ENERGETICS OF ACTIVE TRANSPORT PROCESSES

A. ESSIG

From the Department of Physiology, Boston University School of Medicine, Boston 02118, and the Biophysical Laboratory, Harvard Medical School, Boston, Massachusetts 02115

ABSTRACT Active sodium transport across epithelial membranes has been analyzed by means of linear nonequilibrium thermodynamics. In this formulation the rates of active sodium transport J_{Na} and the associated metabolic reaction J_r are postulated to be linear functions of both the electrochemical potential difference of sodium $-X_{Na}$ and the affinity A (negative free energy) of the metabolic reaction driving transport. Experimental studies in various epithelia demonstrate that both J_{Na} and J_r (oxygen consumption) are indeed linear functions of X_{Na}. Theoretical considerations and experimental studies in other systems suggest the likelihood of linearity in A as well. If so, A may be evaluated. Several observations indicate that the quantity A evaluated from the thermodynamic formalism does in fact reflect the substrate-product ratio of the metabolic reaction which supports transport. This is in contrast to measurements of mean cellular concentrations, which may not reflect conditions at the site of transport. Associated studies of isotope kinetics permit the distinction between effects on the permeability of the active and passive transport pathways. With these combined approaches, it may prove possible to characterize both the energetic and permeability factors which regulate transport. The formulation has been applied to an analysis of the mechanism of action of the hormone aldosterone.

In discussing the nature of active transport from a thermodynamic point of view it is in a sense superfluous to acknowledge the influence of Aharon Katchalsky. I will only say that this work, which was carried out in close collaboration with Roy Caplan, was begun when we were both working in Ora Kedem's laboratory in Aharon's Polymer Department at The Weizmann Institute of Science. Our debt will be evident throughout.

The energetics of active transport is commonly discussed in terms of active sodium transport. There are two reasons for this. For one, sodium transport is very widespread in animal tissues, perhaps ubiquitous. Secondly, very convenient experimental systems are available for the study of this process. Thanks to the work of Ussing with frog skins (1, 2), and later Leaf with toad bladder (3), we have available two relatively simple epithelial membranes which can be used as experimental model systems. When either of these membranes is mounted in an experimental apparatus of the sort shown in Fig. 1, and exposed to physiological saline solutions at each surface, an electrical potential difference is generated. This is the consequence of active sodium transport

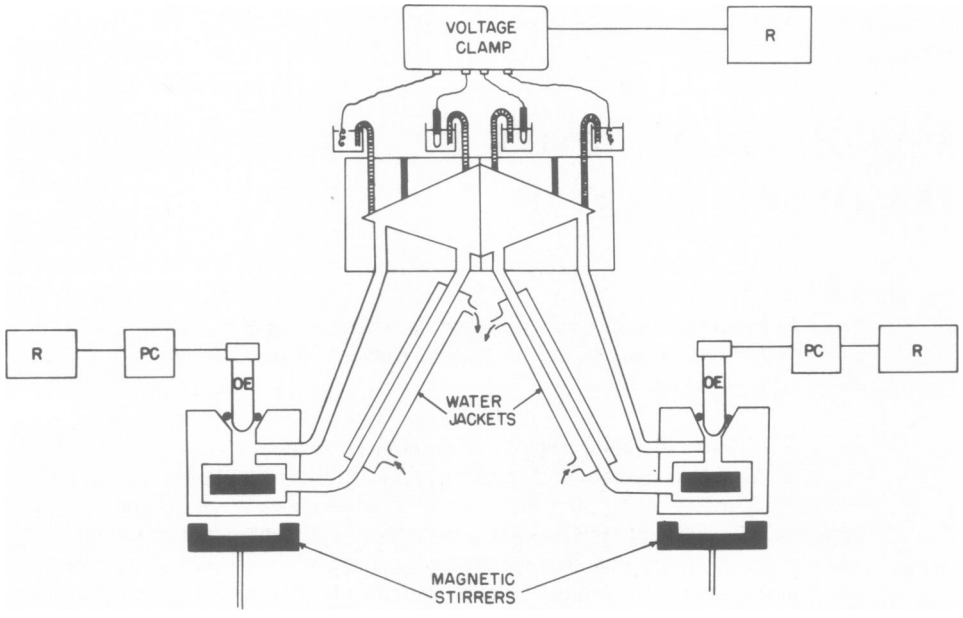

FIGURE 1 Apparatus for the simultaneous measurement of electrical parameters and oxygen consumption. OE, oxygen electrode; PC, polarographic circuit; R, recorder.

from the outer surface of the frog skin (or the urinary surface of the toad bladder) across the tissue into the body fluid. In appropriate species, essentially only sodium is transported actively. Consequently it is possible to manipulate the electrical potential by the use of appropriate circuitry, and to measure the rate of active sodium transport instantaneously and continuously with an ammeter. In this way the effect of experimental manipulations, for example the addition of drugs or hormones, can be observed promptly.

