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Effect of Aldosterone on Active and Passive Conductance and E_{Na} in the Toad Bladder

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Summary. It has been demonstrated previously that aldosterone increases the electrical conductance of the toad bladder in association with the stimulation of active sodium transport. In the present study the concurrent measurement of electrical quantities and ion tracer flux distinguishes effects on active and passive pathways. Lack of an effect on passive Na^+ or Cl^- tracer flux in hemibladders preselected to eliminate large artefactual leaks indicates that aldosterone has no influence on physiological passive conductance. Thus, the enhancement of electrical conductance is entirely attributable to the active pathway. The magnitude of the increase in the active conductance was estimated. The data permitted also the comparison of effects on the flux ratio of Na^+ at short circuit (f_0) and the electrical potential difference adequate to abolish active sodium transport (E_{Na}). Even in membranes with minimal leakage the flux ratio does not reliably reflect E_{Na}. Aldosterone increased mean f_0 from 11 to 22, but did not affect E_{Na}.

Active sodium transport across the toad bladder is believed to comprise two processes: (1) "passive" movement across the apical (mucosal) surface into the cell [13], followed by (2) "active" extrusion at the basal-lateral (serosal) surface [14]. Accordingly, in attempting to explain how aldosterone facilitates sodium transport, two main hypotheses have been advanced. Crabbé [5] and Sharp and Leaf [21, 22] have suggested that aldosterone increases mucosal permeability, permitting more rapid passive inflow and elevation of the intracellular sodium concentration, with resultant enhancement of active transport across the serosal surface. Edelman and his co-workers [12, 20], on the other hand, have urged the importance of energetic factors. In this view aldosterone might increase sodium transport either by increasing the supply of metabolic energy to the sodium "pump," or by facilitating the linkage of metabolism to transport. Recently, it has been suggested that both permeability and energetic factors may be involved [18].

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Certain of the arguments for these views have been derived from characteristics of ion transport. Thus, Porter and Edelman [20] and Fanestil, Porter and Edelman [12] inferred that an increase of the flux ratio of Na^+ following aldosterone suggests an effect on the Na^+ pump, independent of an effect on permeability. Civan and Hoffman [3], on the other hand, have reported that aldosterone decreases the electrical resistance of the toad bladder. Since this was also observed in substrate-depleted tissues and was unaffected by the addition of pyruvate it was felt that the effect was not attributable to energetic factors, but rather to a reduction of the resistance to active sodium transport, either at the pump site or at the series permeability barrier.

In interpreting the above findings it was appreciated that the flux ratio and the electrical conductance reflect ion movement not only by way of the active transport pathway, but also through parallel passive channels. Aldosterone might conceivably act on either or both of these pathways. Porter and Edelman [20] and Fanestil *et al.* [12] found that the back-diffusion of Na^+ from the serosal to the mucosal solutions (i. e., the "passive path") is unaffected by aldosterone, but their studies were performed before recognition of the significant influence of edge damage on passive fluxes [7, 16, 27]. Furthermore, aldosterone might possibly influence the conductance by altering the Cl^- flux, which has been found to account for about half of the passive conductance in the absence of aldosterone.[1]

We have recently reported isotope techniques to characterize the passive ionic channels in the toad bladder and to evaluate their contribution to conductance.[1] In the present study we utilize these techniques in membranes with minimal artefactual leakage to estimate the extent to which the enhancement of conductance by aldosterone is attributable to the active pathway. The data also permit an analysis of the utility of the flux ratio in evaluation of the "electromotive force of sodium transport," E_{Na} [17, 24, 25].

Materials and Methods

Female toads (*Bufo marinus*, from the Dominican Republic; National Reagents, Bridgeport, Connecticut) were partially immersed in 0.6% saline at room temperature for at least 48 hr before use, to suppress the endogenous secretion of aldosterone [22]. All studies were carried out in paired hemibladders from a single toad. The membranes were mounted in modified Ussing-Zerahn Lucite chambers [25] of 7.54 cm^2 cross-sectional area. Each reservoir was filled with 15 to 17 ml of glucose sodium Ringer's solution. The electrical potential difference $\Delta\Psi$ was regulated with a voltage clamp, and the current I [mucosa (M) to serosa (S)] was recorded continuously.

1 T. Saito, P. D. Lief and A. Essig. Conductance of active and passive pathways in the toad bladder. *Amer. J. Physiol.* (*To be published*)

Experimental Protocol

^{22}Na *Studies.* After 20 to 30 min of incubation at short circuit, $\Delta\Psi$ was clamped at 50 mV, with S positive. Five minutes later, 30 μC of ^{22}NaCl was instilled in the serosal solution. Following 15 min of equilibration, and at 60-min intervals thereafter for 8 hr, 100 μliter samples were taken from each bathing solution. After the two initial periods 10 μliters of methanol solution of D-aldosterone were added to the serosal solution of one of the tissues (final concentration 5×10^{-7} M) and 10 μliters of methanol were added to the serosal solution of the control tissue. The chambers used for the control and aldosterone-treated tissues were alternated in successive experiments.

The electrical conductance was measured hourly, 1 min after sampling of the solutions. Otherwise, $\Delta\Psi$ was maintained at 50 mV.

^{36}Cl *Studies.* The protocol was identical to that above except for the omission of ^{22}NaCl and the instillation of 8 μC of Na^{36}Cl into the mucosal solution.

Determination of Tracer Fluxes

The 100 μliter samples were counted with a Packard Tri-Carb liquid scintillation spectrometer. The quantity $-J^x/\Delta c^x$ was computed, where J^x is the tracer flux (activity $cm^{-2}\ sec^{-1}$) and Δc^x is the difference in tracer concentration between the two baths (activity cm^{-3}). For convenience, this quantity was denoted by $(\overleftarrow{J^x/\Delta c^x})_{Na}$ for "outward" ($S \rightarrow M$) Na^+ tracer flux and by $(\overrightarrow{J^x/\Delta c^x})_{Cl}$ for "inward" ($M \rightarrow S$) Cl^- tracer flux.

