EFFECT OF TEMPERATURE ON ELECTROLYTE METABOLISM OF ISOLATED FROG SKIN*

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ABSTRACT

A study is presented on the effect of temperature on unidirectional active ion transport, resting electrolyte equilibrium (electrolyte composition), and oxygen consumption in isolated frog skin. The aims were twofold: first, to find out whether the rate of active transport can be changed without affecting the Na^+ and K^+ balance of skin itself; second, to arrive at minimal $\Delta Na/\Delta O_2$ values by correlating quantitatively inhibition of active ion transport with inhibition of O_2 consumption. NaCl transport was maximal at 20°C. At 28° and at temperatures below 20°, rate of NaCl transport was diminished. In many instances NaCl transport was diminished in skins which maintained their normal Na^+ and K^+ content. In several cases, however, neither rate of transport nor resting electrolyte equilibrium was affected; in other cases, both were.

O_2 consumption decreased when lowering the temperature over the range from 28 to 10°C. From a plot of log Q_{O_2} against $1/T$ an activation energy of $\mu = 13,700$ cal. was calculated, valid for the range from 10 to 20°C. It appeared that μ was smaller for temperatures above 20°C. Working between 10 and 20°, it was found that, on the average, 4 to 5 equivalents of Na^+ were transported for one mole of O_2 consumed in skins with undisturbed resting electrolyte equilibrium.

I

INTRODUCTION

In the mechanism of unidirectional transport of sodium chloride across surviving frog skin, potassium ions play a key role. Potassium-deficient skins are quite inactive (1-6). It has been suggested (7) that the reason for this is that potassium ions must be present for participation in a $K^+ \rightleftarrows Na^+$ ion exchange adsorption reaction which seems to be an essential step in the mechanism of active Na^+ transport across the skin. Irrespective of interpretations, however, it is clear from previous observations (7) that when factors influencing active Na^+ transport are under investigation a chemical analysis of the skin membrane for its sodium and potassium content is desirable. It was

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found (7) that fluoroacetate rather specifically inhibited active Na^+ transport, but had no effect on Na^+ and K^+ content of the skin. Other chemicals, e.g 1×10^{-4} M/liter DNP, also inhibited active Na^+ transport, but this was accompanied by loss of K^+ from and gain of Na^+ by the skin.

In the following, a study is presented on the effect of temperature on maintenance of sodium and potassium in skin, on rate of active NaCl transport across the skin, and on oxygen consumption of skin. Emphasis is placed upon correlations among these metabolic activities.

II

Methods

The studies were carried out during the months of July through December, and the various types of experiments were repeated several times during this period on a rotating schedule. All work was done with skins of *Rana pipiens*. General procedures used here were the same as those described in a previous publication (7). Rates for net NaCl transport across the skin were obtained from estimations of net Cl^- transport during 8 hour experiments, using the *paired bag method*. The salt solutions had the following composition: NaCl, 116 μM/ml.; KCl, 10 μM/ml.; $NaHCO_3$, 2 μM/ml.; pH = 7.4 to 7.5. The solutions were oxygenated before the pH adjustment. Na^+, K^+, Cl^- estimations on solutions and Na^+, K^+, H_2O estimations on skins were done exactly as described previously (7). Na^+ and K^+ in skins are given as microequivalents per gram of dry skin. *Control bags* were kept at 20°C. and *experimental bags* at the desired *experimental temperatures*. These were 28, 16, 13, and 10°C. Temperatures were kept constant within ±0.03°C. All solutions were adjusted to the chosen temperatures before starting the experiments.

The *Warburg technique* as applied to obtaining Q_{O_2}'s of skins was also fully described in the paper already cited (7). Pieces of skins of the legs were used in these measurements. During a period of 2¼ hours at 20°C., three readings were taken. Then, with the manometers kept open towards the atmosphere, the temperature of the bath was changed to and maintained at the experimental temperature. This change-over was accomplished in 10 to 15 minutes. Twenty minutes after the new temperature was reached the manometers were closed again, and three readings were taken at 45 minute intervals. From pooled data regression lines were calculated. The Q_{O_2} values (μl. $\times$ hr.$^{-1}$ $\times$ mg.$^{-1}$ of dry skin) shown in Fig. 1 and Table II are the values for the calculated regression coefficients.

III

RESULTS

1. Sodium and Potassium in Skin. NaCl Transport.—In all studies 20°C. was arbitrarily chosen as the reference temperature. Five different series of experiments were carried out.

In one series (No. 2, Table I) the "left" as well as the "right" bags, forming pairs, were exposed to 20°C. This was done to test the reliability of the paired bag method, which was very good, as can be seen from the data in Table I.

