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Evidence for a Transcellular Component to the Transepithelial Sodium Efflux in Toad Skin

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Summary. The transepithelial efflux of sodium, from the inner to the outer surface, was measured across the isolated toad skin, mostly after abolition of the electrochemical gradient. The effects on this efflux of several agents and manipulations were studied in order to make a distinction between the paracellular component and a hypothetical transcellular one. Amiloride decreased the transepithelial efflux, while ouabain and cyanide increased it. From the known mode of action of those agents, it was inferred that part of the efflux occurred across the cell. Removal of sodium from the external solution interfered apparently with both components of the transepithelial efflux, while the action of external hypertonicity seemed to be restricted to the paracellular shunt pathway. Access of sodium from the internal solution to the active transport pool is thus suggested, with consequent increase in metabolic cost of transport. Yet, compared with the net influx, the amounts involved are very small; consequently, they escape detection by oxygen consumption measurements.

It is generally accepted that sodium transport across epithelial anuran membranes proceeds in two steps: a first one at the apical or luminal border, passive although carrier-mediated, and a second one at the basal-lateral border, active and so critically dependent on a supply of energy. In between those two barriers is located the amount of sodium awaiting transport; this is generally referred to as the "active sodium transport pool". This system would be most efficient if a minimal amount of sodium, or even none at all, diffused from the internal or serosal side back into the transport pool. Indeed, as this sodium would eventually have to be pumped again, additional energy cost for transport activity would result. From manipulations of the sodium concentrations on the internal or serosal side, Beauwens and Al-Awqati (1976), as well as Canessa, Labarca and Leaf (1976) concluded that the basal lateral membrane was impermeable to sodium, in the toad bladder. A similar conclusion had been reached by Civan (1970) on the basis of analysis of the current-voltage relationship in the toad bladder. We decided to measure

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systematically the flux of sodium outward (efflux), using toad skin. Indeed, the paracellular pathway is a well established route for this transepithelial efflux (Ussing & Windhager, 1964) but the question raised is whether additional sodium can move outward across the cells. Advantage was taken of manipulations of drugs meant to specifically modulate one component only of the efflux. As this work was in progress Biber and Mullen (1976, 1977) reported that there is indeed a transcellular efflux of sodium across frog skin.

Materials and Methods

The ventral skin of doubly pithed toads (*Bufo marinus,* from Venezuela) was dissected and divided symmetrically in 2 to 4 fragments that were mounted between the two halves of Lucite Ussing chambers, with an incubation area of 3.14 cm^2; the volume of bathing medium needed to fill the chambers was 2.5 ml on each side.

The preparations were continuously short-circuited throughout the whole experiment by means of a classical voltage-clamp device. Potential difference (PD) was measured on a high input resistance electrometer (Keithley, model 610 C) and short-circuit current (scc) on a DC amperemeter (Norma, model 251). Ohmic resistance was obtained from the ratio of the spontaneous electrical potential difference over short-circuit current; when the latter was small or annihilated, resistance was derived from the change in potential resulting from small current (50–100 μA) briefly imposed to the preparation. Short-circuit current is equivalent to net sodium movement, across toad ventral skin as well as urinary bladder; following amiloride or ouabain treatment, the spontaneous potential difference drops close to zero, never to reach negative values. This can be taken as an indication that the subspecies used is probably the same as the one originating from the Dominican Republic and is thus distinct from *Bufo marinus* from Colombia. (Davies, Martin & Sharp, 1968).

The solution used was the usual "frog Ringer's", arbitrarily designated as Na-R, containing in mM: NaCl, 115; $KHCO_3$, 2.5; $CaCl_2$, 1.0; the pH was 7.8 at room temperature during aeration with atmospheric air and the osmolality, 225 mOsm/Kg H_2O. This solution was used on both sides, except when sodium-free solution was used, arbitrarily designated as Mg-R, in which $MgCl_2$, 57.5 mM, replaced NaCl; sucrose, 57.5 mM, was added to bring the osmolality back to 225 mOsm/Kg H_2O; In a few experiments, NaCl was replaced isoosmotically by KCl or LiCl instead, as will be stated.

All chemicals were reagent grade; amiloride was a gift of Merk, Sharp & Dohme and ouabain was purchased from Sigma (St. Louis, Mo.).

