TRANSEPITHELIAL CURRENT-VOLTAGE RELATIONSHIPS OF TOAD URINARY BLADDER AND COLON

ESTIMATES OF $E_{NA}A$ AND SHUNT RESISTANCE

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ABSTRACT Studies were done to investigate the transepithelial current-voltage (I_T-V_T) relationships of urinary bladder and colon of the toad *Bufo marinus*. Like several other Na transporting epithelia, the I_T-V_T plots characteristically showed a break at voltage E_l, averaging near 124 mV for urinary bladder and 110 mV for colon. With bladders treated with antidiuretic hormone, estimates of E_{Na} and shunt resistance, R_s, were obtained according to a method outlined by Yonath and Civan, 1971 (*J. Membr. Biol.* 5:336–385). Our results not only confirmed their observations, but were consistent with the notion that the values of E_l (I_T-V_T plots) were the same as those of E_{Na}. In addition, the values of R_s were found to be the same as those estimated from the quotient E_l/I_l obtained from the voltage and current coordinates at the break of the I_T-V_T plot of bladders studied in both stretched and unstretched states. Amiloride at concentrations up to 10^{-5} M caused a small decrease of both E_l and E_l/I_l of urinary bladder. Similarly, amiloride caused small but significant changes of E_{Na} and R_{Na} of the colon. For both epithelia, the values of E_l and E_l/I_l of the I_T-V_T plots were the same as those of E_{Na} and R_s estimated by an independent method. In general, these findings are similar to those of several other epithelia where the E_{Na} and R_s can be estimated directly from their I_T-V_T relationships.

INTRODUCTION

Epithelia of diverse origin actively transport Na between the fluids bathing their borders. The mechanism(s) of the transepithelial transport process has remained obscure although it is generally believed that Na is actively extruded from a cellular compartment into the serosal solution after a passive entry into the cells at their mucosal or apical barrier.

In the earliest attempt to apply formalism to the process of active transepithelial sodium transport, Ussing and Zerahn suggested a simple electrical equivalent circuit of the active Na transport pathway (2). The Thévenin equivalent of the active Na transport pathway consisted of a driving force, E_{Na}, in series with a resistor, R_{Na}. Despite the fact that little if anything was known of the manner in which the E_{Na} was generated nor the location of the E_{Na} or R_{Na} within the epithelium, this simple electrical description of active Na transport was fundamentally correct and remains so today.

This laboratory has accumulated evidence that supports the idea that the values of E_{Na} of several epithelia can be estimated simply and directly in studies of their steady-state

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current-voltage relationships (3–7, 23). In particular, examination of the I_T-V_T plots showed distinct "breaks" at a voltage E_1 defined at a transepithelial voltage where the slope resistance changed in the hyperpolarizing region of the I_T-V_T plots. As these values of E_1 were the same as those of E_{Na} estimated by several independent methods, it was concluded that the values of E_1 provided estimates of the E_{Na}. The present studies were done to characterize the I-V relationships of the toad urinary bladder and toad colon, as these tissues are also known to actively transport sodium. In particular, we examined the I_T-V_T relationships of these tissues to ascertain whether they showed breaks such as observed in the frog skin, and if so, whether the voltages at the breaks could be equated with the transepithelial driving force for active sodium transport. Civan had studied the I_T-V_T relationship of the toad urinary bladder and reported a break in the vicinity of 170 and 180 mV that he thought might be equated with the E_{Na} (8). In later studies by Yonath and Civan, values of E_{Na} near 110 mV were estimated by a different method that assumed that vasopressin caused changes of the conductance to active sodium transport with no effects on the E_{Na} and the shunt resistance, R_s (1). Indeed, these values near 110 mV are quite similar to those estimated from the I-V plots of the frog skin and the renal collecting tubule of the rabbit.

In the present work, we studied both the urinary bladder and distal colon of the toad *Bufo marinus* to characterize the steady-state I_T-V_T relationships of these epithelial tissues. Their I_T-V_T plots showed a single break at values of E_1 similar to those observed previously for the frog skin and collecting tubule. In addition, in studies of the urinary bladder with antidiuretic hormone (ADH) we confirm the findings of Yonath and Civan and demonstrate that the values of E_1 can be equated with those of E_{Na} for both the urinary bladder and colon of the toad.

Theoretical Considerations

In general, the steady-state I_T-V_T relationships of the toad urinary bladder and toad colon can be described simply by two regions of linear slope resistance, R_1 and R_2 (see Results) intersecting at a coordinate E_1,I_1 in the hyperpolarizing region of the I_T-V_T plots. Helman and Fisher proposed a simple electrical equivalent circuit which accounted for the break at voltage E_1 observed in studies of frog skin and retained the idea that the values of E_1 approximated those of E_{Na} (5). Their model, shown in Fig. 1, views the active sodium transport pathway with an EMF E_1 in series with a combination of resistors and diodes attributable to the equivalent resistances of mucosal and serosal barriers of the cells. A parallel passive shunt pathway is modeled with a simple ohmic resistor, R_s.

Direct support for such a model was obtained in microelectrode studies of the frog skin where the values of E_1 were observed to be the same as the voltage developed at the serosal barrier when transepithelial Na transport was reduced to zero either with the drug amiloride or by reduction of [Na] of the outer solution (5). Additionally, the changes of slope resistance observed in the I_T-V_T plots could be attributed to a change of electrical resistance at the apical barrier of the cells upon change of its polarity and presumably upon a change in direction of transepithelial current flow via the "E_1 pathway." When Na entry and presumably net charge transfer at the apical barrier was reduced to zero the voltage at the outer barrier was also zero.

Accordingly, if we define the transepithelial electrical resistance of the active E_1 pathway

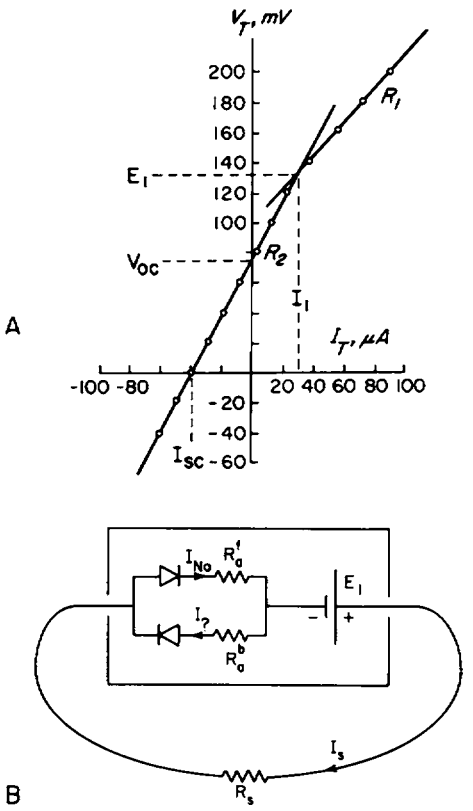

FIGURE 1 (A) Electrical parameters of I_T-V_T plots of urinary bladder and colon of *Bufo marinus*. (B) Simple equivalent circuit. R_1 and R_2 are slope resistances = $\Delta V_T/\Delta I_T$. Break at E_1 is defined at intersection of slope resistances R_1 and R_2. According to the model, the change of slope resistance occurs owing to differences in value of R_a^f and R_a^b and is coincident with reversal of current flow through the "E_1 pathway." At $V_T = E_1$, transepithelial current flow, I_s, is via the shunt pathway and so the $R_s = E_1/I_1$.

as R_a, it can be shown that when $V_T = 0$ (short circuited tissue):

$$I_{sc} = E_1/R_a^f = E_{Na}/R_{Na}, \tag{1}$$

where R_a^f is defined as the transepithelial resistance of the active pathway when current flows from mucosal to serosal solutions and I_{sc} is the short-circuit current.

When $V_T < E_1$:

$$1/R_2 = (1/R_a^f) + (1/R_s). \tag{2}$$

The open-circuit voltage is given by:

$$V_{oc} = (E_1 \cdot R_s)/(R_s + R_a^f). \tag{3}$$

It is obvious from the model that when $V_T = E_1$ the shunt resistance, R_s, is equal to the quotient E_1/I_1. This follows directly from the fact that at $V_T = E_1$ transepithelial current flow via the E_1 pathway is reduced to 0 and so $I_1 = I_s$. Although such a definition is simple, it should be noted that the values of E_1/I_1 encompass all parallel pathways for ion transport and

would include not only extracellular but also transcellular routes in parallel with the E_1 pathway (see Discussion). In a sense, the values of E_1/I_1 represent the equivalent electrical resistance of all pathways in parallel with the E_1 pathway. When $V_T > E_1$:

$$1/R_1 = (1/R_a^b) + (1/R_s). \quad (4)$$

Thus, with the measured values of R_1, R_2, I_{sc}, E_1, and V_{oc}, it is possible to calculate the values of the parameters R_a^f, R_a^b, and R_s of the electrical model.

