

Energetics of Sodium Transport in Frog Skin

II. *The effects of electrical potential on oxygen consumption*

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ABSTRACT Studies were made of the dependence of the rate of oxygen consumption, J_r , on the electrical potential difference, $\Delta\psi$, across the frog skin. After the abolition of sodium transport by ouabain the basal oxygen consumption was independent of $\Delta\psi$. In fresh skins J_r was a linear function of $\Delta\psi$ over a range of at least ± 70 mv. Treatment with aldosterone stimulated the short-circuit current, I_o , and the associated rate of oxygen consumption, J_{ro} , and increased their stability; linearity was then demonstrable over a range of ± 160 mv. Brief perturbations of $\Delta\psi$ (± 30 – 200 mv) did not alter subsequent values of I_o . Perturbations for 10 min or more produced a "memory" effect both with and without aldosterone: accelerating sodium transport by negative clamping lowered the subsequent value of I_o ; positive clamping induced the opposite effect. Changes in J_{ro} were more readily detectable in the presence of aldosterone; these were in the same direction as the changes in I_o . The linearity of J_r in $\Delta\psi$ indicates the validity of analysis in terms of linear nonequilibrium thermodynamics—brief perturbations of $\Delta\psi$ appear to produce no significant effect on either the phenomenological coefficients or the free energy of the metabolic driving reaction. Hence it is possible to evaluate this free energy.

INTRODUCTION

Many people have considered the energetics of ion transport in epithelia (e.g. Ussing and Zerahn, 1951; Zerahn, 1956, 1958; Ussing, 1960; Heinz and Patlak, 1960; Patlak, 1961; Martin and Diamond, 1966; Civan et al., 1966). In general these studies have followed those of Ussing, who treated the system in terms of its electrical analogue. This led him to introduce the concept of "the electromotive force of the active sodium transport," E_{Na} .

Two methods are used for the evaluation of E_{Na} : (a) determination of the electrochemical potential difference of sodium required to reduce its flux to zero, and (b) measurement of the flux ratio at short-circuit. As was stressed by Ussing and others, the E_{Na} determined experimentally should be regarded only as an effective potential, reflecting the mode of evaluation. In particular, leak must decrease its magnitude. Furthermore, the common use of the flux ratio to evaluate E_{Na} requires the questionable assumption that the movements of abundant and tracer species of sodium ions in the active transport pathway are independent (Ussing, 1960). In any event equivalence of the values of E_{Na} obtained by the above two techniques would require that the rate of metabolism be independent of the electrical potential difference across the membrane (Kedem and Essig, 1965; Blumenthal and Kedem, 1969).

In view of the above considerations several authors have attempted a more comprehensive thermodynamic treatment. Since the systems are not at equilibrium, such a treatment must necessarily be based on the use of non-equilibrium thermodynamics (for example, Kedem, 1961; Hoshiko and Lindley, 1967; Essig and Caplan, 1968). In these studies linear relations were assumed between the rates (transport and metabolism) and the forces (electrochemical potential difference of sodium and free energy of the metabolic driving reaction). Such linearity, if it existed, would greatly simplify thermodynamic analysis and would also be of fundamental theoretical significance (Prigogine, 1961). In the present paper we examine the question of whether linearity does in fact obtain between oxygen consumption and electrical potential difference over a significant range.

GLOSSARY

A	affinity of metabolic reaction	J_{r_0}	rate of oxygen consumption in the short-circuited state ($\Delta\psi = 0$) per unit area
E_{Na}	electromotive force of sodium transport	L_{Na}, L_{Na^*}, L_r	phenomenological conductance coefficients
F	Faraday constant	X_{Na}	negative electrochemical potential difference of sodium
I	electrical current density	$\Delta\psi$	electrical potential difference: potential in the inner solution minus that in the outer solution. (Inner and outer refer to the intact animal.)
I_0	electrical current density in the short-circuited state ($\Delta\psi = 0$)		
J_{Na}	rate of sodium transport per unit area		
J_r	rate of oxygen consumption at any given value of $\Delta\psi$ per unit area		

METHODS

Most of the methods used in this study were as described in detail in Vieira et al. (1972). In summary, sodium transport and oxygen consumption were measured in

