COMPONENTS OF SODIUM AND CHLORIDE FLUX ACROSS TOAD BLADDER

MACKENZIE WALSER

From the Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT The effect of transepithelial potential difference (ψ) on Na and Cl flux across toad bladder was assessed by measuring isotopic flux between identical media at various values of ψ. The contribution of edge damage to ionic permeability was eliminated, resulting in relatively high spontaneous ψ (-97 ± 4 mv) and low electrical conductance g. Bidirectional Na fluxes were measured simultaneously. Unidirectional Cl fluxes were measured in paired hemibladders at $\psi = 0$ mv or -97 mv. Net Na flux J_{Na}, at $\psi = 0$ mv, was slightly less than short-circuit current (SCC). At $\psi = -97$ mv, J_{Na} averaged 17% of SCC, and was sometimes zero. $\Delta J_{Na}/\Delta\psi$ ($= g_+$) averaged 60% of g between -97 mv and $+75$ mv; at -150 mv, g_+ fell, indicating rectification. Analysis of unidirectional Na fluxes indicates low passive conductance (1.5 μmho/mg wet weight), a bidirectional, electrically neutral flux of approximately 0.13 μa/mg, and relatively large conductance of the active transport path at $\psi \geq -97$ mv. The absence of appreciable transstimulation of serosal (S)-to-mucosal (M) Na flux (in response to increasing mucosal Na concentration) indicates that the electrically neutral flux is not exchange diffusion in the usual sense. Analysis of Cl fluxes indicates similar values for passive conductance and neutral flux, suggesting linked neutral flux of Na and Cl. Either the electromotive force of the Na pump E, its conductance g_a, or both are strong functions of ψ. The product of these two quantities, Eg_a, is a measure of the "transport capacity" at any given value of ψ, independent of the direct effect of ψ on J_{Na} through the pump path. Eg_a varies with ψ. Hence estimation of the net Na flux or current at any one value of ψ, including $\psi = 0$, fails to reveal the maximal transport capacity of the pump, its resting electromotive force (when $J_{Na} = 0$ through the pump), or the dependence of transport capacity on potential.

INTRODUCTION

Validation of any model of transepithelial sodium transport requires an examination of the relationship between the electrochemical gradient for sodium across the epithelium and the rate of sodium transport. Although Ussing (1960) and his associates conducted some experiments of this type on frog skin while evolving their original model of sodium transport, no systematic study of the interdependence between transport and electrical potential in frog skin or toad bladder appears to have been reported.

One difficulty in obtaining such data is the inherent variability seen in these preparations. Another is the relatively narrow range of potentials that can be examined without passing inordinately high currents and damaging the tissue irreversibly. In recent work from this laboratory, the toad bladder mounted as a sac, with the outer fluid level lowered beneath the mounting ring, has been shown to yield considerably lower conductance and higher spontaneous potential than in conventional plastic chambers (Walser, 1969, 1970 *b*).

Preliminary experiments showed that this preparation could tolerate clamping potentials of -150 mv (mucosa negative) or $+75$ mv without undergoing a substantial change in conductance or decrease in spontaneous potential. The use of such a large range of transepithelial potential greatly facilitates the interpretation of bidirectional flux data because certain components of flux virtually vanish at these extremes.

The results are interpreted in the light of recent theoretical studies concerned with the degree of coupling between transport and metabolism.[1]

SYMBOLS

ψ Transepithelial potential difference, mucosa minus serosa (volts).
F Faraday's constant.
R Gas constant.
T Absolute temperature.
g_a Phenomenological conductance of pump pathway for sodium (reciprocal microhms per milligram wet weight)
g_p Phenomenological conductance of parallel leak pathway for sodium (reciprocal microhms per milligram wet weight).
g_+ Total phenomenological sodium conductance, $g_a + g_p$ (reciprocal microhms per milligram wet weight).
g_- Phenomenological chloride conductance (reciprocal microhms per milligram wet weight).
g Total tissue electrical conductance (reciprocal microhms per milligram wet weight).
I Electrical current from an external source (microamperes per milligram wet weight).
SCC Short-circuit current (microamperes per milligram wet weight).
J Net mucosal-to-serosal flux (microamperes per milligram wet weight).
Φ Unidirectional flux, mucosa to serosa (microamperes per milligram wet weight).
β Unidirectional flux, serosa to mucosa (microamperes per milligram wet weight).
$f_o(\psi)$ A dimensionless function defined as $(\psi F/RT)/[\exp(\psi F/RT) - 1]$.
$f_i(\psi)$ A dimensionless function defined as $f_o(\psi) \exp(\psi F/RT)$.
E Electromotive force of sodium pump (volts).

Subscripts a and p refer to active and passive components of fluxes. Subscript e refers to exchange diffusive flux. Numerical superscripts refer to values of ψ, expressed in millivolts, at which a parameter is evaluated.

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[1] A preliminary report of portions of this work has been published (Walser, M. 1970. *J. Clin. Invest.* **49**:100a).

METHODS

Large Dominican toads (*Bufo marinus*) were obtained from National Reagent Co., Bridgeport, Conn., and kept in sphagnum moss with access to tap water for up to 3 wk until use. They were killed by decapitation and pithing. Hemibladders were mounted as sacs on glass cannulas as described previously (Walser, 1969, 1970 *b*), and gradually filled with 15–25 ml of amphibian Ringer's solutions,[2] depending upon the estimated size of the bladder. Both serosal and mucosal baths were vigorously stirred. All experiments were performed at room temperature (25–27°C).

In every experiment, the presence of edge damage was assessed by observing the effects on spontaneous potential of lowering the outer fluid level below the mounting ring. If no increase in potential occured, the fluid level was raised again to cover the ring. If potential increased with lowering, the fluid level was left about 4 mm below the outer ring, thereby avoiding the contribution of edge damage to transmural isotopic flux (Walser, 1970 *b*). Bladders were rejected in which potential was less than 60 mv or conductance (see below) was greater than 0.4 μmho/mg wet weight. Aeration was not necessary during stirring of both serosal and mucosal baths, as evidenced by the fact that bubbling air through the serosal bath had no effect on the electrical parameters.