MATERIALS AND METHODS

Toads (*Bufo marinus*) originating from either Nicaragua (Pet Farm, Miami, Fla.) or from the Dominican Republic (National Reagents, Bridgeport, Conn.) were used in the present studies. They were stored unfed either on moist San-i-cel (Paxton Processing Co., Inc., Paxton, Ill.) or in tanks with running water for 1–2 wk before use. After the animals were doubly pithed, the urinary bladders and colons were dissected free and rinsed with Ringer solution. The tissues were mounted between chambers (0.72 cm^2) as described previously for studies of isolated frog skin (9). In brief, the tissues were mounted as sheets between gaskets made of Sylgard 184 (Dow Corning Corp., Midland, Mich.). To achieve electrical isolation of mucosal and serosal solutions and to prevent and/or minimize edge damage artifacts, a thin layer of liquid Sylgard was first spread on each face of the gaskets so that thin films of liquid Sylgard separated the tissue from the gaskets. In this way little if any pressure was required to maintain the tissues as sheets between the chambers and to prevent leakage of fluid from the chambers.

General Procedures

After the tissues were mounted they were short circuited continuously with a voltage clamp, and the values of I_{sc} were monitored continuously with a Gould Brush 220 strip chart recorder and displayed digitally to within 0.1 μA (Analog Devices Inc., Norwood, Mass.; AD 2010). In general, the control period encompassed a 3-h period of study during which time the values of I_{sc} became essentially stable for at least 60–90 min before the tissues were treated with either Pitressin (Parke, Davis & Co., Detroit, Mich.) or amiloride (Merck Sharp & Dohme, Div. Merck & Co., West Point, Pa.). The Ringer solution in each chamber (~0.6 ml) was changed at intervals of 10–20 min by flushing fresh solution (5–10 ml) directly into the chambers. The Ringer solution contained in mEq/liter 109 NaCl, 2.4 $NaHCO_3$, 2.5 KCl, 0.9 $CaCl_2$, and 5.5 glucose, and was gassed with 100% O_2. The pH was ~8.2.

$I_T - V_T$ Relationships

During the course of each study, the I_T-V_T relationships were determined at intervals of 10–20 min with identical procedures described previously for studies of the frog skin (3–5, 9). In brief, a voltage clamp was used to change the V_T for intervals of 600 ms over a range of voltages between −100 and +300 mV. The transepithelial current responses, I_T, were viewed directly on a Tektronix 564B storage oscilloscope (Tektronix, Inc., Beaverton, Oreg.). In general, provided that the voltages remained within a range of −40 and +200 mV, the current responses appeared to reach stable values within 10–50 ms and thereafter to remain constant for the duration of the 600 ms period of observation. However, at values of $V_T > 200$ mV or $V_T < -40$ mV, the current most often did not remain stable with time (Fig. 2). Thus, our studies were limited to the range of voltages that could be expected consistently to yield "steady-state" ranges of V_T and I_T. In some preparations it was observed that the steady-state range of V_T was between −100 and +280 mV. In this regard, these observations are similar to those of the frog skin, collecting tubule, and turtle bladder (7) where it has also been noted that steady-state current responses can be observed only over finite ranges of voltage but encompassing the values of E_1. Thus, the observed breaks at voltage E_1 could not be ascribed to time-dependent transients of the current responses to steps of constant voltage. In preliminary studies on these and other epithelia, it was determined that the sequence of voltage clamping did not alter the appearance of the I_T-V_T plots nor did repetition of the same step of voltage alter the magnitude or time-course of the current responses. Thus, we adopted the

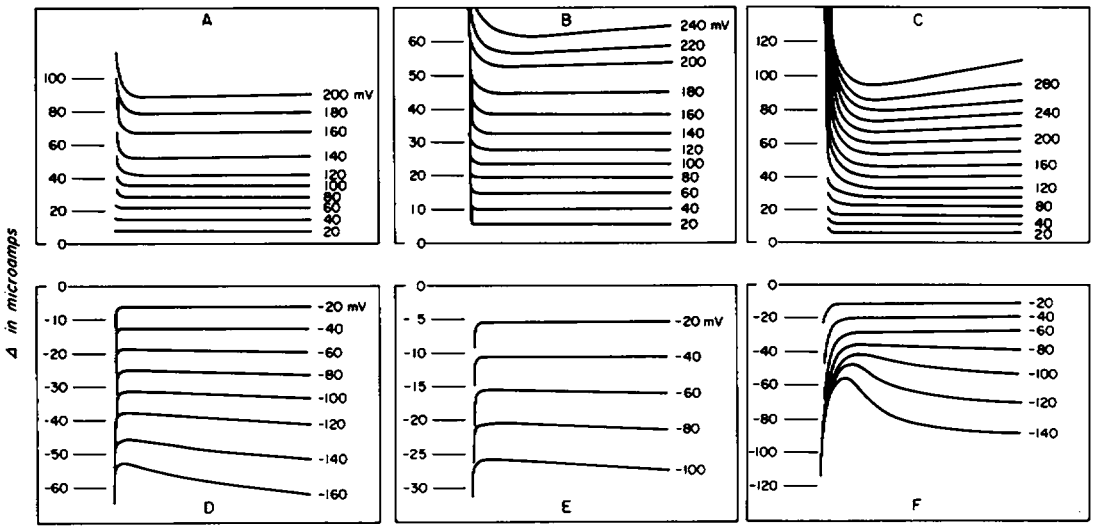

FIGURE 2 Representative current responses to step changes of voltage. Shown in A–F are changes in current in response to voltage clamping of the tissues for 600 ms in increments of 20 mV starting and ending at 0 mV with mucosal solution at ground potential. Between −40 and +200 mV, after a transient of 5–50 ms, the current responses appeared to reach a stable value. Outside this range of voltages, the currents changed continually with time, and in some tissues these changes were marked (see D and F). A sample-hold device was used to read the values of I_T at ~580 ms after onset of the pulse and the "steady-state" values of I_T were used to generate the I_T-V_T plots.

following procedure. As the tissues were short circuited ($V_T = 0$), the changes in V_T began and ended at 0 mV. In sequence, the tissues were polarized first to −20 mV and then to −40 mV followed sequentially in intervals of 20 mV from +20 mV to usually greater than +200 mV, where we verified with the oscilloscope that the current responses no longer represented steady-state values. The values of V_T and I_T were read automatically by a sample hold system at ~580 ms after onset of each pulse and displayed digitally to 0.1 mV and 0.1 μA. The values of V_T and I_T were plotted with a Wang 720B/702 plotter (Wang Laboratories, Tewksbury, Mass.), and the data points fit into what consistently appeared to be two regions of linear slope resistance, R_1 and R_2. The slope resistances were calculated with linear regression analysis (correlation coefficients >0.99) and the coordinate E_1,I_1 calculated at the intersection of the R_1 and R_2 slopes at the voltage and current axes, respectively.

Tissue Handling

TOAD COLON After the colons were rinsed with Ringer solution they were incubated in Ringer solution containing 30 mg/liter papaverine HCl (Eli Lilly Co., Indianapolis, Ind.) for ~30 min to relax the smooth muscle as suggested by Cuthbert (10). The tissues were then mounted as sheets as described above.

TOAD URINARY BLADDER Two groups of studies were done. In the first group the tissues were stretched over a carrier ring to the point where the tissues appeared transparent. As the internal diameter of the carrier ring was larger than the outer diameter of the Sylgard gaskets, the carrier ring with the tissue in place could be positioned between the chambers exposing 0.72 cm^2 of stretched tissue to the solutions. Care was taken not to touch either mucosal or serosal surfaces of the tissue. In a second group of studies, the bladders were similarly placed over the carrier ring, but no attempt was made to stretch the bladders as above. Thus, these "unstretched tissues" studied in the same chambers presented more tissue to the solutions (see Results), and consequently the measured values of I_{sc} were greater and the values of resistance per square centimeter less than the values observed for stretched bladders (see

Results). To normalize these data and to ascertain to what extent stretching of the bladders altered their physiology, the tissue wet weights were determined at the end of each study so that the appropriate parameter values could be expressed on the basis of milligram of wet weight, assuming, however, that the wet weight was constant per milligram dry weight and independent of stretch.