skins (*Rana pipiens*) mounted in standard Lucite chambers. Sodium transport was measured by the short-circuit technique of Ussing and Zerahn (1951). Oxygen consumption was measured by a polarographic method using two oxygen electrodes. A voltage clamp permitted setting the potential difference across the skin at values ranging from 200 to -200 mv, with a compensating circuit providing automatic correction for the potential drop between the voltage-sensing agar-bridge tips and the membrane. Fresh standard glucose-Ringer's solution (110.0 mM NaCl, 2.5 mM KHCO_3 , 1.0 mM CaCl_2 , and 10.0 mM glucose; pH 8.2 and osmolarity 220 mosmol/liter) was used. Streptomycin sulfate was added in a concentration of 0.1 mg/ml to prevent bacterial growth. In the standard protocol oxygen consumption was studied 1.5 hr after mounting the skin. In the aldosterone-aged preparations, *d*-aldosterone-21-acetate was added to the inner solution in 10 μl of methanol to give a final concentration of 10^{-6} M. The system was then aerated for 18 hr. At this time the solutions were replaced by fresh glucose-Ringer's solution and aldosterone was added to the inner solution as above. After 1–1.5 hr the air bubbler was detached and the oxygen electrode-micropump system was attached as shown in Fig. 1 of Vieira et al. (1972). Thereafter, in order to maintain oxygen tension at near physiological levels, both solutions were replaced at approximately 25-min intervals by fresh, aerated glucose-Ringer's solution containing 10^{-8} M aldosterone. Streptomycin sulfate was obtained from Pfizer Labs., Div. Chas. Pfizer & Co., Inc., New York; ouabain was from Sigma Chemical Co., St. Louis, Mo.; and *d*-aldosterone-21-acetate was kindly provided by Dr. Maurice Pechet.

Results are presented as the mean value \pm the standard error (SE) if not otherwise indicated. Straight lines were fitted by the method of least squares.

RESULTS

1. *Effect of the Electrical Potential Difference on the Rate of Oxygen Consumption*

Two different kinds of preparation were used. (a) Fresh skins were studied after 1–1.5 hr of incubation in glucose-Ringer's solution. (b) Aldosterone-aged skins were studied after 18 hr of incubation in glucose-Ringer's solution containing aldosterone.

(A) FRESH SKINS Studies were made of the dependence of the rate of oxygen consumption J_r on the electrical potential difference $\Delta\psi$ imposed across the skin. The magnitude and sequence of the perturbations of $\Delta\psi$ were varied in different experiments. As shown in Fig. 1, a change in $\Delta\psi$ resulted promptly in a change in the rate of oxygen consumption, normally within less than 30 sec. Usually a new steady-state value was reached within less than 2 min. Thus, to ensure stationarity, J_r was evaluated 4–6 min after change of $\Delta\psi$.

Typical relationships between J_r and $\Delta\psi$ are shown in Fig. 2, where each plot represents data obtained within a single 25 min interval. (During this time the skin was stable, as shown by the fact that repeated determinations at a given value of $\Delta\psi$ resulted in closely similar values of J_r .) Positive per-

turbations of $\Delta\psi$, which decreased the rate of sodium transport, reduced the rate of oxygen consumption and vice versa. Characteristically the relationship between J_r and $\Delta\psi$ was linear over a range of at least ± 70 mv, occasionally ± 100 mv.

The addition of ouabain to the inner solution (10^{-3} M) regularly abolished sodium transport almost completely within 30–45 min, and reduced the rate of oxygen consumption to the basal level (Vieira et al., 1972). In these circumstances J_r was no longer dependent on $\Delta\psi$, as can also be seen in Fig. 2. This demonstrates that the influence of the electrical potential differ-

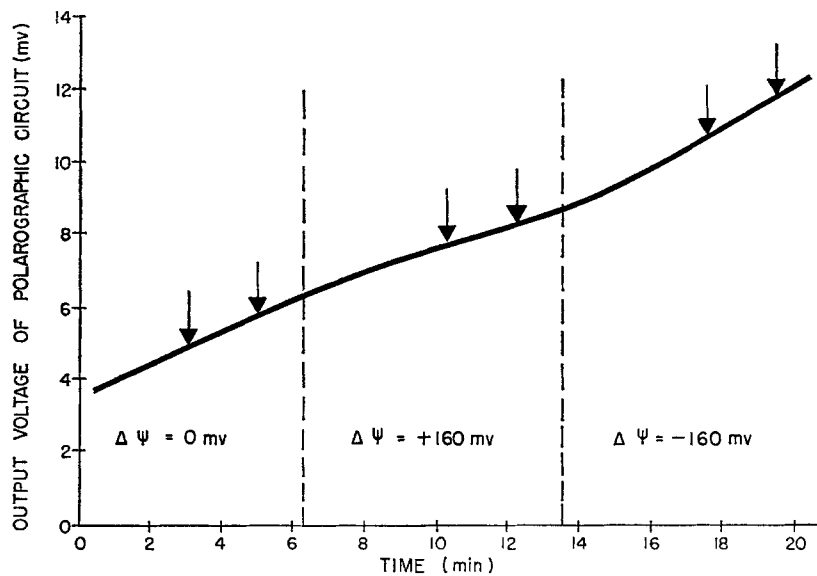


FIGURE 1. Change in the rate of oxygen consumption with perturbation of the electrical potential difference $\Delta\psi$. The rate of oxygen consumption is calculated from the slopes of the curve as described in Vieira et al. (1972). The dashed lines indicate times of alteration of $\Delta\psi$. The calculations were based upon the steady-state slopes during the periods indicated by the arrows.

ence on the rate of oxygen consumption is mediated through an effect on the active transport process.

