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Evaluation of Kinetic and Energetic Parameters of Active Sodium Transport

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Summary. Active sodium transport is classically analyzed in terms of an equivalent circuit, comprising an active conductance κ^a and an electromotive force of sodium transport E_{Na}. Although E_{Na} is commonly considered the driving force of transport, model experiments have suggested that E_{Na} is a composite parameter, incorporating both kinetic and energetic factors. An alternative approach considers both transport and the associated oxidative metabolism in terms of a nonequilibrium thermodynamic (NET) formulation, involving phenomenological coefficients and the affinity A, presumed to represent kinetic and energetic factors, respectively. Model experiments testing the NET formulation suggest that the affinity is indeed an energetic parameter. Calculated values of A in untreated frog skins and toad bladders range from about 20 to 80 kcal per mole of O_2 consumption. Assuming a P/O ratio of 3, this range corresponds to about 3–13 kcal per mole of ATP utilization, values compatible with reported direct measurements. Although brief perturbations of transepithelial electrical potential $\Delta\Psi$ resulted in linear current-voltage relationships, indicating constancy of E_{Na} and κ^a, 15-min perturbations of $\Delta\Psi$ resulted in nonlinearity, indicating changes in E_{Na} and κ^a: perturbations of $\Delta\Psi$ enhancing active transport were associated with decrease of E_{Na} and increase of κ^a; slowing of active transport produced the converse effects.

Ultimately, of course, an important aim of the study of active transport processes is a complete understanding of transport mechanisms at the molecular level. Such understanding, however, may well be in the distant future. Meanwhile, much useful information may be derived from analysis of the kinetic and energetic parameters of transport systems, which determine the rate of transport in a variety of physiological states and in response to pharmacological stimuli. In addition to the importance of such a characterization in its own right, the precise quantification of kinetic and energetic parameters must one day help to evaluate the validity of proposed molecular mechanisms. In the approach to such precise characterization we are all once again indebted to Professor Ussing, who has, as in so many other respects, pointed the way.

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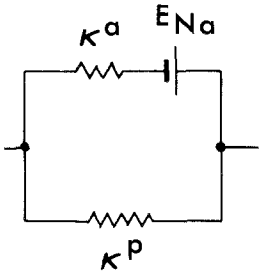

Fig. 1. Equivalent circuit model for active sodium transport

In a first approach to analysis of the parameters of active sodium transport across an epithelial tissue, it has been natural and useful to think in terms of an analogous electric system, i.e., an equivalent circuit model [28], represented in its simplest form in Fig. 1. Here the current in the active pathway I^a represents sodium flow, and thus the rate of active sodium transport is determined by two factors: (i) the electromotive force of sodium transport E_{Na}, and (ii) the conductance of the active pathway, which we call κ^a. The total current also incorporates the flow of various ions in parallel passive pathways, whose conductance is called κ^p. The formal analysis of this system follows immediately from linearity of the current-voltage relationships observed when the transepithelial potential difference $\Delta\Psi$ is perturbed briefly, indicating, when appropriate correction is made for passive flow, that the active conductance is constant under these conditions. Thus, if I^a represents inward sodium flow and $\Delta\Psi$ represents $\Psi^{in} - \Psi^{out}$,

$$-dI^a/d(\Delta\Psi) = \kappa^a, \tag{1}$$

giving on integration,

$$I^a = \kappa^a(E_{Na} - \Delta\Psi), \tag{2}$$

or

$$I^a = (\kappa - \kappa^p)(E_{Na} - \Delta\Psi). \tag{3}$$

Here E_{Na} is the maximum electrical potential difference which can be created and sustained by the active transport system, i.e., the value of $\Delta\Psi$ at which the rate of active sodium transport, and thus I^a, becomes zero. This formulation is easily applied: all that is necessary is to measure the current before and after brief perturbation of $\Delta\Psi$, giving the total conductance κ, and to evaluate κ^p by the use of tracer isotopes [24, 27]

or by the measurement of the conductance after the abolition of active transport [13]. Many workers have used this and/or closely related techniques to evaluate κ^a and E_{Na} in a number of systems and have demonstrated dramatic effects in a variety of states of interest.