In the four other series (Nos. 1, 3, 4, 5, Table I) the experimental skins were exposed to temperatures above or below 20°C., and the respective control skins were kept at 20°C. under otherwise identical conditions. With the exception of series 3, the data are presented in subseries A, B, and C. Lowering the temperature from 20 to 16°C. (series 3) had no significant effect on net rate of transport and electrolytes in skin. In the other series, in which the skin was subjected to greater changes in temperature, there were also several instances (1 A, 5 A) with no change in rate of transport and skin electrolytes, with the exception of 4 A, in which a small but significant loss of K^+ from the

TABLE I

Effect of Temperature on Ionic Composition and Net Salt Transport

Data shown are average values.

Series No.	Temperature. Control and (experiment)	No. of experiment	Composition of skin				Net rate of transport	
			H₂O	[Na⁺]	[K⁺]	[Na⁺]/[K⁺]	H₂O	NaCl
	C.°		per cent	μeq. × gm.⁻¹ dry wt.			μM × cm.⁻² × hr.⁻¹	μM × cm.⁻² × hr.⁻¹
1 A	20 (28)	4	73.3 (73.4)	324 (331)	127 (130)	2.55 (2.54)	74 (136‡)	0.42 (0.46)
1 B	20 (28)	9	73.3 (73.6)	306 (338*)	178 (174‡)	1.72 (1.94*)	180 (128‡)	0.70 (0.41*)
1 C	20 (28)	13	74.2 (74.1)	319 (328‡)	177 (175‡)	1.80 (1.87‡)	185 (148*)	0.79 (0.51*)
2	20 (20)	10	76.0 (75.7)	358 (363‡)	189 (187‡)	1.90 (1.92‡)	119 (117‡)	0.47 (0.48‡)
3	20 (16)	8	75.0 (74.4)	316 (309)	172 (167)	1.84 (1.85)	223 (218)	0.93 (0.93)
4 A	20 (13)	5	74.5 (74.5)	303 (312‡)	163 (153*)	1.86 (2.04*)	186 (125*)	0.72 (0.70‡)
4 B	20 (13)	3	71.8 (71.7)	316 (311)	134 (118)	2.36 (2.64)	88 (35)	0.42 (0.26)
4 C	20 (13)	3	73.0 (72.2)	332 (316)	128 (118)	2.59 (2.68)	127 (51)	0.58 (0.37)
5 A	20 (10)	6	74.5 (74.4)	308 (309)	182 (180)	1.69 (1.72‡)	174 (138‡)	0.74 (0.71‡)
5 B	20 (10)	10	73.6 (73.2)	312 (308‡)	171 (150*)	1.83 (2.05*)	167 (78*)	0.73 (0.45*)
5 C	20 (10)	10	72.8 (72.3)	314 (310‡)	158 (151‡)	1.99 (2.05‡)	155 (44*)	0.67 (0.36*)

* $P < 0.005$.

‡ Not statistically significant.

skin occurred. Net water transport was also somewhat diminished in 4 A. It is fairly certain that the negative findings were not the result of experimental errors. This must be stressed, because in most instances net rate of NaCl transport was significantly diminished when the temperature was changed. Comparing the data of the B and the C series it will be noted that diminished net rate of NaCl transport was found associated with (see B series) and unassociated with (see C series) changes in skin electrolytes. It is these latter cases that are of particular interest.

2. Oxygen Consumption.—Rates of oxygen uptake (Q_{O_2}'s) at various temperatures are shown in Fig. 1. The lines in this illustration are calculated regression lines, and refer to "average" pieces of skin. Indicated below the graph are the number of pieces of skin used in the various series. Solid lines give rates of oxygen uptake during the first 2¼ hour period of respiration studies at 20°C.; broken lines refer to rates of oxygen consumption during the second

$2\frac{1}{4}$ hour period at a different temperature. In order to show the effect of temperature on oxygen uptake more clearly, data of the second period are plotted from the same point of origin. In each series, however, the pieces of skin studied during the two periods were the same.

As can be seen from the graph, rates of oxygen uptake were fairly constant for the periods studied. In the control series (20–20°C.) rate of oxygen uptake