It is of course desirable to examine the relationship between J_r and $\Delta\psi$ in greater detail over a large range. To do so several determinations of J_r must be made, occasionally requiring as long as 4 hr. During these measurements appreciable spontaneous decline of sodium transport and metabolism may occur. Since such instability interferes with the determination of the relationship between J_r and $\Delta\psi$ it is useful to monitor I_o and J_{r_o} ; in a stable preparation both would be nearly constant for long periods. However, in 44 untreated skins studied over an extended period only one was stable, and this at a low level of sodium transport.

(B) ALDOSTERONE-AGED SKINS In order to obtain more comprehensive data a more stable preparation is desirable. We had observed that after several hours of incubation in glucose-Ringer's solution I_o was quite stable; however, its magnitude was small. Since it has been noted that aldosterone increases the rate of sodium transport in epithelia (Crabbé, 1961; Sharp and Leaf, 1964; Porter and Edelman, 1964; Nielsen, 1969; Voûte et al., 1969),

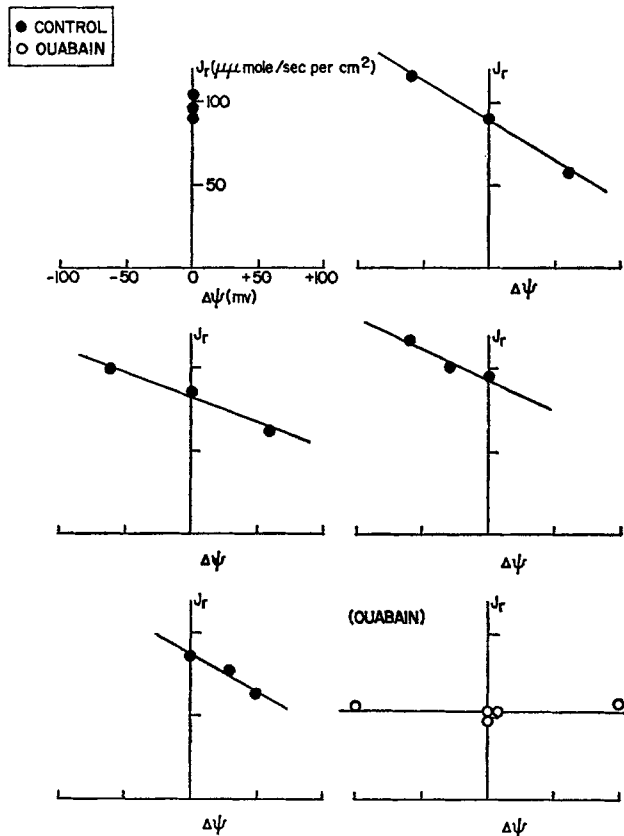


FIGURE 2. Dependence of the rate of oxygen consumption J_r on the electrical potential difference $\Delta\psi$ during several 25-min periods of study of a single skin. The plot in open circles represents data obtained following the elimination of active sodium transport with ouabain.

we used it for this purpose. Prolonged exposure to 10^{-6} M aldosterone in glucose-Ringer's solution resulted in stability, with a significantly larger value of I_o than in an untreated skin from the same animal. Furthermore, J_{r_o} was comparable in magnitude to the rate of oxygen consumption in freshly mounted skins and was also relatively stable. Although the rates of sodium transport and oxygen consumption decreased with time, this effect was much smaller than in the untreated skins. 11 aldosterone-treated skins were studied extensively; of these only one was unstable.

With the use of the more stable preparation, linearity was demonstrable even in long term experiments over a large range of $\Delta\psi$. Figs. 3 and 4, top, show the results of two such experiments. As is seen, linearity was observed over a range of ± 160 mv. The corresponding plots of I_o and J_{r_o} against time in Figs. 3 and 4, bottom, indicate the stability of these preparations.