At the conclusion of each experiment, wet weights of the bladder sacs were obtained after blotting on filter paper, and dry weights by heating overnight at 105°C. The degree of stretch could then be estimated from mucosal sac volume and weight (Walser, 1969). Stretch calculated in this manner varied from 0.07 cm^2/mg wet weight to 0.31 cm^2/mg, averaging 0.14 cm^2/mg. The ratio of dry to wet weight averaged 0.13. The results have been expressed for convenience in relation to wet weight.

The initial volume of the serosal bath was 50 ml. Both bath volumes diminished somewhat during the course of each experiment because of the removal of successive 1 ml samples for counting. This minor degree of reduction in stretch should have negligible effects on sodium transport (Walser, 1970 *a*).

In measuring bidirectional sodium fluxes, ^{22}Na was added first, to either mucosal or serosal bath, at approximately 0.04 μCi/ml. After several minutes of mixing, a sample was obtained of the radioactive bath to assess the contribution of ^{22}Na to the counting of ^{24}Na. This was necessary because of the extremely low fluxes observed in some experiments, coupled with the fact that the gamma spectrometer used (Nuclear-Chicago, Des Plaines, Ill.) did not completely exclude ^{22}Na counts from the ^{24}Na window. ^{24}Na was then added to the other bath at approximately 0.5 μCi/ml. ^{24}Na was counted in a window corresponding to the energy range of 3–4 Mev. ^{22}Na was counted 2 wk later in a window corresponding to the energy range 1.2–1.4 Mev. No radioactivity could be detected after 2 wk in standards containing only ^{24}Na. The efficiency of ^{22}Na counting was 3000 times greater in the ^{22}Na window than in the ^{24}Na window. Accordingly, the magnitude of the correction for ^{22}Na applied to the ^{24}Na results was always less than 10%, and usually negligible. ^{36}Cl as NaCl was used either by itself or in conjunction with ^{24}Na. After decay of ^{24}Na (if present), 1 ml samples were added to 15 ml of Bray's solution for counting by liquid scintillation.

Sampling for flux measurements was not begun until at least 30, or more commonly 60, min after the addition of the isotopes. Three to five samples of both baths were obtained at 10–15 min intervals before altering the clamping voltage.

During each period of observation, the mucosal bath volume used for purposes of calculation was the volume at the midpoint of the period. The measured radioactivity of mucosal samples obtained before or after the midpoint was multiplied by the ratio of actual volume at

[2] NaCl, 111 mM; $NaHCO_3$, 3 mM; $CaCl_2$, 2.7 mM; $MgCl_2$, 2 mM; KCl, 3.4 mM.

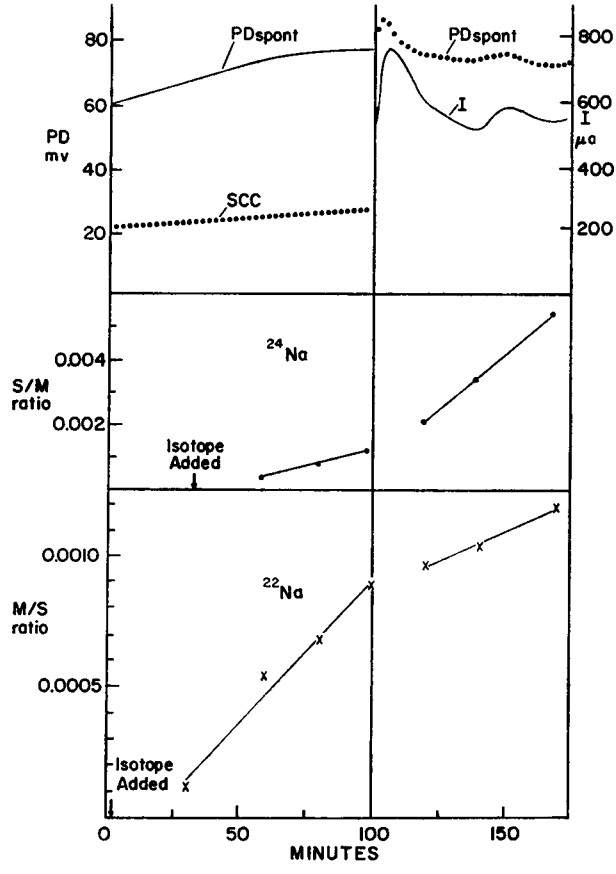

FIGURE 1 Results of a representative experiment. During the first 100 min, the bladder was open-circuited except for brief intervals of observation of SCC (shown as dots). During the next 72 min, sufficient depolarizing current was passed to reverse the potential difference (PD) and clamp it at +75 mv. The magnitude of this current is shown as *I*. During this period open-circuit PD was observed intermittently (shown as dots). Reverse polarization caused a transient rise in both *I* and PD. Mean *I* during the period of flux measurement (120–172 min) was 580 μa, compared with a mean SCC of 240 μa from 58 to 100 min. Mean PD was essentially the same during these two intervals. The lower panels show the sotope concentration ratios for ^{22}Na and ^{24}Na. Bath volumes were (initially) 50 ml (serosal) and 20 ml (mucosal). Bladder wet weight, 245 mg. Experiment No. 903A.

the time of sampling to the midpoint volume, in order to obtain the radioactivity which would have been observed if bath volume had not been reduced. This correction was not necessary in measuring serosal radioactivity because of the large volume of the serosal bath.

Fluxes were calculated from the linear regression of isotope concentration ratios on time, multiplied by the receiving bath volume at midpoint, and by the source sodium concentration (115 mM). Periods in which the concentration ratios did not rise linearly with time were rejected (see Fig. 1). No correction was necessary for falling radioactivity in the bath to which the isotope was added.

The automatic voltage clamp used permitted clamping at any voltage from −200 mv to

+200 mv. At intervals of 120 sec, open-circuit potential was observed for 3–10 sec, and recorded. Current was recorded continuously. During open-circuit observations, SCC was observed every 120 sec for 3–10 sec, and potential was recorded the rest of the time. The accuracy of the instrument was checked by frequent use of a constant voltage source and calibrated resistances.