It is easy to understand why so convenient an experimental apparatus has been widely used in study of the energetics of active transport. Unfortunately, however, the classical approach to this subject, it seems to us, has suffered from fundamental conceptual shortcomings. First of all, studies of the energetics of active sodium transport have often laid emphasis on interpretation of the "flux ratio," the ratio of the two "unidirectional" sodium fluxes across the tissues (1, 2). By analogy with simple passive systems it has been considered that the flux ratio gives a measure of the magnitude of the forces responsible for transport. Various considerations make this point of view suspect, but these would take too long to discuss in detail (2, 4). To mention only one factor, pointed out by Ussing, the epithelial tissues we are considering comprise not only active transport pathways but also parallel leak pathways (1, 2), both physiological and artefactual. Obviously, to the extent that transport occurs by way of passive pathways, and not merely by way of active channels, the unidirectional fluxes cannot reflect the influence only of energetic factors. A second shortcoming of standard

treatments of energetics is that they assume a unique stoichiometric ratio, i.e. a unique ratio of sodium transport to metabolism (e.g. oxygen consumption) irrespective of the specific tissue under study (2, 5, 6). It has been shown however that this is not the case: different frog skins show different Na:O ratios (7). A third consideration relates to the magnitude of the metabolic energy available for the maintenance of transport. This is generally deduced from measurements of the energy released by the metabolism of substrates under conditions differing greatly from those existing in vivo. Most calculations utilize a "calorific value" of oxygen derived from measurements of the heat released in the course of oxygenation of glucose (2, 5, 6). The pertinent variable however is the Gibbs free energy made available by metabolism in the tissue under consideration.

For these reasons and others it seems useful to consider a self-consistent theoretical treatment of active transport which might permit its analysis under a variety of conditions. Hoshiko and Lindley have presented a comprehensive formalism which facilitates the analysis of active salt and water transport in single salt and bi-ionic systems (8). Caplan and I have preferred to restrict ourselves to systems with active transport of only one ion, uncoupled to the flows of other species (9). I will discuss this formulation briefly, but will concentrate mostly on pertinent experimental considerations. The theoretical basis for our work is an extension of the linear nonequilibrium thermodynamic treatment presented originally by Ora Kedem (10).

In analyzing the energetics of active sodium transport it is of course necessary, as in other areas, to start with a simple model. I have already mentioned that in frog skins and toad bladders of appropriate species there appears to be only one significant active transport process, that of sodium, and thus only one significant output for our thermodynamic system. We assume further, despite the great complexity of metabolic processes in biological tissues, that we can isolate one metabolic process which "drives" the active transport process. This model is represented in Fig. 2. Here one input process, the metabolism of substrate, is linked to one output process, the transport of sodium. In this representation the consumption of M and N to produce P and Q provides the free energy which brings about the active transport of sodium from the left side of the membrane to the right side of the membrane.

Similarly, we take a simplified view of the histology of the system (Fig. 3). The process of active transport takes place in the rectangular box. Although it is not necessary for our analysis, in accord with many experimental observations we represent the outer region as a simple passive barrier across which sodium moves down its electrochemical potential gradient. At the inner or basolateral surface is the mechanism responsible for active sodium transport, the so-called sodium "pump." Since the active transport process transports only sodium ions, whereas the tissue as a whole reabsorbs sodium chloride, it is necessary for there to be a pathway across which chloride can move. This is represented as a simple passive channel in parallel with the active transport pathway, which is presumably accessible more or less to all of the ions in the bathing solutions.

Since we have considered active transport to be a two-flow process there are two pertinent flow equations to be considered, one for sodium transport, which we rep-

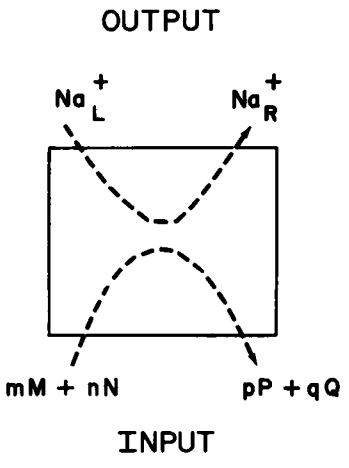

FIGURE 2

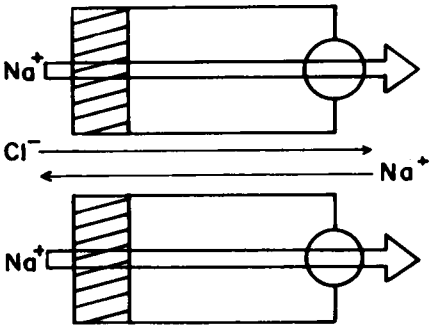

FIGURE 3

FIGURE 2 General scheme for the coupling of metabolism to sodium transport.
FIGURE 3 Model of composite transport system.

resent by J_{Na}, and one for metabolism, which we represent by J_r:

$$J_{Na} = L_{Na}X_{Na} + L_{Nar}A, \tag{1}$$

$$J_r = L_{Nar}X_{Na} + L_rA. \tag{2}$$

Here X_{Na} is the negative electrochemical potential difference of sodium, and A is the affinity of the metabolic reaction which is driving sodium transport. Under the conditions of these experiments the affinity is equivalent to the negative Gibbs free energy change $-\Delta G$ of the driving reaction. The L's are phenomenological coefficients. For simplicity we assume the validity of linear nonequilibrium thermodynamics. J_{Na}, the rate of active sodium transport, is of course a function of the negative electrochemical potential difference X_{Na}, but to the extent that it is coupled to metabolism it is also a function of the affinity A. J_r, the rate of metabolism, here taken as oxygen consumption, is of course a function of A, but to the extent that it is linked to transport it is also a function of X_{Na}. By analogy with a variety of transport processes in nonliving systems (11, 12) the validity of the Onsager reciprocal relation is assumed, and so the cross-coefficients in the two equations are set equal. It may seem optimistic to represent a complex active transport process in such simple terms. Nevertheless, it seemed useful to do so in an attempt to systematize the analysis of the process, to correlate function in different states, and to evaluate the effectiveness of active transport. The appropriateness of the simple thermodynamic formulation can be defended on various theoretical grounds, but I would prefer rather to consider experimental evidence bearing on this point.