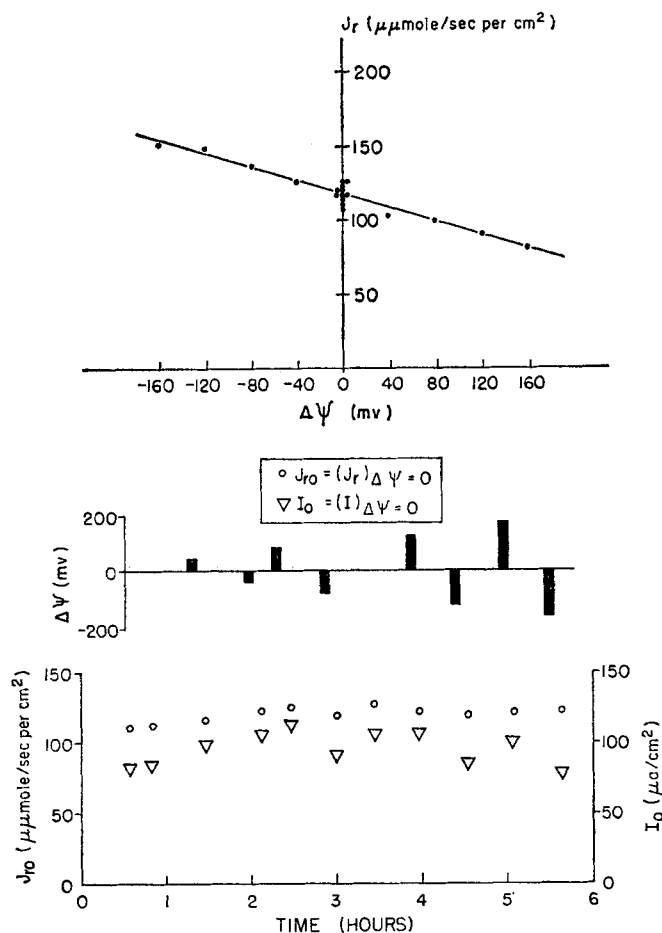


FIGURE 3. *Top*: dependence of the rate of oxygen consumption on the electrical potential difference $\Delta\psi$ in the presence of aldosterone. *Bottom*: plots of I_o and J_{r_o} versus time. These indicate the long-term stability of the preparation. The sequence of perturbations of $\Delta\psi$ was chosen so as to avoid "memory" effects (see Results, section 2).

In these studies we avoided several positive or negative periods in succession, as, for example, 0, -40, -80, -120, -160, -200, followed by the positive values, in order to prevent systematic effects of polarity, as will be discussed in Results, section 2. Providing that this precaution is taken, the sequence of electrical perturbations had no effect on the demonstration of linearity. In the first skin (Fig. 3, top) the sequence was 0, 40, 0, -40, 0, . . . 0, -160 mv;

in the second (Fig. 4, top) it was 0, 40, -40, 0, . . . 0, 200, -200 mv, this sequence being followed twice in succession.

2. The "Memory" Effect

Brief perturbations of $\Delta\psi$ (± 30 -200 mv for a few seconds) did not alter the subsequent values of I_o and $J_{r,o}$. Longer perturbations (here, for 10 min or

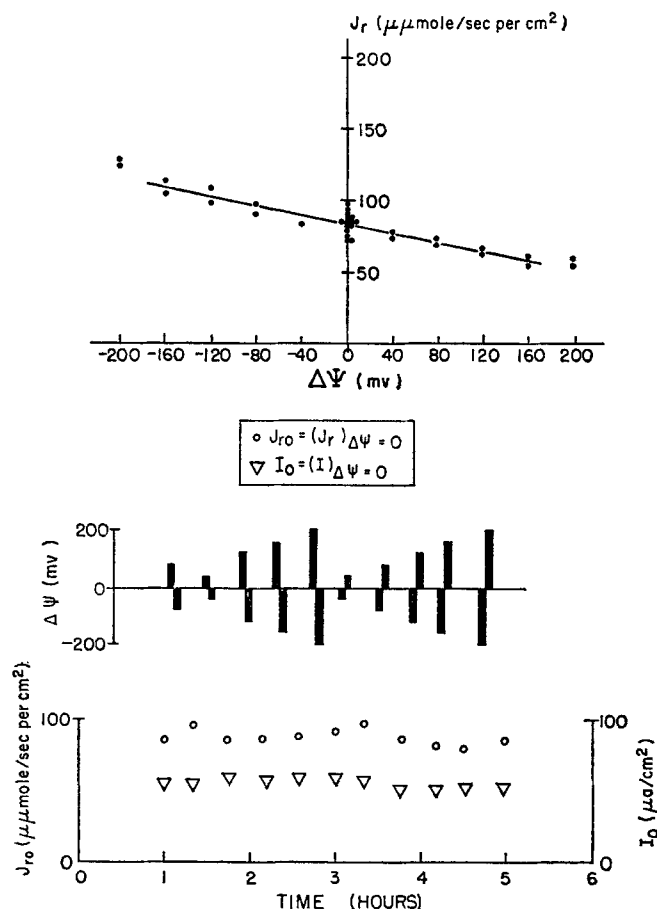


FIGURE 4. *Top:* dependence of the rate of oxygen consumption on the electrical potential difference $\Delta\psi$ in the presence of aldosterone. *Bottom:* plots of I_o and $J_{r,o}$ versus time (see legend, Fig. 3, bottom).