The intermittently observed electrical parameters were not permitted to attain steady-state values. The transients which occurred on changing from one voltage to another were not analyzed in detail, other than to note that they lasted approximately 15 min. This interval represents a small fraction of the period of observation of flux.

The validity of the SCC measurements was further examined in some experiments by replacing the external current-passing agar-KCl bridge with a cylindrical stainless steel mesh electrode, made to fit into and conform to the shape of the beaker holding the serosal bath. There was no difference between the SCC as measured by these two devices. Thus asymmetry of the external electrical field is not a source of error.

Total bladder conductance g was estimated as $I/(\psi_{clamp} - \psi_{spontaneous})$.

The sequence of clamping voltages and the number of different voltages employed varied with the stability of the preparations and practical considerations. In 10 of the 83 bidirectional sodium flux experiments and in 35 additional unidirectional sodium flux experiments at least two different voltages were used.

Unidirectional chloride flux was measured in 70 periods of observations in 27 hemibladders, alternating open- and short-circuit conditions. In 20 of these experiments, ^{24}Na flux was measured simultaneously in the same direction as the ^{36}Cl flux. These 20 experiments were performed on paired hemibladders and thus gave rise to approximate estimates of net sodium and chloride flux in these 10 bladders. In each of these pairs, the difference in open-circuit potential between the two hemibladders was less than 15%.

The effect of mucosal sodium concentration on serosal-to-mucosal sodium flux was studied in 15 open-circuit experiments. After a period of equilibration in normal media, the mucosal bath was exchanged (at constant volume) with one of two low sodium media: in the first, choline chloride and choline bicarbonate replaced NaCl and $NaHCO_3$; in the second, the sodium salts were simply omitted and hence the medium was hypotonic. ^{24}Na or ^{22}Na was added to the serosal bath. After 30–60 min equilibration, three or four 10 min samples were obtained from the mucosal bath. Then normal medium was exchanged, in various quantities, with the mucosal medium, at constant volume, and sampling was continued. In four of the experiments, two successive increments in mucosal sodium concentration were examined in this manner. At the start of each period, a sample of the mucosal bath was obtained for measurement of sodium concentration by atomic absorption spectrophotometry.

RESULTS

Bidirectional Sodium Fluxes

A representative experiment is depicted in Fig. 1. The results are summarized in Table I and Fig. 2. There was no significant difference between the four groups of experiments with respect to open-circuit potential (shown only in one group) or tissue conductance g. The mean conductance, 20 μmho/mg wet weight, corresponds to a resistance of 7000 Ω-cm^2, when expressed in relation to "macroscopic surface," or 15000 Ω-cm^2, when expressed in relation to "ultramicroscopic surface" (Gfeller and Walser, 1971).

TABLE I

BIDIRECTIONAL SODIUM TRANSPORT AT VARIOUS POTENTIALS

No. of observations	ψ	I	g	Φ	β	J_{Na}	Φ/β ratio	J_{Na}/I ratio
	mv	*μa/mg*	*μmho/mg*	*μa/mg*	*μa/mg*	*μa/mg*		
8	75	3.12 ± 0.54	20 ± 3	2.66 ± 0.52	0.14 ± 0.05	2.52 ± 0.50	35.4 ± 8.2	0.81 ± 0.07
31	0	1.90 ± 0.21	22 ± 2	1.75 ± 0.23	0.16 ± 0.02	1.58 ± 0.22	14.4 ± 3.2	0.80 ± 0.04
40	−97 ±4	(2.05 ± 0.21)*	20 ± 2	0.53 ± 0.04	0.21 ± 0.02	0.32 ± 0.04	3.10 ± 0.35	(0.17 ± 0.02)‡
14	−150	−0.77 ± 0.10	15 ± 2	0.14 ± 0.02	0.285 ± 0.05	−0.14 ± 0.04	0.57 ± 0.09	0.17 ± 0.03

* Intermittently observed SCC.

‡ J_{Na} expressed as a fraction of intermittently observed SCC.

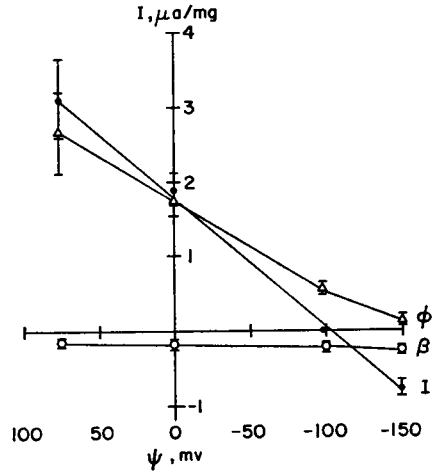

FIGURE 2 Sodium flux and current as a function of PD. Mean Φ, β, and applied current (I) are shown, ±1 SEM, from Table I. β changes only slightly over this 225 mv range. Φ changes 20-fold. At −150 mv, net flux is reversed but is only a small fraction of applied current.

In 31 short-circuited bladders, net sodium flux was 80 ±4% of SCC. This mean differs significantly from 100% ($P < 0.01$), but the range of values overlaps previously reported ranges (Table II). Intermittent opening of the circuit may have contributed to this difference.

During open-circuit conditions, net sodium flux was markedly reduced, in comparison with short-circuit conditions, and sometimes absent (Fig. 3). There is little relationship between SCC and the rate of net transport observed under open-circuit conditions. The reduction in net sodium flux is almost entirely attributable to a decrease in Φ; β is only slightly altered (Table I, Fig. 2).

At +75 mv, Φ is further increased but β is again only slightly decreased. Net sodium flux averages 81% of applied current.

At −150 mv, net flux is reversed, but β is only slightly larger. Net sodium flux accounts for only 17% of applied current.

