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Active Sodium Transport and the Electrophysiology of Rabbit Colon

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Summary. The electrophysiologic properties of rabbit colonic epithelial cells were investigated employing microelectrode techniques. Under open-circuit conditions, the transepithelial electrical potential difference (*PD*) averaged 20 mV, serosa positive, and the intracellular electrical potential (ψ_{mc}) averaged −32 mV, cell interior negative with respect to the mucosal solution; under short-circuit conditions, ψ_{mc} averaged −46 mV. The addition of amiloride to the mucosal solution abolishes the transepithelial *PD* and active Na transport, and ψ_{mc} is hyperpolarized to an average value of −53 mV. These results indicate that Na entry into the mucosal cell is a conductive process which, normally, depolarizes ψ_{mc}.

The data obtained were interpreted using a double-membrane equivalent electrical circuit model of the "active Na transport pathway" involving two voltage-independent electromotive forces (emf's) and two voltage-independent resistances arrayed in series. Our observations are consistent with the notions that:

(a) The emf's and resistances across the mucosal and baso-lateral membranes are determined predominantly by the emf (64 mV) and resistance of the Na entry process and the emf (53 mV) and resistance of the process responsible for active Na extrusion across the baso-lateral membranes: that is, the electrophysiological properties of the cell appear to be determined solely by the properties and processes responsible for transcellular active Na transport. The emf of the Na entry process is consistent with the notion that the Na activity in the intracellular transport pool is approximately one-tenth that in the mucosal solution or about 14 mM.

(b) In the presence of amiloride, the transcellular conductance is essentially abolished and the total tissue conductance is the result of ionic diffusion through paracellular pathways.

(c) The negative intracellular potential (with respect to the mucosal solution) is due primarily to the presence of a low resistance paracellular "shunt" pathway which permits electrical coupling between the emf at the baso-lateral membrane and the potential difference across the mucosal membrane; in the absence of this shunt, the "well-type"

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electrical potential profile characteristic of rabbit colonic cells would be 'converted' into a "staircase-type" profile similar to those reported for frog skin and toad urinary bladder by some investigators.

Previous studies from this laboratory have demonstrated that the spontaneous transepithelial electrical potential difference (ψ_{ms}) and the short-circuit current (I_{sc}) across *in vitro* rabbit colon are attributable entirely to active Na transport from mucosa-to-serosa. Although Cl is also actively absorbed, this process is electrically silent and appears to involve a one-for-one exchange with HCO_3. Transepithelial K movements are entirely diffusional and are probably restricted to paracellular pathways. In addition, we have shown that amiloride, added to the mucosal solution, abolishes active Na absorption and that this can be attributed to inhibition of the unidirectional influx of Na from the mucosal solution into the epithelium across the mucosal membranes. Amiloride also decreases the transepithelial conductance; but, the conductance of the passive diffusional pathways across the epithelium is not affected as evidenced by the lack of an effect of this agent on bidirectional K fluxes or the unidirectional fluxes of Na and Cl from serosa-to-mucosal solution, abolishes active Na absorption and that this can be (Ussing, Erlij & Lassen, 1974), appears to be entirely attributable to an inhibition of the Na conductance across the mucosal membranes. (Frizzell *et al.*, 1975; Frizzell, Koch & Schultz, 1976; Frizzell & Schultz, 1976).

This paper reports the results of a study of the electrophysiologic properties of rabbit colon using microelectrode techniques. The data obtained will be interpreted using an equivalent electric circuit model for Na-transporting epithelia under steady-state conditions (Schultz, Frizzell & Nellans, 1977) and the properties of the "active Na transport pathway" will be defined in terms of equivalent electrical analogues. The results of studies dealing with the effects of imposed transepithelial electrical potential differences on the rate of active Na transport across the tissue will be presented and we will show that these results provide strong, independent support for the equivalent circuit model. The effect of the shunt pathway on the electrical potential profile across the tissue will be examined and we will show that this effect is in complete accord with previous theoretical considerations (Schultz, 1972). Finally, we will present an equivalent electrical analogue of an active Na transport mechanism based on irreversible thermodynamics and network thermodynamics which may provide some insight into the meaning of equivalent electrical analogues (Appendix I).

Materials and Methods

White, male rabbits (2–4 kg) were sacrificed with intravenous pentobarbital and a segment of descending colon was removed, opened along the mesenteric border to form a flat sheet and rinsed free of intestinal contents with an electrolyte solution containing (mM): Na, 140; Cl, 124; HCO_3, 21; K, 5.4; HPO_4, 2.4; H_2PO_4, 0.6; Mg, 1.2; Ca, 1,2; and glucose, 10. This solution has a pH of 7.4 at 37 °C when gassed with a mixture of 95% O_2 and 5% CO_2, and was used in all experiments unless otherwise indicated.

Electrophysiologic Studies

A segment of colon, stripped of the underlying musculature and connective tissue using a glass microscope slide (Frizzell *et al.*, 1976) was mounted mucosal surface up in a lucite chamber as illustrated in Fig. 1 (*T*); the serosal surface was supported by a disc of wide-gauge nylon mesh and the area of tissue exposed to the bathing solutions was 1.25 cm^2. The mucosal (*M*) and serosal (*S*) bathing solutions were continuously circulated through the upper and lower chambers from water jacketed reservoirs which maintained the solutions at 37 °C. To ensure proper temperature control of the circulating fluids the entire apparatus was enclosed in an environmental box which was maintained at 37–39 °C.

The electrical potential difference across the tissue (ψ_{ms}), with respect to the mucosal solution, is monitored by two agar-bridges inserted into the chambers immediately adjacent to the surfaces of the tissue (*P*). These bridges lead to an automatic voltage-clamp apparatus (*VC*; inputs 3 and 4) (Biophysical Laboratories, Harvard Medical

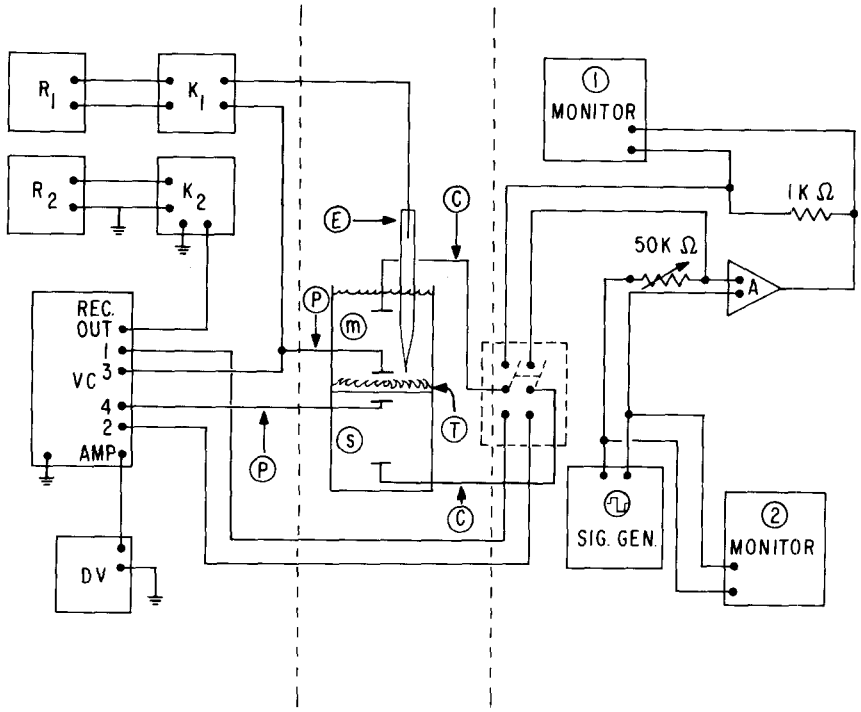

Fig. 1. Schematic of apparatus employed in electrophysiologic studies

School) and in turn through *VC* (Rec. Out) to a Kiethley (Model 602) electrometer (K_2) and to one channel of a Texas Instruments (Servo-riter II) dual-channel, pen recorder (R_2). The electrical potential difference between the microelectrode-tip (*E*) and the mucosal solution (ψ_{mc}) is monitored by another Kiethley electrometer (K_1) and recorded on the second channel of the pen recorder (R_1), as shown.

Current can be passed across the tissue through the agar-bridges (*C*) inserted centrally at the ends of the chambers. Two sources of external current were used. In order to short-circuit the tissue, rapidly and briefly (1–2 sec), sufficient current was automatically passed from *VC* (outputs 1 and 2) through the bridges *C* to reduce ψ_{ms} to zero. Under these conditions, the output of *VC* to K_2 and R_2 is a measure of the I_{sc} (1 mV = 10 μA). At the same time, a digital voltmeter (*DV*) (Digiter 277-O1N) monitors ψ_{ms} through *VC* inputs 3 and 4 to ensure that it has, in fact, been clamped to zero. Alternatively, bipolar square-wave pulses of constant current (40 μA/cm^2, 0.5–1 sec duration) can be passed across the tissue using a signal generator (*SIG.GEN*) (Grass Instruments, Model SD-5) whose output passes through an operational amplifier (*A*) (μ741) in a constant-current configuration. The outputs of *SIG.GEN.* and *A* are monitored using a dual-beam oscilloscope (Monitors 1 and 2). The switch (unlabeled) permits us to choose the voltage-clamping configuration, the constant-current pulsing configuration, or neither (i.e., the open-circuit condition).

Preliminary studies indicated that steady-state values of I_{sc} are achieved within 1 sec following the onset of the external clamping current and that the magnitudes of the deflections in ψ_{ms} and ψ_{mc} also are independent of the duration of the constant current pulse over the range studied (500 msec–1 min). That is, the response to the passage of a square-wave current pulse is a square-wave voltage deflection; any transients must be so brief as to be obscured by the response-time of the pen recorder. Thus, the present techniques yield valid measurements of the steady-state I_{sc} and transepithelial resistance (R_t); as will be discussed below, the data obtained are in excellent agreement with those obtained on tissues mounted in Ussing-type chambers under steady-state conditions (Frizzell *et al.*, 1976)[1].

