to about 25° C, or to cool gradually from an initial high value to about 25° C, while the current, I_0, was monitored. Data obtained from a single skin, and typical of these experiments, are presented in Fig. 1.

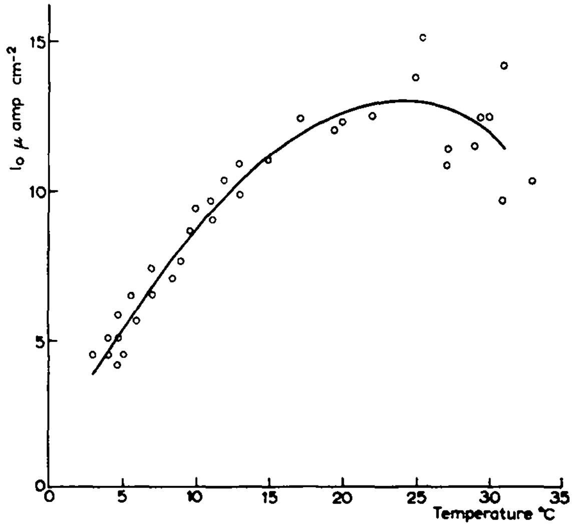

Fig. 1. The net sodium flux, I_0, plotted as a function of temperature. Repeated gradual temperature changes made on a single skin.

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* Cu⁺⁺ has been found to increase the frog skin resistance[15]. The concentration used here is somewhat less than that reported to produce a large effect lasting many hours.

TABLE I

EFFECT OF TEMPERATURE AND SODIUM ION CONCENTRATION ON ACTIVE SODIUM TRANSPORT

Temp. °*C*	% *Sodium Ringer's solution*	*A* $\Delta\varnothing_m$ *mV*	*A* I_0 *μamp/cm²*	*A* Λ_m *mho · 10⁻⁴/cm²*	*B* $\Delta\varnothing_m$ *mV*	*B* I_0 *μamp/cm²*	*B* Λ_m *mho · 10⁻⁴/cm²*
20	100	41	39.3	9.9	48	32.4	6.8
	50	40	36.5	8.9	45	26.5	5.9
	20	35	28.6	8.2	35	18.6	5.3
	10	28	21.0	7.5	25	11.7	4.7
	5	19	12.4	6.5	15	6.5	4.4
	2	9	5.2	5.8	6	2.4	3.7
	1	5	2.8	5.7	2.5	0.7	2.8
10	100	24	19.3	8.0	28	12.4	4.4
	50	21	17.2	8.2	23	10.3	4.5
	20	16	12.4	7.8	18	7.6	4.2
	10	14	9.0	6.4	16	5.5	3.4
	5	9	4.8	5.4	9	2.8	3.1
	2	4	2.1	5.2	3	0.7	2.3
2.5	100	14	8.3	5.9	17	5.5	3.2
	50	13	6.9	5.3	15	4.5	3.0
	20	9	4.8	5.3	11	3.4	3.1
	10	5	2.4	4.8	7	2.1	3.0
	5	2	0.7	3.5	4	0.7	1.8

It is seen that a marked increase in sodium transport took place with increasing temperature in the range of 3–25° C. It is also to be noted that the slope of the transport–temperature curve decreases as the temperature increases; indeed, the slope becomes negative above 25° C. For the range 5–15° C the average increase in sodium flux is 6.9%/degree C, corresponding to a Q_{10} of 2.0. The scatter of data is considerably greater above the temperature of maximum transport (about 25° C) than at lower temperatures.