TABLE II

NET SODIUM FLUX AS A FRACTION OF SHORT-CIRCUIT CURRENT IN TOAD BLADDER, ACCORDING TO VARIOUS AUTHORS

Author	*n*	Mean	SD	SEM
Leaf, Anderson, and Page, 1958	26	1.00	0.18	0.05
" " " " "	16	0.98	0.27	0.07
Leaf, 1960	16	0.98	0.27	0.07
Leaf and Dempsey, 1960	14	1.06	0.21	0.05
Walser, 1969	19	0.99	0.29	0.07
Mendoza, Handler, and Orloff, 1970	130	0.95*	0.24	0.02
Present data	31	0.80*	0.22	0.04

* Significantly less than 1.00.

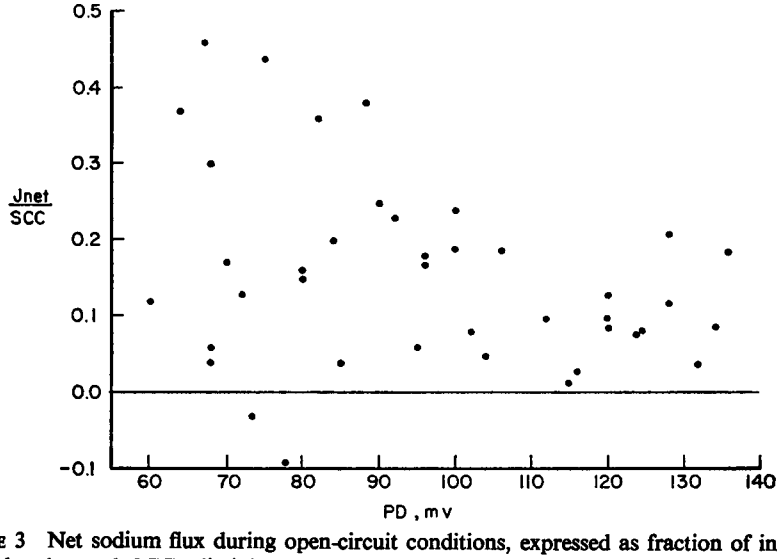

FIGURE 3 Net sodium flux during open-circuit conditions, expressed as fraction of intermittently observed SCC, diminishes with increasing spontaneous PD. Fractions above 0.25 are seen only at PDs smaller than 90 mv. In eight experiments, net flux is less than 5% of SCC, and in two, it is apparently negative.

Between +75 mv and −97 mv the slope $g_+ = \delta J/\delta\psi$ is nearly constant and equal to 12 μmho/mg; between −97 and −150 mv, g_+ is lower, 8.5 μmho/mg.

Unidirectional Sodium Flux

The effect on Φ and β in individual experiments of changing from open-circuit to short-circuit conditions, or the reverse, was assessed in several experiments. Initiation of short-circuiting decreased β insignificantly (0.05 ± 0.03 μa/mg, $n = 7$); initiation of open-circuiting increased it by a similar amount, again in-

significantly different from zero (0.05 ±0.03 μa/mg, $n = 21$). By contrast, Φ increased 0.98 ±0.17 μa/mg ($n = 26$, $P < 0.01$) on starting short circuiting and decreased 0.76 ±0.25 μa/mg ($n = 11$, $P = 0.01$) on changing to open-circuit conditions. Thus the sequence of voltage changes did not alter the results.

Transstimulation of Serosal-to-Mucosal Sodium Flux

The effect of increasing mucosal sodium concentration on β is illustrated in Fig. 4. Initial sodium concentration varied from 0.18 mM to 7 mM. Addition of sodium to the mucosal bath caused a modest increase in β in some experiments but the results were variable. The mean increase was 0.02 μa/mg, or 20%. Since ψ became more negative when sodium was added (by 50 ±7 mv), some increase in β might be expected in accordance with the results shown in Table I. Although these results

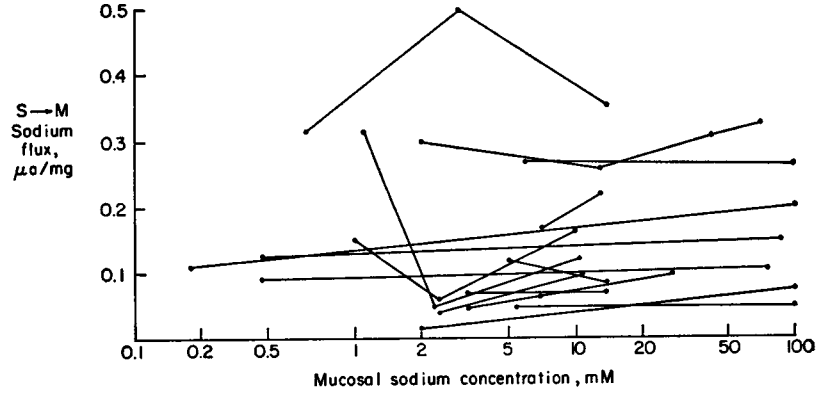

FIGURE 4 Effect of increasing mucosal sodium concentration on serosal-to-mucosal sodium flux. The serosal medium remained normal Ringer. Although there is an upward trend, no consistent transstimulation is seen.

are not unequivocal, they do not support the thesis that most of β is exchange diffusion.

Estimated Net Chloride Flux

Unless other ions are transported, net sodium and chloride fluxes during open-circuit conditions should be equal. As shown in Table III, net sodium flux is about twice as great. Although the paired difference is significant statistically, it is important to note that this comparison is based upon unidirectional flux data in paired hemibladders. The mean difference observed, 0.20 ±0.07 μa/mg, is similar in magnitude but opposite in direction to the difference between net sodium flux and SCC. Movement of any ion along the electrical gradient could account for it.

Frazier and Vanatta (1971) and Ludens and Fanestil (1971) have reported,

TABLE III

NET SODIUM FLUX (J_{Na}) AND NET CHLORIDE FLUX (J_{Cl}) COMPARED DURING OPEN-CIRCUIT CONDITIONS*

	J_{Na}	J_{Cl}	J_{Na}-J_{Cl}
	μa/mg	*μa/mg*	*μa/mg*
	0.26	0.18	0.08
	0.17	0.10	0.07
	0.34	0.30	0.04
	0.21	0.09	0.12
	0.26	0.02	0.24
	0.84	0.31	0.53
	0.63	0.79	−0.16
	0.61	0.20	0.41
	0.16	−0.09	0.25
	0.41	−0.06	0.47
Mean	0.39	0.18	0.20
SEM	±0.07	±0.08	±0.07

* Based upon measurement of forward fluxes of Na and Cl in one hemibladder simultaneously with backward fluxes of Na and Cl in the paired hemibladder.