Although κ^a and E_{Na} cannot yet be interpreted precisely in terms of their biochemical or biophysical determinants, it seems likely that κ^a represents kinetic factors [24]; for the histologically relatively simple toad bladder these presumably are determined at the permeability barriers of the apical and basal-lateral plasma membranes. The interpretation of E_{Na} is more complex. Ussing and Zerahn referred to E_{Na} in operational terms as the electromotive force of sodium transport [28]. Subsequently, however, it has become common to refer to E_{Na} as the driving force of sodium transport. It seems to me that this latter terminology may be somewhat misleading, in that it may tend to cause people to conclude that E_{Na} is a purely energetic quantity, and that by determining whether an agent affects E_{Na} or κ^a one can therefore differentiate between effects on energetic and kinetic factors, respectively. However, there are both theoretical and experimental reasons to believe that E_{Na} is not a purely energetic quantity, but reflects kinetic factors as well. From a theoretical point of view, if E_{Na} were a purely energetic factor, it should be determined solely by the Gibbs free energy change, or affinity A, of the metabolic process driving transport.[1] Thermodynamic analysis indicates however, that E_{Na} depends not only on A, but also on kinetic factors reflecting the coupling of transport to metabolism and the conductance of the active pathway [5, 6, 8]. From the experimental point of view, if E_{Na} were indeed a purely energetic parameter, it should not be affected, at least promptly, by agents though to act only on kinetic factors, but should be depressed by agents which depress transport as a consequence of effects on metabolism. In a recent study, Changgi Hong and I studied three agents which depressed transport by different means. We found that whereas ouabain depressed E_{Na}, amiloride dramatically enhanced E_{Na} (as rapidly as we were able to make the measurement), and the metabolic inhibitor 2-deoxy-glucose had no effect (other than a slight transient increase). All three inhibitors, however, depressed the active conductance [13]. If E_{Na} were a purely energetic parameter, and if, as many believe, mucosal amiloride acts solely on the passive mucosal entry step, amiloride might not be expected to promptly enhance E_{Na}. On the

1 Under usual experimental conditions, i.e., constancy of temperature, pressure, and chemical potential of all reactants, the affinity is equal to the negative Gibbs free energy change per equivalent of reaction.

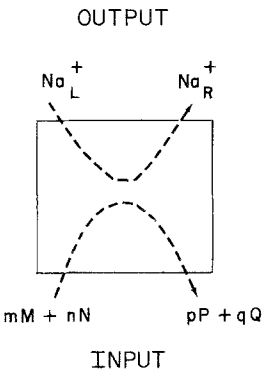

Fig. 2. General scheme for the coupling of metabolism to sodium transport. The consumption of M and N to produce P and Q provides the free energy that results in active transport of sodium across the membrane [8]

other hand, the metabolic inhibitor 2-deoxy-glucose might be expected to depress E_{Na}. Similar findings have been recently reported by Al-Awqati, Mueller, and Steinmetz for the active H^+ transport system of the turtle bladder. In this system the depression of metabolism by deoxygenation, substrate depletion, or the addition of 2-deoxy-glucose produced greater decrease in active conductance than in the protonmotive force PMF; glucose caused a slight decrease in PMF and an increase in active conductance [2]. These various results indicate that E_{Na} and the PMF are not pure energetic parameters. Furthermore, there appear to be important interactions between the kinetic and the energetic parameters of the active transport system.