Microelectrodes were prepared from 1.5–2 mm OD borosilicate glass tubing (A.H. Thomas Co., Philadelphia, Pa.) using a vertical puller (David Kopf, Modell 700 C). The electrodes were filled immediately by boiling in methanol under reduced pressure for 15–30 min. They were then placed in distilled water for 15–30 min and, then, allowed to exchange with 3 M KCl for at least 12 hr. Electrodes were used within 24 hr of filling. Electrodes were selected for a tip resistance between 8–20 MΩ and tip potentials less than 7 mV. Micropuncture was carried out either manually using a Brinkman (Model 06-10-09) micromanipulator or automatically using a David Kopf hydro-drive (Model 607B) with a minimal advance of 1 μ. Criteria for a successful puncture were: (a) an abrupt negative deflection of ψ_{mc} which stabilized quickly to within ± 15 % of the initial value and was maintained for at least 15 sec; and (b) an abrupt return to baseline following withdrawal of the tip with no change in tip potential or resistance. However, for most of the experiments to be reported it was necessary to maintain the microelectrode in the cell for several minutes. This was accomplished very infrequently; however, the values of ψ_{mc} recorded during relatively brief, successful punctures (at least 15 sec) did not differ significantly (*see* below) from those observed during much longer impalements (some-

1 In several studies the voltage deflections in response to the passage of square-wave current pulses of 0.6 sec duration were observed on an oscilloscope; in all instances a square-wave response was observed up to current densities of at least 150 μA/cm^2. Frömter (1972) found that when low current densities are employed there was no difference between the "instantaneous" (< 0.5 sec) current-voltage relation of Necturus gallbladder and that observed after passage of the current for 4 min.

times as long as 10 min) so that we have no reason to believe that results obtained during prolonged impalements are biased or represent a particular cell population.

Flux Studies

The effects of ψ_{ms} on the unidirectional flux of Na from mucosa-to-serosa (J_{ms}^{Na}) and from serosa-to-mucosa (J_{sm}^{Na}) were determined on "partial mucosal strips" of rabbit colon (i.e., the muscle layers of the intact colonic segments were teased-off using fine optical forceps) as described previously (Fig. 1 of Frizzell *et al.*, 1976). Briefly, the "partial mucosal strips" were mounted in the short-circuit apparatus described by Schultz and Zalusky (1964) and, after a 30 min equilibration period, unidirectional fluxes were determined using Na^{22} during the course of three 20-min periods when ψ_{ms} was clamped at +50 mV, 0 mV, or −50 mV. The sequence of clamping was random and each flux period was preceded by a 20 min equilibration period which assured the achievement of a new steady-state. In general, in each experiment, duplicate studies of the effect of ψ_{ms} on J_{ms}^{Na} and on J_{sm}^{Na} were carried out on tissues from the same animal.

Na^{22} was obtained from the New England Nuclear Corp. and amiloride was a generous gift from Merck, Sharpe and Dome (West Point, Pa.). All other reagents were reagent grade.

Results are expressed as the mean ± SEM based on the number of tissues studied. Differences between means were analyzed using the Student's t test; a value of $p < 0.05$ was considered significant.

Results

The Electrical Potential Profile

A histogram of the results of 125 successful impalements on 12 randomly selected tissues is shown in Fig. 2. The spontaneous ψ_{ms} of these tissues averaged 14 ± 1 mV and the values of ψ_{mc} appear to be distributed normally about a mean of −45 mV. In general there appeared to be an inverse relation between ψ_{ms} and ψ_{mc}; that is, low values of ψ_{ms} were associated with large, negative intracellular potentials whereas high values of ψ_{ms} were associated with low intracellular potentials. For example, the highest value of ψ_{mc} recorded was −70 mV and in each instance ψ_{ms} was between 4–10 mV (n = 5); the lowest values of ψ_{mc} observed were −10 to −15 mV (n = 5) and in each instance ψ_{ms} exceeded 30 mV. However, although this is a general impression, suggested by values at the extremes, a plot of ψ_{mc} *vs.* ψ_{ms} discloses so much scatter within the intermediate ranges that it is difficult to draw a firm conclusion that can be supported statistically. Similar findings have recently been reported by Nagel (1976) for isolated frog skin.

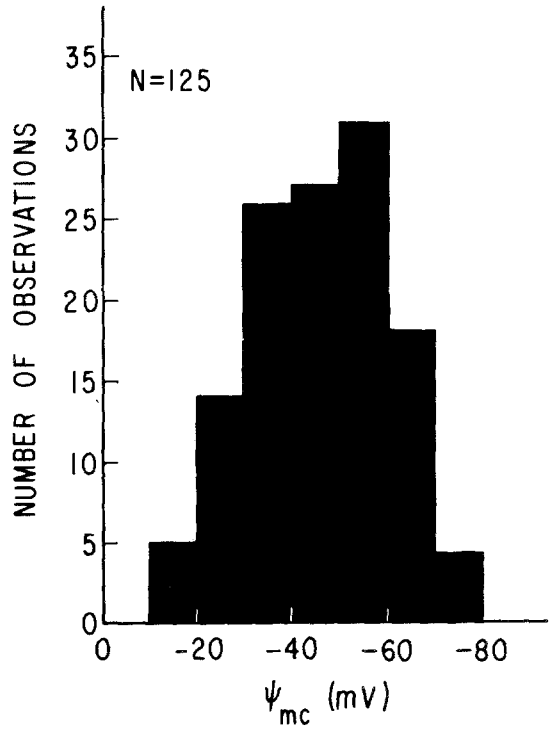

Fig. 2. Histogram of 125 values of ψ_{mc} determined on 12 randomly selected tissues

Effect of Amiloride on the Electrical Potential Profile

As described above, the apparatus employed in these studies permits determination of the transepithelial electrical potential difference (ψ_{ms}), the electrical potential difference across the mucosal membrane (ψ_{mc}), the short-circuit current (I_{sc}), the intracellular potential with reference to that of the mucosal solution under short-circuit conditions ($_0\psi_{mc}$), the transepithelial resistance (R_t) and the ratio of the resistance of the basolateral (serosal) membrane (R_s) to that of the mucosal membrane (R_m)[2] on a

2 As discussed by Frömter (1972), if the amount of an externally applied current which flows across the mucosal membrane is equal to that which flows across the baso-lateral membrane, then

$$\Delta\psi_{ms}/\Delta\psi_{mc} = 1 + (R_s/R_m)$$

where $\Delta\psi_{ms}$ and $\Delta\psi_{mc}$ are the deflections resulting from the passage of a constant current pulse across the tissue. The validity of this "voltage-divider ratio" rests on the assumptions (a) that the length constant of the paracellular pathway is large compared with the length of the pathway (i.e., that most of the current that enters the paracellular pathway remains confined to that pathway); and (b) that the current-pulses are sufficiently brief so that intracellular ionic composition is not altered significantly.

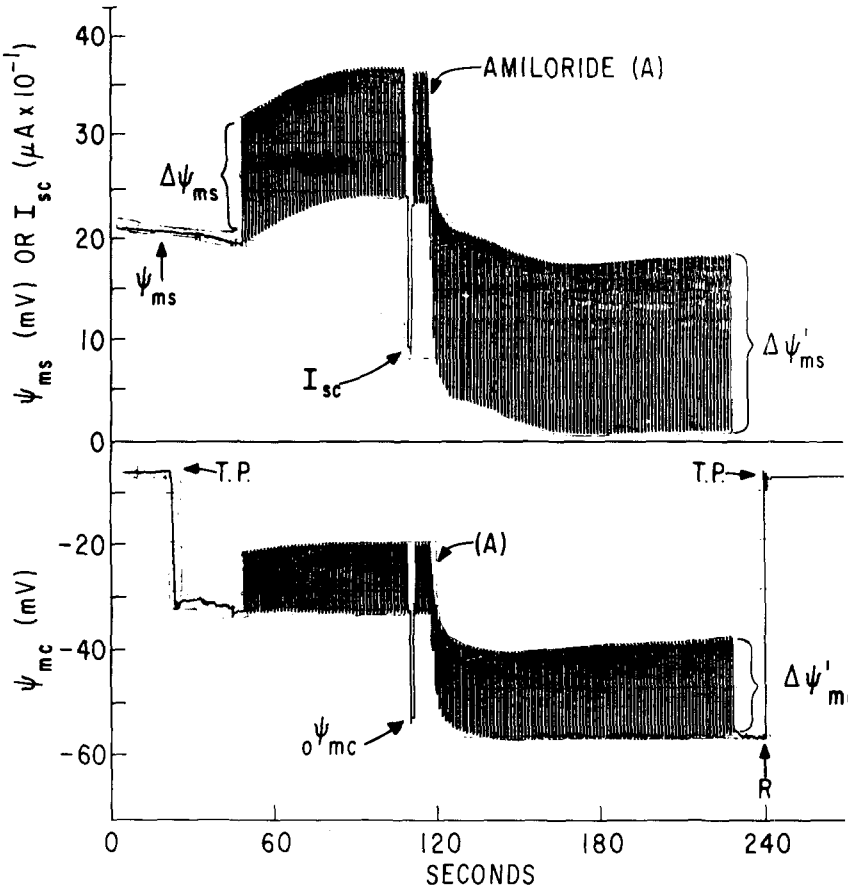

Fig. 3. Reproduction of a typical tracing illustrating the effects of amiloride on the electrical potential profile and tissue resistances. *T.P.* is the tip potential of the microelectrode; all other symbols are defined in the text. The hyperpolarization of ψ_{ms} following the onset of current pulsing was observed in 3 out of 30 experiments and only when unipolar current pulses were employed. Because of its infrequent occurrence, we do not feel that it warrants interpretation

single segment of tissue within a brief period of time. [Data obtained after the addition of amiloride are designated with a prime (′)].