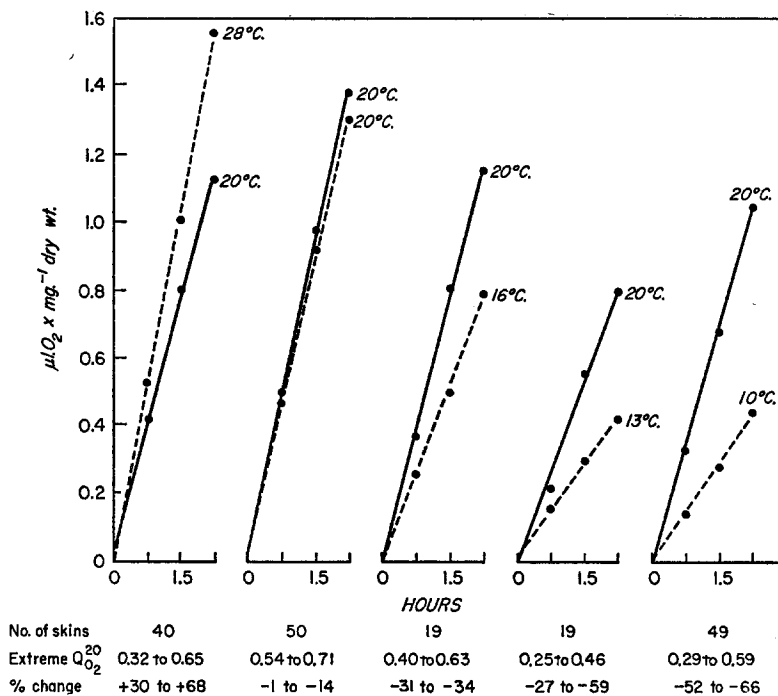| No. of skins	40	50	19	19	49
Extreme $Q_{O_2}^{20}$	0.32 to 0.65	0.54 to 0.71	0.40 to 0.63	0.25 to 0.46	0.29 to 0.59
% change	+30 to +68	–1 to –14	–31 to –34	–27 to –59	–52 to –66

FIG. 1. Effect of temperature on oxygen consumption of skin.

during the second period was slightly lower than the rate of uptake during the first period. As expected lower Q_{O_2} values were found when working at lower temperatures. Fig. 1 shows the decrease of Q_{O_2} for the temperature range from 28 to 10°C.

IV

DISCUSSION

There are a number of reports on the effect of temperature on skin potential (8–14) and O_2 consumption (10, 11). Motokawa (12) made an interesting analysis of his data, and calculated the heat of reaction from the Gibbs-Helmholtz equation. He gives a value of at least 6,600 cal. for 1 Faraday of

electricity moved. Recently Snell and Leeman (14) investigated the effect of temperature on the short-circuit current that can be drawn from skin and which can be regarded as an equivalent of active Na^+ transport. These authors calculated the activation energy for active Na^+ transport and gave it a value of 14,000 to 16,000 cal./mol of sodium transported. None of these studies has taken the ionic balance of the skin into consideration or inquired as to how it may be affected by changes in temperature. It is important to have information on this point. As has been mentioned in the introduction to this paper, certain chemicals inhibit *"unidirectional active ion transport"* without
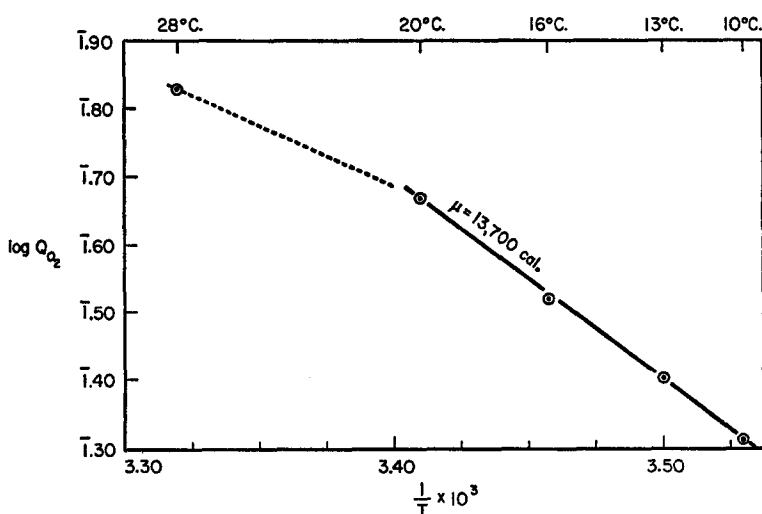

FIG. 2. Arrhenius plot of log Q_{O_2} against $1/T$.

affecting the *"resting electrolyte equilibrium"* of skin, whereas others act on both mechanisms, especially if given in relatively high concentrations (7). Because K^+-deficient skins may show reduced unidirectional active ion transport (without diminished O_2 consumption) (7) it becomes interesting to find out whether temperature can affect unidirectional ion transport without altering the resting electrolyte equilibrium. The data presented in Table I show that this is the case. Unidirectional ion transport is more easily influenced by changes in temperature than the resting electrolyte equilibrium, although both may be affected at the same time. It is planned to continue these studies with the aim of finding under which circumstances unidirectional active ion transport is (or is not) accompanied by a change in resting electrolyte equilibrium. Seasonal factors seem to be involved.