^{22}Na obtained from Amersham, England, was added to the internal solution to reach a specific activity of 6.2 μCi/mEq. After 1 hr the efflux was followed continuously for several hours. External solution was sampled every 45 min with immediate replacement of the samples (1 ml) with the appropriate Ringer's. ^{22}Na was counted in an Autogamma system (Packard Instrument Co., model 3002), so as to reduce the error to $\leq 2\%$.

Edge damage was judged to be minimal because the fluxes were of the same magnitude when different skin fragments of the same animal were studied simultaneously (Table 1); moreover, as will be seen, they compare reasonably with data from investigators who focused on that issue (Biber & Mullen, 1977). With the exception of ouabain and cyanide, the effects of which are slow to be complete and to be eliminated, the experimental period

Table 1. Sodium efflux (peq/cm^2·sec) across different fragments of toad ventral skin

Experiment no.	Fragments			
	A	B	C	D
1	12.2	8.8	12.7	10.0
2	26.6	14.2	25.6	13.0
3	15.3	12.5	10.7	14.2
4	13.4	30.1	14.8	13.2
5	10.7	17.2	13.2	9.3
6	11.4	11.4	15.2	18.0
7	6.4	5.9	10.4	5.8

Transepithelial efflux was determined simultaneously for 4 fragments of the ventral skin of a given animal, thus mounted in 4 different Lucite chambers. Differences when present, probably reflect edge damage.

The data indicate that comparison from one fragment to the other is not completely warranted; therefore, it was judged preferable to use each preparation as its own control.

was bracketed by two control periods; the mean of those two periods will be referred to as control. At any rate, each preparation served as its own control.

Oxygen consumption studies were performed using the polarographic set up described by Noé, Michotte and Crabbé (1977). The chamber was in glass (membrane area: 4.15 cm^2; bathing medium on each side: 4.5 ml) and it was equipped with inlets for electrical potential measurements, short-circuiting, oxygen electrodes (Eschweiler, Inc., West Germany) and taps so as to provide a rapid fluid circulation. Each side has its own reservoir and bubble air-lift device and by opening the taps the pO_2 inside the chamber is brought back to the initial level within a minute. Mixing in the chamber was insured by small motor-driven magnets. The electrodes were kept at room air when not in use, and the Lucite holder of their Teflon membrane was covered with parafilm paper. These slight modifications apparently sufficed to get rid of the absorption phenomenon previously noted in the absence of any tissue. The chamber and all its connections were kept in 10% glutaraldehyde between experiments.

Before use, all the solutions were filtered through a Millipore filter (0.22 μm) and three antibiotics were added: Kanamycin, 50 μg/ml; Gentamycin, 10 μg/ml; and Chloromycetin, 1 mg/ml. Despite those precautions, bacterial proliferation is a continuous potential hazard; frequent renewal of the incubation solutions on both sides of the skin seemed to be effective in this respect. At the end of each experiment, the last solutions used were tested for oxygen consumption with a parafilm paper instead of the skin. Only occasionally were the values observed not negligible; the corresponding experiments were discarded. Also, for these incubations the Na-free solution (or Mg-R) was slightly modified in that sucrose was replaced isoosmotically by polyethylene glycol (PEG-1,000) much more biologically inert. Data are expressed as mean ± standard error (SEM); changes were analyzed statistically by the paired *t*-test.

Results

Control

The transepithelial efflux of sodium was found to average 22.8 ± 1.1 peq/cm^2·sec, with ohmic resistance of $804 \pm 89\ \Omega \cdot cm^2$ for 57 toad skins

studied year round. Biber and Mullen (1977) reported a mean of 8.2 ± 0.8 peq/cm$^2 \cdot$sec ($n = 36$) for frog skin mounted in Lucite chamber specifically designed to avoid edge damage. The minimal efflux rate obtained by Walser (1969) with toad bladder sacs was 11.6 peq/cm$^2 \cdot$sec.