Other experiments were performed in which the temperature was maintained constant while the sodium concentration of the solution bathing the outer skin surface was varied. Each skin was studied at several temperatures and both the sodium flux, I_0, and the potential, $\Delta\varnothing_m^0$, were measured. The data from a typical

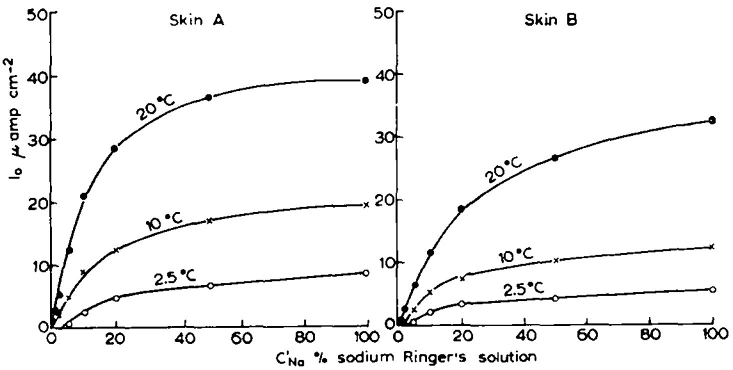

Fig. 2. The net sodium flux, I_0, plotted as a function of the percent sodium Ringer's solution bathing the external surface of the skin, C'_{Na}, at 20° C, 10° C, and 2.5° C.

References p. 320.

experiment of this type are given in Table I. A and B represent halves of the abdominal skin of a single frog. The measurements were made first at the highest temperature (20° C), then at 10° C, and finally at 2.5° C. At each temperature the sodium concentration of the solution bathing the external surface was decreased in steps as tabulated. Time was allowed after each concentration change (about 3–5 min) until reasonably steady behavior was observed. Measurements at each temperature were completed within about an hour. In Fig. 2 the net fluxes, I_0, at the three temperatures are plotted against the sodium concentration of the solution bathing the outer surface.

ANALYSIS OF DATA

It is of interest to analyze the data reported above in terms of a mechanistic model which attempts to describe the transport process with physically interpretable parameters. The model, although patterned after those of LINDERHOLM[12] and KIRSCHNER[13], differs in several respects. Therefore, to insure clarity, especially with respect to the necessary assumptions, the formulation of the model is fully developed here.

The model assumes a simple specific carrier system for the sodium transport, as well as passive diffusion pathways for all constituents, and may be represented as:

$$\begin{array}{r|l} \mathrm{Na}^{+\prime} + \mathrm{X}' \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{NaX}' \rightleftharpoons & \mathrm{NaX}'' \\ \mathrm{A}_i' \rightleftharpoons & \mathrm{A}_i'' \; (i = 1, 2, \ldots) \\ \text{Outside} & \text{Inside} \end{array} \qquad (1)$$

The double vertical line represents the functional barrier of the complex skin. The single prime refers to the outside surface and the double prime to the inside. X represents the carrier, not necessarily identified here as a particular molecular species, but as a composite of the metabolically linked energy-utilizing system specific for the transport of sodium, and existing solely in the membrane phase. NaX represents that state in which the sodium is transported, and k_1 and k_2 represent the specific rate constants for its formation and dissociation. A_i represents the i^{th} constituent which may passively diffuse across the barrier. Per unit area, the net flux, J_i, of the i^{th} species in the x-direction, perpendicular to the plane of the membrane is:

$$J_i = -\omega_i C_i \frac{\partial \bar{\mu}_i}{\partial x} \quad (i = \mathrm{NaX}, 1, 2, \ldots) \qquad (2)$$

where ω_i represents the mobility, C_i the concentration, and $\bar{\mu}_i$ the total chemical potential of A_i or NaX. If activity coefficient gradients are neglected, and if the total chemical potential gradients are assumed to depend only upon the concentration and electrical potential gradients, equation (2) becomes:

$$J_i = -\omega_i RT \frac{\partial C_i}{\partial x} - \omega_i z_i C_i F \frac{\partial \phi}{\partial x} \quad (i = \mathrm{NaX}, 1, 2, \ldots) \qquad (3)$$