Conductance Determinations

Since the current-voltage relationship is linear over the voltage range of present interest,[1] the total conductance κ (mmho cm^{-2}) was evaluated as $-\Delta I/\Delta(\Delta\Psi)$, setting $\Delta\Psi$ first at 60 mV and then at 40 mV for 10 to 15 sec.

Cl^-, I^- and K^+ fluxes, and outward Na^+ flux are thought to be passive. Ratios between any two of these are approximately constant from bladder to bladder, indicating that variations in tracer permeability between different bladders result from variation in the conductance of a common pathway, rather than in the pattern of ion selectivity. Therefore, utilizing an expression developed previously, the determination of the flux of one ion suffices to estimate the total passive conductance κ^p (mmho cm^{-2}).[1] With ^{22}Na, for the solutions employed here,

$$\kappa^p = 4.04 \times 10^5 (\overleftarrow{J^x/\Delta c^x})_{Na,\ \Delta\Psi=50}. \tag{1}$$

With ^{36}Cl,

$$\kappa^p = 3.44 \times 10^5 (\overrightarrow{J^x/\Delta c^x})_{Cl,\ \Delta\Psi=50}. \tag{2}$$

The constants in Eqs. (1) and (2) are obtained by summing the products of the appropriate permeability ratios and concentrations for each ion present.

The conductance of the active pathway κ^a (mmho cm^{-2}) is given by

$$\kappa^a = \kappa - \kappa^p. \tag{3}$$

Materials

The glucose sodium Ringer's solution consisted of 10 mM glucose, and 115.9 Na, 2.5 K, 1.8 Ca, 117.8 Cl and 2.4 HCO_3 mEquiv/liter (pH 7.6, 233 mOsm/kg H_2O), and contained 1.0 mg/ml of streptomycin sulfate (Pfizer Laboratories) and 0.5 mg/ml of penicillin G

(Upjohn). Solutions were prepared daily from concentrated stock solutions. ^{22}Na was obtained from New England Nuclear, Boston, Mass., and ^{36}Cl from Cambridge Nuclear Corporation, Cambridge, Mass. D-aldosterone-21-acetate was kindly provided by Dr. Maurice Pechet.

Analysis of the Data

Results are presented as the mean value $\pm$ the standard error of the mean (SE). Results in paired hemibladders were compared by Student's t test [23].

Results

All experiments were performed in paired hemibladders from a single toad, one serving as the control, the other as the experimental tissue. Membranes were mounted gently to avoid trauma. Pairs for which either hemibladder showed an initial electrical resistance less than 300 Ω or an open-circuit potential less than 15 mV were discarded. About a third of the membranes were rejected on this basis. Each experiment consisted of eight consecutive 1-hr periods, in each of which the electrical current and conductance and either the $S \rightarrow M$ ^{22}Na or $M \rightarrow S$ ^{36}Cl flux were determined. Following the second period, the experimental tissues were exposed to aldosterone. Because passive ionic fluxes in undamaged bladders are small, the accuracy of determination of the tracer fluxes was enhanced by the use of a favorable electrical potential gradient ($\Delta \Psi = 50$ mV, S positive).[1]

Effects on Electrical Current, Total Conductance and Passive Conductance

^{22}Na Studies (n = 14)

Electrical Current. The behavior of the electrical current $I_{\Delta \Psi = 50}$ was similar in the paired tissues prior to the administration of aldosterone (Fig. 1). Despite the unfavorable electrical potential gradient, a positive current was observed 1 hr after the start of the experiment. In the control hemibladders the current rose for 2 hr and then fell gradually. The hormone-treated tissues showed a sustained increase, with a significantly higher level than in the control tissues 3 hr after the administration of aldosterone.

Conductance (κ). As is seen in Fig. 2, there was no significant difference in the conductance of paired hemibladders from the beginning of the experiment until 2 hr after the administration of aldosterone. In control tissues, κ increased slightly but significantly over the first 2 hr and then decreased gradually, reaching the initial level at 8 hr. The conductance of the treated tissues showed a sustained increase, exceeding that of the control tissues 3 hr after the administration of aldosterone. At 8 hr the conductance of the

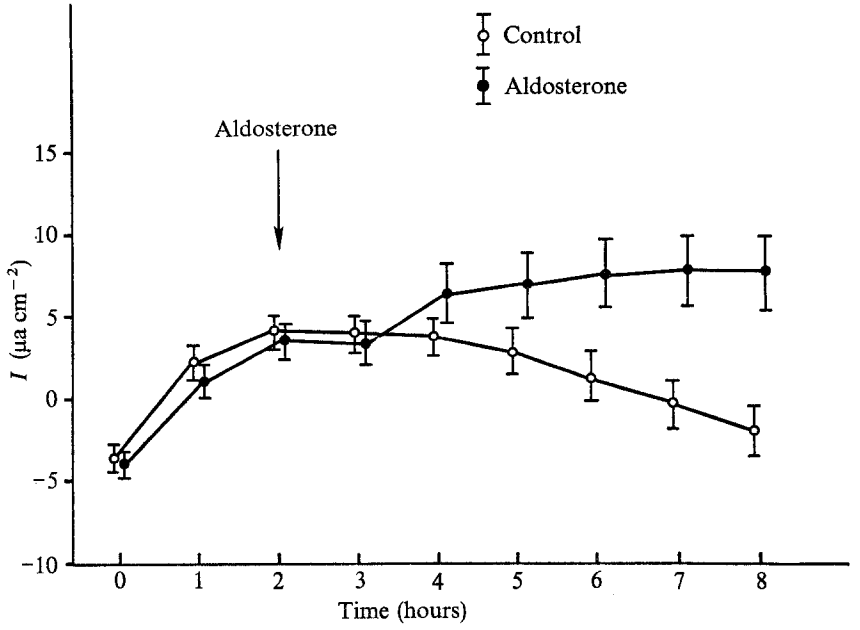

Fig. 1. Effect of aldosterone on the electrical current $I_{\Delta\Psi=50}$ (mean ±SE; n=14). At 5 hr (3 hr following the administration of aldosterone) and thereafter the control and treated tissues differed significantly. p (5) <0.01; p (6), p (7) and p (8) <0.001