more) produced a "memory" effect: positive clamping, which slowed active sodium transport, transiently increased the subsequent values of I_o and $J_{r,o}$; negative clamping induced the opposite effect. The effect was more pronounced with higher magnitudes of $\Delta\psi$. The phenomenon was observed in both fresh and aldosterone-aged skins, but because the effects were small they were more readily demonstrated in stable preparations. Normally the

memory effect is more clearly observed in I_o than in J_{r_o} owing to the greater precision in measuring I_o . Fig. 5 shows the memory effect on I_o in three different skins treated with aldosterone. When large perturbations of $\Delta\psi$ were employed (± 160 mv for some 15–20 min), effects on both I_o and J_{r_o} were observed. Fig. 6 shows the changes in I_o and J_{r_o} between successive short-circuited states; each value of I_o and J_{r_o} was determined 4–6 min after

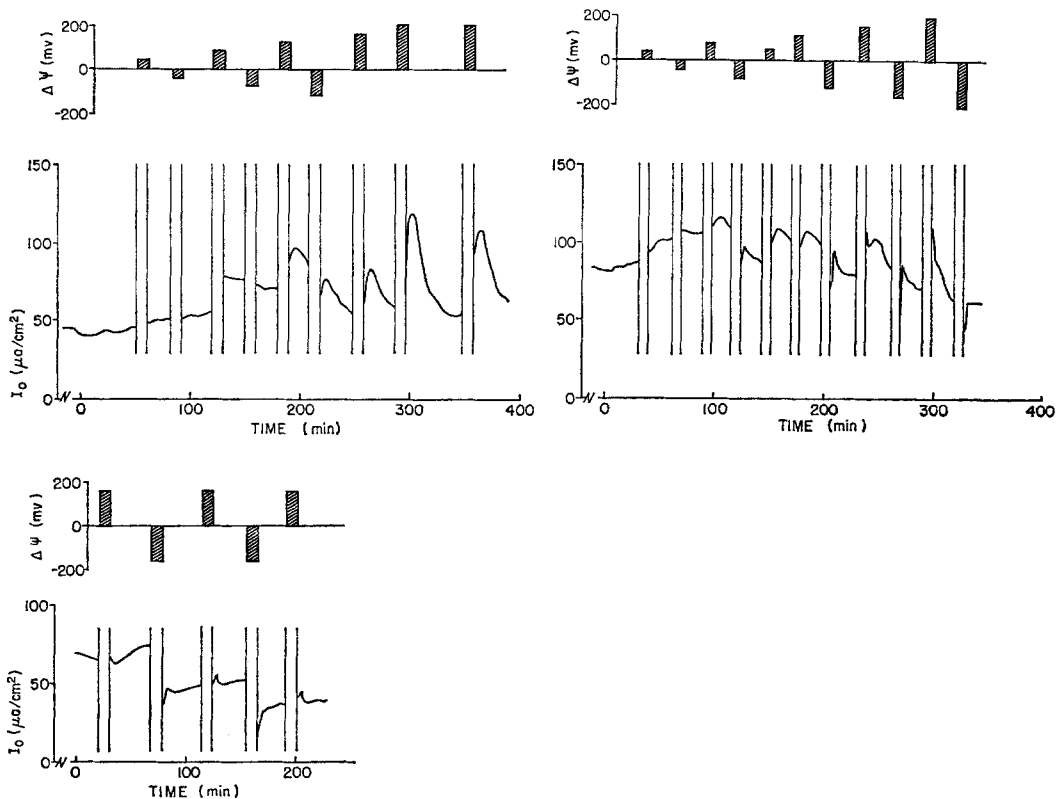


FIGURE 5. Variation of the short-circuit current following perturbation of the electrical potential difference $\Delta\psi$. This study was carried out in the presence of aldosterone.

returning to the short-circuited state. In this case a positive correlation between the two effects was seen.

In Fig. 5 a short-circuited state was interposed between each positive and negative period. In other studies each positive period was immediately followed by a negative period of the same duration and magnitude of potential; in these cases the memory effect was almost completely abolished. The compensating effect of sequential positive and negative periods was seen also in the rate of oxygen consumption, as shown, for example, by Fig. 4, bottom.