Our first study examines the behavior of the transport process. For the use of iden-

tical solutions at each surface there is no concentration difference across the membrane. Thus the electrochemical potential difference for sodium is given by $\mathfrak{F}\Delta\Psi$, where $\mathfrak{F}$ is the Faraday constant and $\Delta\Psi$ is the electrical potential difference across the membrane. If indeed the rate of active sodium transport is a linear function of the forces promoting transport, one might expect to find linear current-voltage relationships. Linear current-voltage relationships have in fact been described for epithelial tissues, but their significance has been unclear because of the possibility that an appreciable fraction of transport was by way of leak pathways. Obviously, to the extent that this is the case linear current-voltage relationships are of little interest, since they are to be expected in dilute aqueous electrolyte solutions. For our purpose it was important to study tissues in which transport by way of leak pathways was minimal. This was accomplished by care to avoid edge damage in mounting the tissues and by the use of a tracer isotope technique for the measurement of passive ion fluxes. This permitted the choice of tissues in which a high fraction of total conductance was by way of the active pathway. The results of such studies, carried out by Doctors Toshikazu Saito and Philip Lief in the toad bladder, are shown in Fig. 4. For the seven tissues represented, an average of some three-fifths of the total conductance was attributable to the active pathway. The finding of a steady-state linear normalized current-voltage relationship in these tissues strongly indicates that the rate of active sodium transport J_{Na} was in fact a linear function of the electrical potential difference (13). (I would point out that in order to demonstrate such linearity we observed the tissues under quasi-steady-state conditions some 15–30 s after the perturbation of $\Delta\Psi$. The observa-

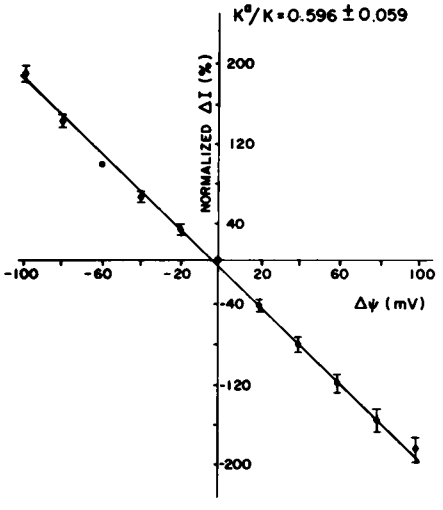

FIGURE 4

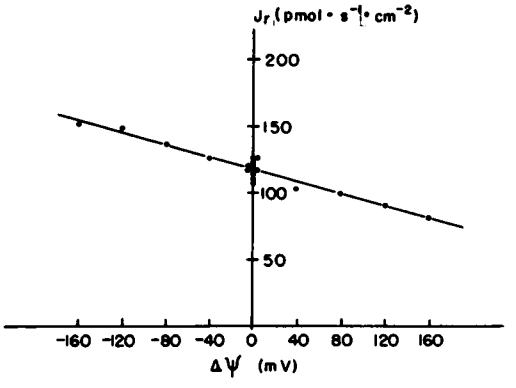

FIGURE 5

FIGURE 4 Normalized current-voltage relationship in the toad bladder.
FIGURE 5 Dependence of the rate of oxygen consumption J_r on the electrical potential difference $\Delta\Psi$ in the frog skin.

tion of transients, on the one hand, or perturbations for many minutes, on the other hand, may well give different results.) The demonstration of linearity of the active transport process is consistent with the use of Eq. 1, and indicates that the phenomenological coefficients and the affinity are unaffected by perturbations of $\Delta\Psi$ of the magnitudes and duration employed.

Studies by Dr. Francisco Vieira of the rate of oxygen consumption J_r in the frog skin are represented in Fig. 5 (14). I will not discuss the technique except to mention that it employs an adaptation of standard Ussing-Zerahn chambers permitting the utilization of oxygen electrodes. Again there was striking linearity, with steady-state J_r being a linear function of $\Delta\Psi$ over a range, in this instance, of +160 to −160 mV. The finding of linearity of J_r in $\Delta\Psi$ is consistent with the validity of Eq. 2, and again indicates constancy of phenomenological coefficients and invariance of the affinity with the perturbations of $\Delta\Psi$ employed.

The studies which I have just described examined the influence of only electrical driving forces on transport and metabolism. Recently, Doctors Danisi and Vieira, of the University of São Paulo, have reported the effects of concentration driving forces. In studies of toad skins in which the transmembrane electrical potential difference was nullified it was found that the rates of both active transport and oxygen consumption were linear functions of the chemical potential difference of sodium across the membranes (15).

Thus, the experimental results to date are consistent with the validity of a linear nonequilibrium thermodynamic approach, but of course the experiments cited have tested only the effects of variation of the electrochemical potential difference. The experimental techniques presently available do not permit a systematic study of the effects of variation of the affinity. Here however theoretical and experimental observations of others are pertinent. It is true that, for an isolated chemical reaction, linearity requires $A \ll RT$, where R is the gas constant and T the absolute temperature. However, as was pointed out by Professor Prigogine years ago, biochemical reactions with large A often consist of a large number of elementary reactions in series (16). In such cases the A's of the elemental reactions may be sufficiently small that the elemental reactions show linearity. Since in the steady state all series reactions occur at the same rate, J_r may then be linear in the overall affinity, which is the sum of the affinities of the individual reactions. A further consideration is the fact that under certain circumstances the kinetics of enzymatic reactions are compatible with linear nonequilibrium thermodynamic equations over an appreciable range (17).