where R is the gas constant, T the absolute temperature, F the Faraday, ϕ the electrical potential, and z_i the valence of the i^{th} species, reckoned positive for cations and negative for anions. Equation (3) is integrated in that steady state in which J_i is not a function of x (J_i is non-divergent), with the assumption that $\partial \phi/\partial x$ also is a constant $(\Delta\emptyset/\Delta x)$, independent of x [16]. The boundary conditions are then, $x = 0$, $C_i = C'_i$ and $x = \Delta x$, $C_i = C''_i$. With the additional relation that $I_i = z_i F J_i$, where I_i is the current due to the flux of A_i or NaX, integration leads to:

$$\left(I_i + \omega_i z_i^2 C'_i F^2 \frac{\Delta\emptyset}{\Delta x}\right) e^{-\frac{z_i F \Delta\emptyset}{RT}} = I_i + \omega_i z_i^2 C''_i F^2 \frac{\Delta\emptyset}{\Delta x} \quad (i = \text{NaX}, 1, 2, \ldots) \tag{4}$$

The exponential is expanded as a power series. As an approximation valid for small values of the exponent, all terms with powers greater than unity are dropped. Equation (4) then becomes:

$$I_i = \Omega_i [(C'_i - C''_i) - \xi_i C'_i] \quad (i = \text{NaX}, 1, 2, \ldots) \tag{5}$$

where $\Omega_i = \frac{\omega_i z_i FRT}{\Delta x}$ and $\xi_i = \frac{z_i F \Delta\emptyset}{RT}$. The total current across the skin is:

$$I = \sum_i I_i = \sum_i \Omega_i [(C'_i - C''_i) - \xi_i C'_i] \quad (i = \text{NaX}, 1, 2, \ldots) \tag{6}$$

It is now assumed that $C'_{\text{NaX}} + C'_{\text{X}} = X_t$, where X_t is a constant at a particular temperature. If the reaction governed by k_1 and k_2 is assumed to be in equilibrium, and C''_{NaX} is negligible in comparison to C'_{NaX}, then,

$$C'_{\text{NaX}} = \frac{X_t C'_{\text{Na}}}{C'_{\text{Na}} + K} \quad \left(K = \frac{k_2}{k_1}\right) \tag{7}$$

C'_{Na} is the sodium concentration at $x = 0$, assumed to be that of the bathing solution. Equation (7) is to be identified in form with the classical Michaelis-Menten expression for the concentration of an enzyme–substrate complex[17]. With equation (7), (6) becomes:

$$I = \frac{\Omega_{\text{NaX}} C'_{\text{Na}} X_t}{C'_{\text{Na}} + K} (1 - \xi_{\text{NaX}}) + \sum_{i=1} \Omega_i [(C'_i - C''_i) - \xi_i C'_i] \tag{8}$$

This is the general equation for the current flow across the skin, in terms of this model system. The first term represents that contribution to the current due to the active transport of sodium, expressed as a function of the sodium concentration of the solution bathing the external surface and the electrical potential difference across the skin. The second term represents that contribution to the current from the passive fluxes. An expression for the total conductance across the skin may be obtained by differentiating equation (8) with respect to $\Delta\emptyset_m$.

$$\Lambda_m = -\frac{\mathrm{d}I}{\mathrm{d}(\Delta\emptyset_m)} = \frac{\lambda_{\text{NaX}} C'_{\text{Na}} X_t}{C'_{\text{Na}} + K} + \sum_{i=1} \lambda_i C'_i \tag{9}$$

$$\lambda = \frac{\Omega_i z_i F}{RT} = \frac{\omega_i z_i^2 F^2}{\Delta x} \quad (i = \text{NaX}, 1, 2, \ldots)$$

The first term on the right of equation (9) represents the conductance, Λ_t, of the active transport system, and the second term the conductance Λ_p, due to the passive fluxes. The first term is comparable to that obtained by LINDERHOLM[12], but differs in that his contains an added constant in the numerator and thus is not a hyperbolic function of C'_{Na}.