Amiloride

This diuretic rapidly inhibits sodium transport in many epithelia and its action is thought to be localized at the luminal border (Bentley, 1968; Ehrlich & Crabbé, 1968). When the drug, 10^{-5} M, was added to the external side, short-circuit current rapidly declines to zero and transepithelial efflux decreased from 31.3 to 21.0 peq/cm$^1 \cdot$sec (Δ: 10.3 ± 2.0; $n = 17$; $P < 0.001$), while ohmic resistance increased from 651 to 1449 $\Omega \cdot$cm^2 (Δ: 798 ± 113; $n = 17$; $P < 0.001$). This indicates either that amiloride acts on the paracellular shunt pathway or alternatively that there is a transcellular component to the efflux. If the latter interpretation is favored, it implies that the Na carriers in the apical membrane of amphibian epithelia are blocked in both directions by amiloride, as had already been suggested by Morel and Leblanc (1973) and by Rick, Dörge, Bauer and Thurau (1976).

Ouabain

This cardiac glycoside is a well-known inhibitor of the Na pump located at the basal lateral membrane (Herrera, 1968; Handler, Preston & Orloff, 1972). It was added to the internal solution (5×10^{-4} M), and one hour was allowed for completeness of its action. At that time electrical potential was always less than 8 mV, with residual current below 3 μA/cm^2. Both efflux and resistance increased from 11.9 to 21.0 peq/cm$^2 \cdot$sec ($\Delta = 9.1 \pm 1.4$; $P < 0.001$; $n = 9$) and from 531 to 1353 $\Omega \cdot$cm^2 ($\Delta = 822 \pm 209$; $P < 0.005$; $n = 8$), respectively. An action of ouabain limited to the paracellular shunt pathway is considered unlikely, and the increase in the efflux could suggest that ouabain, by blocking the Na pump, did enhance transcellular efflux, otherwise minimized by immediate recirculation through the pump.

In order to test this hypothesis, another set of experiments was performed to study to what extent amiloride would block this increased efflux. In 4 experiments, the effect of amiloride was tested between two

control periods as explained in the Methods; then 60 to 90 min were allowed for completeness of ouabain action and the action of amiloride was re-evaluated in the presence of the glycoside. In two additional experiments the sequence was reversed; ouabain was used first, and control periods were documented after removal of ouabain. It must be added that although 90 min were allowed for the electrical parameters to recover, they did so only partially, values approximating 50% of control (20 mV for potential, 15 $\mu A/cm^2$ for current). The results of all data are presented in Table 2: addition of amiloride to ouabain-treated preparations brought the efflux to a value similar to the one observed when amiloride alone was added, thus again supporting the contention that part of the efflux is transcellular.

Cyanide

This drug is a strong inhibitor of cytochrome *c* oxydase, hence of mitochondrial energy production, and so it leads to decreased sodium

Table 2. Effect of amiloride on transepithelial sodium efflux across toad skin before and after ouabain treatment

	Efflux ($peq/cm^2 \cdot sec$)			
	Control	Ouabain	Δ	P ($n=6$)
No amiloride	27.8 ± 6.9	33.0 ± 7.3	5.2 ± 2.4	<0.05
Amiloride (10^{-5} M)	22.2 ± 4.6	23.8 ± 5.9	1.5 ± 2.8	>0.6

Transepithelial sodium efflux in the presence of amiloride was the same whether or not the skin had been treated with ouabain. This residual value is thought to represent the paracellular component of the efflux.

Table 3. Effects of cyanide on the isolated toad skin

	Control	NaCN	Recovery
scc ($peq/cm^2 \cdot sec$)	283	104	180
Resistance ($\Omega \cdot cm^2$)	591	1974	987
Efflux ($peq/cm^2 \cdot sec$)	13.0	18.4	16.1

The effects of sodium cyanide treatment are summarized for short-circuit current (scc), ohmic resistance and transepithelial sodium efflux. 45 min were allowed for the effect of cyanide to be considered complete, as well as for its recovery, which was only partial. Δ and P values are reported in the text. ($n=6$).

transport. Na-cyanide, 2 mM, was added isohydrically to the internal solution, after two control periods. Within 45 min, the short-circuit current dropped to approximately one third of its initial value (Table 3), and it remained stable thereafter for the next 90 min. Both efflux and resistance increased significantly by 5.4 ± 1.2 peq/cm^2·sec ($P < 0.005$; $n = 6$) and by $1383 \pm 330\ \Omega \cdot \text{cm}^2$ ($P < 0.01$; $n = 6$), respectively. Forty-five minutes after removal of cyanide, each of the parameters studied had recovered to a certain extent only (Table 3).