Dr. Hagai Rottenberg has recently studied these matters in some detail from both a theoretical and experimental point of view (18). Rottenberg showed that for the case of mitochondrial oxidative phosphorylation the rates of both oxidation and phosphorylation are linear functions of both the affinity of the oxidation reaction and the affinity of phosphorylation in regions quite far removed from equilibrium. Furthermore, his system provided a striking example of the validity of the Onsager reciprocal relation.

Given the above support for the possibility of linear phenomenology from two

theoretical points of view, and Rottenberg's experimental results, it seems reasonable to pursue the possibility that active transport in epithelial tissues might indeed show linearity in both the electrochemical potential difference of sodium and the affinity of the metabolic driving reaction. If so, we can evaluate an "apparent" affinity by use of the two phenomenological equations above:

$$A = -I_0/(\mathrm{d}J_r/\mathrm{d}\Delta\Psi). \tag{3}$$

Here the numerator represents the current measured in the absence of both an electrical potential difference and a concentration difference across the membrane, the so-called "short-circuit current," and the denominator is the slope of the plot relating the rate of metabolism J_r (here oxygen consumption) to the electrical potential difference $\Delta\Psi$. In this model A represents the free energy change (per mole of O_2) for a characteristic region of the metabolic chain for which A remains constant on perturbation of $\Delta\Psi$. Admittedly, for the present this affinity is a rather vague quantity, being evaluated only in abstract thermodynamic terms. Nevertheless, it is of physiological interest, since it must reflect the substrate-product concentration ratio of some critical reaction in the metabolic pool which supports the active transport process. This is in contradistinction to estimates of substrate-product concentration ratios derived from chemical tissue analyses; although attempts have been made to study the driving forces for transport by this means, concentration ratios applicable to the tissue as a whole may well depend importantly on tissue functions other than transepithelial transport.

Clarification of the significance of the affinity calculated by the thermodynamic method above must await experiments correlating thermodynamic studies with a variety of biochemical procedures. Meanwhile, several experimental studies appear to support the validity of attempting to evaluate the affinity by the means described. The first, a phenomenon studied by Dr. Vieira in the frog skin, has been termed a "memory" effect (14). This involves the observation of the short-circuit current I_0 and the concomitant rate of oxygen consumption J_{r0}, before and after perturbation of the electrical potential. If we perturb the potential so as to much enhance sodium transport for an extended period, we find that on return to the short-circuit state both I_0 and J_{r0} are less than initially. These results are shown by the open circles of Fig. 6. As you see, after permitting the skin to function at $\Delta\Psi = -160$ mV for some 15–20 min, which substantially enhances the rates of transport and metabolism, on return to the short-circuit state, I_0 and J_{r0} have decreased (i.e. ΔI_0 and ΔJ_{r0} are negative). The black circles show the converse effect, noted after temporarily setting $\Delta\Psi$ at $+160$ mV, thereby depressing transport and metabolism. Both of these sets of observations are consistent with the interpretation that although brief perturbations of $\Delta\Psi$ have relatively little influence on tissue metabolite levels, sufficiently long perturbations of significant magnitude change substrate and product concentrations appreciably. The resultant change in the substrate-product ratio, and thus presumably in the affinity, manifests itself on return to the short-circuited state by alterations in the magnitudes of I_0 and J_{r0}.

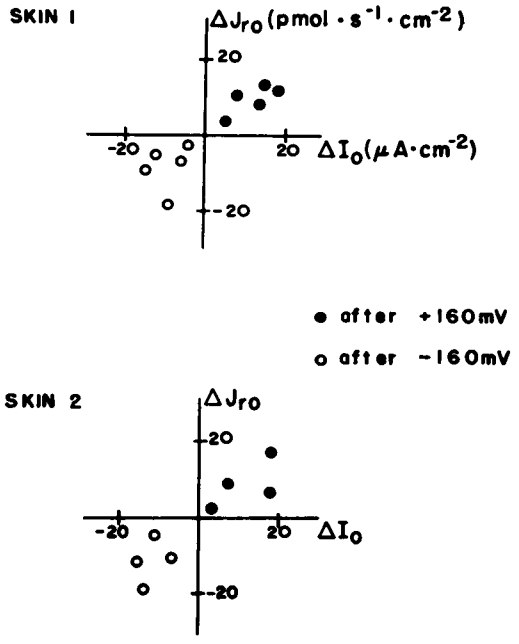

FIGURE 6

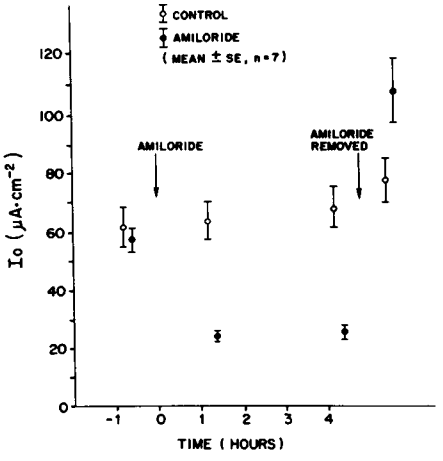

FIGURE 7

FIGURE 6 Effect of electrical potential perturbations on subsequent values of short-circuit current I_0 and oxygen consumption J_{r0} in the frog skin.
FIGURE 7 Effect of amiloride on short-circuit current in the frog skin.