In applying equation (8) to the experiments reported above, in which the current was measured with $\Delta\emptyset_m = 0$ and in which the only concentration variable was C'_{Na}, the equation reduces to:

$$I_0 = \frac{\Omega_{\mathrm{NaX}} C'_{\mathrm{Na}} X_t}{C'_{\mathrm{Na}} + K} + \Omega_{\mathrm{Na}}(C'_{\mathrm{Na}} - C''_{\mathrm{Na}}) \qquad (10)$$

It is assumed that $\Omega_{\mathrm{choline}} = 0$. Equation (10) is formally identical with that obtained by KIRSCHNER[13] (equation (11), p. 67). The first term on the right is the usual rectangular hyperbola of Michaelis-Menten kinetics, and the second a correction for the back flux of Na^+. As seen in Fig. 2, the smooth curve drawn through the experimental points appears to approach the origin and thus if this model applies, the second term must be very small. This is in agreement with the conclusions of KIRSCHNER[13]. The conventional reciprocal plots of the data, C'_{Na}/I_0 *vs.* C'_{Na}, are seen in Fig. 3. The constant, K, and the product, $\Omega_{\mathrm{NaX}} X_t$, may be evaluated from the slope and intercept of the straight line. The product, $\Omega_{\mathrm{NaX}} X_t$, is the theoretical maximum short-circuit current.

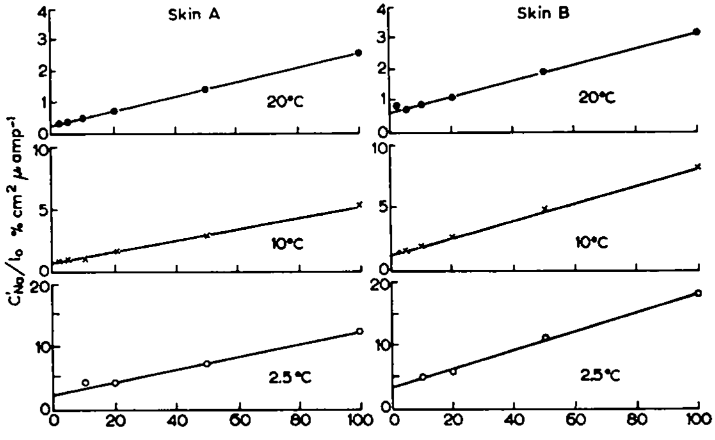

Fig. 3. The percent sodium Ringer's solution divided by the net sodium flux, C'_{Na}/I_0, plotted as a function of the percent sodium Ringer's solution, C'_{Na}, at 20° C, 10° C and 2.5° C. The intercept at $C'_{\mathrm{Na}} = 0$ is $K/\Omega_{\mathrm{NaX}} X_t$ and the slope is $1/\Omega_{\mathrm{NaX}} X_t$. See text for further explanation.

Since the passive flux of Na^+ was seen to be small, Λ_p, the last term of equation (9), may as an approximation be considered a constant. With K determined, equation (9) may be rearranged to give a straight line function.

$$\Lambda_m(C'_{\mathrm{Na}} + K) = (X_t \lambda_{\mathrm{NaX}} + \Lambda_p)C'_{\mathrm{Na}} + \Lambda_p K \qquad (11)$$

Plots of the left hand member, evaluated from the data, against C'_{Na}, are presented in Fig. 4. It is seen that the experimental points are well represented by straight lines which may be taken as further evidence that the passive flux of Na^+ is small in these experiments. From these plots and equation (9), Λ_p and Λ_t may be estimated.