In order to characterize energetic determinants of active transport more precisely it is necessary, of course, to study not only transport, but also metabolism, and to relate it to thermodynamic considerations. The simplest preliminary approach is indicated in Fig. 2, in which a single metabolic process is presumed to drive a single transport process [5, 8]. Thus, if J_r represents the rate of metabolism, and A represents the affinity, $J_r A$ is the rate of input of metabolic energy. If J_{Na} represents the rate of active sodium transport and X_{Na} represents the negative electrochemical potential difference of sodium across a membrane, $-J_{Na} X_{Na}$ is the rate of output of electroosmotic work. The second law of thermodynamics tells us that the input must exceed, or at least equal, the output. Therefore, if we evaluate the rate of metabolism in terms of the rate of oxygen consumption we have

$$A \geqq -(J_{Na}/J_{O_2}) X_{Na}, \tag{4}$$

where A is expressed in terms of kcal-mole^{-1} of O_2 consumed. If we now assume that J_{Na} and J_{O_2} are in a fixed stoichiometric ratio, irrespective of the rate of transport, this relationship allows us to estimate the magnitude of A. This is most readily done by setting X_{Na} at the limiting value consistent with sodium transport against an electrochemical potential difference, in the absence of a concentration difference, i.e., $X_{Na} = -F(\Delta\Psi)_{J_{Na}=0} = -FE_{Na}$. At this limit, for a system in which transport and metabolism are stoichiometrically coupled, the equality holds in Eq. (4). Hence we have

$$A = (J_{Na}/J_{O_2})(FE_{Na}). \tag{5}$$

If we now assume commonly cited average values for anuran epithelia in standard solutions, e.g., $(J_{Na}/J_{O_2})_{\Delta\Psi=0} \equiv (J_{Na}/J_{O_2}) \simeq 18$ [18] and $E_{Na} \simeq 100\,\text{mV}$ [11, 32], we calculate that $A \simeq 42\,\text{kcal} \cdot \text{mole}^{-1}\ O_2$. Since it is thought that active transport in these tissues is driven by ATP, we would like to re-express this affinity in terms of ATP utilization, but are hampered by lack of knowledge of the stoichiometry of oxidative phosphorylation in intact epithelia. If, however, we assume a P/O ratio of 3, as may be observed in healthy mitochondria, each mole of O_2 consumed will result in the synthesis (and in the steady state, the utilization) of 6 moles of ATP, giving a mean value of A of about $7\,\text{kcal} \cdot \text{mole}^{-1}$ ATP.

This "ball park" mean value is of some interest, but cannot be considered precise, not only because we have assumed specific values for J_{Na}/J_{O_2} and E_{Na}, both of which may vary appreciably [1, 29], but more fundamentally, because we have assumed here for simplicity that the rates of oxidation and phosphorylation, and the rates of active sodium transport and oxygen consumption, are in a fixed stoichiometric relationship, irrespective of the rate of transport. To use Kedem and Caplan's term, we have assumed that oxidation and phosphorylation, as well as transport and oxidative metabolism, are completely coupled [15]. Studies in both frog skin and toad bladder suggest, however, that this is not the case [16, 17]. If transport and metabolism are not completely coupled, the utilization of metabolic energy may be appreciably less efficient, and in this case Eq. (5) may significantly underestimate the magnitude of the affinity necessary to sustain active transport. Under these circumstances it would be desirable to employ a more complete and more precise formulation. Hopefully, in addition to allowing the evaluation of the affinity, such a formulation should allow us to correlate and therefore predict the behavior of the transport system under a wide variety of circumstances.

In an attempt to find such a useful general formalism, Roy Caplan and I and our colleagues have for several years been testing the utility of a linear nonequilibrium thermodynamic (NET) formulation [5, 8, 16, 17, 21, 25, 30], based on the work of Ora Kedem [14]. As mentioned above, we assume a 2-flow system and consider first, for simplicity, a frog skin or toad bladder exposed to identical solutions at each surface, so that there is no concentration gradient influencing transport. We would expect the rate of sodium transport J_{Na} to be influenced by the electrical potential difference $\Delta\Psi$ across the membrane, but since sodium transport is driven by metabolism we would expect it also to be influenced by the affinity A. Similarly, we expect the rate of metabolic reaction J_r to be determined largely by A, but since transport and metabolism are coupled, we expect J_r to be influenced also by $\Delta\Psi$. There is a range in which these dependencies will be linear; we postulate that this range may be sufficiently large to be experimentally useful. Accordingly we write