A second study employs the diuretic amiloride. This pharmacological agent increases the excretion of salt by depressing the reabsorption of sodium from the kidney filtrate. Studies in the toad bladder demonstrate that amiloride depresses active sodium transport by interfering with the passive entry process at the outer (urinary) surface (19, 20). In our view of the mechanisms influencing the metabolic pools, we would anticipate that marked depression of passive entry would cause a continuing accumulation of the intermediary metabolites which drive transport, with a gradual increase in the value of the affinity. On removal of the drug this might be expected to cause an "overshoot," with higher rates of transport than prior to exposure to amiloride. Such overshoot has in fact been reported (21). Fig. 7 shows this effect again in our studies (22). Here the open circles represent "control" tissues and the solid circles represent paired "experimental" tissues to be treated with amiloride. For each pair the control and experimental tissues are derived from the same animal, assuring good matching of function prior to administration of the drug. Before the administration of amiloride the magnitude of I_0 is the same in the paired tissues. Following initial observations, amiloride was applied to the outer surface in concentrations sufficient to depress I_0 to about a third of the initial level for 4 h. On subsequent removal of the amiloride, I_0 in the treated tissues rose to a level significantly higher than in the initial

period and higher than observed simultaneously in the paired tissues. The question as to whether this behavior can be attributed to effects on the affinity is considered in Fig. 8. Before the administration of amiloride the apparent affinity "A" was the same in the control and experimental tissues, just as was the case for I_0. 1 h after the administration of amiloride "A" was not affected demonstrably. 4 h after the administration of amiloride, however, "A" in the treated tissues was significantly greater than initially and significantly greater than simultaneously in the paired control tissues.

A third study uses the cardiac glycoside ouabain, an inhibitor of the sodium-potassium-ATPase generally identified with the sodium pump. When ouabain is administered in a concentration adequate to eliminate active sodium transport there is, as expected, abolition of the dependence of oxygen consumption on the electrical potential difference. Since ouabain should have no direct effect on tissue metabolite levels, it would not be expected to have a prompt effect on the affinity. This prediction was recently tested by Dr. Albert Owen, in studies of frog skin (23). In order to apply our formulation for the evaluation of the affinity, it is necessary to depress active sodium transport substantially, but not completely, so that the quantity A in Eq. 3 will remain determinate. This was accomplished by the use of a low concentration of ouabain, 10^{-7} M. Within $2\frac{1}{2}$ h of exposure to this concentration of ouabain the short-circuit current in the treated or "experimental" hemiskin had gradually fallen to a level about half that in the paired "control" hemiskin. This was accompanied by a significant depression of the sensitivity of oxygen consumption to perturbation of $\Delta\Psi$, as is shown in Fig. 9, representing a typical result in nine experiments. As might be expected, incomplete inhibition of sodium transport for a relatively short period was not associated with a significant effect on the affinity.

Finally, Dr. Owen studied also the effect of 2-deoxyglucose, an inhibitor of carbohydrate metabolism (23). In view of its effects in other tissues, 2-deoxyglucose might

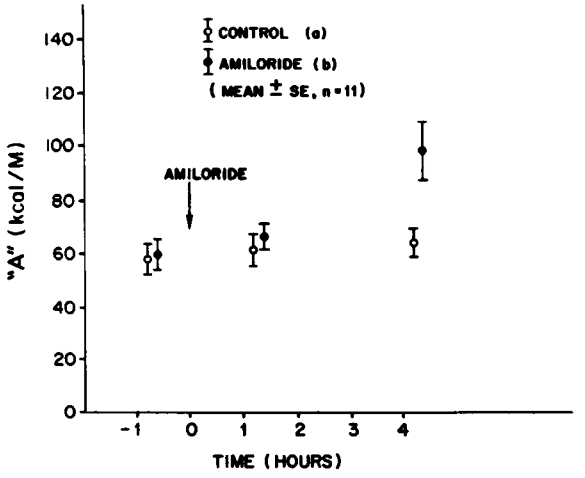

FIGURE 8 Effect of amiloride on the apparent affinity "A" in the frog skin.