Hormones and Drugs

In some studies the effects of ADH and amiloride on the I_T-V_T plots of the bladder and colon were observed. ADH (Pitressin, Parke, Davis & Co.) was added to the serosal solution at a concentration of ~40 mU/ml. The effects of amiloride were studied at concentrations ranging between 10^{-7} and 10^{-4} M when added to the mucosal solution.

All studies were done at room temperature (23–25°C). Statistical analyses were done in the usual way and the Student's t test was used to test for differences between means. Values are reported as mean ±SE (n).

RESULTS

I_T-V_T Relationships

Characteristically, despite considerable spontaneous variability among bladders and colons, their I_T-V_T relationships appeared as shown in Fig. 3. (see also Figs. 4, 8, 9, 12) In a range of V_T usually between −40 and +200 mV (and occasionally between −100 and +280 mV) the data points fell into two regions of linear slope resistance. When deviations from linearity were observed at the extremes of V_T, observations of the oscilloscopic tracings confirmed the fact that the current responses were not in a steady state for the 600-ms periods of observation. Accordingly, in the present studies only steady-state values were included in the analysis of the I_T-V_T plots. Despite considerable spontaneous differences in the values of I_{sc}, V_{oc}, and resistances, and despite the changes caused by vasopressin and amiloride (see below) the steady-state I_T-V_T plots consistently showed two regions of slope resistance from which the values of E_1 and E_1/I_1 were estimated. In contrast to the frog skin where the I_T-V_T relationships show two breaks at voltages E_1 and E_2 (5, 9), the I_T-V_T relationships of the colon and bladder possessed only a single break at the voltage E_1. In this respect, these I_T-V_T relationships are the simplest observed so far in this laboratory.

A summary of results of the control parameters is shown in Table I. The control period for

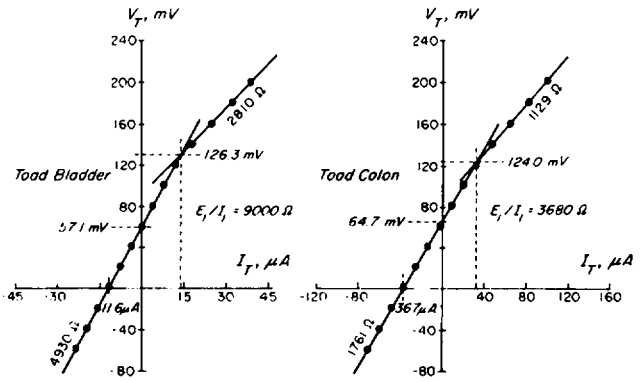

FIGURE 3 Representative I_T-V_T plots of urinary bladder and colon of *Bufo marinus*.

TABLE I

SUMMARY OF I-V PARAMETERS OF TOAD URINARY BLADDER AND COLON (*Bufo marinus*)

	I_{sc}	V_{oc}	R_1	R_2	R_1/R_2	E_1	E_1/I_1	R_a^f	R_a^b	V_{oc}/E_1	Wet weight
	$\mu A/cm^2$	mV	Ωcm^2	Ωcm^2		mV	Ωcm^2	Ωcm^2	Ωcm^2		mg/cm^2
Toad bladder	12.5	38.7	2,280	3,280	0.67	124.5	5,230	13,250	4,810	0.32	6.8
(stretched) (15)	± 1.6	± 5.1	± 280	± 310	± 0.05	± 4.7	± 700	± 2,000	± 740	± 0.04	± 0.7
Toad colon (8)	61.5	70.8	743	1,200	0.63	109.5	3,535	1,890	955	0.65	—
	± 5.8	± 3.7	± 38	± 81	± 0.03	± 4.6	± 365	± 164	± 47	± 0.04	

data collection encompassed the time interval between 120 and 180 min from the time the tissue was first placed in the chamber and short circuited and when the I_{sc} and other parameters appeared to be stable. I_T-V_T relationships were determined at intervals of 10–20 min from which the control parameters were estimated by averaging the 3–6 values determined from the I_T-V_T plots. In both tissues the values of R_1 were less than R_2 in every I_T-V_T plot, and their ratio averaged near 0.65. The values of E_1 averaged 124.5 mV and 109.5 mV for the bladders and colons, respectively, and these values are similar to those observed in studies of the I_T-V_T relationships of the frog skin, cortical collecting tubule, and the turtle bladder (3–7, 9). The values of R_s estimated from the values of E_1/I_1 averaged 5,230 and 3,535 Ω cm^2, respectively, for the stretched bladders and colons. Also summarized in Table I are the values of R_a^f which were considerably larger for the bladder (13,250 Ω cm^2 vs. 1,890 Ω cm^2), owing most likely to the fact that the mean values of I_{sc} were considerably less (12.5 μA/cm^2) for the bladders than the colons (61.5 μA/cm^2).

As noted above, the change of slope resistance from R_2 to R_1 is thought to be a consequence of a change of resistance of the E_1 pathway when the $V_T > E_1$. Resistance R_a^b was found to average 4,810 and 955 Ω cm^2, respectively, for the bladders and colons, and these values are considerably less than their corresponding values of R_a^f.

Effect of Stretch of Urinary Bladder

It should be noted that in the above studies the bladders were studied in their stretched state yielding on the average 6.8 mg wet wt/cm^2. Since it has been observed that the conductance of the bladder increases with the degree of stretch (11, 12) a second group of studies was done as above except the bladders were studied in a minimally stretched state. As shown in Table II, these bladders yielded 26.1 mg wet wt/cm^2. To permit a comparison of the parameter values

TABLE II

EFFECT OF STRETCH ON I-V PARAMETERS OF TOAD URINARY BLADDER

Toad bladder	I_{sc}	V_{oc}	R_1	R_2	R_1/R_2	E_1	E_1/I_1	R_a^f	R_a^b	V_{oc}/E_1	Wet weight
	$\mu A/mg$	mV	$K\Omega mg$	$K\Omega mg$		mV	$K\Omega mg$	$K\Omega mg$	$K\Omega mg$		mg/cm^2
Stretched (15)	2.00	38.7	15.7	21.8	0.67	124.5	35.8	74.6	33.7	0.32	6.8
	± 0.29	± 5.1	± 2.6	± 2.9	± 0.05	± 4.7	± 5.8	± 6.2	± 7.1	± 0.04	± 0.7
Unstretched (9)	1.42	66.2	40.3	52.8	0.78	134.1	118.8	111.6	68.3	0.50	26.1
	± 0.21	± 6.6	± 5.6	± 6.7	± 0.06	± 3.0	± 21.4	± 14.0	± 10.2	± 0.05	± 4.1

of stretched and unstretched preparations, the data were normalized per milligram of wet weight determined at the conclusion of each study. These data are summarized in Table II. The mean value of V_{oc} was increased from 38.7 to 66.2 mV when unstretched bladders were studied, and the mean I_{sc} appeared to somewhat less (1.42 vs. 2.00 μA/mg). Although this latter observation would be in accordance with the effects of stretch on the I_{sc} noted by Walser (11), the difference of I_{sc} between groups was not significantly different. As the values of E_1 (that we interpret to give estimates of E_{Na}, see below) were essentially the same, the values of R_a^f calculated from the quotient E_1/I_{sc} are not significantly different from each other.

To test for differences of parameter values attributable to differences among populations of toads, four additional studies were done where paired bladders from the same toads were studied in stretched and unstretched states. As can be observed for a typical pair of I_T-V_T plots shown in Fig. 4, the E_1 was 126 mV for stretched and unstretched preparations. However, the slope resistances R_1 and R_2 were considerably lower for the stretched bladders compared to the unstretched bladders. Such a decrease of transepithelial resistance could be attributed primarily to a marked decrease of the R_s from 193 to 45.5 KΩ mg, estimated from the values of E_1/I_1. A summary of four studies is shown in Table III. When the parameter values of unstretched and stretched bladders were compared, no consistent difference was observed for I_{sc}. However, for unstretched bladders the open-circuit voltage, V_{oc}, was twice the value observed for stretched bladders. Because the values of E_1 did not vary with stretching of the bladders, the differences of V_{oc} are probably due to differences of the R_s. The transepithelial resistances, R_1 and R_2, were also considerably larger for unstretched bladders, and this, too, would be expected if the shunt resistances varied markedly between groups. This is reflected in the values of E_1/I_1 that averaged 2.9 for unstretched/stretched bladder pairs. Although the origin and ionic dependency of the R_a^b is unknown, it was of interest to note that large increases of its value when unstretched bladders were studied despite the relative constancy of the values of R_a^f.