Fig. 3 is a reproduction of a recording that illustrates the effect of amiloride on the electrical properties of rabbit colon. The salient points to be noted are: (a) following the addition of amiloride (A) there is a prompt decline in ψ_{ms} which reaches a value of zero within 20 sec.;[3] (b)

3 In these experiments, circulation of buffer through the mucosal chamber was stopped immediately after a successful cell impalement and amiloride was then added directly into the mucosal chamber. Circulation of the mucosal solution was resumed after the amiloride effect was complete.

Table 1. Effect of amiloride on electrical properties of rabbit colon

	ψ_{ms} (mV)	ψ_{mc} (mV)	R_t (ohm cm^2)	G_t (mmhos/cm^2)	R_s/R_m	$_0\psi_{mc}$ (mV)	I_{sc} μA/cm^2
Control[a]	20±1	−31±2	265±15	3.77	0.20	−46±3	82±8
Amiloride	4±1	−50±3	323±19	3.10	0.10	−53±3	14±4
n	30	30	21	21	21	21	21
Control[b]	20±2	−32±2	286±13	3.50	–	−46±3	70±9
Amiloride	1±1	−53±3	345±15	2.90	–	−53±3	0±3
n	16	16	10	10		10	10

[a] All experiments.
[b] Selected experiments in which the final values of ψ_{ms} after treatment with amiloride were in the range ±3 mV.

the decline in ψ_{ms} is accompanied by a hyperpolarization of ψ_{mc} and the value of the intracellular potential when ψ_{ms} is abolished by amiloride ($_0\psi'_{mc}$) is less (more negative) than when ψ_{ms} is abolished by the short-circuit current ($_0\psi_{mc}$); and, (c) following the addition of amiloride there are prompt increases in R_t and R_m/R_s as indicated by the increased values of $\Delta\psi_{ms}$ and $\Delta\psi_{mc}$ in response to brief, constant-current pulses (40 μA/cm^2, 800 msec). The effect of amiloride on ψ_{ms}, ψ_{mc}, R_t and R_m are completely reversed within several minutes following perfusion of the mucosal surface with an amiloride-free solution.

The compiled data from all such experiments are given in Table 1 (upper section); for reasons that will be discussed below we have tabulated the results of all experiments in which ψ_{ms} fell to values in the range ±3 mV separately (lower section). The values of ψ_{ms}, I_{sc} and R_t reported in Table 1 are in excellent agreement with those reported previously (Frizzell *et al.*, 1976). Several points should be noted. (a) In response to amiloride, ψ_{ms} decreased by 16±1 mV and ψ_{mc} hyperpolarized by 19 ±4 mV. Although the average values of $(\psi_{ms}-\psi'_{ms})$ and $(\psi_{mc}-\psi'_{mc})$ do not differ significantly, in every instance $(\psi_{ms}-\psi'_{ms}) \leqq (\psi_{mc}-\psi'_{mc})$ and the paired ratio $(\psi_{ms}-\psi'_{ms})/(\psi_{mc}-\psi'_{mc})=0.84$ is significantly less than unity ($p<0.02$). (b) Amiloride brought about a decrease in the tissue conductance (G_t) of 0.6–0.7 mmhos/cm^2. (c) $_0\psi_{mc}$ resulting from the passage of a short-circuit current (I_{sc}) across the tissue is significantly less negative than ψ_{mc} observed when ψ_{ms} was reduced to zero by amiloride ($_0\psi'_{mc}$) (lower section); although the mean values shown do not suggest a highly significant difference (i.e., −46±3 *vs.* −53±3) *in every instance* the cell interior was electrically more negative with respect to the mucosal

solution following amiloride than during passage of the short-circuit current. Using a paired analysis ${}_0\psi'_{mc} < {}_0\psi_{mc}$ at $p < 0.001$; the significance of this finding will be discussed below. (d) Under control conditions, the resistance of the baso-lateral membrane (R_s) is, on the average, only 20% that of the mucosal membrane (R_m) and this decreases to less than 10% following the addition of amiloride to the mucosal solution. Thus, these findings are consistent with the finding that amiloride inhibits Na influx across the mucosal membrane (Frizzell *et al.*, 1975) and with the notion that amiloride preferentially increases the resistance of the mucosal membrane. However, it should be stressed that the *mean* values of R_s/R_m cannot be assigned heavy quantitative significance. As shown in Table 2, the frequency distribution of $R_m/(R_m+R_s)$ is highly skewed and most values fall between 0.9 and 1.0 under control conditions as well as in the presence of amiloride. In this range, the difference between $\Delta\psi_{ms}$ and $\Delta\psi_{mc}$ in response to moderate constant-current pulses is less than 1 mV and cannot be resolved accurately with our recording techniques. Hence we can only conclude that (i) the effective resistance[4] of the mucosal membrane is much greater than that of the baso-lateral membrane; and, (ii) amiloride appears to increase the relative resistance of the mucosal membrane preferentially.

Six studies were performed when the tissue was bathed on both surfaces with a Na-free, choline Ringer's. Under these conditions $\psi_{ms}=0$ and ψ_{mc} averaged -49 ± 4 mV. This value is significantly greater than ψ_{mc} in the presence of Na (140 mM) but does not differ significantly from ${}_0\psi'_{mc}$ observed in the presence of amiloride. Further, amiloride had no effect on ψ_{ms} or ψ_{mc} when the tissue was bathed by the Na-free buffer.

Table 2. Frequency distribution of voltage-divider ratios

$R_m/(R_m+R_s)$	Control	Amiloride
0.4–0.5	1	2
0.5–0.6	2	0
0.6–0.7	2	0
0.7–0.8	3	2
0.8–0.9	3	3
0.9–1.0	10	14
n	21	21

4 Since the relative resistance is not corrected for relative surface area a distinction must be made between the "effective resistance" and the "specific resistivity" of the membranes (*see* footnote 12).

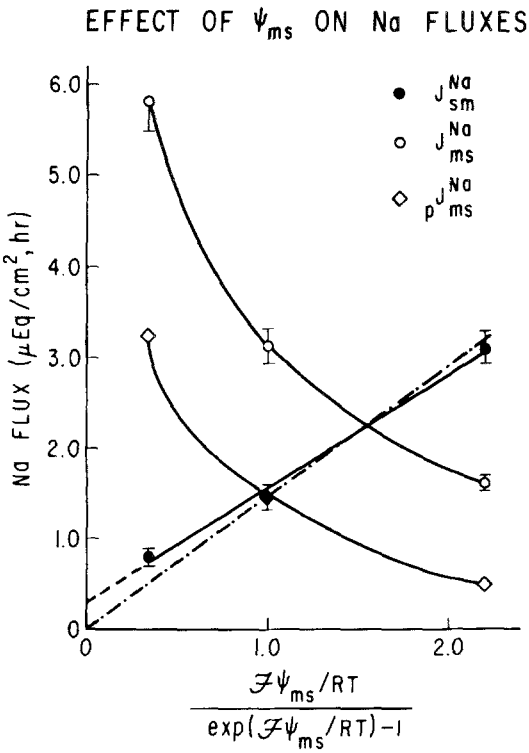

Fig. 4. The effect of ψ_{ws} on J^{Na}_{sw} (●), J^{Na}_{ws} (○) and the calculated value of $_pJ^{Na}_{ws}$ (◇). The dashed (- · -) line drawn through origin does not differ significantly from the solid line which describes the least squares linear fit to the data. Data was from 10 experiments involving 30 flux determinations at each ψ_{ms}. The average tissue resistance was 231 ± 5 ohm $\times$ cm^2 and was independent of ψ_{ms}

The Effect of ψ_{ms} on Na Fluxes

As discussed previously by Schultz & Zalusky (1964), if the unidirectional flux of an ion across a barrier is strictly diffusional then

$$J_{ik} = {}_0J_{ik}(z\mathcal{F}\psi_{ik}/RT)/[\exp(z\mathcal{F}\psi_{ik}/RT) - 1] \quad (1)$$

where J_{ik} is the unidirectional flux from compartment i to compartment k; $\psi_{ik} = \psi_k - \psi_i$; ${}_0J_{ik}$ is the unidirectional flux when $\psi_{ik} = 0$; and, z, $\mathcal{F}$, R and T have their usual meanings. More recently we have demonstrated that, in general, this relation is valid only if the diffusional pathway involves a single barrier (Schultz & Frizzell, 1976); if transepithelial flows of a given ion involve strictly diffusional movements across *two mem-*

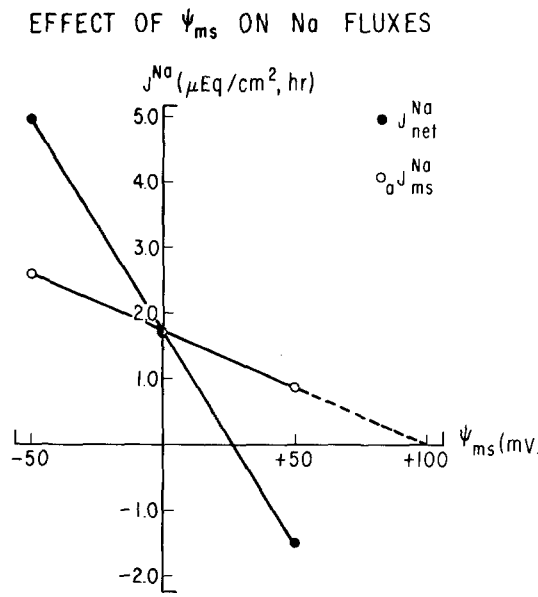

Fig. 5. Effect of ψ_{ms} on active and passive transepithelial fluxes of Na. A negative value of J^{Na} designates net flow from serosa to mucosa

branes arranged in series, Eq. (1), in general, does not hold and the relation between J_{ik} and $(z\mathcal{F}\psi_{ik}/RT)/[\exp(z\mathcal{F}\psi_{ik}/RT)-1]$ will not be linear.