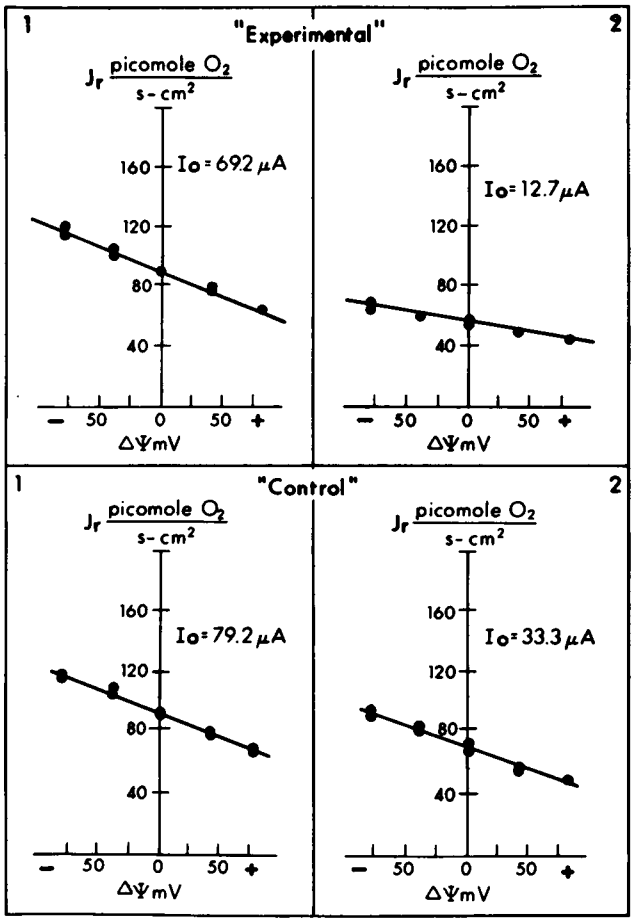

FIGURE 9 Effect of ouabain on the dependence of the rate of oxygen consumption J_r on the electric potential difference $\Delta\Psi$. Initially the slopes $dJ_r/d(\Delta\Psi)$ in paired hemiskins ("Control" 1 and "Experimental" 1) differed insignificantly. Following the administration of ouabain, $|dJ_r/d(\Delta\Psi)|$ was significantly less in the treated tissue ("Experimental" 2) than in the untreated tissue ("Control" 2).

be expected to depress the affinity of the metabolic reaction driving sodium transport. In 10 studies of frog skins exposed to 1 mM glucose, a concentration of 16 mM 2-deoxyglucose depressed active sodium transport, as measured by the short-circuit current, to an average of 58% of the control level. As expected, this was associated with a significant decrease in the affinity, in this case to 53% of control level.

The various lines of evidence which I have just discussed give us some tentative confidence in our interpretation of the nature of the metabolic pools driving transport and the significance of the affinity. On this basis it seems appropriate to utilize the thermodynamic formalism to investigate the mechanisms of action of substances which influence transport by unknown means. An appropriate substance for first studies is the

important hormone aldosterone. Aldosterone promotes substantial and stable enhancement of the rate of active sodium transport for extended periods. Because of its considerable importance much study has been directed at the mechanism of its action. Three main possibilities which have been considered are pictured in Fig. 10, adapted from Fanestil et al. (24). Mechanism 1 is facilitation of sodium movement across the outer passive permeability barrier. This mechanism has been suggested by Sharp and Leaf, Crabbé, and others (25, 26). Mechanism 2 is facilitation of the linkage between oxidative metabolism and the phosphorylation of ADP, resulting in enhancement of the ATP/ADP ratio. In our terms, enhancement of the ATP/ADP ratio (or the ratio of some other pair of appropriate reactants) corresponds to an increase in the affinity. Mechanism 3 involves a direct effect of aldosterone on the active transport mechanism, resulting in the more rapid pumping of sodium.

The results of studies of Dr. Saito concerning the mechanism of aldosterone action in the frog skin are shown in the next two figures. Prior to the administration of aldosterone, the short-circuit current in paired control and experimental tissues was the same. Following overnight exposure to physiological concentrations of aldosterone, I_0 in the control tissues was much the same as initially, whereas I_0 in the treated tissues was appreciably greater. These results are essentially the same as described by many others. Fig. 12 shows the effect of aldosterone on the apparent affinity. As with I_0, the value of "A" was initially the same in control and experimental tissues. Following overnight exposure to aldosterone, "A" was appreciably greater in the treated tissues than in the untreated tissues. These results suggest that one means whereby aldosterone may enhance the rate of active sodium transport is by increasing the affinity of the driving metabolic reaction, as in mechanism 2 above.

This does not appear however to be the sole means by which aldosterone can influence transport. It has been pointed out previously by Civan and Hoffman that aldosterone increases the electrical conductivity of toad bladders (27). This effect is shown in Fig. 13, representing the results of studies of Saito in the toad bladder (28).

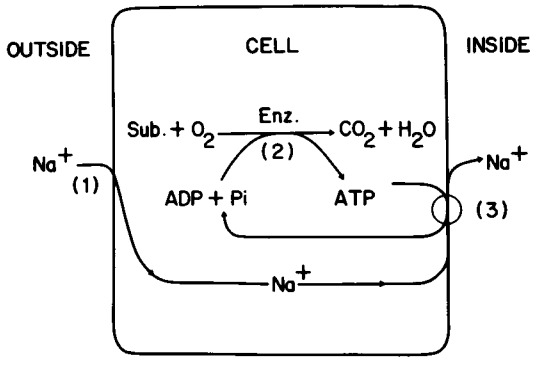

FIGURE 10 Model of the active sodium transport system; possible mechanisms of regulation of transport (adapted from Fanestil et al. [24]).