In support of the results obtained by Snell and Leeman (14) our data show that the optimal temperature for active ion transport in frog skin is not far

from 20°C. When the temperature was raised to 28° or lowered to 13°, the rate for active ion transport was significantly diminished.

From the five sets of data shown in Fig. 1, it can be seen that the rates of oxygen consumption at 20° (solid lines) varied during the 6 months of investigations from 0.25 to 0.71 μl. $\times$ hr.$^{-1}$ $\times$ mg.$^{-1}$ dry skin. The average value is 0.49. The assumption is now made that within this range of observations relative changes in oxygen consumption as a result of temperature changes are not related to the absolute value for Q_{O_2}. All experimental Q_{O_2} values for higher or lower temperatures were then expressed in proportion to the same average $Q_{O_2}^{20°}$, namely 0.49. When these new figures were used for an Arrhenius plot, the graph shown in Fig. 2 was obtained. Over the temperature

TABLE II

Minimal $\Delta Na/\Delta O_2$ Ratios

Series (see Table I)	Temperature. Control and (experiment)	[Na+]/[K+] for skin	Net Cl- (Na+) transport		Q_{O_2}		$\dfrac{\Delta Na}{\Delta O_2}$	
			At 20°	At 13°; 10° respectively	At 20°	At 13°; 10° respectively	Average	Range of values
	C.°		$\mu eq. \times hr.^{-1} \times mg.^{-1}$	$\mu eq. \times hr.^{-1} \times mg.^{-1}$	$\mu M \times hr.^{-1} \times mg.^{-1}$ dry skin			
4 B	20 (13)	Slight increase	63×10^{-3}	38×10^{-3}	15.3×10^{-3}	8.3×10^{-3}	3.6	
4 C	20 (13)	No change	82×10^{-3}	48×10^{-3}	15.3×10^{-3}	8.3×10^{-3}	4.9	
5 B	20 (10)	Slight increase	102×10^{-3}	62×10^{-3}	19.5×10^{-3}	8.6×10^{-3}	3.7	1.8 to 6.2
5 C	20 (10)	No change	100×10^{-3}	54×10^{-3}	19.5×10^{-3}	8.6×10^{-3}	4.2	1.0 to 9.2

range from 10 to 20°C. the activation energy for oxygen consumption is $\mu = 13,700$ cal. The graph suggests that for the range from 20 to 28°C. μ is smaller. This may be related to the decrease in rate of active NaCl transport at 28° as compared to the rate at 20°.

Some of the data already presented in Table I and Fig. 1 are shown again in Table II with $\Delta Na/\Delta O_2$ ratios on which more recently several investigators have focused attention (7, 14–17). $Q_{O_2}^{13°}$ and $Q_{O_2}^{10°}$ were corrected for the small decrease in O_2 consumption which was noticed when skins were kept at 20°C. over a period of several hours (see Fig. 1). It can be seen from Table II that with no change in resting electrolyte equilibrium, average $\Delta Na/\Delta O_2$ ratios of 4.9 and 4.2 were obtained by calculation. The latter value is based on ten, the former on only three experiments. It must be emphasized that although transport studies and Q_{O_2} measurements were carried out during the same seasons, they were performed on skins of different animals, and in the calculations average values were used. Lowest and highest ratios (last column of Table II) were obtained from lowest and highest ΔNa and average Q_{O_2} values. The average $\Delta Na/\Delta O_2$ values given under 4 B and 5 B, where the skins had lost some K+ and gained some Na+ (see Table I), are smaller than the values under 4 C and 5 C, but the differences are not significant.

A similar approach to $\Delta Na/\Delta O_2$ ratios was tried in previous studies in which decrease of NaCl transport and decrease in O_2 consumption in fluoroacetate-poisoned skins were related. It is interesting to note that in this situation average $\Delta Na/\Delta O_2$ ratios of 3.2 to 8.7 were found, varying with inhibitor concentration. In any case, such values must be considered as minimal because it is unlikely that, when working with whole skin, application of fluoroacetate or lowering of temperature affects only oxidative processes directly related to active ion transport.

Another analogy between effect of temperature and metabolic poison may be seen in the observation that either elevating the temperature from 20 to 28°C. or poisoning the skin with 2,4-DNP (7) increased O_2 consumption, but decreased active NaCl transport. At present neither of these effects is understood. Since 2,4-DNP is known to uncouple oxidative phosphorylation, it would seem that elevating the temperature likewise inhibits this metabolic process and thereby diminishes the efficiency of active Na^+ transport. The inverse relation between temperature and efficiency of the Na^+ transport process has also been pointed out by Snell and Leeman (14).

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