Since the conductances Λ_t and Λ_p are in parallel in the equivalent electric circuit of the skin, it becomes possible to estimate the transport potential, $\varnothing_t$, with the relations:

$$\varnothing_t = \frac{\Lambda_t + \Lambda_p}{\Lambda_t} \cdot \Delta\varnothing_m^0 = \frac{I_0}{\Lambda_t} \qquad (12)$$

The values of the various parameters have been calculated and are collected in Table II. It may be seen that the values of K show considerable variability with no apparent relation to temperature. This variation may be due in part to the errors of the method of estimation, which weights most heavily just those data which are the most difficult to obtain accurately; *i.e.*, the small currents at low concentrations of sodium. The values, however, are in essential agreement with those reported by KIRSCHNER[13], obtained in the species *Rana temporaria*. Also of interest is that the

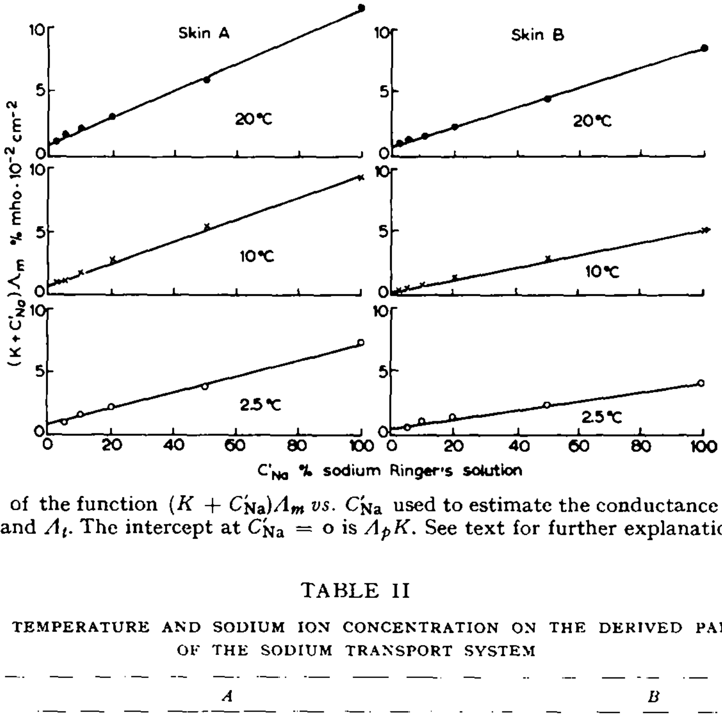

Fig. 4. Plots of the function $(K + C'_{Na})\Lambda_m$ *vs.* C'_{Na} used to estimate the conductance parameters Λ_p and Λ_t. The intercept at $C'_{Na} = 0$ is $\Lambda_p K$. See text for further explanation.

TABLE II

EFFECT OF TEMPERATURE AND SODIUM ION CONCENTRATION ON THE DERIVED PARAMETERS OF THE SODIUM TRANSPORT SYSTEM

		A					*B*				
Temp. °*C*	% *Sodium Ringer's solution*	*K* %	$\Omega_{NaX}X_t$ $\frac{\mu amp}{cm^2}$	Λ_p $\frac{mho \cdot 10^{-4}}{cm^2}$	Λ_t $\frac{mho \cdot 10^{-4}}{cm^2}$	$\varnothing_t$ *mV*	*K* %	$\Omega_{NaX}X_t$ $\frac{\mu amp}{cm^2}$	Λ_p $\frac{mho \cdot 10^{-4}}{cm^2}$	Λ_t $\frac{mho \cdot 10^{-4}}{cm^2}$	$\varnothing_t$ *mV*
20	100	17	52.4	5.3	4.6	88	23	40.0	3.4	3.4	96
	50				3.6	99				2.5	106
	20				2.9	99				1.9	98
	10				2.2	95				1.3	90
	5				1.2	103				1.0	66
	2				0.5	105				0.3	77
	1				0.4	71					
10	100	16	22.4	4.1	3.9	49	14	14.1	2.4	2.0	62
	50				4.1	42				2.1	49
	20				3.7	34				1.8	42
	10				2.3	39				1.0	54
	5				1.3	37				0.7	40
	2				1.1	19					
2.5	100	22	10.0	2.3	3.6	23	24	6.9	1.4	1.8	30
	50				3.0	23				1.6	28
	20				3.0	16				1.7	20
	10				2.5	10				1.6	13
	5				1.2	6				0.4	18

transport potential, $\emptyset_t$, is reasonably independent of the sodium concentration, C'_{Na}, at each temperature. Here again the values at the lower transport rates are subject to greater experimental error. Finally, it is to be noted that the magnitudes of the various parameters differ in the two halves of the same skin. This difference is not unusual in our experience; no serious attempt has been made to evaluate its significance.