TABLE IV

CHANGE IN UNIDIRECTIONAL Cl FLUXES, EXPRESSED AS PER CENT OF CHANGE IN APPLIED CURRENT, DURING OPEN ("OFF") VS. SHORT ("ON") CIRCUIT

	On to off	Off to on	Combined
$\Delta\Phi$: Mean	−0.4%	9.3%	3.3%
SEM	±1.5%	±2.8%	±1.5%
n	31	19	50
p	n.s.*	<0.01	<0.05
$\Delta\beta$: Mean	2.4%	3.7%	2.6%
SEM	±1.1%	±2.9%	±1.2%
n	30	14	44
p	n.s.	n.s.	n.s.
Total ($\Delta\Phi + \Delta\beta$):	2.0%	13.0%	5.9%

* n.s. = not significant.

since these experiments were completed, that net flux of hydrogen ions from serosa to mucosa can occur in toad bladder in the presence of CO_2. In previous studies, aeration has usually been continuous and hence P_{CO_2} of the bathing media has probably been very low. In our experiments, the same low bicarbonate medium is used and is exposed to air, but not bubbled. Hence some accumulation of CO_2

in the media doubtless occurs, derived from metabolism. This may have been sufficient to promote active or passive hydrogen secretion.

In order to estimate the contribution of net chloride flux to current, the changes in both unidirectional chloride fluxes on changing from open- to short-circuit (or vice versa) were expressed as a fraction of the change in applied current (Table IV). If chloride flux responded as expected, all such fractions should be positive.

The sum of the changes in unidirectional fluxes should be an estimate of the change in net chloride flux. As the table shows, only small and inconsistent changes in chloride fluxes usually occurred, but the over-all contribution of net chloride flux amounts to about 6% of the applied current.

Net chloride flux, during short-circuit conditions, did not differ significantly from zero.

Unidirectional Chloride and Sodium Fluxes

β_{Cl} and β_{Na} were measured simultaneously in 14 experiments during short-circuiting and in 12 experiments during open-circuit conditions (Fig. 5). A positive correlation is evident under both conditions. In short-circuit conditions, the mean β_{Cl} (0.26 $\pm$0.05 μa/mg) exceeds mean β_{Na} (0.21 $\pm$0.04 μa/mg) and the mean difference, though small, (0.06 $\pm$0.02 μa/mg), is significantly greater than zero. As expected, the mean β_{Cl} in open-circuit conditions is less than the mean β_{Na}, and the difference is statistically significant.

The means of all of the unidirectional Cl fluxes are shown in Table V. The estimation of passive and exchange components is explained in the discussion.

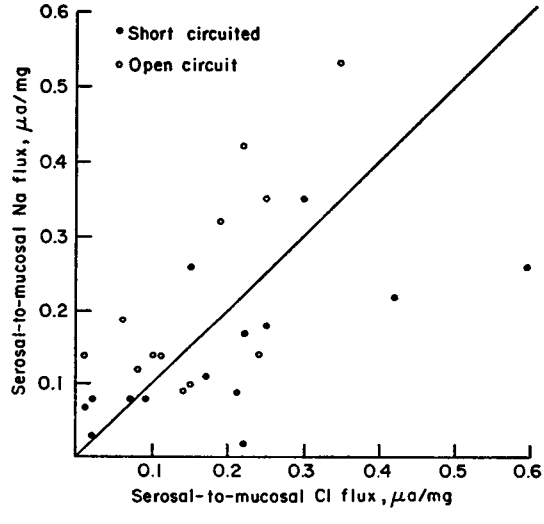

FIGURE 5 Unidirectional Na and Cl flux measured simultaneously, during open- and short-circuit conditions. The diagonal line indicates equality.

TABLE V
UNIDIRECTIONAL CHLORIDE FLUX ACROSS TOAD BLADDER DURING OPEN- AND SHORT-CIRCUIT CONDITIONS

Condition	ψ	Φ^-	β^-	Φ_p^-	β_p^-
	mv	*μa/mg*	*μa/mg*	*μa/mg*	*μa/mg*
Short circuit		0.15 ± 0.04 (22)*	0.20 ± 0.04 (17)	0.04	0.09
Open circuit	−75 ±4	0.24 ± 0.04 (17)	0.12 ± 0.03 (13)	0.13	0.01

* Number of observations in parentheses.

ANALYSIS AND DISCUSSION

The results obtained in these experiments differ markedly from those that would be anticipated from a simple "pump-and-leak" model of Na transport. Serosal-to-mucosal Na flux is only slightly altered over a 225 mv range of transepithelial potential, while mucosal-to-serosal flux changes almost as much as current over the same range. Before possible interpretations are examined it is necessary to consider whether the data may be erroneous.

Possible sources of error include:

(*a*) Incomplete mixing or nonsteady-state conditions. Both baths were vigorously stirred. An average of four samples was obtained during each period, and the resulting isotope concentrations were linear with time. The lowest net sodium flux (at −150 mv), −0.8 neq/min per mg, when compared with the mucosally labeled "transport pool" of sodium, 0.8 neq/mg (Gfeller et al., 1971), suggests a half-time of this pool of the order of 40 sec. Even if this estimate is wrong by an order of magnitude, significant accumulation or depletion of tissue sodium (sufficient to invalidate fluxes estimated from transepithelial movement) could not persist for more than a small fraction of the period of observation.

(*b*) Systematic overestimation or underestimation of one flux or the other or of current. ^{24}Na and ^{22}Na were used interchangeably, and no difference could be discerned between the results when ^{24}Na was moving M → S as compared with S → M. There is no apparent reason why such a systematic error should vary with potential; however, a constant error in the electrical measurements would invalidate the comparison of absolute values of net flux with current. The latter error has been discussed previously with respect to this technique (Walser, 1969) and shown to be negligible.