The effects of ψ_{ms} on J^{Na}_{sm} and J^{Na}_{ms} are given in Fig. 4; in this figure we have plotted J^{Na}_{ik} as a function of $(\mathcal{F}\psi_{ms}/RT)/[\exp(\mathcal{F}\psi_{ms}/RT)-1]$ when ψ_{ms} was clamped at +50, 0 or −50 mV. J^{Na}_{sm} (solid circles) is a linear function of $(\mathcal{F}\psi_{ms}/RT)/[\exp(\mathcal{F}\psi_{ms}/RT)-1]$ and the intercept (0.32 ± 0.24) does not differ significantly from zero ($p>0.1$). These findings are in complete agreement with those published previously (Frizzell *et al.*, 1976) and suggest that the serosa-to-mucosa flux of Na is entirely diffusional and is restricted to a paracellular route. The values of J^{Na}_{ms} observed when ψ_{ms} is clamped at +50, 0 and −50 mV are designated by the open circles.

The notion that J^{Na}_{sm} is the result of paracellular diffusion is consistent with other evidence (*see* below) that transcellular Na transport is rectified in the mucosa-to-serosa direction. Accordingly we may subdivide the total J^{Na}_{ms} into active and passive components (designated by the subscripts a and p) so that

$$J^{Na}_{ms} = {}_aJ^{Na}_{ms} + {}_pJ^{Na}_{ms}. \tag{2}$$

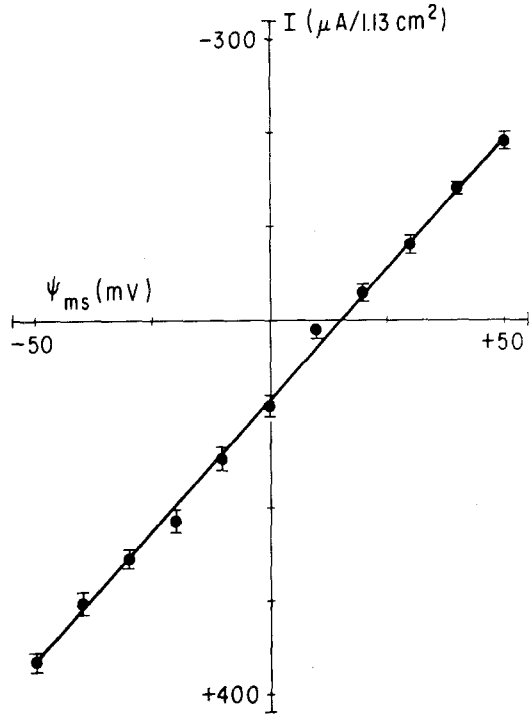

Fig. 6. Effect of an external current (I) on ψ_{ms}

Further, the passive (diffusional) component at any ψ_{ms} may be calculated from the relation

$$ {}_pJ^{Na}_{ms} = {}_0J^{Na}_{sm} \cdot (-\mathcal{F}\psi_{ms}/RT)/[\exp(-\mathcal{F}\psi_{ms}/RT) - 1] \quad (3) $$

or the Ussing flux ratio equation (Schultz & Zalusky, 1964). The calculated values of ${}_pJ^{Na}_{ws}$ at 50 and -50 mV are given in Fig. 4 ($\diamond$).

Fig. 5 gives the relation between the net Na flux ($J^{Na}_{net} = J^{Na}_{ms} - J^{Na}_{sm}$), the active Na flux (${}_aJ^{Na}_{ms} = J^{Na}_{ms} - {}_pJ^{Na}_{ms}$) and ψ_{ms}. Clearly both J^{Na}_{net} and ${}_aJ^{Na}_{ms}$ are linear functions of ψ_{ms} over the range studied.

Finally, as shown in Fig. 6, the tissue behaves as an ohmic resistor over the range ± 50 mV.

Discussion

The Nature of Na Entry Across the Mucosal Membrane

The findings that amiloride brings about a marked hyperpolarization of ψ_{mc}, increases R_m and, at the same time, inhibits Na influx across the

mucosal membranes (Frizzell *et al.*, 1975) indicates that Na entry is conductive and normally exerts a depolarizing effect on ψ_{mc}. The findings that ψ_{mc} in the presence of a Na-free medium does not differ significantly from that observed in the normal buffer containing amiloride, and is unaffected by amiloride, are entirely consistent with this conclusion. Finally, the observations that J_{mc}^{Na} conforms to saturation kinetics and that it is competitively inhibited by amiloride, (Frizzell & Schultz, *unpublished observations*) suggests that Na entry is a *rheogenic*, carrier-mediated process although the possibility of diffusional entry through pores or channels cannot be excluded (Lindemann & Voute, 1976; Fuchs *et al.*, 1977). The apparent inverse relation between ψ_{ms} and ψ_{mc} is also consistent with this notion. Thus, a rapid rate of rheogenic Na entry into the cell is associated with a high ψ_{ms} and I_{sc} but a low ψ_{mc}; conversely, when the rate of Na entry is low, ψ_{ms} and I_{sc} are low but the intracellular negativity is hyperpolarized. In short, these results are consistent with a rheogenic (conductive) Na entry process which, under some circumstances, is rate-limiting for transepithelial active Na transport.

In contrast with rabbit colon and some other "leaky" epithelia the intracellular potential in toad urinary bladder is positive with respect to the mucosal solution (Frazier, 1962; Reuss & Finn, 1974). The situation is somewhat unclear for the case of amphibian skin. Several investigators have reported a "staircase-type" profile where the intracellular potential is positive with respect to the outer solution (*cf.* Schultz, 1972; Schultz *et al.*, 1977) but more recently Nagel (1976) and Helman and Fischer (1976) reported negative intracellular potentials in frog skin compared to the outer solution; the reasons for the differences between these recent findings and earlier observations are unclear. Nevertheless, regardless of whether these amphibian epithelia display "staircase" or "well-type" electrical potential profiles, the effect of amiloride is similar; in all instances the cell interior becomes more negative (or less positive) (Larsen, 1973; Reuss & Finn, 1975; Flemström & Sachs, 1975; Helman & Fischer, 1976). Higgins and Frömter (1974) have reported that in *Necturus* urinary bladder $\psi_{mc} > 0$ when $\psi_{ms} > 95$ mV and $\psi_{mc} < 0$ when $\psi_{ms} < 90$ mV; amiloride brings about a large increase in intracellular negativity and in the presence of this agent ψ'_{mc} is approximately -75 mV. Finally, Lewis, Eaton and Diamond (1976) have reported that $\psi_{mc} < 0$ in rabbit urinary bladder and becomes less negative (but never positive) with increasing I_{sc} or G_m; when I_{sc} approaches zero (as R_m increases), ψ_{mc} approaches a value of approximately -50 mV. Thus it seems likely that Na entry into all of these epithelia is conductive and direct evidence in

support of this notion has been presented for frog skin by Biber and Sanders (1973).

An Equivalent Electric Circuit Model

We have recently described a double-membrane, equivalent electrical circuit model for Na-transporting epithelia[5] under steady-state conditions (Schultz *et al.*, 1977). The underlying feature of this model is that current due to the flow of a given ionic species across the mucosal membrane is linked in series to the flow of that species across the basolateral or serosal membrane so that a steady-state characterized by constancy of the intracellular composition is assured. Fig. 7 illustrates one limb of the model, namely, the transcellular pathway responsible for *active* Na transport from mucosa-to-serosa. E^m_{Na} and R^m_{Na} are the electromotive force (emf) and resistance associated with the Na current across the mucosal membrane and E^s_{Na} and R^s_{Na} are the analogous

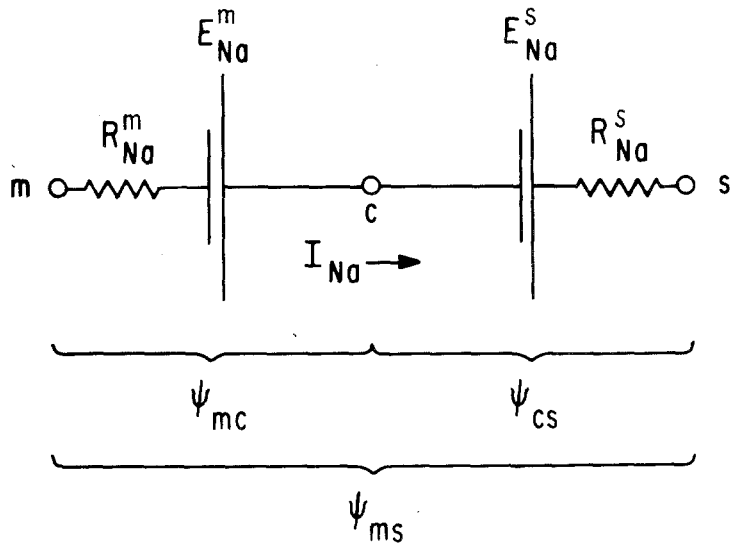

Fig. 7. Limb or branch of a total equivalent electrical circuit model which only represents the pathway through which the active Na current flows from mucosa to serosa (Schultz *et al.*, 1976); *m*, *c* and *s* designate the mucosal solution, cell interior, and serosal solution, respectively

5 The phrase "Na-transporting epithelia" applies to epithelia, such as frog skin and toad urinary bladder, where active transport of ions other than Na is absent or minimal. However, some of the conclusions of the model described by Schultz *et al.* (1977) are valid if the I_{sc} can be attributed to active Na transport even if other ions are also actively transported.

parameters for the serosal membrane. It is assumed that the emfs and resistances are voltage-independent particularly during brief perturbations; this assumption will be justified below. It should be stressed that the limb shown refers only to the active Na current across the transcellular pathway which as discussed below appears to be rectified in the mucosa-to-serosa direction. *Transepithelial* flows of Na attributable *entirely* to diffusional movements are not included in this pathway but can be represented by other limbs that traverse transcellular and/or paracellular routes (Schultz *et al.*, 1977)[6].