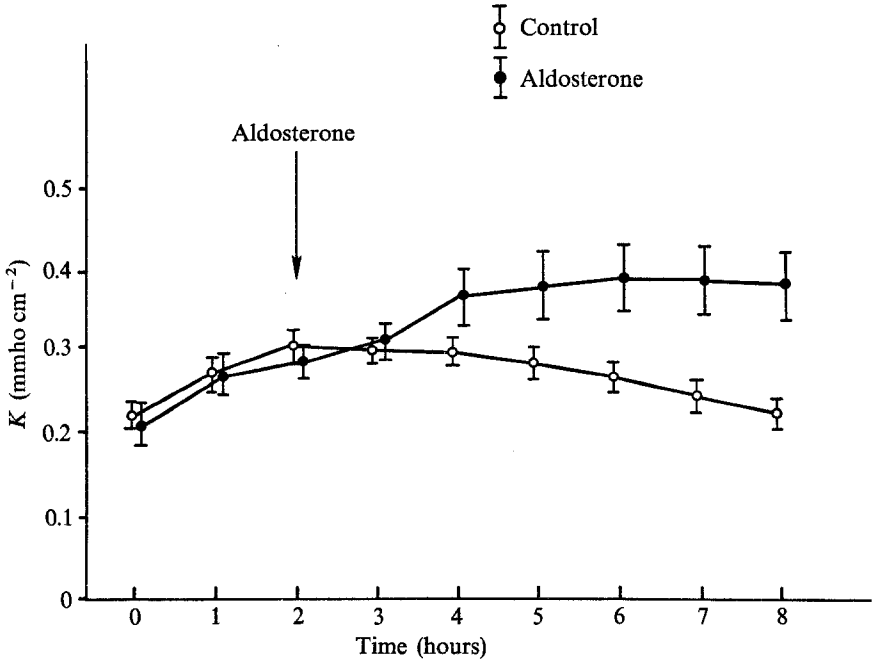

Fig. 2. Effect of aldosterone on the electrical conductance κ (mean ±SE; n=14). p (5)<0.025; p (6) <0.01; p (7)<0.005; p (8)<0.001

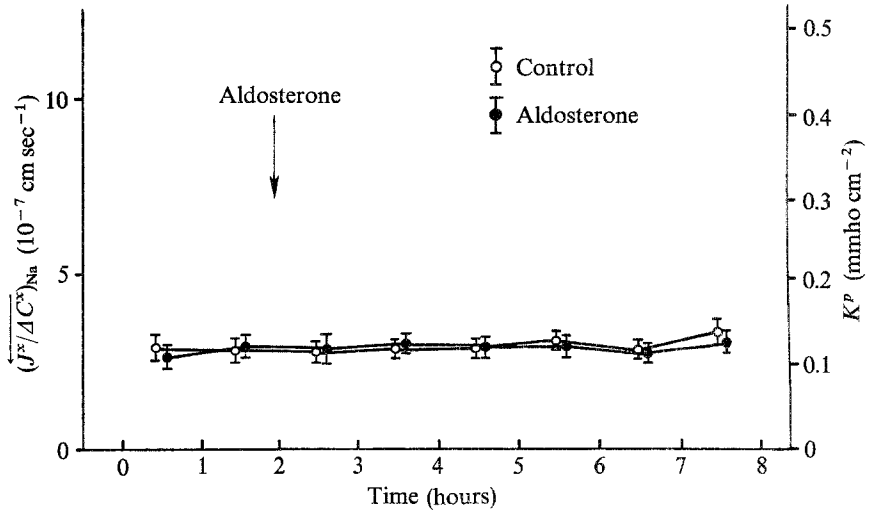

Fig. 3. Effect of aldosterone on $(\overleftarrow{J^x/\Delta c^x})_{Na,\,\Delta\Psi=50}$ and the passive conductance κ^p calculated from ^{22}Na flux [Eq. (1)] (mean $\pm$SE; n = 11 to 14). All p's were >0.05

aldosterone-treated tissues was 70% greater than that of the control tissues. The effects on electrical conductance paralleled those on the current in both time course and magnitude. These results are closely similar to those reported by Civan and Hoffman [3].

Passive Na^+ *Tracer Flux and Conductance of the Passive Pathway* (κ^p). The mean values of the tracer permeability $(\overleftarrow{J^x/\Delta c^x})_{Na,\,\Delta\Psi=50}$ are shown in Fig. 3. (Five values out of 224 involved an obvious sampling error and therefore the corresponding five pairs of data were omitted from the analysis.) There was no significant difference between the control and experimental tissues either before or after the administration of aldosterone.

On the basis of a previous study,[1] the above data may be used to calculate the conductance of the passive pathway [Eq. (1)]. The mean values of κ^p obtained in this way can be read on the scale at the right of Fig. 3.

^{36}Cl Studies (n = 10)

Previous studies have indicated that in the absence of aldosterone passive Na^+ and Cl^- tracer fluxes are proportional.[1] Therefore, it is possible to use either to evaluate the passive conductance. In attempting to use the Na^+ tracer flux for this purpose in the present studies, however, we were concerned that aldosterone might alter the permeability to Cl^- selectively. If so, observations of Na^+ tracer flow and the application of Eq. (1) would fail to evaluate κ^p.