(*c*) Bias introduced by the sequence in which the experiments were performed. As noted above, the flux changes with potential were reversible, and the sequence of potential changes did not alter the results. Furthermore, the data presented have been (for the most part) obtained from experiments in which a single potential was employed, after a standard period of equilibration.

(*d*) Random error. The standard error of the linear regression coefficients of isotope concentration on time averaged 8% of the coefficients. Random error in

flux must be the same percentage, since the other components of this calculation (time, volume, and sodium concentration) have far smaller errors.

Are these results in conflict with earlier data? There are few comparable studies of toad bladder in the literature. Leaf (1965) gives partial data from a single experiment in which unidirectional sodium fluxes were measured in paired bladders during short-circuit and open-circuit (−50 mv) conditions. β changed threefold while Φ changed sevenfold. Schwartz and Snell (1968) give data from two experiments in which bidirectional transepithelial fluxes were measured open- and short-circuited, but do not report ψ. J in open-circuit conditions was one-half of SCC, in contrast to the present ratio of 0.17. Pendleton et al. (1968) measured bidirectional sodium fluxes during open-circuit conditions in a series of experiments. Φ/β averaged 4, close to our value, but J/SCC averaged 0.8. ψ in these experiments averaged about 44 mv. They did not compare fluxes during open- and short-circuit conditions.

It should be noted that all of these experiments were performed on low resistance preparations, in which edge damage was probably considerable (Walser, 1970 *b*).

Schilb (1969) measured bidirectional sodium fluxes in open-circuit (−80 mv) and short-circuit conditions in turtle bladder, with mean results that conform closely to the flux-ratio equation. The two means were obtained from different sets of experiments. Nevertheless these results are more at variance with the present data than any others.

In frog skin, Ussing and Zerahn (1951) measured unidirectional sodium fluxes at various values of potential between open circuit and zero. Influx changed more than efflux, but flux ratios could not be calculated, as they lacked bidirectional flux data. Linderholm (1952) gives results of two experiments in which either efflux or influx of sodium was measured, first open- and then short-circuited. ψ was about −40 mv in both experiments. Influx increased about 70%, but outflux decreased only about 30%. Biber et al. (1966) measured simultaneous influx and outflux with low sodium medium on the outside, either open- or short-circuited in several experiments with $\psi = -49 \pm 1$ mv. In no experiments were fluxes measured under both conditions. Mean outflux was 46% greater during open-circuit conditions, while mean influx was reduced 87%. In another group of these experiments, in which ψ's were not given, influx changed 60% but outflux remained constant. Candia (1970) has recently reported that outflux is quite insensitive to potential, as in the present data.

In interpreting these results, it is tempting to view Φ and β as distinct processes, since they respond so differently; however this would imply two independent and irreversible transport mechanisms operating in opposite directions, a most unlikely situation.

We are therefore compelled to interpret the results in terms of the usual pump-and-leak model, or some variant of it. The parameters of this model are E, the

electromotive force of the sodium pump, g_a, the conductance of the pump pathway, and g_p, the sodium conductance of the parallel leak pathway. All three parameters may conceivably vary with potential. In addition, a constant exchange flux J_e may be present, but cannot contribute to net flux. Other "abnormal" fluxes might of course be present, but will not be considered, in view of the complexities of fitting the data to this admittedly simplified model. In addition, we assume that the mean fluxes at each potential are representative of the results which could (in theory) be obtained in a single bladder studied at all four potentials. Heterogeneity of parameters is ignored.

First the data can be examined from the view that $J_e = 0$, as suggested by the absence of significant transstimulation of serosal-to-mucosal sodium flux. Shapiro and Candia (1971) have recently shown how bidirectional flux data at any potential can be analyzed to yield E, g_a, and g_p, provided that $g_+ (= g_a + g_p)$ is known; however, g_+, the phenomenological coefficient of conductance of sodium transport, is not given by $\delta J_{Na}/\delta\psi$ unless J_r, the flow of reaction, is constant (Essig and Caplan, 1968). In frog skin, and evidently in toad bladder too, oxygen consumption is increased by depolarization (Voûte and Ussing, 1968; Ussing, 1958; Vieira et al., 1972; Zerahn, 1958; Parisi and Bentley, 1970). Hence any value of g_+ less than $\delta J_{Na}/\delta\psi$ is possible, depending upon the degree of coupling between transport and metabolism. Measurement of $\delta J_r/\delta\psi$ in the preparation used in the present experiments will be required before the procedure proposed by Shapiro and Candia (1971) can be applied with a known value of g_+. These authors also give a procedure by which the parameters E, g_a, g_p, and J_e can be calculated from flux data at two different potentials, assuming that J_e and either g_a, g_p, or both are the same at both potentials; but again knowledge of g_+ is required.

If we permit all of the parameters to vary with potential, except that $J_e = 0$, and consider g_+ as unknown, but limited by $g_+ < 13$ (since $\delta J_{Na}/\delta\psi < 13$), it is possible to use the procedure of Shapiro and Candia (1971) to obtain the parameters E, g_a, and g_p as a function of g_+ at each of the four potentials. The resulting functions for $\psi = 0$, -97, and -150 mv are shown in Fig. 6. At $\psi = 75$ mv, no solutions are obtained because a quantity, termed "A_{exp}" by these authors, lies outside of limits which this model would dictate. Clearly no single value of g_+ fits the data at these three ψ's. Constant g_a and variable g_p fit the data (except at $\psi = 75$ mv), but seem highly unlikely.

A more plausible assumption is that g_p is constant and $J_e > 0$. The absence of transstimulation of β may not be conclusive evidence that $J_e = 0$, because saturation of the exchange flux could occur at very low sodium concentration. Furthermore, coupled neutral flux of Na and Cl might fail to exhibit transstimulation.