$$J_{Na} = L_{Na}(-F\Delta\Psi) + L_{Na,r}A \tag{6}$$

$$J_r = L_{Na,r}(-F\Delta\Psi) + L_r A. \tag{7}$$

Here F is the Faraday constant and the Ls are phenomenological coefficients. In assigning the same phenomenological cross-coefficient $L_{Na,r}$ to each flow we are assuming the validity of the Onsager reciprocal relation, which has been widely tested in a variety of systems [5, 20, 23]. It should be noted that if, indeed, the linear phenomenological equations are valid, the affinity A is of considerable physiological interest, since it must represent the substrate-product concentration ratio of some critical reaction in the metabolic pool which supports active transport. In this regard it differs from quantities such as the phosphate potential calculated from direct chemical analyses of ATP, ADP, and P_i. This quantity, in addition to involving numerous experimental difficulties, may importantly reflect tissue functions other than transport.

It will be noted that Eqs. (6) and (7) represent the simplest general thermodynamic formulation possible, consistent with the function of an active transport system. This being so, it is perhaps pertinent here to comment on a common misconception, namely that NET "predicts" a given result. Of course NET does not and cannot predict *a priori* the result of a given biological experiment; all that it can do is describe and correlate the behavior of a system, presuming that it obeys the phenomenological relations, within the domain of their validity. To quote Prigogine, "It is clear that the existence of such relations is an extra-

thermodynamic hypothesis and it is quite conceivable that in some particular cases the relationship between flow and [force] may not be linear" [22].

Space does not permit an exhaustive analysis of theoretical considerations relative to the range of validity of linear phenomenological equations. Suffice it to say that for brief perturbations of transepithelial electrical potential, both J_{Na} and J_r have been shown to be linear functions of $\Delta\Psi$ [5]. The dependence of the flows on the affinity A is a more difficult issue, which it has not yet been possible to approach directly. Although for a simple chemical reaction linearity of the rate in the affinity is not found unless $A \ll RT$, there are both theoretical and experimental reasons to believe that in complex biological systems this need not be the case [4, 22, 23]. Again to be brief, I shall mention only that studies in isolated mitochondria have demonstrated linearity of rates of both oxidation and phosphorylation in the affinities of both oxidation and phosphorylation [12, 23].

As for the question of linearity in A for transport processes, a definitive answer must await the development of a suitable model system in which the pertinent affinity can be modulated as desired. Meanwhile, we must enquire as to the plausibility of values of A calculated from the linear NET formulation in model experiments. The results to date are encouraging, in that the behavior of A following the administration of ouabain, amiloride, or 2-deoxy-glucose is consistent with these agents' established biochemical and electrophysiological effects [5, 21, 25]. Also deserving consideration is the plausibility of the magnitude of values of A calculated from the linear formalism. Values in untreated frog skins and toad bladders have ranged from some 20–80 $kcal \cdot mole^{-1}$ of O_2 consumed [17, 21, 25, 30]. Again assuming a P/O ratio of 3, so that utilization of 1 mole of O_2 corresponds to the synthesis and utilization of 6 moles of ATP, these values of A correspond to some 3–13 $kcal \cdot mole^{-1}$ ATP. Two comments are noteworthy: first, the values calculated here from the linear formalism are in the same neighborhood as the approximate value of A calculated above from the second law of thermodynamics, independent of the validity of linear NET; and second, the calculated values are in the range of directly measured values, namely some 7.6 $kcal \cdot mole^{-1}$ ATP for ATP hydrolysis under standard conditions, and Wilson *et al.*'s 11.4 $kcal \cdot mole^{-1}$ ATP for ATP utilization under conditions obtaining in isolated rat liver cells [31]. Considering that the free energy may well vary with cell pH, Mg^{++}, and Ca^{++} [3], and that the P/O ratio and $[ATP]/[ADP][P_i]$ ratio may well vary in

different tissues and with changes in experimental conditions, the values of the affinity calculated for the frog skin and toad bladder seem quite reasonable, presuming that the transport process is indeed driven by ATP. Of course, more conclusive tests must be awaited.