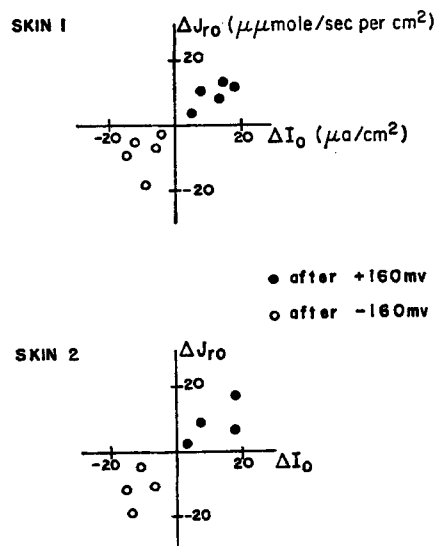


FIGURE 6. Effect of extended electrical potential perturbations on the subsequent values of short-circuit current I_0 and rate of oxygen consumption J_{r_0} . The change in oxygen consumption is plotted against the change in short-circuit current. The perturbations last some 15-20 min.

DISCUSSION

1. General Considerations

A linear relationship between the rate of oxygen consumption and the electrical potential difference is interesting per se, but takes on added significance in attempts to understand the fundamental mechanisms of active transport. In principle any consistent relationship between flows and forces would be of value in correlating behavior in a variety of conditions. Obviously a linear relationship would have the greatest utility.

Accordingly, several authors have attempted to consider active transport from the viewpoint of linear nonequilibrium thermodynamics. Kedem (1961) showed that the formalism permits the correlation of results of different measurements. Hoshiko and Lindley (1967) extended the methods of Kedem in single salt and bi-ionic systems and outlined procedures for the evaluation of the requisite 10 or 15 coefficients. Essig and Caplan (1968) treated the transport of a single cation driven by a single metabolic reaction, and showed that a composite system comprising a "pump," a series barrier, and a leak pathway may be described by linear equations, providing that each element shows linearity. In the present study we have carried out initial experiments with the aim of determining the extent to which active sodium transport and the associated oxidative metabolism in the frog skin may in fact be analyzed in the framework of a linear nonequilibrium thermodynamic model.

In order to analyze data in terms of this model it is necessary that certain preliminary requirements be satisfied. Firstly, data must be obtained in the steady state, which means that all pertinent parameters must be constant with time. This requirement can of course only be approximated. In order to determine the extent to which the steady-state requirement is satisfied, we monitored intermittently the rates of sodium transport and oxygen consumption in the short-circuited state. (It has been shown that both in the absence of aldosterone and in the steady state following administration of aldosterone the short-circuit current in frog skins is electrically equivalent to net sodium transport [Ussing and Zerahn, 1951; Nielsen, 1969].) In freshly mounted skins I_o and $J_{r,o}$ were stable only rarely. In general, both declined appreciably with time, and in addition showed spontaneous fluctuations. Skins which were aged in the presence of aldosterone and glucose were usually much more stable than fresh skins. A second requirement is that a new steady state be reached promptly after perturbation of $\Delta\psi$. This condition was clearly satisfied, since constant values of J_r were established within 2 min of the electrical perturbations, whereas measurements were made after 4–6 min. Another requirement is that the results of variation of $\Delta\psi$ must be specific, reflecting intrinsic changes in the function of the active transport system. Specificity was shown by the insensitivity of J_r to $\Delta\psi$ after sodium transport had been blocked by ouabain. It is also necessary that electrical clamping be harmless in the voltage range and for the duration employed. This was demonstrated in stable preparations by the return of $J_{r,o}$ and I_o to their previous values after electrical perturbations, provided that the memory effect was small or compensated by two periods of identical length and opposite polarity.

As discussed in Vieira et al. (1972) the rate of oxygen consumption after the blockage of active sodium transport by ouabain is a good estimate of the basal rate of oxygen uptake unrelated to transepithelial sodium transport. Therefore the results obtained following administration of ouabain indicate that the basal rate of oxygen consumption was unaffected by changes in the electrical potential difference across the skin.

2. Linearity

The linear current-voltage relationship commonly observed in the frog skin may well reflect linearity of the intact sodium transport system, but is not completely convincing because of the undefined contribution of artifactual leak pathways introduced by the mounting procedure. However, studies in the toad bladder restricted to tissues in which two-thirds of the total conductivity was by way of the active transport pathway showed a linear relation between current and potential difference (A. Essig and P. D. Lief, manuscript in preparation).

The present study enables us to make precise statements about the relationship between J_r and $\Delta\psi$ in frog skin. Although we did not always observe linearity, in those cases where the skin was stable, as shown by constancy of J_{r_0} with time, linearity was impressive over a wide range. In the absence of aldosterone linearity was demonstrable over a range of ± 70 mv; after incubation with aldosterone linearity was demonstrable over as much as ± 160 mv in stable skins.