The assumption of constant g_p sets a lower limit on J_e. Since $J^{-97} > 0$, $E^{-97} > 97$ mv. Hence $E^{75} > 97$ mv, and $E^{75} + \psi > 172$ mv (assuming that depolarizing current does not reduce E). Since $f_0(172)F/RT = 0.00021$, $\beta_a^{75} =$

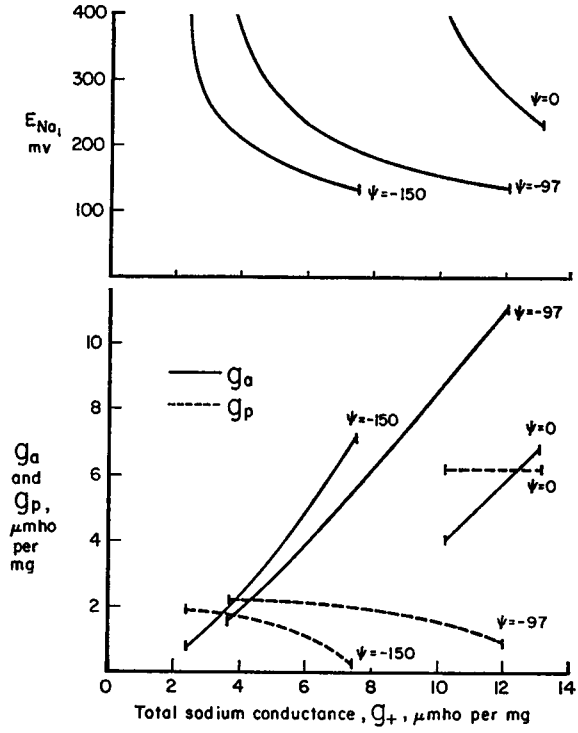

FIGURE 6 Calculated parameters of sodium transport plotted as a function of total sodium conductance at each of three values of potential, assuming exchange diffusion to be absent. The left-hand limits of each curve are determined by (*a*) $E < 400$ mv and the right-hand limits by (*b*) $g_+ \leq \Delta J_{Na}/\Delta\psi$, or (*c*) the limits of g_+ dictated by ψ, J_{Na}, and Φ (Shapiro and Candia, 1971).

0.00021 g_a^{75} (Shapiro and Candia, 1971). Thus β_a^{75} is negligibly small in comparison with β_p^{75}. $f_0(75)F/RT = 0.00425$. Hence $\beta_p^{75} = 0.00425\ g_p$. But $\beta^{75} = 0.14 = \beta_p^{75} + J_e$. Therefore $g_p = (0.14 - J_e)/0.00425$. Now $\beta^{-150} = 0.285 = \beta_a^{-150} + \beta_p^{-150} + J_e$. Since $\beta_a^{-150} > 0$, $0.285 - \beta_p^{-150} - J_e > 0$. $f_0(-150)F/RT = 0.1504$. Hence $\beta_p^{-150} = 0.1504\ g_p$. Substituting for g_p, $\beta_p^{-150} = 0.1504\ (0.14 - J_e)/0.00425 = 4.95 - 34.4\ J_e$. Hence $0.285 - (4.95 - 35.4\ J_e) - J_e > 0$. Therefore $J_e > 0.1356$ and $g_p < 1.03$. Table VI shows the fluxes corrected for exchange diffusion of this magnitude, and the resulting limits placed upon β_p, β_a, and J_a.

The chloride data can also be used in a similar way to obtain an estimate of the magnitude of exchange diffusion for this ion, J_e^-. Owing to the absence of simultaneous bidirectional fluxes and the smaller number of observations, this estimate is less precise. We assume that only passive diffusion and exchange diffusion occurs. Φ is taken as equal to $J_e + (RT/F)g_-f_0(\psi)$ and β as $J_e + (RT/F)g_-f_i(\psi)$. g_- is given by $(\Phi - \beta)/\psi$. The resulting estimate of J_e^-, 0.11 μa/mg, is close to the value obtained for sodium. This observation points to the possibility that the exchange fluxes of sodium and chloride are coupled.

TABLE VI

COMPONENTS OF SODIUM FLUX AT VARIOUS POTENTIALS, CALCULATED ON THE BASIS OF $g_p < 1.03$ AND $J_e > 0.1356$

ψ	Φ	Φ-J_e	Φ_p	Φ_a	β	β-J_e	β_p	β_a	J_a	E	g_a
mv	*μa/mg*	*μa/mg*	*μa/mg*	*μa/mg*	*μa/mg*	*μa/mg*	*μa/mg*	*μa/mg*	*μa/mg*	*mv*	*μmho/mg*
+75	2.66	<2.52	<0.08	2.44–2.52	0.14	<0.0044	<0.0042	0–0.0044	2.44–2.54	>87	<15.1
0	1.75	<1.614	<0.026	1.59–1.61	0.16	<0.024	<0.0256	0–0.024	1.57–1.61	>108	<14.5
−97	0.53	<0.394	<0.002	0.392–0.394	0.21	<0.074	<0.10	0–0.074	0.32–0.39	>140	<7.4
−150	0.14	<0.004	<0.0004	0.0036–0.0040	0.285	<0.149	<0.15	0–0.149	−0.14–+0.0036	>55	<1.5

ψ: transepithelial potential difference.
Φ, Φ_p, Φ_a : total, active, and passive fluxes of sodium from mucosa to serosa.
β, β_p, β_a : total, active, and passive fluxes of sodium from serosa to mucosa.
J_e : exchange diffusion flux of sodium.
J_a : net sodium flux via active transport pathway.
E: electromotive force of sodium pump.
g_a : conductance of active transport pathway for sodium.
g_e : conductance of leak pathway for sodium.

Minimal values of E at each potential are next obtained from the relationship:

$$(RT/F) \log_e \Phi_a/\beta_a = E + \psi,$$

using the lower limit for Φ_a and the upper limit for β_a (Shapiro and Candia, 1971). Maximal values of g_a are then obtained from the relationship:

$$J_a = (E + \psi)g_a,$$

using the maximal values of J_a and minimal values of E. The results are shown in Table VI.