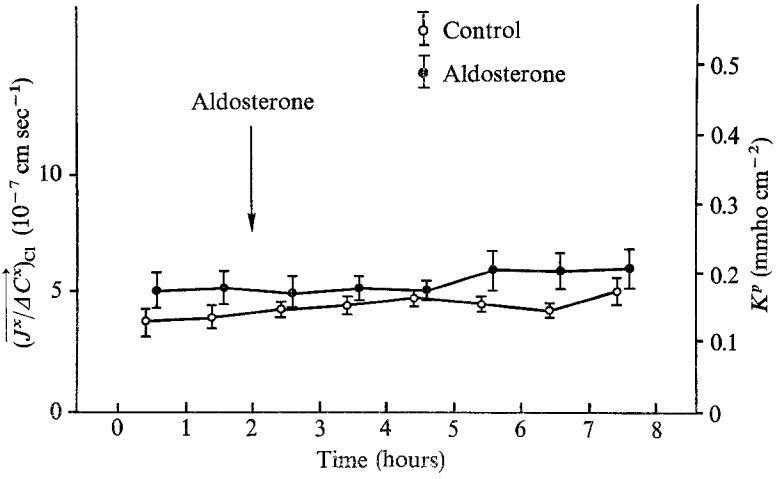

Fig. 4. Effect of aldosterone on $(\overrightarrow{J^x/\Delta c^x})_{Cl,\,\Delta\Psi=50}$ and the passive conductance κ^p calculated from ^{36}Cl flux [Eq. (2)] (mean $\pm$SE; n = 10). All p's were >0.05

To test this possibility we performed experiments identical to those above, except for the evaluation of Cl^- influx $(\overrightarrow{J^x/\Delta c^x})_{Cl,\,\Delta\Psi=50}$ rather than Na^+ efflux $(\overleftarrow{J^x/\Delta c^x})_{Na,\,\Delta\Psi=50}$. In 10 pairs of membranes the behavior of the electrical current and the conductance was similar to that in the Na^+ series, although the magnitudes of κ and κ^p were somewhat greater and there was more scatter. Values of $(\overrightarrow{J^x/\Delta c^x})_{Cl}$ in the control and experimental tissues differed insignificantly both before and after aldosterone (Fig. 4).

Since aldosterone has no effect on Cl^- tracer flux it is appropriate to use either Eq. (1) or Eq. (2) to evaluate κ^p. The results of the application of Eq. (2) to the Cl^- tracer flux data can be read from the scale on the right of Fig. 4.

Conductance of the Active Pathway (κ^a)

Since aldosterone has no significant effect on passive conductance its effects on total conductance are entirely attributable to the active pathway. The magnitude of these effects may be estimated with the use of Eqs. (1)–(3). As is shown in Fig. 5 for the Na^+ tracer data, the time course of κ^a in both treated and untreated tissues was closely similar to that of the electrical current and the total conductance. Six hours following the administration of aldosterone, κ^a in the treated tissues was 2.18 times that in the controls. The response of κ^a deduced from observations of ^{36}Cl flux is similar to that in the studies with ^{22}Na (Fig. 6).

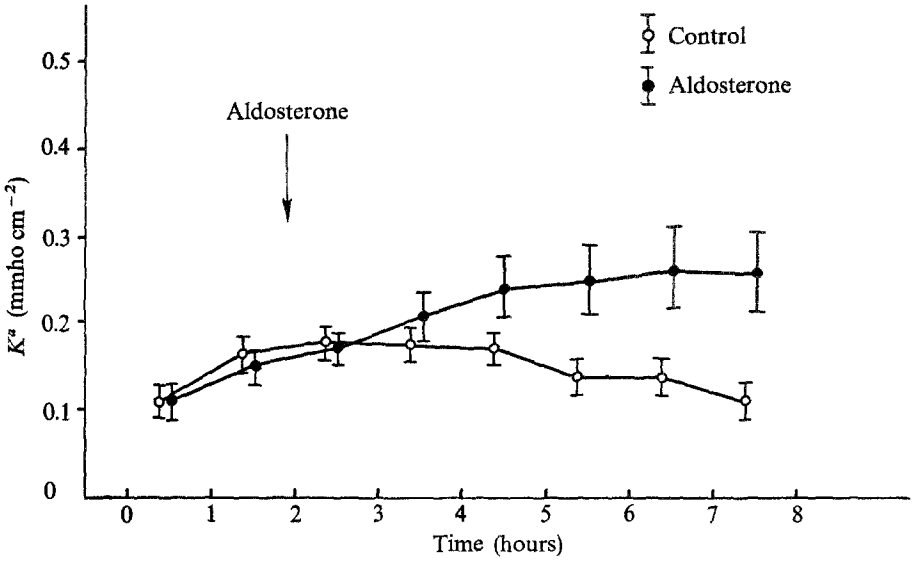

Fig. 5. Effect of aldosterone on the active conductance κ^a calculated from ^{22}Na tracer flux (mean $\pm$SE; n = 12 to 14). For each period the total conductance was taken as the arithmetic mean of the values of κ at the beginning and end, so as to correspond temporally to the value of κ^p calculated from tracer flux [Eq. (1)]. κ^a was then given by $\kappa - \kappa^p$ [Eq. (3)]. p (4, 5) and p (5, 6) <0.05; p (6, 7) <0.01; p (7, 8) <0.005