While awaiting definitive answers, we have attempted to apply the NET formalism to important problems not easily approached by more traditional means. For example, in the study of hormonal modulation of transport processes an important and disputed issue is whether aldosterone stimulates active sodium transport by affecting kinetic or energetic factors, or both [7, 9, 19, 26]. An analysis based on NET suggests both types of effects [24, 25].

It is possible, of course, that the two mechanisms of aldosterone action are independent, reflecting discrete effects on cellular function. However, in attempting to analyze a transport process in such terms it is important to remember that a polar epithelial cell is presumably constrained to regulate not only transepithelial transport of sodium, but also the intracellular electrochemical potential and concentration of sodium. If aldosterone were to enhance transepithelial active sodium transport solely as a consequence of enhancing passive entry at the mucosal surface, the cellular sodium concentration would tend to rise, whereas if aldosterone were to enhance transepithelial sodium transport solely as a consequence of effects on energetic factors at the basal-lateral membrane, the intracellular sodium concentration would tend to fall. If, however, aldosterone were to act by a dual mechanism, increasing both the active conductance and the thermodynamic affinity, substantial enhancement of transepithelial sodium transport could occur without significant effects on cellular sodium content or concentration. Given the present state of knowledge, we can only speculate as to whether the effects of aldosterone are independent or the components of an integrated mechanism. Nevertheless, the nature of the response to aldosterone, and other results alluded to above, point to the importance of investigating possible interactions between various fundamental parameters, taking the point of view that such interactions may influence not only transepithelial transport, but also the state of sodium within the cell.

These issues have been approached in a recent study with Daniel Wolff, in which we examined the effects of perturbing transport on the parameters of the equivalent circuit model. In this study the rate of active sodium transport in the toad bladder was altered by perturbing the transepithelial electrical potential difference $\Delta\Psi$. When $\Delta\Psi$ was perturbed at 30-sec intervals in the symmetrical sequence $0 \rightarrow \pm 100\,\text{mV}$,

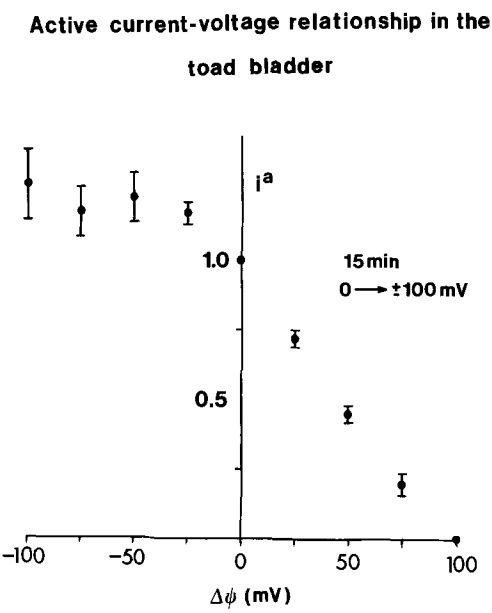

Fig. 3. Active current-voltage relationship in the toad bladder ($n=11$). The value of active current I^a at a given value of $\Delta\Psi$ was related to the initial value at $\Delta\Psi=0$ and that at $\Delta\Psi=100$ mV to give the reduced quantity $i^a=(I^a_{\Delta\Psi}-I^a_{100})/(I^a_0-I^a_{100})$ (Wolff & Essig, *unpublished results*)