Removal of Sodium from the Outside Medium

Isoosmotic replacement of sodium in the outside solution by $MgCl_2$ and sucrose induced a large decrease in the efflux, from 28.9 to 13.1 peq/cm^2·sec ($\Delta = 15.8 \pm 2.3$; $P < 0.001$; $n = 9$) and an increase in the ohmic resistance from 842 to $2155\ \Omega \cdot \text{cm}^2$ ($\Delta = 1313 \pm 212$; $P < 0.001$; $n = 9$). On the one hand, the transcellular component was apparently involved as ouabain now failed to bring about any significant rise in the efflux (Table 4). The decrease in transcellular efflux is attributed to the fact

Table 4. Effects of amiloride and ouabain on transepithelial sodium efflux and resistance upon exposure of external surface of isolated toad skin to sodium free solution

	Mg-R	Mg-R with ouabain	Δ	P	n
Efflux	7.1	8.3	1.2 ± 0.8	>0.1	8
Resistance	2204	3780	1576 ± 725	$=0.05$	5
	Mg-R	Mg-R with amiloride	Δ	P	n
Efflux	12.5	8.9	3.6 ± 1.5	<0.05	7
Resistance	1764	2508	744 ± 346	<0.05	10
	Mg-R	Na-R with amiloride	Δ	P	n
Efflux	11.4	24.5	13.1 ± 3.0	<0.001	17
Resistance	1920	1254	666 ± 257	<0.01	13

Na-R: usual frog Ringer's; Mg-R solution in which NaCl has been replaced isoosmotically by $MgCl_2$ and sucrose on the outside. Amiloride (10^{-5} M) was added to the external solution; ouabain (5×10^{-4} M) to the internal solution, 1 hr prior to measurements. Efflux is expressed in peq/cm^2·sec and resistance in $\Omega \cdot \text{cm}^2$.

that no positive charge in the outside solution be readily available to substitute for intracellular sodium ions. The fact that amiloride in the absence of external sodium produced a small additional drop in the efflux (Table 4) might reflect a residual transcellular component in this condition. This residual transcellular efflux could be explained by the fact that even after several rinsings external sodium is not totally removed, as indicated by flame photometry measurements; furthermore, the concentration of residual sodium in the unstirred layers adherent to the tissue was probably higher.

On the other hand, it is likely that the manipulation brought about a decrease in the paracellular component of the efflux as shown in Table 4. Indeed, when the alleged transcellular component of the efflux is blocked by amiloride, a further reduction in the overall efflux was seen after removal of external sodium, while the resistance further increased. This is in agreement with direct electrical potential measurements (Reuss & Finn, 1975).

The nature of the substitute seemed irrelevant since NaCl replacement by KCl led also to a significant decrease in efflux, from 8.2 to 6.0 peq/$cm^2 \cdot sec$ ($\Delta = 2.2 \pm 0.4$; $P < 0.001$; $n = 6$). However, when the substitute is an ion capable of entering the cell, such as lithium, the efflux was less influenced, since values decreased from 16.7 to 13.9 peq/$cm^2 \cdot sec$ ($\Delta = 2.8 \pm 1.0$; $P < 0.02$; $n = 6$); it could be proposed that in the latter case, only the paracellular route was affected, as at the apical border sodium can exchange for lithium.

Hypertonicity of the External Solution

This manipulation results in a drastic opening of the paracellular shunt pathway as demonstrated by Ussing and Windhager (1964). In addition, a cellular action has been postulated by these authors, by Erlij and Martinez-Palomo (1973) and by Bindslev, Tormey, Pietras and Wright (1974); this does not seem of major import for the Na transport system since the short-circuit current did not change following exposure of the external surface of the toad skin to a hypertonic solution (Table 5). Hypertonicity was realized by adding 225 mM urea to the usual Ringer's solution on the outside. As seen in Table 5, the resistance thus dropped by one half, while sodium efflux increased more than sevenfold, presumably only or at least essentially at the expense of the paracellular component. Removal of external sodium (Table 6)

Table 5. Effects of hypertonic solution on the isolated toad skin

	Control	Hypertonicity of external solution	Δ ($n=10$)
scc (peq/cm^2·sec)	287.7	284.3	3.4 ± 27.6
Resistance (Ω·cm^2)	832	406	426 ± 75
Efflux (peq/cm^2·sec)	15.3	111.7	96.3 ± 14.0

Hypertonicity of the external solution was obtained by adding 225 mM urea to the usual Ringer's. A marked effect is seen in the efflux as in the resistance with no change in the short-circuit current (scc).