It should be noted that the mean values of V_{oc} observed in the present studies of the toad

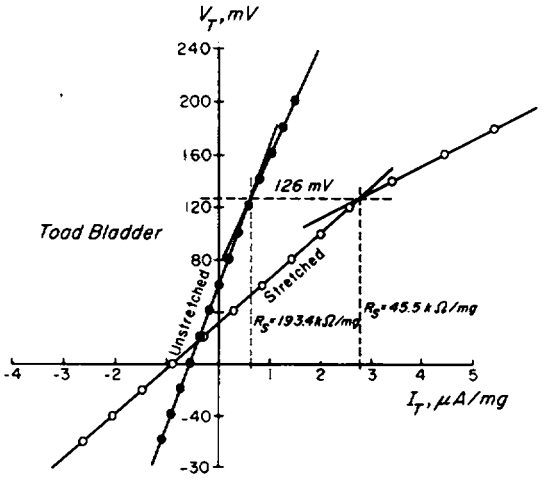

FIGURE 4 I_T-V_T plots of a bladder pair mounted stretched and unstretched. Values of I_T normalized per milligram wet weight. Note similar values of E_1 and difference of $E_1/I_1 = R_s$.

TABLE III
EFFECT OF STRETCH ON I-V PARAMETERS OF PAIRED TOAD URINARY BLADDERS

	I_{sc}	V_{oc}	R_1	R_2	R_1/R_2	E_1	E_1/I_1	R_s^I	R_s^{II}	V_{oc}/E_1	Wet weight
Unstretched/ stretched (4)	1.01	2.02	3.62	2.09	1.56	1.05	2.90	1.10	3.84	1.91	2.52
	± 0.12	± 0.50	± 1.57	± 0.51	± 0.45	± 0.06	± 0.54	± 0.16	± 1.96	± 0.42	± 0.49

bladder are considerably less than observed by Walser (sac preparations) (13) and by Finn and Hutton (chamber preparations) (14) in their studies with nonedge-damaged bladders, and we can't rule out that edge effects may have contributed in part to the resistance of the shunt pathway, thereby resulting in lower values of V_{oc}. However, the values of R_2 are quite similar to those values of transepithelial resistance reported by Finn and Hutton (~27 KΩ mg, 10–15 mg/cm^2) and by Walser (50 KΩ mg), and so it remains possible that other unknown differences may exist that could influence the values of V_{oc} and I_{sc}. Nevertheless, it is clear that if edge effects exist, their contributions to the value of R_s are included in the values of E_1/I_1 reported here.[1]

Effects of ADH on Urinary Bladder

If we accept as a working hypothesis that the values of E_1 and E_1/I_1 approximate E_{Na} and R_s, then it would be reasonable to expect that such values determined from the I_T-V_T plots would be the same as those of E_{Na} and R_s estimated by other methods. In particular, Yonath and Civan suggested that by virtue of the ability of ADH to alter the electrical resistance of the mucosal barrier of the Na transporting cells it is possible to obtain estimates of E_{Na} and R_s provided that ADH exerts little or no effect on the E_{Na} and R_s and that it increases the I_{sc} by acting on the R_{Na} (1). According to their idea:

$$G_2 = 1/R_2 = (I_{sc}/E_{Na}) + G_s. \tag{5}$$

Consequently, from a plot of transepithelial conductance against I_{sc}, the E_{Na} can be estimated from the slope of the I_{sc} vs. G_2 relationship and the shunt conductance $G_s = 1/R_s$ estimated from the intercept at the ordinate.

At the end of each control period, Pitressin (40 mU/ml) was added to the serosal solution. As shown in Fig. 5, the I_{sc} increased after a delay of ~30 s to peak values ~190% above control values. In stretched bladders, the response to ADH was considerably faster than observed for unstretched bladders. During the rising phase of the I_{sc} response to ADH, G_2 was determined

[1]If we assume a value of E_{Na} = 125 mV and take the reported data of Finn and Hutton (14), we calculate a value of R_s near 70 KΩ mg for nonedge-damaged bladders that lies between the values reported here for stretched and unstretched preparations. Thus, if we accept their evidence of the independence of the electrical parameters on the edge:area ratio of the preparation, it seems most likely that changes of the R_s can be attributed to the epithelium and not to edge artifacts. We note also that in those studies by Finn and Hutton, the I_{sc} averaged 2.88 μA/mg, a value considerably larger than observed here. Consequently, if E_{Na} is similar between studies, the values of R_{Na} would be expected to be larger in our studies, and so the values of V_{oc} would be less than reported by Finn and Hutton. We note further that no selection of bladders was made in the present studies. Accordingly, if quantitative differences exist between laboratories, we believe they can be attributed primarily to differences either in the degree of stretch or to differences of the values of I_{sc}.

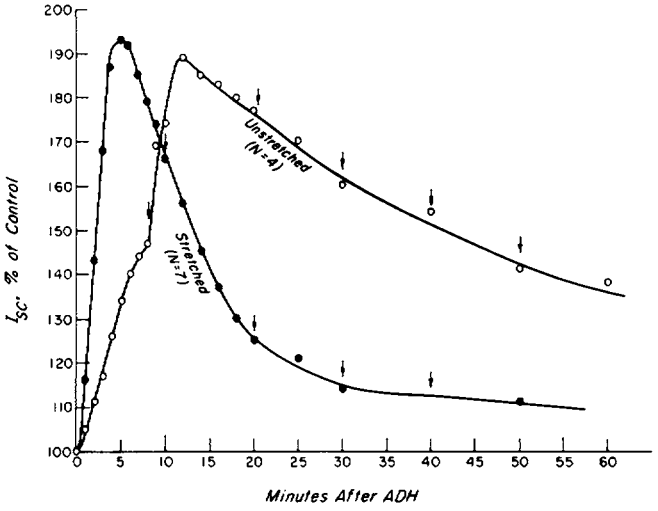

FIGURE 5 Mean time-course of I_{sc} response to addition of Pitressin to serosal solution. Bathing solutions were flushed at the arrows.

at intervals of 0.2 min from the values of ΔI_T observed upon voltage clamping the bladders to ± 20 mV. Owing to the rapidity of the I_{sc} response, it was not possible with the voltage clamps available to determine the entire I_T-V_T plots during this time. Nevertheless, because the I_T-V_T relationships are linear near $V_T = 0$, the values of G_2 could be estimated from the $\Delta V_T/\Delta I_T$.

A typical I_{sc} vs. G_2 plot is shown in Fig. 6 confirming the findings of Yonath and Civan (1). The data points could be fit to a straight line with linear regression analysis from which the values of E_{Na} and R_s were estimated. A summary of the data is shown in Fig. 7 and Table IV. Indeed, for stretched and unstretched bladders, the values of E_1 and E_{Na} were the same, as

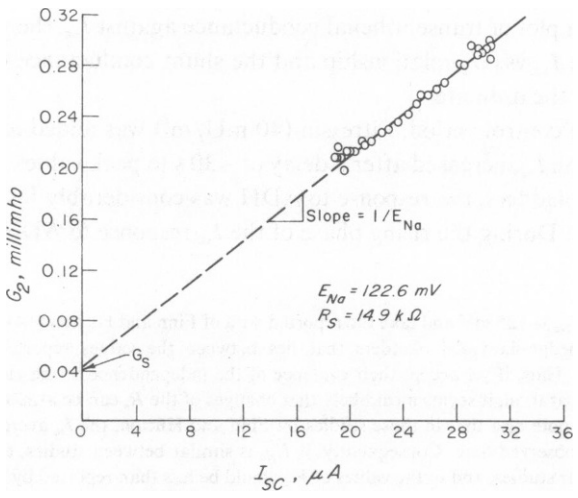

FIGURE 6 G_2-I_{sc} plot determined during rising phase of I_{sc} response to ADH. Slope was determined with linear regression analysis (correlation coefficient >0.99).

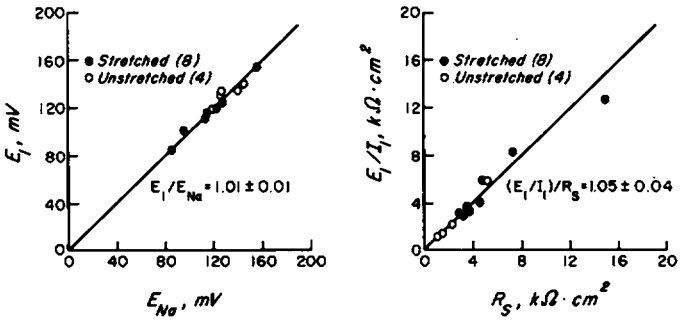

FIGURE 7 Paired comparison of E_{Na} with E_I and R_s with E_I/I_I in studies of stretched and unstretched bladders. Slope is line of identity.

were the values of R_s and E_I/I_I. Accordingly, it was concluded that the values of E_{Na} and R_s estimated with the method of Yonath and Civan could be estimated equally well from the values of E_I and E_I/I_I of the I_T-V_T plots.