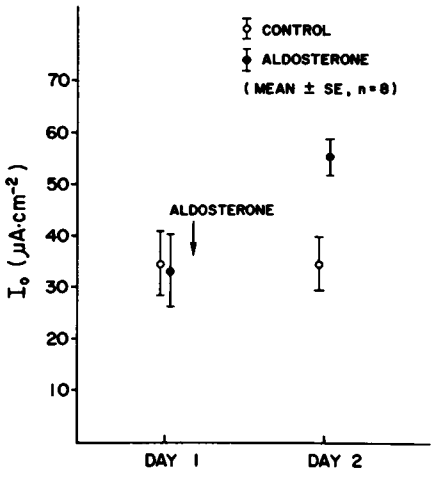

FIGURE 11

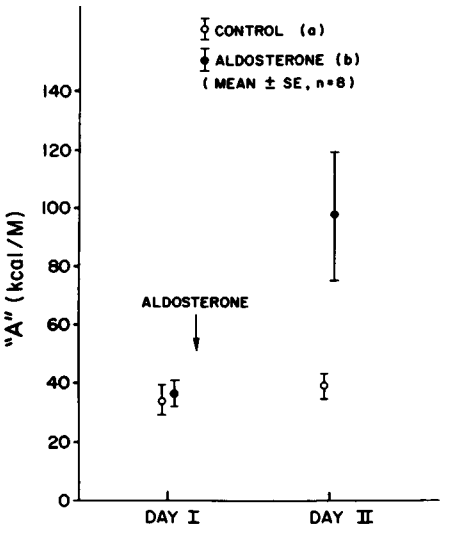

FIGURE 12

FIGURE 11 Effect of aldosterone on short-circuit current I_0 in the frog skin.
FIGURE 12 Effect of aldosterone on the apparent affinity "A" in the frog skin.

Paired control and experimental tissues showed closely similar values of electrical conductance κ during the 3 h prior to the administration of aldosterone. Some 2 h after the administration of aldosterone, at a time when stimulatory effects on the current were observed, the conductance of the aldosterone treated tissues had increased comparably. These increments in current and conductance increased progressively during the final 6 h of the experiment. This observation is of interest, since thermodynamic analysis indicates that it is unlikely to be attributable to any increase in the

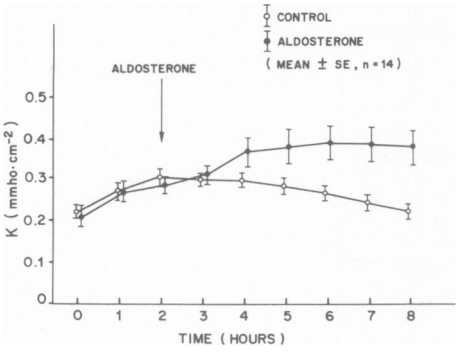

FIGURE 13

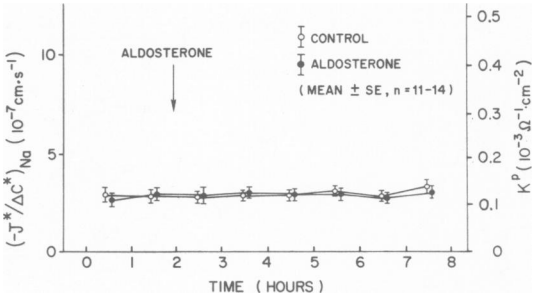

FIGURE 14

FIGURE 13 Effect of aldosterone on the electrical conductance of the toad bladder.
FIGURE 14 Effect of aldosterone on the passive conductance of the toad bladder.

affinity which might be occurring concurrently. Rather it suggests an independent effect on permeability factors.

In order to determine whether the observed effects on conductance are in fact attributable to movement of sodium by way of the active pathway it was necessary to evaluate the effect of aldosterone on the conductance of the passive pathway κ^p. Such studies were carried out by means of isotopic techniques, κ^p being evaluated from passive tracer sodium permeability $(-J^*/\Delta c^*)_{Na}$. The results are shown in Fig. 14. The passive conductances of paired control and experimental tissues were identical both before and after the administration of aldosterone. Thus the influence of aldosterone on conductance must be attributed to effects on the active pathway. This is demonstrated in Fig. 15. At the time when aldosterone enhances sodium transport there is enhancement of the active conductance κ^a in the treated tissues, but not in the control tissues.

In attempting to relate these effects of aldosterone on conductance to its earlier demonstrated effects on the affinity it must be remembered that the two studies were carried out in different tissues, and at different intervals following the administration of aldosterone. Nevertheless, the results suggest that aldosterone may have a dual effect on the active sodium transport mechanism, acting to increase both the affinity and permeability. This possibility has been suggested by Lipton and Edelman on the basis of measurements of sodium concentration in isolated epithelial cells of the toad bladder (29). Presuming that there is indeed a dual effect, it is of interest to speculate whether this is a specific manifestation of aldosterone function, or an example of a more general phenomenon such that any factor which alters pump function will produce parallel effects on permeability, thereby regulating entry into the sodium active transport pool. Such a mechanism might serve to stabilize intracellular sodium. We are now doing experiments to clarify this matter.

In summary, our results to date lead us to the tentative conclusion that it may be possible to characterize active sodium transport in representative model tissues in terms of a simple linear nonequilibrium thermodynamic treatment. If further studies establish this to be the case we may be able to gain important insights which are un-

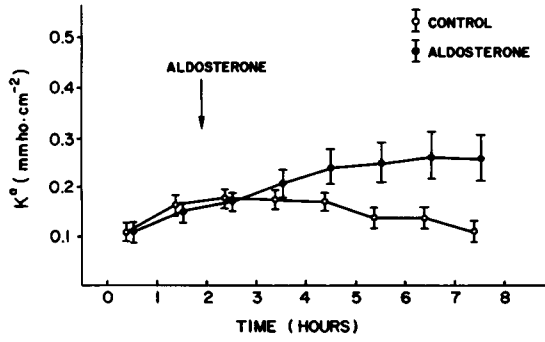

FIGURE 15 Effect of aldosterone on the active conductance of the toad bladder.

available with present approaches. Obviously, if we are to have confidence in our ability to do so, we must obtain a variety of information correlating biochemical and thermodynamic considerations.