Table 6. Effect of sodium free solution—hypertonic or isotonic—on the outside solution of isolated toad skin

	Control	Mg-R	Hypertonicity		
			Control	Mg-R	Control
scc (peq/sec·cm^2)	320	0	320	115	409
Resistance (Ω·cm^2)	754	2858	349	773	737
Efflux (peq/sec·cm^2)	16.4	8.0	112.2	36.6	27.6

Changes between columns are all significant statistically ($P<0.05$; $n=6$).

while keeping the solution hypertonic resulted in a highly significant drop in the efflux while the resistance increased, which supports the contention that both maneuvers—hypertonicity and sodium withdrawal—affect the paracellular pathway in opposite ways. Interestingly, in those conditions the short-circuit current is not nil, possibly on account of a recycling of sodium from the internal to the external solution through the shunt pathway of much larger magnitude than at normal tonicity.

Oxygen Consumption

Since flux measurements led to the conclusion that part of the sodium efflux across the toad skin is transcellular, we repeated metabolic measurements when the preparations were bathed with normal Ringer's and after removal of sodium from the internal solution. The idea was that, were the recycling of sodium to be of quantitative significance, this should lead to a drop in oxygen consumption.

In four skins of different toads, oxygen consumption and short-circuit current were measured simultaneously for 10 to 12 consecutive periods of 20 min duration; at the end of each period both the external and the internal sides were washed and the bathing media were replaced with appropriate solutions: "Na-R" or "Mg-R" as explained in the *Methods* section. Removal of sodium from the internal solution led to a 50% change in the short-circuit current. It was assumed that, as is the case with the toad bladder (Frazier, Dempsey & Leaf, 1962), the short-circuit current in this condition is still a valid index of sodium influx. However, the marked changes observed indicate that the maneuver is clearly not as benign as hoped; the reason for this change in active sodium transport is, to our knowledge, unknown. Be it as it may, for a given short-circuit current value no difference was noted in oxygen consumption whether sodium was present or absent in the internal bathing medium (Table 7). In the absence of sodium in the external solution, the short-circuit current is, of course, zero, and the active sodium transport pool should reach its minimal value. If internal sodium also contributed to this pool, the entrance of sodium at the basal lateral membrane should be maximal in this condition, because of a more favorable electrochemical gradient (Nagel, 1976; Helman & Fisher, 1977). Yet, as appears from Table 7, no significant change in oxygen consumption resulted when, in the absence of external sodium, internal sodium was removed, too.

Thus, why did those studies fail to show anything even though flux measurements indicated a recycling of sodium? We think the answer lies in the low rate of recycling. Even if all the transcellular component of the efflux gained access to the transport pool—an assumption that can be questioned—it amounts to a small fraction (usually less than 5%) of the short-circuit current, and therefore the change in metabolic rate to be expected is by far too small to be picked up with methods for oxygen consumption or CO_2 production thus far available.

Table 7. Effect of removal of internal sodium on oxygen consumption (pM/cm^2·sec)

Na-outside	Na-inside		Δ	P ($n=4$)
	Present	Absent		
Present	50.7±5.0	49.3±6.7	1.4±2.0	>0.2
Absent	35.8±3.3	37.0±3.3	1.2±2.8	>0.35

For further explanation, *see text.*

Discussion

In epithelia, passive ionic movement occurs to a large extent between cells: this applies even to "tight" preparations such as amphibian skin and urinary bladder (Ussing & Windhager, 1964; Ussing, 1966, 1969; Di Bona & Civan, 1973; Wade, Revel & Di Scala, 1973). Electrophysiological studies of Mandel and Curran (1972) as well as of Saito, Lief and Essig (1974) have suggested that this pathway is the only one available for sodium leaking outward across such epithelia. Yet the values reported were substantially higher than those obtained with sac preparation of toad bladder, which raises the issue of edge damage effect. Moreover, those studies were not designed to test the possibility that part of this efflux was transcellular. Recently Biber and Mullen (1976, 1977) provided strong support in favor of a transcellular component to the transepithelial efflux in the case of frog skin. These investigators indeed showed that the efflux is characterized by saturation as a function of sodium activity on the internal side; also they evaluated the effect of inhibitors of sodium transport on this efflux, pretty much in the manner dealt with here.