ADH on I-V Relationships of Urinary Bladder

After ~30 min of exposure to ADH when the I_{sc} had returned to a more stable rate of decline, I_T-V_T relationships were determined again. Typical examples of the I_T-V_T plots are shown in Fig. 8, and a summary of parameter values estimated from the average values of the 30–60-min post-ADH period is given in Table V. As observed, the values of E_I remained unchanged from control despite the transient behavior of the I_{sc}. This independence of the values of E_I on ADH in urinary bladder is the same as reported previously for studies of the frog skin (4). Consequently, it would be reasonable to believe that changes of the I_{sc} are mediated by changes of the R_{Na}. In contrast to the frog skin, in toad bladder ADH appeared to have little or no consistent effect on the R_s as measured 30–60 min after ADH treatment of the bladders. With unstretched bladders, E_I/I_I appeared not to change, whereas with stretched bladders E_I/I_I decreased ~24%. The reason for the difference of behavior of the R_s at 30–60 min post-ADH is unknown. We presume that during the increasing phase of the I_{sc} response, ADH exerted little or no consistent effect on the R_s of both stretched and unstretched bladders since the values of E_I/I_I of pretreated bladders were the same as those of R_s (ADH method) in both groups of tissues.

Whereas it is clear that changes of the slope resistance R_2 of the I_T-V_T plots correlate with

TABLE IV
COMPARISON OF METHODS OF DETERMINATION OF E_{NA} AND R_S OF ISOLATED TOAD URINARY BLADDER

E_I	E_{Na}	E_I/E_{Na}	E_I/I_I	R_S	$(E_I/I_I)/R_S$
mV	*mV*		*Ωcm²*	*Ωcm²*	
124.0	122.4	1.01	4,730	4,628	1.05
± 5.3	± 5.7	± 0.01	± 947	± 1,057	± 0.04

Values are means ± SE. n = 12.

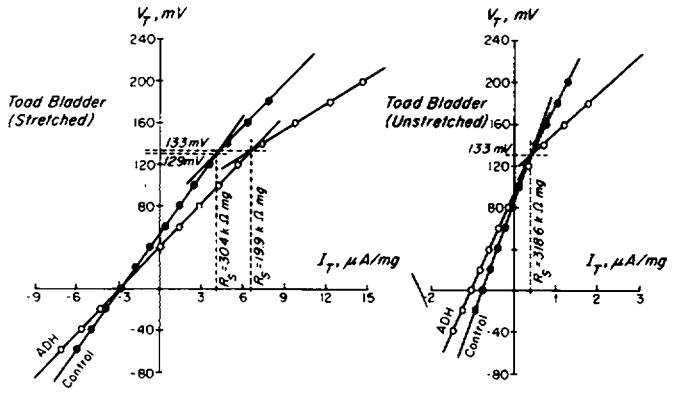

FIGURE 8 Representative I_T-V_T plots of bladders treated with ADH (30–60 min) compared to pretreated controls. E_1 and R_s remained essentially unchanged when unstretched bladders were studied. However (see Table V), R_s decreased considerably with no change of E_1 when stretched bladders were studied.

changes of the I_{sc}, essentially nothing is known of the factors that govern the behavior of slope resistance R_1 in this or other epithelial tissues (5). In this regard, we noted that ADH caused large decreases of R_1, and in most preparations (see Fig. 8) the break at E_1 was more pronounced after treatment of the bladders with ADH. The reason for this is unknown. Of interest, however, is the fact that despite the changes of the values of slope resistances, the voltage E_1 at the break remained essentially constant.

TABLE V
SUMMARY OF I-V PARAMETERS OF TOAD URINARY BLADDER 30–60 MIN AFTER ADH

	I_{sc}	V_{oc}	R_1	R_2	R_1/R_2	E_1	E_1/I_1	R_a^f	R_a^b	
	$\mu A/mg$	mV	$K\Omega\ mg$	$K\Omega\ mg$		mV	$K\Omega\ mg$	$K\Omega\ mg$	$K\Omega\ mg$	mg/cm^2
Stretched bladders (n = 7)										
Control	2.57	36.6	12.8	15.3	0.78	118.9	26.2	58.4	33.0	5.40
	± 0.55	± 9.2	± 3.0	± 2.7	± 0.07	± 8.3	± 6.1	± 7.8	± 12.6	± 1.14
ADH	2.73	35.3	7.3	12.5	0.56	120.6	20.6	49.4	12.6	
	± 0.43	± 8.3	± 1.6	± 2.5	± 0.06	± 3.8	± 4.9	± 6.2	± 3.1	
ADH/control	1.18	1.04	0.59	0.82	0.73	1.05	0.76	0.89	0.50	
	± 0.14	± 0.12	± 0.04	± 0.07	± 0.06	± 0.08	± 0.06	± 0.10	± 0.09	
Unstretched bladders (n = 4)										
Control	1.34	57.3	42.2	52.4	0.85	133.0	112.5	122.9	72.7	36.9
	± 0.39	± 9.0	± 8.2	± 13.0	± 0.07	± 4.4	± 5.8	± 24.3	± 13.0	± 3.8
ADH	1.83	68.7	23.2	43.2	0.59	125.7	106.0	82.2	33.6	
	± 0.44	± 10.8	± 3.4	± 9.9	± 0.09	± 4.3	± 5.5	± 19.9	± 7.2	
ADH/control	1.46	1.21	0.59	0.85	0.69	0.95	0.95	0.70	0.51	
	± 0.25	± 0.13	± 0.09	± 0.05	± 0.07	± 0.05	± 0.04	± 0.11	± 0.09	

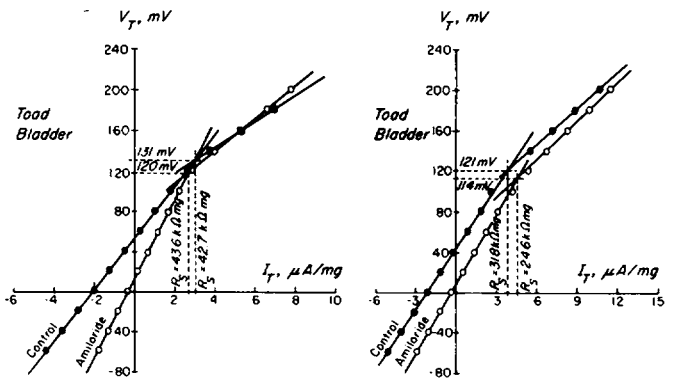

FIGURE 9 Effect of 10^{-5} M amiloride (mucosal solution) on I_T-V_T plots of urinary bladder.

Amiloride on Urinary Bladder

In many Na transporting epithelia, including the toad urinary bladder and colon, amiloride is thought to inhibit active transepithelial Na transport by acting at the mucosal barrier to increase its resistance to Na entry. In frog skin studied with microelectrodes, it was observed that the effect of amiloride was rather selective, increasing the values of R_0^f and having lesser, if any, effect on the values of R_0^b ($V_T > E_1$) or on the values of E_1' of the inner barrier of the Na transporting cells (5). In this regard, it was of interest to examine the effects of amiloride on the I_T-V_T plots of the urinary bladder and colon of the toad.

Shown in Fig. 9 are typical I_T-V_T plots of urinary bladders exposed to 10^{-5} M amiloride at their mucosal surface. As before, the I_T-V_T plots showed two regions of linear slope resistance intersecting at voltage E_1. Characteristically, inhibition of the I_{sc} was accompanied by small decreases of the E_1 of ~9% (see Table VI). In some cases the E_1/I_1 seemed to remain essentially constant but in others the R_s fell by ~20% with increasing amiloride concentration (see Table VI). Taken at face value, these data are consistent with the view that inhibition of the I_{sc} with amiloride can be attributed primarily to an increase of the R_{Na}.