3. Phenomenological Description

The observation of linearity indicates that the phenomenological coefficients characterizing the system and the affinity A of the metabolic driving reaction must be nearly invariant on perturbation of $\Delta\psi$. The alternative possibility, that the coefficients and/or the affinity may vary so as to produce the observed linearity, seems unlikely¹. Accordingly, the behavior of the system may be described by standard equations of nonequilibrium thermodynamics, namely:

$$J_{Na} = L_{Na} X_{Na} + L_{Na,r} A \quad (1)$$

$$J_r = L_{Na,r} X_{Na} + L_r A. \quad (2)$$

Here J_{Na} represents net sodium flux, X_{Na} is the negative electrochemical potential difference of sodium across the skin, and the L 's are phenomenological coefficients. With identical solutions at each surface $X_{Na} = -F\Delta\psi$. In writing the cross-coefficient as $L_{Na,r}$ in both equations 1 and 2 the validity of the Onsager reciprocal relation has been assumed.²

4. Evaluation of the Affinity

The apparent validity of equations 1 and 2 justifies the application of an equation derived previously (Essig and Caplan, 1968). For linear systems obeying the Onsager reciprocal relation between phenomenological coefficients

$$A = - \frac{I_0}{(\partial J_r / \partial \Delta\psi)_A}. \quad (3)$$

¹ It is conceivable that A might be a linear function of $\Delta\psi$ (Essig and Caplan, 1968). However, since the memory effect evaluated at short-circuit is small, it would seem that A could not be a strong function of $\Delta\psi$.

² Since A appears to be constant in these studies, we cannot rule out the existence of higher order terms in the affinity. For the present purposes this is immaterial; all of our conclusions are unaffected by this consideration. In a previous publication (Essig and Caplan, 1968) the phenomenological equations were presented in terms of resistance coefficients rather than conductance coefficients for reasons cited. In the present context the conductance formulation is intuitively more meaningful. Although the Onsager relation has been widely tested, its validity for active transport is as yet unknown. In a model system in which the enzymatic hydrolysis of an amide results in current flow simulating active transport the relation was found to hold (Blumenthal et al., 1967).

Values of A calculated in this way are given in Tables I and II. These affinities represent the negative free energy of an oxidative metabolic reaction which "drives" sodium transport. It is to be emphasized that the values presented are those appropriate for the skins under the conditions obtaining at the time

TABLE I
RESULTS OF REPLICATE STUDIES IN FIVE UNTREATED FRESH SKINS

Skin	I_o	$\Delta\psi$	$-\partial J_r/\partial\Delta\psi$	A	Mean A
	$\mu\text{amp}/\text{cm}^2$	mv	$\mu\text{mole}/\text{sec per cm}^2 \text{ per mv}$	kcal/mole	kcal/mole
I	63.4	-30, 30, 0	0.364	41.6	48.3±2.9
	60.6	-30, 30, 0	0.293	49.4	
	40.8	-60, 60, 0	0.234	41.7	
	26.1	-90, 90, 0	0.119	52.3	
	26.1	-90, 90, 0	0.111	56.4	
II	59.2	-30, 30, 0	0.345	41.0	50.9±2.9
	70.4	30, -30, 0	0.299	56.2	
	45.1	-60, 60, 0	0.217	49.7	
	56.3	60, -60, 0	0.224	60.0	
	35.2	-90, 90, 0	0.187	45.1	
	35.2	90, -90, 0	0.156	53.8	
III	63.4	0, -30, 0	0.306	49.5	42.1±2.8
	54.9	-60, -30, 0	0.371	35.4	
	52.1	0, 30, 60	0.338	36.8	
	59.4	60, 30, 0	0.299	47.5	
	52.8	60, 0, -60	0.306	41.3	
IV	66.9	0, 50, 0	0.370	43.2	57.1±6.1
	65.1	0, -50, 0	0.223	69.9	
	57.0	-50, 0, 50	0.293	46.5	
	52.8	-50, 0, 50	0.280	45.0	
	51.7	50, 0, -50	0.211	58.6	
	52.8	-50, 0, 50	0.159	79.5	
V	20.7	0, -50, 0, 50	0.246	20.1	22.7±2.9
	17.6	0, -100, 0, 100	0.223	18.9	
	13.8	0, 50, 0, -50	0.105	31.3	
	12.7	0, 100, 0, -100, -100	0.147	20.7	
Mean	46.6±3.4		0.247±0.016		44.2±13.2

Values of I_o , $-\partial J_r/\partial\Delta\psi$, and the affinity A were calculated from equation 3 (untreated skins). Mean values are given \pm SE.

of their observation, and are not to be confused with the often cited negative free energies evaluated under standard conditions.

Table I shows the results of replicate studies in five untreated fresh skins. The mean affinity value, 44.2 ± 13.2 kcal/mole of oxygen, is to be compared with the value of some 116 kcal/mole cited for glucose oxidation under

physiological conditions (Davies and Ogston, 1950). Table II shows the results of studies in seven aldosterone-treated skins, each representing a period of some 2 hr or less. The high values of the correlation coefficients indicate linearity. The mean affinity value, 107.1 ± 51.5 kcal/mole of oxygen, is higher than in the untreated skins. Since adequate control studies were not performed we cannot conclude that aldosterone was responsible for this difference.