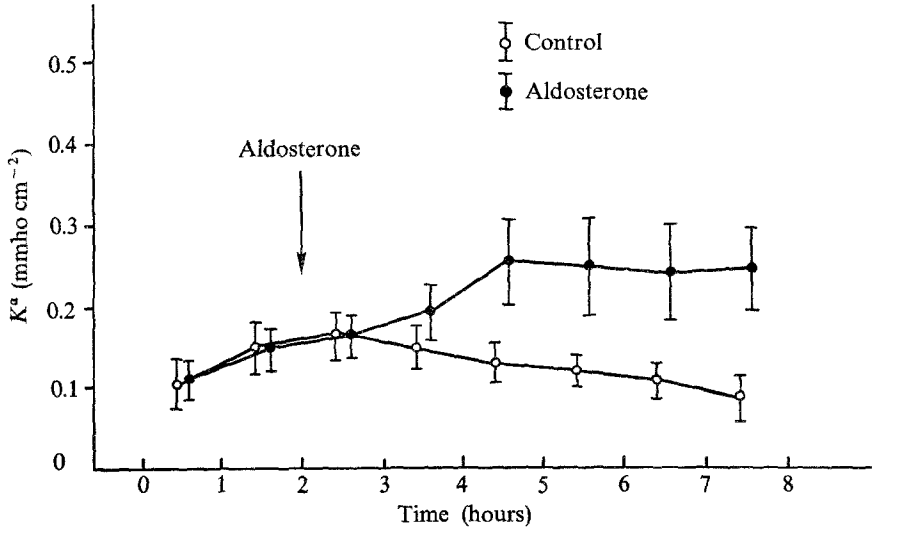

Fig. 6. Effect of aldosterone on the active conductance κ^a calculated from ^{36}Cl tracer flux (mean $\pm$SE; n = 10). *See* legend of Fig. 5. p (7, 8) <0.05

Discussion

The significance of the electrical behavior and permeability patterns of epithelial tissues is often obscure, owing to ignorance of the routes of ion movement. The techniques presented here distinguish between effects on passive and active pathways, thereby clarifying the influence of permeability factors in active sodium transport. The approach also permits analysis of the usefulness of the flux ratio for evaluation of the electromotive force of sodium transport.

To study toad bladders in this manner, however, it is necessary to minimize ion movement by way of artificial channels.

Edge Damage

It has recently been emphasized that the *in vitro* permeability of tissues is often largely artefactual, reflecting edge damage [7, 16, 27]. Such damage is absent in sac preparations [27]. Helman and Miller [16] have shown that with a special mounting technique and the use of a sealant it is possible to eliminate chamber edge damage almost completely. We have found that it is possible to minimize the damage associated with the standard techniques.[1] For this purpose we employ chambers of large cross-sectional area (7.54 cm^2), giving a favorably small edge/surface ratio (1.29 cm^{-1}). Extra large toads provide bladders sufficiently large to be mounted without stretching, even in experiments requiring two membranes from a single animal. Screening of the initial electrical resistance eliminates severely damaged tissues, permitting the study of membranes in which passive permeation is largely by way of physiological channels.

Effect of Aldosterone on the Permeability of the Physiological Passive Pathway

The failure of aldosterone to influence $S \rightarrow M$ Na^+ tracer flux has been reported previously by Edelman and co-workers [12, 20], but artefactual leakage was likely to have been significant in those studies (*see* footnote 2).

2 Several considerations suggest that artefactual leakage was likely to have been more significant in the studies of Edelman and co-workers [12, 20] than in the present study: The use of circular chambers with a cross-sectional area of 2.54 cm^2 would give an edge/surface ratio of 2.22 cm^{-1}, as compared with our 1.29 cm^{-1}. Porter and Edelman [20] found flux ratios of 2.9 and 4.4 in their control and aldosterone-treated tissues, respectively; the corresponding (derived) values for the present study are 11 and 22. Fanestil *et al.* [12] found unidirectional $S \rightarrow M$ Na fluxes of 5.9 to 7.3 μequiv/2.54 cm^2-hr at $\Delta\Psi =$ 100 mV (their Fig. 4). Our values of $(\overleftarrow{J^x/\Delta c^x})_{Na,\,\Delta\Psi=50}$ were 0.86 to 6.13 $\times 10^{-7}$ cm sec^{-1}. Presuming that increasing $\Delta\Psi$ from 50 to 100 mV does not alter the permeability characteristics of the membrane, this range of values would correspond to $S \rightarrow M$ flux $\overleftarrow{J}_{Na,\,\Delta\Psi=100}$ of 0.16 to 1.14 μequiv/2.54 cm^2-hr.[1]

In the present study, tracer permeabilities were low and an appreciable fraction of total conductance was attributable to the active pathway: κ^a/κ was initially 0.519 ± 0.043 (n = 26) in the ^{22}Na experiments and 0.390 ± 0.057 (n = 20) in the ^{36}Cl experiments. These findings suggest minimal edge damage. Hence the lack of an effect of aldosterone on either $(\overleftarrow{J^x/\Delta c^x})_{Na, \Delta\Psi=50}$ or $(\overrightarrow{J^x/\Delta c^x})_{Cl, \Delta\Psi=50}$ indicates strongly that aldosterone does not affect the permeability of the physiological passive pathway.

Effect of Aldosterone on the Permeability of the Active Pathway

The present results are relevant to Civan and Hoffman's [3] demonstration of a fall in electrical resistance in the toad bladder following the administration of aldosterone. This was interpreted as suggesting that aldosterone stimulates transepithelial sodium transport by reducing the resistance to movement of sodium ions. However, since aldosterone increased the short-circuit current by 90%, while reducing the electrical resistance by only 18%, the following question arose: Could the data be attributed exclusively to an effect on Na^+ entry? As Civan and Hoffman [3] pointed out, the discrepancy is explicable to the extent that the resistance reflects flux through both passive and active channels, but not knowing the contributions of each, this factor could not be analyzed quantitatively.

This matter is clarified by our study of $S \rightarrow M$ ^{22}Na fluxes, where over half of the initial conductance was attributable to the active pathway. Six hours after the administration of aldosterone, when the ratio of mean short-circuit currents $\overline{I_0\,(\text{aldo})}/\overline{I_0\,(\text{control})}$ was 2.75, the ratio of mean conductances $\overline{\kappa\,(\text{aldo})}/\overline{\kappa(\text{control})}$ was 1.70, and the ratio of mean conductances in the active pathway $\overline{\kappa^a\,(\text{aldo})}/\overline{\kappa^a\,(\text{control})}$ was 2.18 ± 0.52. These results support Civan and Hoffman's [3] interpretation of the significance of nonspecific leak pathways, and their conclusion that aldosterone stimulates transepithelial sodium transport by reducing the resistance to sodium movement in the active pathway.