Clearly, the Na current through the active transport pathway is simply

$$I_{Na}=(E^m_{Na}-\psi_{mc})/R^m_{Na}=(E^s_{Na}-\psi_{cs})/R^s_{Na} \tag{4}$$

where $\psi_{mc}=\psi_c-\psi_m$, $\psi_{cs}=\psi_s-\psi_c$ and $\psi_{ms}=\psi_s-\psi_m=\psi_{mc}+\psi_{cs}$. When $\psi_{ms}=0$ (e.g., the short-circuit conditions or after treatment with amiloride), ${}_0\psi_{mc}=-{}_0\psi_{cs}$ so that from Eq. (4) we obtain

$${}_0\psi_{mc}=(R^s_{Na}E^m_{Na}-R^m_{Na}E^s_{Na})/R_{Na} \tag{5}$$

where R_{Na} is simply $(R^m_{Na}+R^s_{Na})$. It should be stressed that Eq. (5) describes the steady-state condition for Na and is generally valid regardless of whether other ions are actively transported or not.

It can also be shown (Schultz *et al.*, 1977) that if $I_{sc}={}_0I_{Na}$ (i.e. the short-circuit current is entirely attributable to active Na transport)[7]

$$\psi_{ms}=(R_t/R_{Na})(E^m_{Na}+E^s_{Na}). \tag{6}$$

Finally, if R_t (the total transepithelial resistance) is independent of ψ_{ms} (i.e., the tissue behaves as an ohmic resistor)[8], (Fig. 6), then

$$I_{sc}=\psi_{ms}/R_t=E_{Na}/R_{Na} \tag{7}$$

where $E_{Na}=(E^m_{Na}+E^s_{Na})$. It should be noted that Eqs. (6) and (7) are identical to those that can be derived from a single-membrane equivalent electrical circuit model assuming voltage-independent analogues (e.g., Ussing & Zerahn, 1951; Yonath & Civan, 1971).

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6 The term "transepithelial" must be stressed inasmuch as we do not wish to exclude the possibility of diffusional entry of Na into the cell across the mucosal membrane.

7 *See* Appendix *II*.

8 Ohmic behavior (i.e., a linear current-voltage relation) is necessary only over the "physiologic range" between the spontaneous or open-circuit ψ_{ms} and the short-circuit condition. Deviations from linearity beyond this range would not invalidate Eq. (7).

It is generally agreed (Ussing *et al.*, 1974) that in the presence of maximally effective concentrations of amiloride R^m_{Na} approaches ∞. In this case $\psi'_{ms} \cong 0$ [Eq. (6)], $I'_{sc} \cong 0$ [Eq. (7)] and ${}_0\psi'_{mc} \cong -E^{s'}_{Na}$ [Eq. (5)]. Further, we see [Eq. (5)] that ${}_0\psi'_{mc}$ (i.e., in the presence of amiloride) will be more negative than ${}_0\psi_{mc}$ (the short-circuit condition), as found in the present studies (Table 1).

Finally, R_{Na} can be determined from the tissue conductances before and after treatment with amiloride from the relation(s)

$$1/R_{Na} = (1/R_t) - (1/R'_t)$$

or

$$G_{Na} = G_t - G'_t. \tag{8}$$

Equations (8) assume that amiloride has no effect on any resistive properties of the tissue other than R^w_{Na}. This assumption is supported by the finding that amiloride does not affect J^{Na}_{sm}, or the bidirectional fluxes of Cl or K (Frizzell *et al.*, 1976). Further, as will be shown below, the value of R_{Na} determined from the data given in Table 1 is in good agreement with independent data derived from the studies of the effect of ψ_{ms} on ${}_aJ^{Na}_{ms}$ (Fig. 5).

The relations given in Eqs. (5), (6), (7) and (8) permit the calculation of R^m_{Na}, E^m_{Na}, R^s_{Na} and E^s_{Na} from the measurements of ψ_{ms}, I_{sc}, ${}_0\psi_{mc}$, ${}_0\psi'_{mc}$, R_t and R'_t given in Table 1 (lower section) *providing* E^s_{Na} *is not affected by amiloride.* There are no direct data bearing on this point. Yonath and Civan (1971) reported that amiloride does not affect E_{Na} in toad urinary bladder so that either (a) neither E^m_{Na} nor E^s_{Na} is affected or (b) both are affected in opposite directions and the changes cancel. On the other hand, Hong and Essig (1976) found that amiloride increases E_{Na} in toad urinary bladder and the same has been reported by Larsen (1973) for isolated toad skin. Thus, there is some conflict on this point perhaps related to the fact that different methods were employed to determine E_{Na}. For the present we will assume that amiloride does not affect E^s_{Na} in rabbit colon; experimental justification for this assumption will be presented below and a theoretical rationale for this assumption is presented in Appendix I.

The following values are derived from Eqs. (5)–(8) and the data given in Table 1 (lower section):

Effective Resistances (ohm · cm^2): $R_{Na} = 1670$; $R^m_{Na} = 1570$; $R^s_{Na} = 100$.
Electromotive Forces (mV): $E_{Na} = 117$; $E^m_{Na} = 64$; $E^s_{Na} = 53$.

Significance of these Findings

The concept of E_{Na} was originally introduced by Ussing and Zerahn (1951); it may be operationally defined as the "overall emf" responsible for driving the Na current through the transcellular active transport pathway or, alternatively, as the imposed ψ_{ms} necessary to abolish the current *through the active transport pathway.* The meaning of E_{Na} in terms of biochemical and molecular events is obscure; for the present, E^m_{Na} and E^s_{Na} may be viewed as phenomenologic, electrical analogues which essentially distribute the "overall emf" across the two limiting membranes. Possible interpretations of these analogues are presented in Appendix I.

Several approaches have been employed to determine E_{Na} in tight epithelia such as frog skin and toad bladder but all are rendered difficult or uncertain by the presence of finite shunt or passive conductance pathways that permit transepithelial Na diffusion. Nevertheless, using a variety of techniques and models the E_{Na} for toad urinary bladder has been estimated to be between 105–158 mV (Yonath & Civan, 1971; Saito, Leaf & Essig, 1974; Chen & Walser, 1975) and that for frog and toad skin approximately 120 mV (Ussing & Zerahn, 1951; Ussing, 1960; Larsen, 1973; Helman, O'Nell & Fisher, 1975); with the exception of the high value of 158 mV reported by Chen and Walser (1975) most estimates are approximately 100–120 mV in excellent agreement with that found in the present study for a leaky, mammalian epithelium.

As discussed by Schultz (1972) and Schultz *et al.* (1977), direct determination of E^m_{Na} and E^s_{Na} in Na-transporting epithelia using microelectrode techniques is compromised by the presence of finite passive conductance shunts (either transcellular or extracellular). However, when the conductance of these shunt pathways is maximally reduced or abolished (a condition which is most closely *approximated* in frog skin through the use of a Na_2SO_4-Ringer's) active transepithelial Na transport is essentially abolished and under steady-state conditions net Na entry into the active transport pathway and extrusion from the active transport pathway must also approach zero. Under these conditions both the entry and the exit processes are operating at "near static head" (Kedem & Caplan, 1965; Essig & Caplan, 1968) and

$$\psi_{ms} \cong E^m_{Na} + E^s_{Na} \quad \text{where } E^m_{Na} \cong \psi_{mc} \text{ and } \quad E^s_{Na} \cong \psi_{cs}$$

(Schultz, 1972; Schultz *et al.*, 1977). This conclusion follows directly from Eq. (4) when $I_{Na} = 0$. In essence, under these conditions ψ_{mc} must oppose

the emf of the Na entry mechanism and ψ_{cs} must oppose the emf of the Na exit mechanism.

An intuitive feeling for the reason why ψ'_{mc} is a measure of $E^{s'}_{Na}$ when Na transport and ψ_{ms} are abolished by amiloride can be gained from the following considerations. Treatment of frog skin (Dörge & Nagel, 1970; Nagel & Dörge, 1970) and toad urinary bladder (Handler, Preston & Orloff, 1972; Macknight, Civan & Leaf, 1975) with amiloride results in a decline in intracellular Na content, and evidence has been presented suggesting that this is a result of a decrease in the size of the Na transport pool. Thus, when Na entry into the transport pool is blocked, a lower than "normal" intracellular Na pool is maintained through the unimpaired action of the active Na extrusion mechanism at the basolateral membranes in spite of the fact that net transepithelial Na transport (I_{Na}) has been abolished. Thus, the serosal active extrusion mechanism is operating at "static head"; that is, it functions to maintain an electrochemical potential gradient for Na in the absence of net Na flow. Under these conditions the emf of this mechanism, $E^{s'}_{Na}$ must be precisely balanced by the opposing ψ'_{cs} which, in turn, is equal to $-\psi'_{mc}$.

When frog skin is bathed by a SO_4-Ringer's, ψ_{mc} and ψ_{cs} are each approximately 50–70 mV (Engbaek & Hoshiko, 1957; Hoshiko, 1961; Ussing & Windhager, 1964; Cereijido & Curran, 1965) and similar values have been reported for toad skin (Whittembury, 1964; Larsen, 1973). To the extent that the shunt pathway across frog skin is only sparingly permeable to Na, these values may be considered reasonable (minimal) estimated of E^{m}_{Na} and E^{s}_{Na} and are in reasonable agreement with the values of 64 and 53 mV calculated for rabbit colon. From the data of Lewis *et al.* (1976) on rabbit urinary bladder one may estimate that when R^{m}_{Na} becomes very large ψ_{mc} approaches -50 mV, in excellent agreement with our value of ${}_0\psi'_{mc} = -53$ mV. On the basis of these similarities it is tempting to suggest that similar mechanisms are responsible for active transepithelial Na transport by these diverse epithelia. [As noted above we have no explanation for the differences in electrical potential profile reported for amphibian skin; however, an explanation for the finding of a "well-type" profile in some tight epithelia and the transition between "well-type" and "staircase" observed by Higgins and Frömter (1974) has been suggested (Schultz *et al.*, 1977)].