Thus, agents or maneuvers that hopefully interact with one pathway specifically were tested: inhibitors of active sodium transport and hypertonicity of the external solution. Transepithelial efflux decreased following amiloride, and this was interpreted as resulting from the nullification of a transcellular component: it had been shown that amiloride blocks sodium movement at the apical border of amphibian epithelia in both directions.

Blocking, in turn, the sodium pump with ouabain induced an increase in sodium efflux which was taken as an indication that in the usual short-circuited condition, there is some recycling of sodium; that is, sodium from the internal solution diffuses back into the cell and is pumped again, of course, at the expense of metabolic energy. Therefore, blocking the sodium pump with ouabain will enhance the total transcellular efflux. Addition of amiloride to ouabain-treated skins brought the efflux back to what it was with amiloride alone. Assuming that in the presence of amiloride, the residual sodium efflux represents the paracellular component, the respective contributions of the paracellular *vs.* the transcellular components could be evaluated: the transcellular component amounts to approximately one half of the paracellular one.

Analysis of our data rests on the assumption that neither amiloride nor ouabain interfered with a paracellular shunt pathway. This assumption is borne out only indirectly. In frog skin ouabain does not affect

the shunt pathway, as would appear from urea flux measurements (Mandel & Curran, 1972). With toad skin, ouabain and amiloride are devoid of effect on the shunt pathway estimated by mannitol and sulfate fluxes (Hviid Larsen, 1973). More recently Biber and Mullen (1977) did not observe any significant alteration in the transepithelial flux across frog skin for several nonelectrolytes (sucrose, mannitol, PEG 900) following amiloride or ouabain treatment. It should be pointed out that those substances could affect the shunt conductance only for electrolytes or even specifically for sodium. However, if the changes in transepithelial sodium efflux observed with amiloride and ouabain were ascribed to influences of these drugs on the shunt conductance, then amiloride and ouabain should have opposite effects on that pathway. This should not be expected if the link between active sodium transport and the shunt conductance rested only on intercellular accumulation of sodium achieved locally by pumping.

Sodium cyanide, which decreases the energy supply to the sodium pump, acts on the transepithelial efflux in a manner essentially similar to that of ouabain. The percentage increase was clearly less than was seen with ouabain, which correlates with the relative effect of both drugs on active sodium transport. Thus, cyanide also reveals that part of the transcellular efflux which is otherwise masked by immediate recirculation through the pump.

Hypertonicity of the external solution is understood as opening the paracellular shunt pathway: actually short-circuit current was not influenced while ohmic resistance decreased. The increase in sodium efflux observed in these conditions is thus attributed mainly, if not exclusively, to an increase in the paracellular component of this flux. The present data do not provide any evidence regarding additional action of hypertonicity on the transcellular component.

Removal of external sodium results in probably complex changes of the efflux. Indeed the latter was significantly more depressed in this condition than when active sodium transport was blocked with amiloride. There is probably a decrease in the transcellular component, because no ion is readily available in the external solution to substitute for intracellular sodium. Indeed in the absence of external sodium, addition of ouabain did not bring about any increase in efflux, supporting the assumption that the transcellular component is essentially blocked in this condition. The fact that amiloride still had a small significant action would be in keeping with the presence of residual external sodium. However, the decrease in efflux observed following removal of external sodium

was higher than expected if only the transcellular component was blocked. This was demonstrated by the fact that the efflux rate dropped for a given skin when the conditions were changed from amiloride in the presence of sodium to removal of sodium from the external solution. It is thus likely that in the latter condition, the further decrease in efflux probably involves the paracellular component, as the transcellular flux was already blocked. Reuss and Finn (1975), measuring directly the shunt resistance, had concluded similarly.