the plot of the reduced "active current" i^a *vs.* $\Delta\Psi$ was linear, as observed previously, indicating that κ^a and E_{Na} were unaffected by brief perturbations of $\Delta\Psi$. However, when $\Delta\Psi$ was perturbed in the same sequence, but at 15-min intervals, the plot became strikingly nonlinear in the region of enhanced transport at large negative values of $\Delta\Psi$ (serosa negative), as is seen in Fig. 3. On the face of it, it might seem that such a nonlinear plot cannot be analyzed in terms of the linear formalism of the equivalent circuit model [Eqs. (1)–(3)]. However, it was found that even membranes which had been maintained for long periods at large negative values of $\Delta\Psi$ showed short-term linear current-voltage relationships when $\Delta\Psi$ was perturbed briefly and symmetrically (i.e., for 10 sec by ± 20 mV) relative to the long-term reference value. Hence κ^a was well defined, permitting the application of the linear equations of the equivalent circuit model to evaluate E_{Na}, as well as κ^a, at each setting of $\Delta\Psi$. When this is done, interesting behavior is observed. As is seen at the top of Fig. 4, E_{Na} varied systematically with perturbation of $\Delta\Psi$, falling to low levels after the maintenance of high rates of active transport at negative settings of $\Delta\Psi$, and rising to high levels after the slowing of

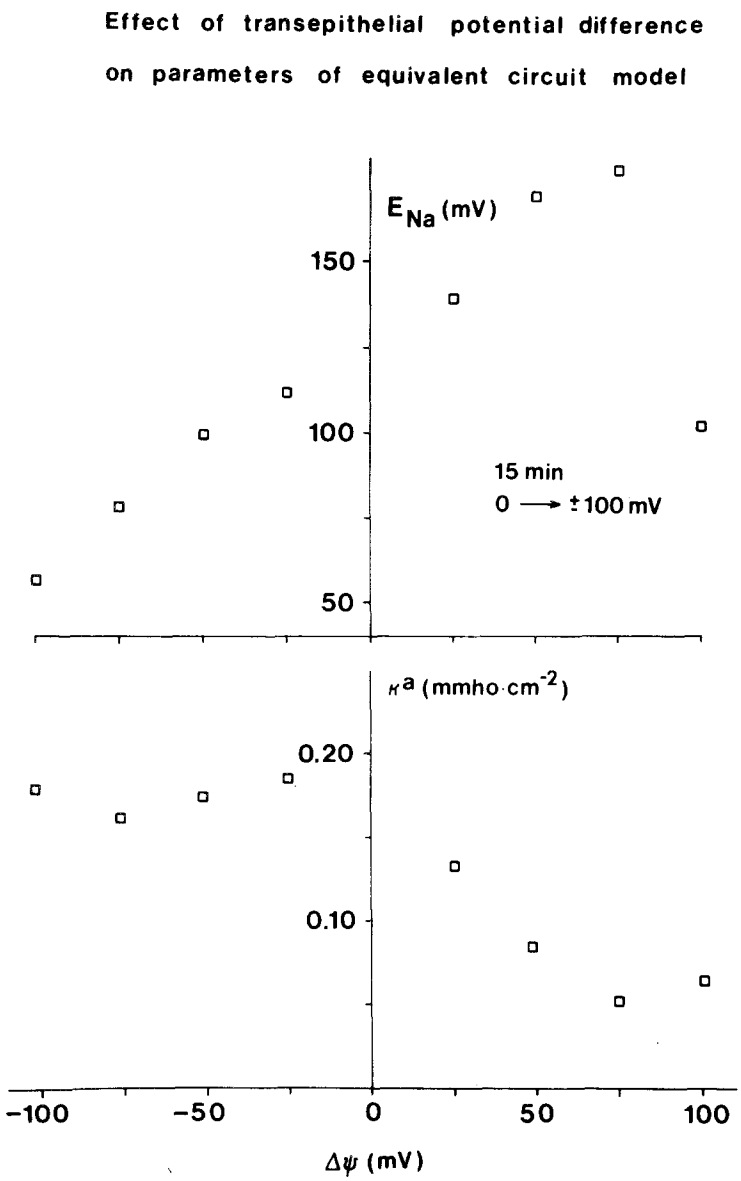

Fig. 4. Effect of transepithelial potential difference on parameters of equivalent circuit model ($n=4$) (Wolff & Essig, *unpublished results*)

active transport by large positive settings of $\Delta\Psi$. These findings are consistent with Feig, Wetzel, and Frazier's recent report of an inverse relationship between the rate of active sodium transport and E_{Na} when transport was altered by the administration of amiloride or antidiuretic hormone, or variation of the concentration of sodium in the bathing