These limits are not in themselves very informative except in one respect: g_a^{-150} is apparently much smaller than g_a at greater values of ψ. This suggestion of rectification is in accord with the lesser value of $\Delta J_{Na}/\Delta\psi$ seen between −97 and −150 mv as compared with other potentials (Fig. 2).

The data from Table VI can now be used to plot E as a function of g_a for all possible values of the latter, given the limits already placed on E. The results are shown in Fig. 7. The only single value of E applicable to all of the data is 140 mv. In this event, g_a^{-97} and g_a^{-150} are both about 8.5 μmho/mg, but g_a^0 and g_a^{75} are both considerably larger. In other words, depolarization causes a substantial increase in the conductance of the pump pathway, but hyperpolarization has little or no effect.

The degree of coupling q_a between the metabolic reaction, J_r and active sodium transport J_a is a function of the phenomenological coefficients that characterize the relationship between these two flows and the two driving forces, viz., the electrochemical gradient for sodium X^+ and the affinity of the driving reaction A. As Essig and Caplan (1968) have shown, $\delta J_a/\delta\psi_{J_r} = g_a$, but $\delta J_a/\delta\psi_A = g_a/$

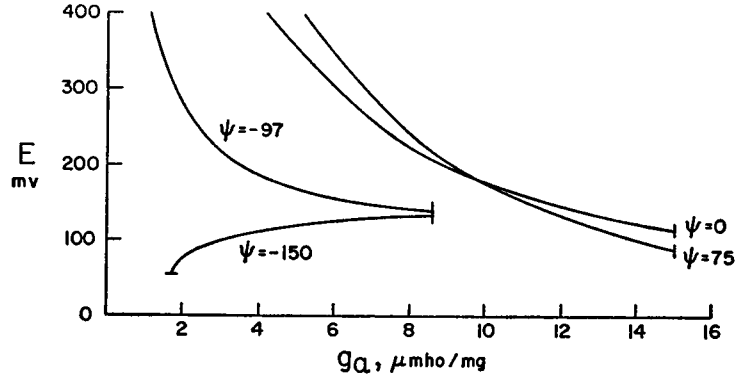

FIGURE 7 Calculated values of E_{Na} as a function of g_a at each ψ. The limits of each curve are determined by $E < 400$ mv and by the limits shown in Table VI. The only value of E that is on all of the curves is ~140 mv.

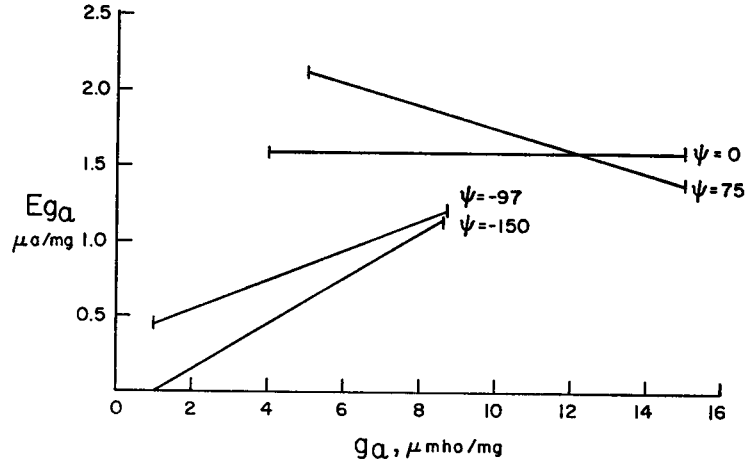

FIGURE 8 Sodium transport capacity Eg_a as a function of g_a for all possible values as determined by the limits shown in Table VI and the condition $E < 400$ mv. No single value of Eg_a is found on all four curves.

$(1 - q_a^2)$. (An additional component of coupling is introduced by the parallel leak pathway.) The reason that $g_a + g_p$ may be $< \delta J_{Na}/\delta\psi$ in the toad bladder is that J_r may vary with ψ (see above). $\delta J_{Na}/\delta\psi$ evaluated at $\psi = 0$ is 13 μmho/mg (Fig. 2). Hence choice of any value of $g_a + g_p$ less than 13 leads to a value of q_a^0, the degree of coupling between active transport and its driving reaction, at this particular value of ψ. The derivative cannot be evaluated at other values of ψ from these data.

Alternatively, we may consider E as variable. In doing so, it is useful to calculate the product Eg_a. This quantity, termed "virtual level-flow influx" by Shapiro and Candia (1971) is a measure of the transport capacity of the pump at

any given value of ψ, independent of the direct effect of ψ on flux through the pump pathway. It is the SCC which would be observed if ψ could be reduced to zero without changing E or g_a in the process. At $\psi = 0$, it is equal to SCC.

Transport capacity estimated in this way is shown as a function of g_a for these same estimates (from Table VI) in Fig. 8. We have arbitrarily omitted consideration of values of $E > 400$ mv. Again it is apparent that no single value of Eg_a fits all of the data. Eg_a^{-150} and Eg_a^{-97} must be less than Eg_a^0 and Eg_a^{75}. Thus transport capacity, defined in this way, must be increased by depolarization. It follows that SCC ($= Eg_a^0$) measures only one particular value of transport capacity. More important, SCC is not (necessarily) a measure of maximal transport capacity, and does not reveal the dependence of this quantity on ψ.

A third characteristic of the pump pathway is the value of E at which $J_a = 0$, i.e. the E.M.F. of the pump when operating reversibly in a thermodynamic sense. This value is not indicated by any of the observations herein, and in general cannot be obtained simply by measuring bidirectional fluxes of sodium. We can infer from Fig. 7 that it probably lies in the vicinity of 140 mv, but a lower value is also possible. A higher value is not, given the assumptions on which this analysis is based.

It must be reemphasized that all of the inferences drawn from Table VI and Figs. 7 and 8 depend upon the validity of the assumptions of constant g_p and J_e.

Previous observations on Cl flux in toad bladder have indicated much higher permeability, probably because of edge damage (Jamison, 1961; Leaf and Hays, 1962; Finn et al., 1967). The last-named authors also found evidence for active transport of Cl from mucosa to serosa, but only after removal of serosal potassium.

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