The Effect of ψ_{ms} on Active and Passive Na Fluxes

The model of the *active Na transport pathway* illustrated in Fig. 7 consists of voltage-independent emf's and resistors linked in series, and

one may resonably question whether these analogues are appropriate or whether other electrical analogues such as voltage-dependent emf's and/or resistors (Finkelstein, 1964) or constant-current sources (Boulpaep, 1976; Fuchs *et al.*, 1977) might not be more appropriate. The "overall" behavior of the limb illustrated in Fig. 7 is given by

$$I_{Na} R_{Na} = E_{Na} - \psi_{ms}. \tag{9}$$

Thus, if R_{Na} and E_{Na} are voltage-independent, I_{Na} (or ${}_aJ^{Na}_{ms}$) must be a linear function of ψ_{ms}. The data illustrated in Fig. 5 indicate that this is, in fact, the case. Thus, ${}_aJ^{Na}_{ms}$, J^{Na}_{net} and, by inference, ${}_pJ^{Na}_{net}$ (the passive net movements of Na) are linear functions of ψ_{ms} [9]. Further we see that $I_{Na} = 0$ when $\psi_{ms} = E_{Na} \cong 100\,mV$; this independent estimate of E_{Na} is in excellent agreement with the value of 117 mV derived indirectly above. In addition, the slope of the line gives $R_{Na} = 2200$ ohm cm^2 ($G_{Na} = 0.45$ mmhos/cm^2); this value is in good agreement with the value of 1670 ohm cm^2 (0.6 mmhos/cm^2) determined from the change in R_t following the addition of amiloride [10]. Thus, the results of the studies of the effect of ψ_{ms} on Na fluxes provide independent support for the equivalent circuit model and some of the derived data. In particular it is of interest that the values of E_{Na} and R_{Na} estimated from experiments in which ψ_{ms} is briefly perturbed are in good agreement with those estimated from experiments in which ψ_{ms} is clamped over the range ± 50 mV for periods exceeding one hour. Finally, it should be stressed that the value of $E_{Na} \cong 100$ mV determined from these studies strictly applies only over the range of these studies (i.e., ± 50 mV) which includes the "physiological range" [7]. It is entirely possible that when tissues are clamped at voltages approaching or exceeding E_{Na}, backflux of Na through the active transport pathway may become significant (Chen & Walser, 1975) and ${}_aJ^{Na}_{ms}$ (as calculated) may differ from the rate of active Na transport (i.e., the notion that J^{Na}_{sm} is entirely diffusional and paracellular applies only over the range of ± 50 mV).

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9 As mentioned above, and discussed by Schultz and Frizzell (1976), the finding that the net diffusional flow of Na is a linear function of ψ_{ms} strongly suggests that the flow is paracellular; in general, linearity would not be observed if transepithelial diffusion were the result of diffusional flows across both limiting membranes of the epithelial cells.

10 It should be noted that in the experiments whose results are illustrated in Fig. 5 the rate of active Na transport (1.7 μEquiv/cm^{2}hr) was significantly less than that reported in Table 1 (2.6 μEquiv/cm^{2}hr). This difference cannot be attributed entirely to a decrease in E_{Na} but is in large part due to an increase in R_{Na}. Thus, these findings are consistent with the notion that the lower rate of active Na absorption is the result of a higher resistance to Na entry across the mucosal membrane (R^m_{Na}). This notion is in accord with the findings of Lewis and Diamond (1076), Lewis *et al.*, (1976) and Higgins *et al.* (1975).

The active Na currents across frog skin (Vieira, Caplan & Essig, 1972*a*, *b*) and toad bladder (Saito *et al.*, 1974; Chen & Walser, 1975) also appear to be linear functions of the transepithelial *PD* so that both tissues may be modeled electrically by voltage-independent emf's and resistors (Ussing & Zerahn, 1951; Yonath & Civan, 1971). The implications of such linear behavior have been discussed by Essig and Caplan (1968). It should be stressed, however, that although the *overall* tissue behaves as a constant source of emf in series with a constant resistor, it may be hazardous to extrapolate from these findings to the current-voltage relations characteristic of the entry mechanism or the active pump mechanism at the baso-lateral membranes. Since $R^m_{Na} = \partial(E^m_{Na} - \psi_{mc})/\partial I_{Na}$ and $R^s_{Na} = \partial(E^s_{Na} - \psi_{cs})/\partial I_{Na}$, both resistances are likely to be functions of the Na activity in the intracellular transport pool, $[Na]_c$, which, in turn, may be influenced by ψ_{ms}. Thus it is possible that the overall ohmic behavior reflects the sum of two nonlinear $I-V$ relations. Not enough is known about the effects of ψ_{ms} on $[Na]_c$ and the relation between $[Na]_c$ and the properties of the entry and exit mechanisms to warrant further speculation on this point.

Finally, the findings that (a) J^{Na}_{sm} is attributable to strict passive diffusion, probably through a paracellular route; (b) the unidirectional influx into the cells (J^{Na}_{mc}) is equal to I_{sc} or ${}_aJ^{Na}_{ms}$ (Frizzell & Schultz, *unpublished observations*); and (c) when J^{Na}_{mc} is inhibited with amiloride the inhibition is equal to the decrease in I_{sc} (Frizzell & Schultz, *unpublished observations*), suggest that transcellular Na movement is "rectified" in the mucosa-to-serosa direction and that this is due in part, if not entirely, to "rectification" at the entry step. That is, there does not appear to be any detectable Na backflux across the mucosal membranes[11]. A possible explanation for this finding stems from the observations of Fuchs *et al.* (1975; 1977) on frog skin (*see also* Lindemann & van Driessche, 1977). These investigators have obtained evidence consistent with the notion that Na entry into frog skin is diffusional and that the saturating behavior of the entry process is the result of an inverse

11 Biber (1971) reported close agreement between the unidirectional influx of Na across the outer barrier of frog skin and the I_{sc} and these findings have been recently confirmed by Rick, Dörge and Nagel (1975) using a different technique; these findings are consistent with the notion that the unidirectional flux of Na from the inner to the outer solution traverses an extracellular route (Mandel & Curran, 1972) and that entry into the tissue is rectified. Finally, Civan (1970) has provided electrophysiologic evidence for rectification of active Na transport by toad urinary bladder and Saito *et al.* (1974) and Chen and Walser (1975) have arrived at a similar conclusion from studies of isotope fluxes in response to transepithelial *PD*s.

relation between the Na permeability of the outer membrane and the concentration of Na in the outer solution. If the same is true for rabbit colon the absence of a detectable backflux of Na across the mucosal membrane is entirely predictable inasmuch as: (a) As discussed in Appendix I, if Na entry is diffusional the value of $E^m_{Na} = 64\,mV$ means that the activity of Na in the intracellular Na pool is approximately one-tenth that in the mucosal solution; and (b) ψ_{mc} is approximately $-30\,mV$ (Table 1). Thus, using the Ussing flux-ratio equation it can be readily shown that the predicted backflux (J^{Na}_{cm}) should be only 3% of the influx (J^{Na}_{mc}) so that no difference between ${}_0J^{Na}_{mc}$ and the I_{sc} would be detectable experimentally.

Alternatively, it is possible that Na entry into rabbit colon is the result of a downhill carrier-mediated process. The solutions of relatively simple kinetic models of rheogenic carrier-mediated processes indicate that when $[Na]_c = 0.1\,[Na]_m$ and $\psi_{mc} = -30\,mV$, J^{Na}_{cm} may be very small compared to J^{Na}_{mc} (Schultz & Frizzell, *in preparation*). Thus the finding that ${}_0J^{Na}_{mc} \cong I_{sc}$ may be entirely explicable without the need to postulate "true" rectification at the mucosal membrane.

Electrophysiologic Properties of the Epithelium

The purpose of this final section is to attempt to relate these and previous findings to the electrophysiologic properties of the epithelium using the simplified circuit model illustrated in Fig. 8. E_m and R_m are the emf and resistance across the mucosal membrane, respectively, E_s and R_s are the analogous parameters for the serosal membrane, and R_L is the resistance of the passive conductance pathways across the epithelium. The solutions of this model are:

$$\psi_{ms} = R_L(E_s + E_m)/(R_m + R_s + R_L) \tag{10}$$

and

$$\psi_{mc} = [E_m(R_s + R_L) - E_s R_m]/(R_m + R_s + R_L). \tag{11}$$

It should be noted that the orientations of the emf's as shown in Fig. 8 are included in the solution so that absolute values are employed. It should also be noted that the orientation of E_m is opposite from that which might be intuitively expected from the finding that $\psi_{mc} < 0$.

The total conductance of the tissue is the sum of the conductance due to active ion transport and that due to diffusional ionic movements (Frizzell *et al.*, 1976) so that

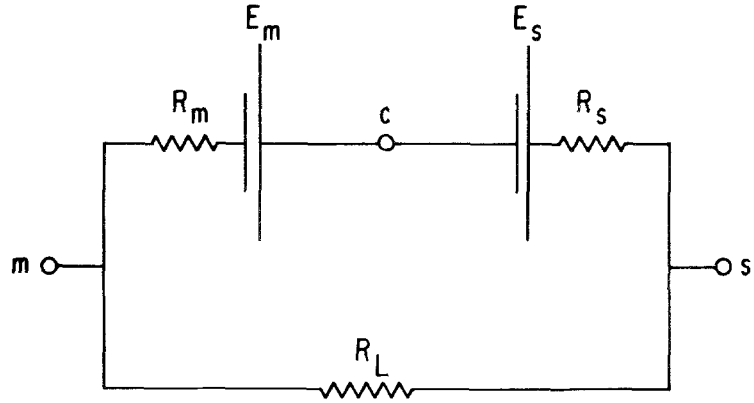

Fig. 8. Simplified equivalent electrical circuit model of the epithelium involving the resistances and electromotive forces of the transcellular pathway and the resistance of a paracellular pathway

$$G_t = G_{Na} + \sum_i {}_pG_i \tag{12}$$

where ${}_pG_i$ is the partial ionic conductance of species i due to transepithelial diffusion.