Thus, the present data support the existence of two components in the efflux: a paracellular and a transcellular one. Sodium entering the cells from the inside can probably gain access to the active transport pool, since there occurs an increase in efflux following ouabain (and cyanide) treatment. The amplitude of this sodium movement potentially involved in recycling across the pump is quite small relative to the short-circuit current; the resulting metabolic energy cost is therefore below detection by standard oxygen consumption measurements.

The results observed are in several respects similar to those of Biber and Mullen (1977); yet two important differences have to be pointed out. First, these authors observed a reduction of sodium efflux with amiloride only at a sodium concentration of 6 mM (both sides), while at higher concentration (100 mM) they actually observed an increase in the efflux. The reason for opposite effects at the two sodium concentrations was not commented upon, and the present work does not provide any explanation for this discrepancy, since we uniformly observed decrease in efflux, either with 115 mM on both sides or with 115 mM inside and 0 mM outside. Second, the action of ouabain, though qualitatively the same, was quantitatively much larger in their study (nine times the control efflux in their study against twice the control, as reported here). We like to stress that the ouabain effects on toad skin are almost totally reversible as shown earlier by Crabbé, Fanestil, Pelletier and Porter (1974), unlike what applies to frog skin (Voûte, 1973).

Earlier studies of Fanestil, Porter and Edelman (1967) had already suggested the existence of a transcellular component in the transepithelial sodium efflux with the toad bladder. Yet, their study bears all the uncertainties of active sodium transport pool measurement, as outlined by MacKnight, Civan and Leaf (1975).

Several investigators, relying on metabolic or electrical methods, failed to substantiate a recycling of sodium at the basal-lateral membrane. This doubtless results from the small magnitude of the transcellular component in the transepithelial efflux detectable by direct approach,

such as used here. Thus, the permeability to sodium of the basal-lateral membrane is not nil, although obviously much lower than that of the apical border of the cells. The existence of this transcellular efflux is particularly relevant when transepithelial electrochemical gradient for sodium is allowed to develop and when the ratio of unidirectional fluxes is relied upon to evaluate the electromotive force of the sodium pump (Kedem & Essig, 1965).

Finally the electrical resistance data can be interpreted in the context of the equivalent circuit model proposed by Ussing and Zerahn (1951) (Fig. 1). The increase in resistance after amiloride probably reflects an increased resistance at the apical barrier; the decrease induced by hypertonicity of the external solutions is essentially due to an influence on the paracellular shunt pathway; removal of external sodium increased both the paracellular and the transcellular resistances, while it looks reasonable to admit that in the cases of cyanide and ouabain the increase in resistance is restricted to the cellular pathway. By nullification of E_{Na} battery, ouabain would reveal the latter's internal resistance, i.e., a resistance located at the basal lateral membrane, and not encountered by sodium influx. It should be added that the resistance at the apical

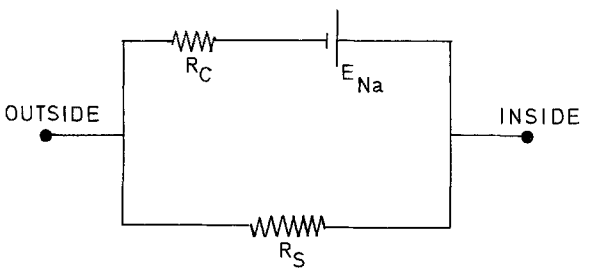

Fig. 1. Equivalent circuit for the toad skin.

Manipulation	Resistance ($\Omega \cdot cm^2$)		Δ	n
	Control	Experimental		
Amiloride	651	1449	798±113	14
Hypertonicity	832	406	426± 75	10
Sodium free	842	2155	1313±212	14
Ouabain	531	1353	822±209	8
Cyanide	591	1974	1383±330	6

R_C stands for the cellular resistance in series with a battery (representing the sodium pump). Its electromotive force is E_{Na}. R_S corresponds to the paracellular resistance (shunt pathway). Control values were obtained with sodium Ringer's outside. For further details, *see text*

border has been shown to increase following ouabain and cyanide treatment (Hviid Larsen, 1973); this could result from a coupling between the two barriers (Finn, 1976). The proposed interpretation of the localization of the resistances' changes seems to be supported by direct measurements with microelectrodes (Reuss & Finn, 1975; Sudou & Hoshi, 1977).

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