In three bladders, we examined the effects of amiloride on the I_T-V_T relationship in a range

TABLE VI
SUMMARY OF EFFECTS OF AMILORIDE ON THE I-V RELATIONSHIP OF TOAD BLADDER

	I_{sc}	V_{oc}	R_1	R_2	R_1/R_2	E_1	E_1/I_1	R_a^f	R_a^b
10^{-7} M Amiloride/ control	0.72 ± 0.03	0.76 ± 0.04	1.02 ± 0.02	1.03 ± 0.08	0.54 ± 0.06	0.93 ± 0.03	0.95 ± 0.07	1.29 ± 0.04	1.07 ± 0.06
10^{-6} M Amiloride/ control	0.34 ± 0.03	0.40 ± 0.06	1.04 ± 0.05	1.16 ± 0.15	0.51 ± 0.10	0.92 ± 0.01	0.91 ± 0.08	2.74 ± 0.27	1.22 ± 0.19
10^{-5} M Amiloride/ control	0.16 ± 0.01	0.20 ± 0.04	1.13 ± 0.01	1.20 ± 0.18	0.56 ± 0.11	0.91 ± 0.01	0.85 ± 0.09	5.60 ± 0.38	1.58 ± 0.30
10^{-4} M Amiloride/ control	0.11 ± 0.03	0.14 ± 0.04	1.14 ± 0.04	1.22 ± 0.18	0.55 ± 0.10	0.94 ± 0.01	0.82 ± 0.08	9.62 ± 2.78	1.57 ± 0.20

Values are means ± SE. $n = 3$.

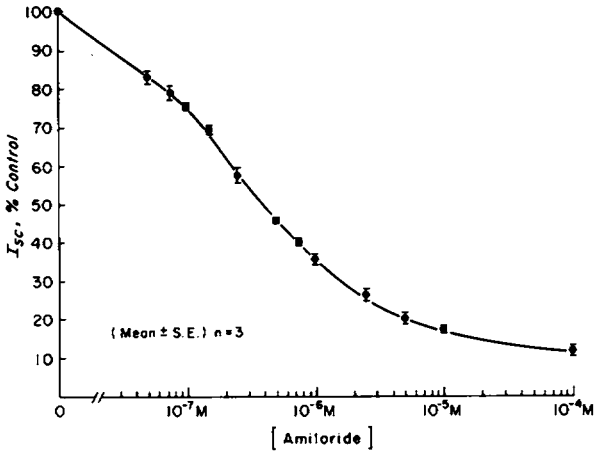

FIGURE 10 Response of I_{sc} of urinary bladder to amiloride in the mucosal solution.

of concentrations between 5×10^{-8} and 10^{-4} M. Shown in Fig. 10 is the response of the I_{sc} to increasing amiloride concentration. 50% inhibition of the I_{sc} occurred at $\sim 3 \times 10^{-7}$ M. Table VI is a summary of the effects of amiloride on the I_T-V_T parameters. It was observed, as noted by others, that the decrease of the I_{sc} or V_{oc} occurred concurrently with an increase of the R_2. Since the E_1 was decreased by <10% even at 10^{-4} M amiloride, the decrease of the I_{sc} could be attributed to an increase of the R_a^f that at 10^{-4} M averaged 9.6 times its control value. In contrast, the values of R_a^b ($V_T > E_1$), although increased by amiloride, remained essentially unchanged especially as compared to the effects of amiloride on the R_a^f. In this regard, these data are similar to those of the frog skin (5, 7).

Although the data are not shown here, we plotted the values of G_2 vs. I_{sc} at each step of increasing amiloride concentration. This yielded curvilinear plots as would be expected if the

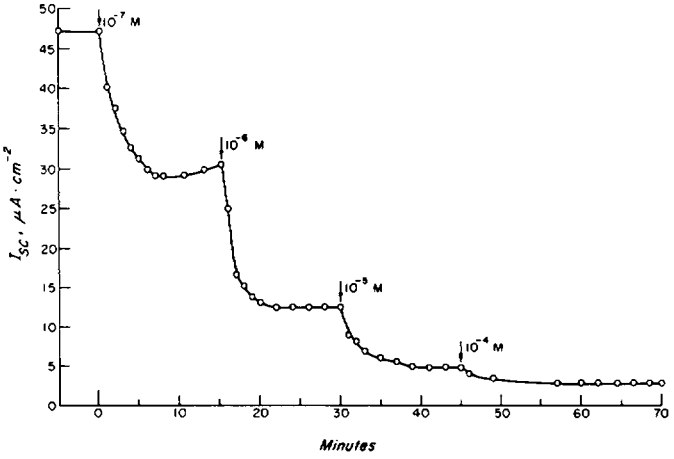

FIGURE 11 Response of I_{sc} of colon to amiloride in the mucosal solution. Representative plot showing slow response compared to the virtually immediate effect observed with urinary bladder.

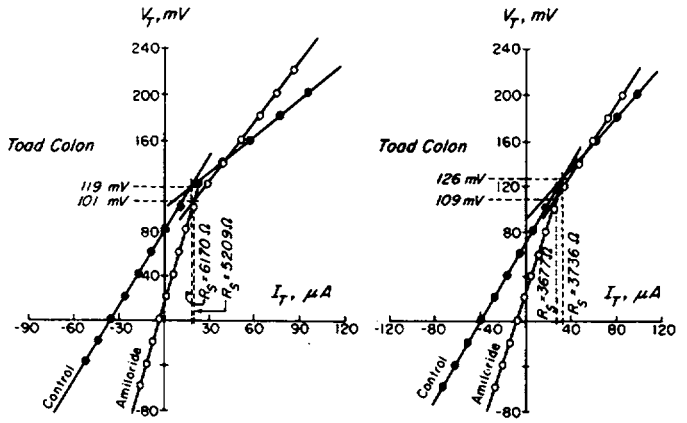

FIGURE 12 Representative I_T-V_T plots of colon exposed to 10^{-4} M amiloride (mucosal solution) as compared to the pretreated control I_T-V_T plot. Compare with Fig. 9 (urinary bladder). Note small decreases of E_1.

E_{Na} and/or the R_s were changed by the amiloride. Consequently, we did not pursue this method of estimating E_{Na} and R_s from the relationships between G_2 and I_{sc} (see Discussion).

Amiloride on Colon

It was also of interest to examine the effects of amiloride on the I_T-V_T relationships of toad colon. In contrast to the immediate effects of amiloride on the I_{sc} of urinary bladder, the response of the I_{sc} of colon is slow as shown in Fig. 11 and is similar to observations of others (10). When the I_{sc} appeared to be stable, I_T-V_T plots were determined at concentrations of amiloride ranging between 10^{-7} and 10^{-4} M. Typical plots are shown in Fig. 12. Characteristically, as in the urinary bladder, amiloride caused inhibition of the I_{sc} with relatively small but consistent decreases of the E_1. Thus, the changes of R_2 and the I_{sc} can be attributed to increases of the R_{Na}. In some cases the values of E_1/I_1 appeared to remain essentially constant, but as shown in summary Table VII, the E_1/I_1 was increased at 10^{-7} M amiloride by ~29%, falling to 87% of control at higher concentrations of amiloride. Since the R_s of the colon compared to the bladder contributed less to the values of transepithelial resistances R_1 and R_2 (see Table I), the increases of their values with amiloride were, as expected, greater

TABLE VII
SUMMARY OF EFFECTS OF AMILORIDE ON THE I-V RELATIONSHIP OF TOAD COLON

	I_{sc}	V_{oc}	R_1	R_2	R_1/R_2	E_1	E_1/I_1	R_s^f	R_s^b
10^{-7} M Amiloride/ control (4)	0.74 ± 0.06	0.98 ± 0.03	1.08 ± 0.06	1.24 ± 0.05	0.88 ± 0.02	0.96 ± 0.02	1.29 ± 0.03	1.21 ± 0.07	1.03 ± 0.07
10^{-6} M Amiloride/ control (8)	0.35 ± 0.03	0.60 ± 0.05	1.36 ± 0.07	1.75 ± 0.13	0.80 ± 0.06	0.93 ± 0.02	1.09 ± 0.12	2.73 ± 0.19	1.56 ± 0.15
10^{-5} M Amiloride/ control (8)	0.16 ± 0.02	0.33 ± 0.02	1.48 ± 0.05	2.11 ± 0.23	0.76 ± 0.08	0.88 ± 0.02	0.95 ± 0.07	5.67 ± 0.51	1.88 ± 0.18
10^{-4} M Amiloride/ control (8)	0.11 ± 0.02	0.21 ± 0.02	1.46 ± 0.04	2.21 ± 0.31	0.74 ± 0.08	0.88 ± 0.03	0.87 ± 0.07	9.62 ± 1.70	1.95 ± 0.17

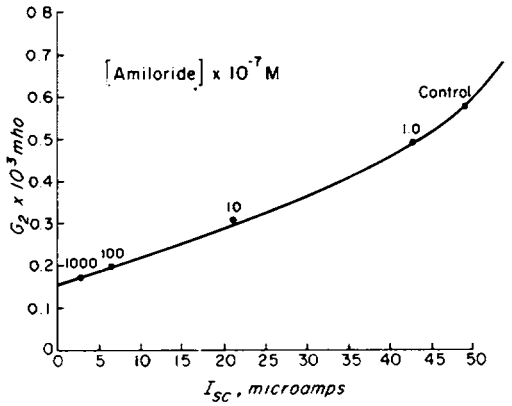

FIGURE 13 G_2-I_{sc} plot of colon generated with mucosal amiloride inhibition of the I_{sc}.

than observed in the studies with urinary bladders (compare data of Tables VI and VII). Consistent with the observations of the bladders, amiloride appeared to exert a dominant effect on the values of R_a^f with a lesser effect on the values of R_a^b.