The temperature coefficients for each parameter expressed as percent changes per degree centigrade are collected in Table III. These coefficients are the averages for the entire range of temperature studied. That of the overall transport process, as represented by I_0 with $C'_{\mathrm{Na}} = 100\%$, as well as those of $\emptyset_t$ and the calculated maximum for I_0, show rather large values, while the temperature coefficients of the conductance parameters are smaller.

TABLE III

TEMPERATURE COEFFICIENTS OF THE VARIOUS MEASURED QUANTITIES AND PARAMETERS AS PERCENT CHANGE PER DEGREE

Skin	I_0 *(100% Na)*	$\Omega_{NaX}X_t$	*K*	Λ_m	Λ_p	Λ_t	$\emptyset_t$
A	8.9	9.5	—	3.0	4.6	1.5	7.4
B	10.0	10.0	—	4.2	4.7	3.6	6.5

DISCUSSION

The method used in this study for the determination of net sodium flux across the frog skin depends upon the assumption that this flux is equivalent to the short-circuit current. This equivalence has been shown by USSING and his collaborators[14, 18], and confirmed by others[11, 12], under conditions in which the skin was bathed on both sides with sodium Ringer's solution. KIRSCHNER[13] has shown that choline may be substituted for sodium in the solution bathing the outer surface without contributing significantly to the ionic flux.

The data presented in Fig. 1 show that for the sodium transport system of the frog skin there is a temperature of maximum flux. This temperature of about 25° C is similar to that observed by FRANCIS[6] for the maximum current through the frog skin when shunted with a 1500 ohm circuit. BARNES[7], however, reported a continued increase in the frog skin potential with temperatures up to 40° C. The 25° C maximum reported here appears to agree well with the maxima observed in certain phenomena in frog muscle, such as the resting potential[19], tension, and degree of shortening[20]. The scatter of our data at temperatures above 25° C may be indicative of decreased stability at these temperatures and although these effects here appear to be reversible, injurious effects of more elevated temperatures have been described[4].

The simplified mechanistic model with which the experimental data have been analyzed requires little further discussion. No claims are made that the model represents the actual mechanisms even in part, although the data are easily and quantitatively portrayed with a minimum of interpretable parameters. Such a model, aside from new experimental approaches that it may suggest, has the advantage of simplicity. One outcome, moreover, is the fact that the two main conductance components, that associated with the active sodium transport and that associated with the passively diffusing ions, may be easily separated, the total conductance having been measured as a function of the sodium concentration. This, together with the short-circuit current, allows the transport potential to be estimated. The estimation of both the transport potential and the transport conductance, as well as the derived parameter, K, is valuable in assessing the effect of any variable on the transport process. The relative constancy of this transport potential, $\o_t$, with varying C'_{Na} is in agreement with the findings of Linderholm[12], who arrived at this conclusion assuming that the passive conductance of sodium is proportional to that of chloride, and that all other passive conductances are negligible. The magnitude of the transport potential at 20° C agrees fairly well with those values reported by others who have utilized isotopic fluxes to estimate the resistance of the active transport process[2,12,14]. It should be emphasized that the transport potential as represented in equation (12) is purely an equivalent electrical potential. Due to the simplicity of the model, a source or a sink for the energy is not explicitly recognized, and thus an interpretation of the transport potential based on equation (8) has little meaning. With the absence in the model of a specified cyclic return of X to the outer surface, one must assume that z_{NaX} has a positive value, although it is not necessary to specify its magnitude.