Although many fundamental questions remain, we can be confident that our eventual understanding will be enhanced by Aharon Katchalsky's earlier devotion to these matters. (Tragically, subsequent to the presentation of this paper it has become most appropriate to acknowledge a similar debt to the organizer of this symposium, Peter Curran.)

I am grateful to Doctors S. R. Caplan and R. C. DeSousa for comments on this manuscript.

This work is supported by USPHS Grant HL 14322 to the Harvard-MIT Program in Health, Sciences, and Technology, NSF Grant GB-40704, and USPHS Grant AM 17817.

REFERENCES

1. Ussing, H. H., and K. Zerahn. 1951. Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* **23**:110.
2. Ussing, H. H. 1960. The Alkali Metal Ions in Biology. Springer-Verlag, Berlin.
3. Leaf, A., J. Anderson, and L. B. Page. 1958. Active sodium transport by the isolated toad bladder. *J. Gen. Physiol.* **41**:657.
4. Kedem, O., and A. Essig. 1965. Isotope flows and flux ratios in biological membranes. *J. Gen. Physiol.* **48**:1047.
5. Zerahn, K. 1956. Oxygen consumption and active sodium transport in the isolated and short-circuited frog skin. *Acta Physiol. Scand.* **36**:300.
6. Zerahn, K. 1958. Doctoral thesis. Universitetsforlaget I Aarhus, Aarhus, Denmark.
7. Vieira, F. L., S. R. Caplan, and A. Essig. 1972. Energetics of sodium transport in frog skin. I. Oxygen consumption in the short-circuited state. *J. Gen. Physiol.* **59**:60.
8. Hoshiko, T., and B. D. Lindley. 1967. Phenomenological description of active transport of salt and water. *J. Gen. Physiol.* **50**:729.
9. Essig, A., and S. R. Caplan. 1968. Energetics of active transport processes. *Biophys. J.* **8**:1434.
10. Kedem, O. 1961. *In* Membrane Transport and Metabolism. A. Kleinzeller and A. Kotyk, editors. Publishing House of the Czechoslovak Academy of Sciences, Prague, Czechoslovakia. 87.
11. Miller, D. G. 1960. Thermodynamics of irreversible processes. The experimental verification of the Onsager reciprocal relations. *Chem. Rev.* **60**:15.
12. Blumenthal, R., S. R. Caplan, and O. Kedem. 1967. The coupling of an enzymatic reaction to transmembrane flow of electric current in a synthetic "active transport" system. *Biophys. J.* **7**:735.
13. Saito, T., P. D. Lief, and A. Essig. 1974. Conductance of active and passive pathways in the toad bladder. *Am. J. Physiol.* **226**:1265.
14. Vieira, F. L., S. R. Caplan, and A. Essig. 1972. Energetics of sodium transport in frog skin. II. The effects of electrical potential on oxygen consumption. *J. Gen. Physiol.* **59**:77.
15. Danisi, G., and F. L. Vieira. 1974. Nonequilibrium thermodynamic analysis of the coupling between active sodium transport and oxygen consumption. *J. Gen. Physiol.* **64**:372.
16. Prigogine, I. 1961. Thermodynamics of Irreversible Processes. John Wiley & Sons, Inc., New York.
17. Caplan, S. R. 1971. Nonequilibrium thermodynamics and its application to bioenergetics. *Curr. Top. Bioenerg.* **4**:1.
18. Rottenberg, H. 1973. The thermodynamic description of enzyme-catalyzed reactions. *Biophys. J.* **13**: 503.
19. Bentley, P. J. 1968. Amiloride: a potent inhibitor of sodium transport across the toad bladder. *J. Physiol. (Lond.).* **195**:317.
20. Handler, J. S., A. S. Preston, and J. Orloff. 1972. Effect of ADH, aldosterone, ouabain, and amiloride on toad bladder epithelial cells. *Am. J. Physiol.* **222**:1071.
21. Robbie, D. R. 1971. Ph.D. thesis. Harvard University, Cambridge, Mass.

22. SAITO, T., A. ESSIG, and S. R. CAPLAN. 1973. The effect of aldosterone on the energetics of sodium transport in the frog skin. *Biochim. Biophys. Acta.* **318**:371.
23. OWEN, A., S. R. CAPLAN, and A. ESSIG. 1974. Effect of ouabain and 2-deoxy-D-glucose on thermodynamic variables in frog skin. *Fed. Proc.* **33**:215.
24. FANESTIL, D. D., G. A. PORTER, and I. S. EDELMAN. 1967. Aldosterone stimulation of sodium transport. *Biochim. Biophys. Acta.* **135**:74.
25. SHARP, G. W. G., and A. LEAF. 1966. Mechanism of action of aldosterone. *Physiol. Rev.* **46**:593.
26. CRABBÉ, J. 1963. Site of action of aldosterone on the bladder of the toad. *Nature (Lond.).* **200**:787.
27. CIVAN, M. M., and R. E. HOFFMAN. 1971. Effect of aldosterone on electrical resistance of toad bladder. *Am. J. Physiol.* **220**:324.
28. SAITO, T., and A. ESSIG. 1973. Effect of aldosterone on active and passive conductance and E_{Na} in the toad bladder. *J. Membr. Biol.* **13**:1.
29. LIPTON, P., and I. S. EDELMAN. 1971. Effects of aldosterone and vasopressin on electrolytes of toad bladder epithelial cells. *Am. J. Physiol.* **221**:733.