Frizzell *et al.* (1976) have shown that the diffusional flows of Na, K, Cl, and HCO_3 account for approximately 85% of the total tissue conductance and there is reason to believe that most if not all of these diffusional movements are paracellular (Schultz & Frizzell, 1976). Since $G_t = 3.5\,\text{mmhos/cm}^2$ (Table 1), we calculate, using Eq. (12), that $\sum_p G_i = 3.0\,\text{mmhos/cm}^2$ and $G_{Na} = 0.5\,\text{mmhos/cm}^2$; this value for G_{Na} is in excellent agreement with that obtained in the present studies from the amiloride-induced decrease in conductance (Table 1) ($0.6\,\text{mmhos/cm}^2$). *Thus, the transcellular conductance derived by subtracting the paracellular conductance from the total conductance is equal to the decrease in tissue conductance observed in the presence of amiloride.*

If this line of reasoning is correct it implies that the transcellular conductance (e.g., $1/(R_m + R_s)$) is dominated by the conductance of the active Na transport pathway; a similar conclusion has been reached by Saito *et al.* (1974). In addition we note that R^s_{Na}/R^m_{Na} determined *indirectly* from the equivalent circuit model is 0.06. The average value of R_s/R_m determined *directly* from the voltage-divider ratio is 0.17 but as pointed out above (Table 2) most of the values are between 0 and 0.1. This rough agreement supports the notion that R_m and R_s are determined predominantly by R^m_{Na} and R^s_{Na}, respectively, and that in the presence of

amiloride R_m or $R^m_{Na} \to \infty$ and the residual conductance is almost entirely paracellular[12, 13].

We now enquire whether the electrical potential profile across the tissue can be accounted for *entirely* by the properties of the active Na transport pathway shunted by a paracellular conductance? Using the data in Table 1, we set $R_L = (1/2.9) = 345$ ohm cm^2 and $R_t = (R_m + R_s) R_L / (R_m + R_s + R_L) = 286$ ohm cm^2. Further we assign: $R_m = R^m_{Na} = 1570$ ohm cm^2; $R_s = R^s_{Na} = 100$ ohm cm^2; $E_m = E^m_{Na} = 64$ mV; and $E_s = E^s_{Na} = 53$ mV.

Using these data, Eq. 10 predicts a value of $\psi_{mc} = 20$ mV which is precisely that observed (Table 1) and Eq. 11 predicts a value of $\psi_{mc} = -27$ mV which is in good agreement with the observed value of -32 mV. The fact that the predicted value of ψ_{ms} agrees precisely with the observed value is not surprising since ψ_{ms} was employed in the calculation of E_{Na} and R_{Na} (Eq. 6); this agreement is entirely predictable and lends no independent support to our interpretations. *However, the experimentally observed value of ψ_{mc} was not employed in any of the equations* (5–8) *used to calculate* E^m_{Na}, E^s_{Na}, R^m_{Na} or R^s_{Na}. Thus, the good agreement between the predicted ψ_{mc} and the observed value provides strong, independent support for the internal consistency of our equivalent electrical circuit model, the experimental data and our interpretations.

If this line of reasoning is correct, the "well-type" electrical potential profile observed in rabbit colon is primarily due to the presence of a low resistance shunt pathway which permits current from E^s_{Na} to influence ψ_{mc} and counteract the effect of E^m_{Na}. As discussed previously (Schultz, 1972) the fact that $R_m \gg R_s$ is important, but only permissive; in the absence of a low resistance shunt, the electrical potential profile across this tissue would be a "staircase" similar to that reported for toad urinary bladder and, by some, for frog skin.

Finally, we will consider the changes in ψ_{mc} and ψ_{ms} brought about by the addition of amiloride to the mucosal solution which are illustrated in Fig. 9. We have already demonstrated that the calculated values of E^m_{Na}, E^s_{Na}, R^s_{Na}, R^m_{Na} and R_L can satisfactorily account for the electrical potential profile under control conditions. It can be readily shown that, if

12 Since R_m and R_s are "effective resistances" uncorrected for membrane area, the fact that $R_s \ll R_m$ could be due to extensive infoldings of the baso-lateral membrane so that its area is considerably larger than that of the mucosal membrane.

13 Helman (1972) has reported that studies of the voltage-divider ratio in isolated rabbit cortical collecting tubules indicated that the pertubular membrane resistance was "immeasurably small" compared to that of the luminal membrane and Lewis and Diamond (1976) and Lewis *et al.* (1976) have made similar observations on rabbit urinary bladder under certain conditions.

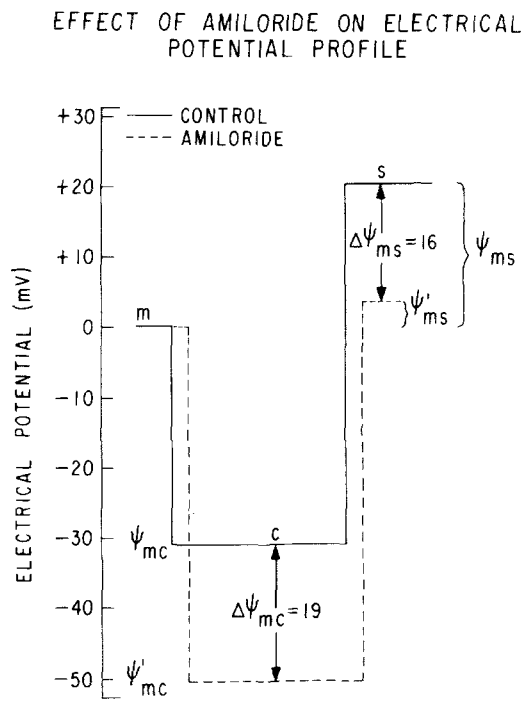

Fig. 9. Electrical potential profiles across rabbit colon under control conditions and in the presence of amiloride

the effect of amiloride is only to increase R^m_{Na} (or R_m), the change in ψ_{ms} divided by the change in ψ_{mc} is given by (Rose & Schultz, 1971)

$$\Delta\psi_{ms}/\Delta\psi_{mc}=1/[1+(R_s/R_L)]. \tag{13}$$

Using the values $R_s=R^s_{Na}=100\,\text{ohm cm}^2$ and $R_L=345\,\text{ohm cm}^2$, the predicted $\Delta\psi_{ms}/\Delta\psi_{mc}$ is 0.78. This predicted value does not differ significantly from the observed value of $(16/19)=0.84$. Since the relation between $\Delta\psi_{ms}$ and $\Delta\psi_{mc}$ was not employed in any of the calculations described above, the agreement between the predicted and observed values is another *independent* confirmation of the internal consistency of the data and interpretations.[14]

14 In particular, the good agreement between the observed and predicted values of ψ_{mc} and $\Delta\psi_{ms}/\Delta\psi_{mc}$ following amiloride supports the assumption that E^s_{Na} is not markedly affected by amiloride since the calculated values of E^m_{Na}, R^m_{Na} and R^s_{Na} depend on this assumption. For example if the value of E^s_{Na} in the absence of amiloride were 60 mV, rather than 53 mV, the calculated value of R^s_{Na} would be 200 ohm cm^2 and the predicted value of $\Delta\psi_{ms}/\Delta\psi_{mc}$ would be 0.63. The value of R_s necessary to give the observed $\Delta\psi_{ms}/\Delta\psi_{mc}=0.84$, is 86 ohm $\times$ cm^2 in excellent agreement with the value of $R^s_{Na}=100$ ohm $\times$ cm^2.

In summary, the values of E^m_{Na}, R^m_{Na}, E^s_{Na} and R^s_{Na} calculated from the data given in Table 1 *and* the equivalent circuit model of the active Na transport pathway illustrated in Fig. 7 [using Eqs. (5)–(8)] are consistent with the observations on the effect of ψ_{ms} on net and active Na fluxes across the epithelium. In addition, assuming that $E_m \cong E^m_{Na}$, $R_m \cong R^m_{Na}$, $E^s_{Na} \cong E_s$, $R_s \cong R^s_{Na}$ and that R_L $(=1/\Sigma_p G_i)$ is paracellular, the equivalent circuit model illustrated in Fig. 8 can account for (a) ψ_{mc} and (b) $\Delta\psi_{ms}/\Delta\psi_{mc}$ in response to amiloride; these agreements are entirely independent of the equations used to derive the values of the electrical analogues illustrated in Fig. 7. Thus, all of our observations, taken together, strongly suggest that (a) transepithelial passive conductance (diffusional) pathways are extracellular; (b) the conductance of the transcellular route and across each of the limiting membranes is determined predominantly by the resistances to flow through the active Na transport pathway; (c) the electromotive forces acting across the two limiting membranes are determined predominately by the mechanisms responsible for Na entry into and exit from the intracellular Na transport pool; and (d) under steady-state conditions, the entire system can be represented by an equivalent electrical circuit model comprised of voltage-independent emf's and resistors; there does not appear to be any need to postulate more complex, nonlinear models.

Finally, the notion that $R_m \cong R^m_{Na}$ and that $E_m \cong E^m_{Na}$ is entirely consistent with the evidence that the mucosal membrane is virtually impermeable to K (Frizzell & Schultz, 1976) and with the likelihood that Cl and HCO_3 movements across this barrier are mediated by a nonconductive, coupled exchange process (Frizzell *et al.*, 1976). It is more difficult to interpret the physical meanings of E^s_{Na} and R^s_{Na} since Na extrusion from the cell across the baso-lateral membrane is the result of a carrier-mediated process coupled to a supply of metabolic energy. Until more is known about this "pump", these values must be simply viewed as "phenomenologic parameters" that describe the relation between the Na current across the baso-lateral membrane and ψ_{cs} (Appendix I).