Unlike the urinary bladder, the colon is unresponsive to ADH (10), at least under the conditions of the present studies. Moreover, if changes of E_1 and E_1/I_1 are taken to indicate changes of the E_{Na} and R_{Na}, it would not be surprising to find that plots of G_2 vs. I_{sc} would yield curvilinear relationships. Indeed, as shown in Fig. 13, the data points could not be fit to a straight line. Such plots would be consistent with the notion that either the E_{Na} and/or R_s were changed by amiloride. Consequently, no attempt was made to obtain estimates of the E_{Na} from such plots in studies of the colon or as above in studies of the bladders.

Regardless of the manner in which the G_2-I_{sc} relationships approach the ordinate at $I_{sc} = 0$, the value of conductance at the ordinate should give an estimate of the shunt conductance when the resistance of the active pathway, $R_a^f \rightarrow \infty$ and so $R_2 \rightarrow R_s$.

If we assume, moreover, that changes of E_{Na} and R_s occur primarily at concentrations of

TABLE VIII
COMPARISON OF METHODS FOR DETERMINATION OF E_{Na} OF ISOLATED TOAD COLON

No.	R_S (ordinate)	V_{oc}	I_{sc}	E^*_{Na}	E_1	E_1/E^*_{Na}
	Ωcm^2	mV	$\mu A/cm^2$	mV	mV	
1	2,668	15.7	5.1	91.8	88.4	0.96
2	1,600	9.3	4.7	93.4	92.6	0.99
3	4,000	13.2	2.8	81.6	86.8	1.06
4	2,334	19.0	7.2	99.7	94.1	0.94
5	4,800	15.6	2.8	104.1	99.2	0.95
6	2,571	15.2	5.0	101.1	101.0	1.00
7	3,789	13.6	3.0	95.6	101.9	1.07
8	2,667	18.3	6.0	105.8	109.6	1.04
Mean ± SE	3,054 ± 370	15.0 ± 1.1	4.6 ± 0.6	96.6 ± 2.8	96.7 ± 2.7	1.00 ± .02

The values of V_{oc}, I_{sc}, and E_1 were estimated from the I–V relationship of colons treated with 10^{-4} M amiloride. E^*_{Na} was calculated with Eq. 6.

amiloride $<10^{-4}$ M, then with values of V_{oc} and I_{sc} measured with colons exposed to 10^{-4} M amiloride, it is possible to calculate E_{Na} assuming that R_s (ordinate) is the same as the R_s of 10^{-4} M-treated colons.

$$E^*_{Na} = (V_{oc}R_s)/[R_s - (V_{oc}/I_{sc})]. \quad (6)$$

A summary of eight studies is shown in Table VIII. E^*_{Na} estimated in this way averaged 96.6 ± 2.8 mV. Correspondingly, the values of E_1 determined also with 10^{-4} M amiloride-treated colons averaged 96.7 ± 2.7 mV. Since the ratio of E_1/E_{Na} was not different from unity, we concluded that E_1 approximated the E_{Na} of the toad colon. Thus, despite the changes of E_{Na} and E_1/I_1 caused by amiloride, the identity between E_1 and E_{Na} appears valid under these conditions. When the values of R_s (ordinate) = 3,049 ± 367 (n = 8) were compared with those of E_1/I_1 observed with 10^{-4} M-treated colons, their ratio $(E_1/I_1)/R_s$was 1.00 ± 0.01 (n = 8).

DISCUSSION

The present studies were done to investigate the current-voltage relationships of the urinary bladder and distal colon of the toad, *Bufo marinus*. In part, these studies followed previous investigations of the *I-V* relationships of other epithelia, notably the frog skin and renal cortical collecting tubule, where evidence has accumulated in support of the idea that the E_{Na} of Ussing and Zerahn could be estimated directly in studies of the observed breaks or discontinuities of the slope resistance of the I_T-V_T plots. As reported here, a single break was observed in the steady-state I_T-V_T plots of both the urinary bladder and the colon, and these I_T-V_T plots are the simplest observed so far in this laboratory. Provided that the current responses to step changes of voltage remained stable for up to 600 ms, the date points relating V_T and I_T can be fit into two linear regions of slope resistance intersecting at voltage E_1. In preliminary studies, voltage increments of 5 or 10 mV were used to generate the I_T-V_T plots. As the appearance of the plots was the same as in those generated with pulses of 20-mV increments, we used 20-mV increments routinely in the present studies, as this facilitated the collection of the data and minimized the time required to collect the data for each I_T-V_T plot. We note also the fact that for each I_T-V_T plot, regardless of the sequence of voltage-clamping of the tissues, the value of I_T at any value of V_T is the same, and so the I_T-V_T plots represent the "steady-state" I_T-V_T relationships.

We wish to emphasize the fact that at the extremes of hyperpolarization and depolarization of the tissues ($V_T > +200$ mV or $V_T < -40$ mV), the current responses did not remain stable over the 600-ms periods of observation, and so with values of I_T recorded at ~500 ms after onset of a voltage step, deviations from linearity were observed as expected, owing to the time-dependent changes of current. To the extent that such time-dependent phenomena are not understood, and moreover, as such phenomena do not lend themselves to an analysis of the ohmic properties of the tissue, the present studies were confined to the range of transepithelial voltages where it could be established that from the electrical point of view the tissues achieved a steady state for at least 500–600 ms. With adherence to this criterion, we observed consistent changes of slope resistance from R_2 to R_1 as the V_T was increased above E_1 regardless of the magnitudes of the short-circuit currents, open-circuit voltages, and transepithelial resistances between and among tissues. In this regard, Civan studied the I_T-V_T

relationships of toad urinary bladder and reported the existence of discontinuities in the hyperpolarizing region of the I_T-V_T plots of toad urinary bladder. Examination of his data showed that some but not all of the plots exhibited discontinuities at voltages similar in value to those of E_1 reported here (130–150 mV). (see Figs. 3–6, 8, 9 of reference 8) He also observed discontinuities at values between 172 and 184 mV that were similar in value to those of E_{Na} estimated previously by Civan et al. (15). Accordingly, it was suggested that the break at 172 and 184 mV might be equated with that of the E_{Na}. However, in later studies by Yonath and Civan using the ADH method for estimating E_{Na}, the values of E_{Na} varied over a large range (74–186 mV) averaging 105 ± 2.9 mV. Others working with control tissues using this method have estimated the E_{Na} to average 117.7 ± 7.2 mV (16) and near 85–90 mV in the studies by Feig et al. (17). Other techniques based upon tracer flux measurements have been used to estimate the E_{Na} (18, 19). In studies by Saito et al. (18), the E_{Na} averaged 114 ± 13 mV. Accordingly, if all methods yield valid estimates of E_{Na}, there appears to be a considerable range of values of E_{Na} that can be attributable in part to possible differences of the physiological status of the tissue, its environment of study, species, origin, and other unknown factors. We do not consider such variability among tissues a difficulty with the interpretation of the E_{Na} (20, 21), because for tissues involved in the regulation of their external environment, it would not be unexpected nor surprising to find that parameters like the E_{Na} and/or R_{Na} are subject to variation. The more interesting question to be resolved, as noted by Hong and Essig, deals with problems such as the apparent constancy of the E_{Na} in their studies with 2-deoxyglucose and in our present and our past studies with ADH, amiloride, and [Na] reduction of the mucosal (outer) solution of frog skin and cortical collecting tubules (4, 5, 16).

The results of the present studies with urinary bladder, extended to those of the colon, are in agreement with and confirm the findings of Yonath and Civan. The I_{sc}-G_2 plots generated with ADH during the increasing phase of the I_{sc} response were linear and thus consistent with the notion that the E_{Na} and R_s remained essentially constant during this time. Hong and Essig have noted that such linearity does not preclude the possibility that both E_{Na} (and R_s) may change with time of response in such a way that the I_{sc}-G_2 plot appears linear. However, in view of the rather excellent agreement of values of E_{Na} and R_s and those of E_1 and E_1/I_1, it would be difficult for us to believe that the estimates of E_{Na} are consistently biased by the same amount as those of E_1. Thus we believe that the assumption that R_s and E_{Na} remain essentially constant upon stimulation of urinary bladders with ADH is reasonable. Indeed, even at 30–60 min of the post-ADH response, the values of E_1 were the same as those estimated during the control period and the same as those of E_{Na} estimated from the I_{sc}-G_2 plots. Thus, these data indicate that ADH exerts little or no effect on the E_{Na} of the toad urinary bladder, and this finding is similar to that reported previously from studies of the frog skin studied acutely or long term (4).