Mechanism of Effect on κ^a—Increase in Number of Active Units?

In interpreting the effect of aldosterone it is pertinent to ask whether it increases κ^a and sodium transport simply by increasing the number of active transport units acting in parallel. Recent observations of the dependence of the rate of oxygen consumption J_r on the electrical potential difference $\Delta\Psi$ in the frog skin suggest that this cannot be the sole mechanism. If aldosterone

were to act only in this way, $-dJ_r/d\Delta\Psi$ should increase in proportion to the short-circuit current. In fact, there was a tendency for $-dJ_r/d\Delta\Psi$ to decrease.[3]

Mechanism of Effect on κ^a—Energetic Factors?

Could a change in the conductance of the active pathway be a manifestation of energetic factors? This question may be approached with the formalism of linear nonequilibrium thermodynamics [9]. In this view, with identical solutions at each surface the rates of active sodium transport and the associated oxidative metabolic reaction are expressed respectively as

$$J^a_{Na} = L_+(-F\Delta\Psi) + L_{+r}A, \tag{4}$$

and

$$J^a_r = L_{+r}(-F\Delta\Psi) + L_r A. \tag{5}$$

Here F is the Faraday constant, the L's are "phenomenological coefficients," functions of permeability and kinetic factors, and A is the affinity (negative free energy) of the oxidative metabolic reaction which is "driving" active Na transport. In these terms

$$\begin{aligned}\kappa^a &= -(d/d\Psi)(FJ^a_{Na}) \\ &= F(FL_+ - L_{+r}\,dA/d\Psi).\end{aligned} \tag{6}$$

Thus, to the extent that the free energy of metabolic reaction is altered by perturbation of the electrical potential, κ^a would reflect energetic factors. However, it has been demonstrated that J^a_{Na} (in the toad bladder) and J^a_r (in the frog skin) are both linear functions of $\Delta\Psi$, both in the presence and absence of aldosterone, indicating that for brief perturbations of $\Delta\Psi$, $dA/d\Psi = 0$ [3, 26] (*see also* footnotes 1 and 3). Therefore, it seems that the enhancement of κ^a by aldosterone is not a direct consequence of energetic factors.

Site of Effect on κ^a

The above considerations indicate that aldosterone has a direct effect on the permeability of individual transport units. This might occur at either of the two main permeability barriers, one at the apical (mucosal) surface, the other at the basal-lateral surface, in the region of the sodium "pump". An increase in apical permeability has been suggested on the basis of radio-

3 T. Saito, A. Essig and S. R. Caplan. The effect of aldosterone on the energetics of sodium transport in the frog skin. *Biochim. Biophys. Acta.* (*To be published*)

isotope labeling [21, 22], the dependence of aldosterone-mediated substrate utilization on mucosal Na^+ [22], mucosal effects of the polyene antibiotic amphotericin B [22], and the Na^+ content of isolated epithelial cells [15]; but the significance of these data has been disputed [11, 18, 22]. Moulting of frog skin and toad skin has been shown to be temporally associated with an increase in short-circuit current following aldosterone [19], but we are not aware of an analogous effect in the toad bladder. If the permeabilities of the apical and basal-lateral barriers were sufficiently different the present results might be helpful in establishing the site of aldosterone action. However, since some 54% of cell resistance is attributable to the basal-lateral plasma membrane [2], the demonstration of approximate doubling of κ^a is consistent with an effect on either or both.

The Electromotive Force of Sodium Transport E_{Na} and the Flux Ratio

Various workers have studied energetics in terms of the model of Ussing and Zerahn [25], who represented the frog skin as an equivalent electrical circuit, with active transport driven by the "electromotive force of sodium transport," E_{Na}. The definitive method employed to evaluate E_{Na} is to determine the electrochemical potential difference necessary to abolish active sodium transport. Alternatively, it has been proposed that in the absence of concentration and electrical potential gradients E_{Na} could be evaluated from the ratio of the influx to the outflux of Na^+ [24, 25]. Although it was appreciated that the E_{Na} defined in this way is an effective active transport potential, incorporating the effects of leaks [24], measurements of the flux ratio were utilized to infer the mode of action of agents which modify the rate of sodium transport.

Porter and Edelman [20] pointed out that an increase in the flux ratio of Na^+ in the toad bladder from 2.9 ± 0.4 to 4.4 ± 0.6 following aldosterone might suggest a direct effect on the Na^+ pump or on the supply of high energy intermediates. However, because of concern that a rise in intracellular Na^+ consequent to an increased rate of mucosal entry might have evoked an increase in E_{Na}, Fanestil *et al.* [12] carried out further studies using a 1:5 mucosal to serosal concentration gradient and a 100-mV electrical potential difference (serosa positive) to reverse the direction of active transport. It was felt that under these circumstances an increase in mucosal permeability would facilitate the movement of Na^+ from the cells into the lumen, so that there would be no increase in the intracellular Na^+ concentration, and therefore no increase in pump activity and the flux ratio. Accordingly, the finding that aldosterone did increase the flux ratio, with a threefold enhancement of

influx but no effect on efflux, was considered consistent with an effect on the pump or other energetic factors.