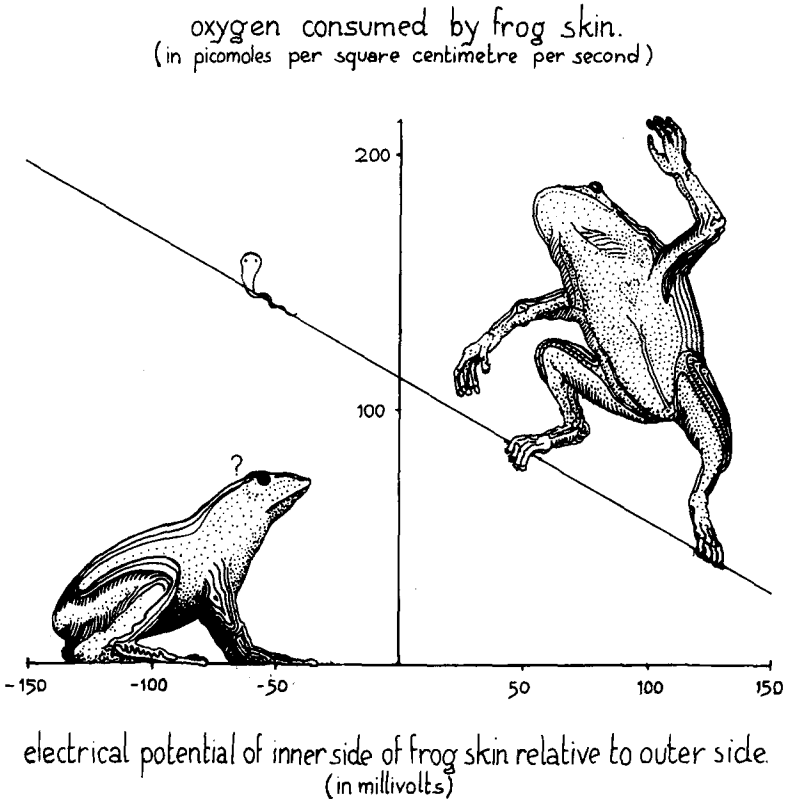

Fig. 5. Artist's conception of linear relationship between J_r and $\Delta\Psi$ in frog skin (courtesy of Hans Van Leeuwen and S.R. Caplan; reproduced from *REHOVOT*, Vol. 7, No. 4, Winter 1974–1975)

mediums [10]. Also of interest is the finding, shown at the bottom of Fig. 4, that the behavior of the active conductance is roughly the converse of that of E_{Na}, with κ^a falling to low levels at positive values of $\Delta\Psi$, but rising to high levels with high rates of transport at negative values of $\Delta\Psi$.

These various results are very reminiscent of the "memory effect" observed earlier by Vieira *et al.* in frog skin: in this system prolonged large perturbations of $\Delta\Psi$ resulted in marked effects on both the current and rate of oxygen consumption on return to the short-circuit reference state [30]. The precise nature of the memory effect and the recently observed effects of $\Delta\Psi$ on κ^a and E_{Na} remains to be determined. Also of importance is the systematic investigation of the behavior of the parameters of the NET formulation; this must await methodology permitting the monitoring of metabolism with a higher degree of temporal resolution than is possible at present.

Clearly, these various dynamic effects point to significant regulatory phenomena deserving of thorough study. Furthermore, they indicate the need for care in the design and interpretation of experiments which aim at the quantification of kinetic and energetic parameters, lest experimental perturbations alter the very parameters which it is desired to characterize. We remain encouraged, however, that if we are sufficiently circumspect, our animals seem willing to "toe the line".

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