Although the present studies were done to characterize the I_T-V_T plots of the bladder and colon, it was also of interest to examine the effects of stretch of the bladder on the I_T-V_T plots. In contrast to reports by others (11, 12), we could not demonstrate a stimulation of the I_{sc} when bladders were studied in their stretched state. The reason for the differences of observation is unknown, but could be due in part to the fact that the bladders in the present studies were allowed to equilibrate and stabilize for 2–3 h. At these times we did not observe

consistent differences of the I_{sc} between groups or between pairs of bladders. Of interest, however, was the observation that despite considerable differences in the degree of stretch, the values of E_1 and those of E_{Na} (ADH method) were the same. Consequently, since the values of I_{sc} were also not significantly different, the lower values of V_{oc} observed for stretched bladders could be attributed to lower values of R_s in bladders studied in their stretched state. Examination of the values of E_1/I_1 showed that compared to unstretched bladders the mean value of R_s was 35.8 KΩ mg or ~30% of the value estimated for unstretched bladders. Whether such behavior reflects an effect of stretch on the physiological shunts is unknown, since it remains possible that edge effects, or (as suggested by Finn and Hutton) damage due to tissue handling, may in part be responsible for the differences in value of R_s (14). Nevertheless, since the values of E_{Na} and I_{sc} were not different, it can be concluded that stretch alone is without effect on the active Na transport pathway, at least under the conditions of the present studies.

As noted above, when I_T-V_T plots were determined 30–60 min after ADH treatment of the bladders, the values of E_1 were not changed from control. In studies of unstretched bladders the values of E_1/I_1 also were the same as control, and this observation is consistent with the interpretation that ADH did not affect the R_s. However, in studies with strecthed bladders, we noted consistently that the values of E_1/I_1 were decreased from control by ~24% at 30–60 min. The reason for the difference of behavior between stretched and unstretched bladders is unknown. In frog skin, where the I_{sc} response to ADH is considerably slower, ADH was observed to cause a consistent decrease of the R_s with no change of the E_{Na}. This ruled out the use of the Yonath-Civan method to estimate the E_{Na}, and other precedures were adopted (4). Whereas amiloride has little or no effect on the R_s of control skins, the drug caused profound changes of the R_s of ADH-treated skins. Thus, although no a priori reasons exist that permit us to expect similar observations between tissues, it may well be that the response or lack of a response of a particular parameter of interest may depend not only on the nature of the stimulus but also on the physiological and biochemical status of the tissue at the time studied. Such observations as those above underscore the need for caution in assuming that either E_{Na} and/or R_s can be considered constant and independent of procedures that either stimulate or inhibit the I_{sc}. In this regard, Feig et al. (17) concluded that the E_{Na} of urinary bladder varies with mucosal Na concentration. Also, Feig et al. (17), in agreement with Hong and Essig, but in contrast to the findings of Yonath and Civan (1), reported that E_{Na} is increased by amiloride. Such observations, in part, led Feig et al. to conclude that such observations were incompatible with the assumption of the original model proposed by Ussing and Zerahn (2), and moreover, that enrichment of the model is unlikely to enhance its utility. Such a conclusion is indeed controversial, and, in our opinion, unjustified. Since it is not possible to rule out differences of species, metabolic status, differences of handling and other such factors, there could exist differences of methodology or other uncontrolled factors that might explain the differences of observations between laboratories. It remains possible that with the ADH method of Yonath and Civan, the assumption of constancy of E_{Na} and/or R_{Na} is not valid under all experimental conditions. We note in particular the studies of Hong and Essig (16) and those of Feig et al. (17) who presented as evidence of the constancy of R_s the constancy of the unidirectional tracer fluxes of Na and Cl. O'Neil and Helman reported on studies with ADH of frog skin that indicated tracer fluxes of Cl, SO_4, urea, and sucrose

remained essentially constant despite considerable changes of the R_s determined electrically. We emphasize again that the transepithelial shunt pathways of epithelia conceivably arise not only from simple extracellular pathways but also from transcellular pathways that retain an asymmetry of ionic permeability of their apical and basolateral membranes. If such passive transcellular shunt pathways exist, then by virtue of series barriers of differential ionic permeability the R_s measured electrically from $\Delta V/\Delta I$, or in our case by E_1/I_1, would not be the same as the resistance of the extracellular shunt pathways where presumably transepithelial tracer fluxes (and their electrical resistance) could remain constant despite changes of resistance of transcellular shunt pathways. We believe objections such as these need to be ruled out before it can be concluded that the electrical values of R_s remain constant. If we take at face value the unidirectional flux data of O'Neil and Helman, then according to the criteria of Hong and Essig and Feig et al., the R_s would have been assumed to be constant, and consequently, the E_{Na} would have been found to change with treatment of the skins with ADH and amiloride. O'Neil and Helman argued, on the contrary, that despite the relative constancy of the tracer fluxes, R_s determined electrically varied markedly, especially when amiloride inhibited the I_{sc} of ADH-treated skins. This led to the speculation that the shunt pathways were not likely to consist simply of extracellular pathways alone. We believe it most reasonable for the moment to leave the question open.

Our interpretation of the values of E_1 and E_1/I_1 rests on the idea that at values of $V_T = E_1$, current flow via the active E_1 pathway is zero, and so the current I_1 represents the flow of current via the shunt pathway. As such, the nature and location of the shunt pathway is undefined. Thus, no inferences can be made of its relationship to the anatomical routes of ion transport nor to the ionic species subserving the current, I_1. In frog skin, the sum of the partial conductances of Na, Cl, and $SO_4^{=}$ determined in short-circuited preparations was observed to be considerably less than estimated electrically from the E_1/I_1 (22). Such an observation is consistent with the view stated before (4, 22) and above.

In view of the rather remarkable agreement of the values of E_1 of the I_T-V_T plots of the frog skin and those of E_1' determined more recently in microelectrode studies of this tissue, we are of the opinion that the values of E_1 or E_{Na} are not abstract parameters, but rather may represent phenomena occurring at the basolateral borders of Na transporting cells, at least in the frog skin. Consequently, the abstract nature of a Thévenin emf, defined as E_{Na} by Ussing and Zerahn, is a parameter readily identified and associated with the behavior of the basolateral barrier of the cells of frog skin. Except for the similarity of behavior of the frog skin and the other epithelia studied in this laboratory, we have no compelling reason to believe that ideas derived from studies of the skin can readily be extrapolated to other epithelia. Nevertheless, the similarity of electrical behavior among tissues of diverse origin including frog skin, toad bladder and colon, turtle bladder, rabbit collecting tubule, and snake distal nephron (23),[2] at least for the E_1 pathway viewed with the Thévenin equivalent, is quite remarkable. It is entirely possible that the mechanisms subserving such behavior are different among tissues, but we think this unlikely. Our bias on the interpretation of the E_{Na} is weighted heavily by the microelectrode findings in frog skin by Nagel (24) and by Helman and Fisher

[2]Beyenbach, K. W., B. M. Koeppen, W. H. Dantzler, and S. I. Helman. Reduction of Na transport by high luminal Na concentrations in the distal nephron. Submitted for publication.

(5) that suggest that the E_{Na} is developed at the basolateral borders of the epithelial cells at a time when transepithelial Na transport is reduced to zero. Consequently, given that the voltage at the inner barrier is dependent on the K concentration gradient as well as the Na-K-ATPase system (the pump), it would seem obvious that the E_{Na} is not a parameter expressing the nature of the "Na pump" alone (20, 21). Indeed, the values of E_{Na} could be influenced not only by energetic and kinetic factors of the ATPase system but also by changes of the passive properties of the inner barrier that could be consequent to alterations of K, Na, and Cl conductances and their concentration gradients. Clearly, resolution of such involvements must await further study. It seems clear that changes of E_{Na} can be mediated by factors other than those directly implicated in the "active pump mechanism" itself. Thus, E_{Na} would not be expected to be a biological constant related to any particular thermodynamic equivalent. We believe its utility rests as defined by Thévenin and used by Ussing and Zerahn and others as the effective transepithelial driving force for active transepithelial Na transport. Moreover, we believe that as more detailed models of the process are developed, they must be compatible with the estimates of E_{Na}.

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