The Flux Ratio; Influence of Leakage

It is well known that several considerations prevent the use of the flux ratio for the precise evaluation of energetic parameters [4, 6, 10, 12, 17, 20, 24, 25]. It is perhaps not generally appreciated however that, even when leakage is minimal and unaffected by experimental procedures, passive tracer flux may make the flux ratio grossly misleading. This is the case for example in the toad bladder, where even if the major part of conductance is active, the *back-flux* must be preponderantly passive, since it has not been possible to demonstrate back-flux through the active pathway[1] (*see also* ref. [1]).[2]

The pertinence of these considerations may be shown precisely: the flux ratio f^a of the active pathway is given by

$$f^a = \vec{J}^a / \overleftarrow{J}^a, \tag{7}$$

where $\vec{J}^a$ and $\overleftarrow{J}^a$ are the active influx and efflux, respectively, whereas the observed flux ratio is given by

$$f = \vec{J} / \overleftarrow{J} = (\vec{J}^a + \vec{J}^p)/(\overleftarrow{J}^a + \overleftarrow{J}^p). \tag{8}$$

(Here the superscript p refers to the passive pathways.) We assume that $\vec{J}^a > \vec{J}^p$, whereas $\overleftarrow{J}^p > \overleftarrow{J}^a$.[4] Under these circumstances an agent which acted only on permeability, so as to increase $\vec{J}^a$ and $\overleftarrow{J}^a$ commensurately without altering f^a, would increase the numerator of Eq. (8) relatively more than the denominator, and therefore produce an increase in the observed flux ratio. (This is true irrespective of the relative magnitudes of $\vec{J}^a$ and $\overleftarrow{J}^a$, i.e. irrespective of whether the direction of net active Na^+ transport is reversed.) Therefore, it cannot be assumed that an increase in the observed flux ratio f repre-

4 Under the conditions of Fanestil *et al*'s [12] experiments the 100-mV potential difference would of course decrease the value of $\vec{J}^a$ much below that in the short-circuited state. In a previous study in toad bladder, imposing a +100-mV potential difference decreased total $M \rightarrow S$ sodium flux to 15.4% of the short-circuit value (n=20) [8]. However, $\vec{J}^p$ would also be expected to decrease markedly, to 8.0% of control value (*see* footnote 1). Similarly, diluting the mucosal medium fivefold would be expected to decrease $\vec{J}^p$ fivefold, while affecting $\vec{J}^a$ less (*see* ref. [13], Fig. 3). Even with $\Delta\Psi = 100$ mV it has not been possible to demonstrate measurable active back-flux [Lief & Essig, *unpublished observations*], while $\overleftarrow{J}^p$ is of course greater than at short circuit.

sents an increase in f^a, the flux ratio of the active pathway, and thus in the electromotive force of sodium transport E_{Na}.

This point is substantiated by the present study, which permits a direct comparison of the effects of aldosterone on f and E_{Na}.

Effect of Aldosterone on f

Since $I_{\Delta\Psi=50}$ increased following aldosterone, whereas passive Na^+ and Cl^- flux were unaffected, it can be shown that aldosterone increased the flux ratio $f_{\Delta\Psi=50}$. However, it is more instructive to consider the flux ratio at short circuit, f_0 ,which may be calculated from the following relations:

$$f_0=\vec{J}_0/\overleftarrow{J}_0, \tag{9}$$

and

$$J_0=\vec{J}_0-\overleftarrow{J}_0. \tag{10}$$

In the short-circuited state the rate of net sodium transport is simply related to the current. Although the short-circuit current was not measured in this study it can be calculated.

$$J_0=I_0/F=(I+\kappa\Delta\Psi)/F=(I_{\Delta\Psi=50}+50\kappa)/F. \tag{11}$$

Also, it has been shown previously[1] that in toad bladders mounted in standard chambers the effect of a 50-mV electrical potential difference on $S\rightarrow M$ sodium tracer flux differs insignificantly from that which would obtain in free solution:

$$\overleftarrow{(J^x/\Delta c^x)}_{Na,\,\Delta\Psi=50}=2.27\,\overleftarrow{(J^x/\Delta c^x)}_{Na,\,\Delta\Psi=0}. \tag{12}$$

Since the undirectional flux $\overleftarrow{J}$ is the product of the concentration of Na^+ and the tracer permeability.

$$f_0=(J_0+\overleftarrow{J}_0)/\overleftarrow{J}_0$$

$$=\frac{(I_{\Delta\Psi=50}+50\kappa)/F+(c_{Na}/2.27)\,\overleftarrow{(J^x/\Delta c^x)}_{Na,\,\Delta\Psi=50}}{(c_{Na}/2.27)\,\overleftarrow{(J^x/\Delta c^x)}_{Na,\,\Delta\Psi=50}}. \tag{13}$$

Fig. 7 shows the values of the flux ratio f_0 calculated in this manner for the Na^+ tracer study above. The stimulation of active sodium transport was associated with an increase in the flux ratio from an initial value of 11 to a value of 22, 6 hr following the administration of aldosterone. If this were an