With respect to the temperature coefficients reported above, the value of 9–10%/°C for I_0 with 100% sodium Ringer's solution bathing both skin surfaces corresponds to an activation energy of 14–16 kcals/mole. This is sufficiently large to be entirely consistent with metabolically dependent steps. The conductance parameters, A_p and A_t, however, show considerably smaller temperature coefficients than do I_0 and the transport potential, $\o_t$. Nevertheless, the temperature coefficients of these conductances are larger than that exhibited by the conductance of electrolytes in aqueous solution, which in general is about 2%/°C[21]. The dependence of ionic conductance on temperature is to a large extent determined by the solvent viscosity changes with temperature. Therefore, it is not unreasonable to expect that passive flux across a complex structure such as the frog skin would portray greater temperature dependence than would free diffusion, unless this flux were to occur only through large aqueous channels. Other evidence also suggests that such large channels are not involved[22–24].

The amount of energy ($\o_t I_0$) dissipated by the sodium transport process is of interest in that this energy is the minimum used to overcome the resistive forces of the system. Any dissipation of energy in its supply to the transport system must be added to this minimum and would represent a degree of inefficiency. The minimum energy dissipated per mole of sodium transported has been estimated by Zerahn[10], in conjunction with his studies of the oxygen consumption of the frog skin. He used the ratio of sodium influx to outflux to determine $\o_t$ ($= E_{\text{Na}}$), and calculated the energy dissipation to be 1340–2680 calories per mole of sodium transported. Our values of $\o_t$ at 20° C, with $C'_{\text{Na}} = 100\%$, give 2000 and 2200 calories per mole for skins A and B, respectively. Of greater interest perhaps is the fact that at the lower temperatures the energy dissipation decreases, amounting to an average of about 1000 cals per mole of sodium at 10° C and 400 cals per mole at 2.5° C. If one arbitrarily assumes a 50% efficiency of the transport system, *i.e.*, that the energy dissipated in the actual transport represents one half of the total supplied to the system, then at 20° C almost 24 moles of sodium could be transported per mole of oxygen utilized (assuming 100,000 cals per mole of oxygen). This agrees well with ZERAHN's measurement[10] of 16–20 moles of sodium transport per mole of net oxygen consumption, and also with the 18 moles of sodium per mole of oxygen found by LEAF AND RENSHAW[11] to be the ratio of increased sodium transport to increased oxygen consumption in pituitrin stimulated skins. Again using our assumptions with respect to efficiency and energy equivalence of oxygen, our data indicate that at 10° C 50 moles of sodium, and at 2.5° C 125 moles of sodium may be transported per mole of oxygen.

SUMMARY

A study has been made of the temperature dependence of the active sodium transport system of the isolated abdominal skin of *Rana pipiens*. The method of measuring the current across the short-circuited skin has been used to determine the net sodium flux. In addition, potential measurements were made on the unshunted skin. Typical experiments illustrative of the observed temperature effects are presented.

A skin, bathed on both sides by sodium Ringer's solution, was subjected to repeated gradual changes in temperature from 0–30° C while the current was monitored. The current was found to have a temperature coefficient of 6.9%/°C in the range 5–15° C, corresponding to a Q_{10} of 2.0, but above 25° C the coefficient was negative and the transport system appeared relatively unstable.

In other experiments the sodium concentration of the solution bathing the external skin surface was varied (substituting choline) from 0–100% sodium Ringer's solution, while the temperature was maintained constant at 20°, 10° and 2.5° C. The net sodium flux with 100% sodium Ringer's solution showed temperature coefficient of 9–10%/°C. Analyzing the data in terms of a model carrier system, the stoichiometric dissociation constant of the carrier–sodium complex, the passive conductance, the active transport conductance, and the transport potential were computed. The dissociation constant shows no consistent temperature dependence. The two conductances show positive temperature coefficients of 4–5%/°C and 2–4%/°C respectively. The transport potential was found to increase with temperature by 7%/°C.

The energy dissipation of the transport system is discussed with reference to reported oxygen consumption measurements of the frog skin. Our data suggest that the transport process becomes increasingly efficient as the temperature is lowered.

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