It is to be expected that with the passage of time the affinity will decrease at a rate determined by substrate utilization, and a rapid decline in the affinity may well account for a rapid decrement in I_o and $J_{r,o}$ as was sometimes observed. However, since the techniques employed here for the evaluation of

TABLE II
RESULTS OF STUDIES IN SEVEN ALDOSTERONE-TREATED SKINS

Skin	I_o	$J_{r,o}$	n_o	$-\partial J_r / \partial \Delta \psi$	n	r	A
	$\mu\text{amp}/\text{cm}^2$	$\mu\text{mole}/\text{sec per cm}^2$		$\mu\text{mole}/\text{sec per cm}^2 \text{ per mv}$			kcal/mole
VI	32.1 ± 1.1	81.9 ± 1.0	4	0.156 ± 0.010	20	0.963	49.2 ± 3.8
VII	116.5 ± 1.1	88.8 ± 1.3	4	0.137 ± 0.013	20	0.928	203.7 ± 16.2
VIII	96.3 ± 0.4	86.6 ± 1.2	5	0.179 ± 0.012	21	0.957	128.5 ± 8.6
IX	99.6 ± 1.3	115.5 ± 1.4	6	0.233 ± 0.017	14	0.969	102.2 ± 6.4
X	51.1 ± 0.6	90.2 ± 3.3	4	0.212 ± 0.041	11	0.865	57.5 ± 10.9
XI	74.0 ± 0.5	86.3 ± 0.7	5	0.191 ± 0.008	21	0.985	92.4 ± 3.9
XII	85.8 ± 0.8	84.7 ± 0.9	4	0.177 ± 0.009	20	0.976	115.9 ± 6.0
Mean	79.3 ± 29.4	90.6 ± 11.3		0.184 ± 0.032			107.1 ± 51.5

Values of I_o , $J_{r,o}$, $-\partial J_r / \partial \Delta \psi$, and the affinity A were calculated from equation 3 (aldosterone-treated skins). The observations were made at 6-min intervals; n_o is the number of simultaneous determinations of I_o and $J_{r,o}$. Perturbations of potential were made in 40-mv steps ranging from -160 to $+160$ mv. Mean values are given \pm SE; n is the total number of observations and r the correlation coefficient. For $n \geq 11$ a value of $r \geq 0.684$ is significant at the 0.01 level.

A necessitated the use of stable preparations, and since the studies of oxygen consumption were carried out for only limited periods, we were not able to demonstrate the time-dependence of A . In the studies shown in Table I, the short-circuit current varied more than the affinity. This may possibly reflect variations in membrane permeability.

5. Significance of the "Memory" Effect

In view of the evidence above for near-constancy of the affinity it is of interest that positive clamping, which slows active sodium transport, transiently increases subsequent values of I_o and $J_{r,o}$, and that negative clamping induces the opposite effects. These phenomena might of course reflect transient effects of the electrical potential on tissue permeability coefficients or rate constants, but this seems unlikely, particularly in view of the small magnitudes of the perturbations which we often employed. Another possibility is

that changes in the rates of transport resulting from perturbations of $\Delta\psi$ might slightly alter the concentrations of metabolic intermediates so as to induce the small changes observed. Such behavior is consistent with a model for the active transport system discussed previously (Essig and Caplan, 1968, Appendix I). It might seem as though short-term variations of A resulting from perturbations of $\Delta\psi$ would invalidate our attempts to evaluate an affinity characteristic of each skin. However, the sequence of perturbations was chosen so as to minimize the memory effect. Furthermore, the clear demonstration of the memory effect requires longer perturbations than were employed in the studies of J_r vs. $\Delta\psi$.

6. Concluding Remarks

Considerable interest attaches to the mechanisms whereby various substances alter the rate of sodium transport (Porter and Edelman, 1964; Sharp and Leaf, 1964; Sharp et al., 1966; Fanestil et al., 1967). In principle such substances may act by effects on (a) permeability, (b) coupling between transport and metabolism, or (c) modification of the affinity of the metabolic driving reaction. The present means of evaluating the affinity may be useful in differentiating between these possibilities.

The efficiency of active transport is often evaluated in terms of "the calorific value of 1 eq. of oxygen" (Zerahm, 1958). It seems more appropriate to utilize the free energy of the metabolic driving reaction measured *in vivo* rather than an enthalpy derived from bomb calorimetry.

As mentioned above, equivalence of the two methods used for the evaluation of the "electromotive force" of sodium transport E_{Na} requires constancy of the rate of metabolism. However, the present study shows that J_r varies markedly with $\Delta\psi$.

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