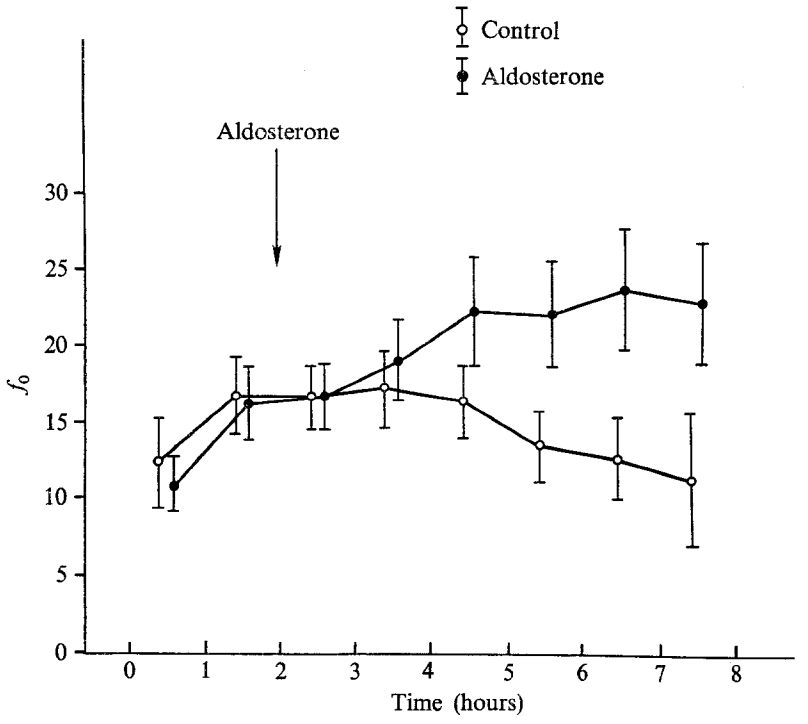

Fig. 7. Effect of aldosterone on the flux ratio at short circuit, calculated with Eq. (13) (mean $\pm$ SE; n = 12 to 14). p (4, 5) <0.02; p (5, 6), p (6, 7), p (7, 8) <0.005

indication of enhancement of the flux ratio f_0^a of the active pathway it would be expected that there would also be an increase in E_{Na}.

Effect of Aldosterone on E_{Na}

With identical solutions at each surface, E_{Na} is readily evaluated from the magnitude of $\Delta\Psi$ adequate to abolish active transport [17, 25].[5] Given the linear relationship between the rate of active sodium transport J_{Na}^a and $\Delta\Psi$ (*see* footnote 1),

$$FJ_{Na}^a = I_0 - \kappa^a \Delta\Psi. \tag{14}$$

At $\Delta\Psi = E_{Na}$, $J_{Na}^a = 0$, and therefore

$$E_{Na} = I_0/\kappa^a. \tag{15}$$

5 It is not possible to state the precise relation between f_0^a and E_{Na}, since the extent of interaction between abundant and tracer Na^+ flows in the active pathway is unknown [17]. Assuming the absence of such isotope interaction, an increase in f_0^a from 11 to 22 would correspond to a 29% increase in E_{Na}.

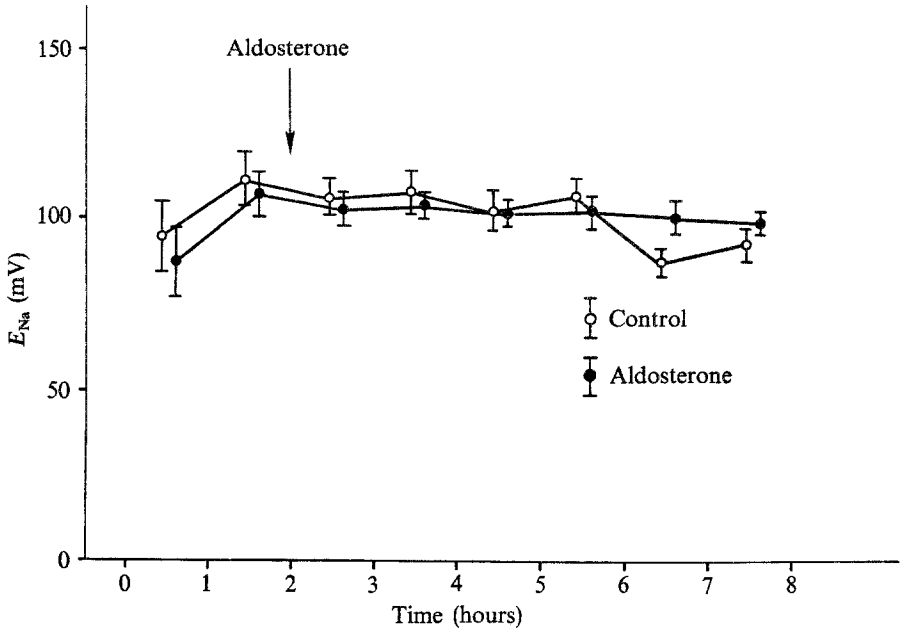

Fig. 8. Effect of aldosterone on E_{Na} calculated with Eqs. (11) and (15); ^{22}Na studies (mean $\pm$SE; n = 10 to 14). For each period I_0 was taken as the arithmetic mean of the values at the beginning and end, so as to correspond temporally to the values of κ^a. All p's were >0.05, except for p (6, 7) <0.025. (For period 1, in three tissues κ^p was too close to κ to permit the accurate calculation of κ^a, giving values of E_{Na} very significantly different from the mean [$p<0.001$]; these values were rejected)

Values for E_{Na} for the Na^+ tracer studies, calculated from Eqs. (11) and (15), are shown in Fig. 8. There is no significant variation over the entire period of observation. Values of E_{Na} in control and experimental tissues were 91.7 ± 10.4 and 84.4 ± 10.5 mV, respectively initially, and 91.0 ± 5.5 and 91.7 ± 2.6 mV, respectively, 6 hr following the administration of aldosterone. Thus, despite the use of bladders in which transport through passive pathways is minimal, an increase in the flux ratio was unassociated with an increase in E_{Na}.

Significance

It should not be concluded that failure to demonstrate an effect on E_{Na} necessarily means that aldosterone has no direct effect on energetics. As discussed in detail elsewhere, E_{Na} reflects both permeability and energetic factors [9, 10, 17].

On the basis of finding no effect of aldosterone on the Na^+ content of isolated bladder epithelial cells, Lipton and Edelman [18] have suggested

that aldosterone may stimulate sodium transport by enhancing both metabolism and permeability. Handler, Preston and Orloff [15], however, using similar techniques, found evidence only for an effect on mucosal entry. Recent studies in the frog skin suggest that overnight exposure to aldosterone may increase the negative free energy of an oxidative reaction which "drives" sodium transport.[3] Taken in conjunction with the present results, this would seem to support Lipton and Edelman's view of a dual effect of aldosterone. However, firm conclusions cannot be drawn from studies in different species under different temporal conditions.

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