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Appendix I

An Interpretation of Equivalent Electrical Circuit Analogues of the "Active Na Transport Pathway"

Equivalent electrical circuit models are most appropriate for describing transmembrane ion movements that can be attributed entirely to simple diffusion because the terms in the Nernst-Planck equation can be precisely and unambiguously represented by electrical analogues. Thus, as discussed by Finkelstein and Mauro (1963), under steady-state conditions, the Nernst-Planck electrodiffusion equation can be written in the form of Ohm's law:

$$I_i R_i = (\mathscr{E}_i + \Delta\psi), \tag{1A}$$

where: I_i is the current carried by the diffusional flow of $i(J_i)$ and is simply $z_i \mathscr{F} J_i$; $\mathscr{E}_i$ is the electromotive force (emf) given by the Nernst equation, i.e., $\mathscr{E}_i = (RT/z_i\mathscr{F})\,\Delta \ln c_i$ where $\Delta \ln c_i$ is the natural logarithm of the ratio of the activities of i in the two bathing solutions; $\Delta\psi$ is the observed electrical potential difference across the barrier; and R_i is the "integral resistance" of the membrane given by $\int_0^{\Delta x} [dx/(z_i^2 \mathscr{F}^2 u_i c_i)]$ where u_i is the mobility of i at point x, c_i is the activity of i at the point and Δx is the thickness of the membrane. Thus, the diffusional flow can be represented by a source of emf in series with a resistance where the two analogues are functions of ion activities and membrane properties. Note that Eq. (1A) has the same form as Eqs. (4) and differs only because of the polarity convention for $\Delta\psi$ employed in the text.

Attempts to represent "carrier-mediated" transport processes, particularly those coupled to a source of metabolic energy, by equivalent electrical analogues are confronted with the facts that the underlying mechanisms are poorly understood and that there are, at present, no generally accepted formal descriptions of such processes analogous to the equations of electrodiffusion. Consequently, at present, the most general equivalent electrical representations of carrier-mediated ion transport processes must be entirely phenomenologic; they must deal with the observable system as a "black box" independent of underlying mechanisms.

In this section we will present an equivalent electrical circuit analogue of a Na "pump" based on the theories of linear, irreversible thermodynamics and network thermodynamics. In essence we will pres-

ent an electrical analogue that corresponds to the irreversible thermodynamic equations which phenomenologically describe coupling between an exergonic chemical reaction and ion transport, analogous to the treatment of Finkelstein and Mauro (1963) for diffusional ion flows. This treatment closely follows that of Essig and Caplan (1968) and may provide some insight into the possible meanings of E^m_{Na}, E^s_{Na} and E_{Na}.

Coupling between the transport of Na across a membrane and a chemical reaction can be described using the formalism of irreversible thermodynamics as follows (Katchalsky & Curran, 1965):

$$X_{Na} = R_{Na} J_{Na} + R_{r,Na} J_r$$

and (2A)

$$A = R_{r,Na} J_{Na} + R_r J_r$$

where A is the "affinity" of the reaction which, under conditions of constant temperature and pressure is equal to the decrease in Gibbs free energy; J_{Na} and J_r are the flows of Na and of the chemical reaction, respectively, expressed in moles/cm^{2}sec; R_{Na} and R_r are the "straight" phenomenological coefficients; $R_{r,Na}$ is the "cross coefficient" (it is assumed that Onsager's reciprocity is obeyed); and

$$X_{Na} = -\Delta\tilde{\mu}_{Na} = -(RT\Delta \ln c_{Na} + \mathcal{F}\Delta\psi). \tag{3A}$$

Equations (2A) can be readily transformed into "electrical terminology" so that

$$X_{Na}/\mathcal{F} = R_{Na} I_{Na} + R_{r,Na} I_r \tag{4A}$$

and

$$\mathcal{A} = R_{r,Na} I_{Na} + R_r I_r \tag{5A}$$

where

$$(X_{Na}/\mathcal{F}) = -[(RT/\mathcal{F})\Delta \ln c_{Na} + \Delta\psi] = -(\mathcal{E}_{Na} + \Delta\psi) \tag{6A}$$

and $\mathcal{A} = A/\mathcal{F}$.

I_{Na} and I_r are now expressed in A/cm^2; the generalized resistances (R's) are in ohm$\cdot$cm^2; and, $(X_{Na}/\mathcal{F})$ and $\mathcal{A}$ are in volts.

Combining Eqs. (4A), (5A) and (6A) we obtain:

$$I_{Na} = \frac{-R_r(\mathcal{E}_{Na} + \Delta\psi) - R_{r,Na}\mathcal{A}}{R_{Na}R_r - R^2_{r,Na}} \tag{7A}$$

We now define I_{Na} as positive when $(\mathcal{E}_{Na} + \Delta\psi) = 0$. Accordingly, $R_{r,Na} < 0$ ("positive coupling") and since $R_r R_{Na} \geqq R^2_{r,Na}$ (Katchalsky & Curran,

1965), I_{Na} will be positive when $|R_{r,Na}\mathscr{A}/R_r| > |\mathscr{E}_{Na} + \Delta\psi|$. That is, under this condition, Na will be driven across the membrane against its electrochemical potential difference. Eq. (7A) is very similar to one derived by Essig and Caplan (1968) in their analysis of the energetics of active ion transport processes [their Eq. (6)].

Solving Eqs. (4A), (5A) and (6A) for I_r we obtain

$$I_r = \frac{R_{Na}\mathscr{A} + R_{r,Na}(\mathscr{E}_{Na} + \Delta\psi)}{R_r R_{Na} - R_{r,Na}^2} \tag{8A}$$

so that $I_r < 0$ when $|\mathscr{E}_{Na} + \Delta\psi| > |R_{Na}\mathscr{A}/R_{r,Na}|$. That is, under this condition, $\Delta\tilde{\mu}_{Na}$ can drive the chemical reaction "backward". Garrahan and Glynn (1967) have demonstrated reversal of the Na-K ATPase activity in erythrocytes in the presence of large Na-gradients, and the general question of reversible coupling between transport and chemical reactions has been discussed by Mitchell (1970).

Recently it has been shown that the expressions of irreversible thermodynamics can be transformed into terms of network theory using either electrical circuit analogues (Peusner, 1970) or bond-graph notation (Oster, Perelson & Katchalsky, 1971, 1973). Using the former approach, coupled interactions under steady-state conditions can be represented by two circuits that share a common resistance. It can be readily shown that the electrical circuit illustrated in Fig. 10 is described by Eqs. (4A) and

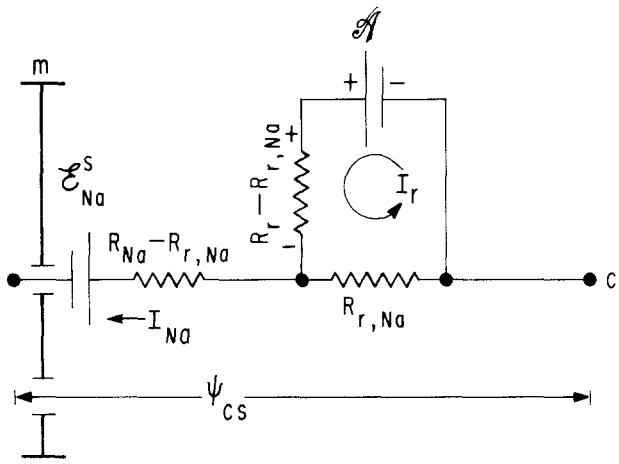

Fig. 10. Equivalent electrical analogue of a Na pump. The vertical heavy line designated *m* represents the baso-lateral membrane; *s* and *c* designate the serosal solution and intracellular compartment, respectively. The arrows indicte the positive directions of the currents I_{Na} and I_r

(5A) and hence is the equivalent electrical analogue of Eq. (7A). In this figure, s represents the serosal solution and c the intracellular compartment; $\psi_{cs}=\psi_s-\psi_c$ and $\mathscr{E}^s_{Na}=(RT/\mathscr{F})\ln([Na]_s/[Na]_c)$ where [Na] is the Na activity of the compartment. The Na current is displayed as flowing from c to s and is defined as positive in that direction.

To examine the meaning of E^s_{Na} of Fig. 7 in terms of the analogue illustrated in Fig. 10 we write

$$I_{Na}=\frac{(E^s_{Na}-\psi_{cs})}{R^s_{Na}}=\frac{-R_r(\mathscr{E}^s_{Na}+\psi_{cs})-R_{r,Na}\mathscr{A}}{R_{Na}R_r-R^2_{r,Na}}. \tag{9A}$$

Clearly, solving equation 9A for E^s_{Na} yields a complex function of the terms in the right-hand expression and R^s_{Na} which is not very informative. However, when I_{Na} is abolished by amiloride

$$E^{s'}_{Na}={}_0\psi'_{cs}=-{}_0\psi'_{mc}=-(R_{r,Na}\mathscr{A}/R_r)'-\mathscr{E}^{s'}_{Na}. \tag{10A}$$

[*Note:* we continue to use primes (′) to designate values in the presence of amiloride.]

Thus, the "static head" E^s_{Na}, designated by $E^{s'}_{Na}$, is determined by the affinity of the driving reaction ($\mathscr{A}$), the resistance to the flow of that reaction (R_r), the coupling coefficient between the chemical reaction and the flow of Na($R_{r,Na}$) and the chemical potential difference of Na across the baso-lateral membrane between the serosal solution and the Na transport pool ($\mathscr{E}^s_{Na}$). ${}_0\psi'_{mc}$ will be negative when $|(R_{r,Na}\mathscr{A}/R_r)'|>|\mathscr{E}^{s'}_{Na}|$ which is the necessary condition for uphill transport of Na out of the cell or the maintenance of a static-head electrochemical potential difference.

From Eqs. (4A) and (6A) it is clear that if Na *entry* into the cell is energized solely by its conjugate driving force (i.e., $R_{r,Na}=0$), $E^m_{Na}=(RT/\mathscr{F})\ln([Na]_m/[Na]_c)$ regardless of whether the flow is strictly diffusional or carrier-mediated (e.g., facilitated diffusion). Thus, the observed value of $E^m_{Na}=64$ mV is consistent with a Na activity within the transport pool that is approximately one-tenth that in the mucosal solution.

From Eq. (10A) and the above considerations, when $[Na]_m=[Na]_s$ (so that E^m_{Na} always equals $\mathscr{E}^s_{Na}$),

$$E'_{Na}=E^{m'}_{Na}+E^{s'}_{Na}=-(R_{r,Na}\mathscr{A}/R_r)' \tag{11A}$$

or that

$$E^{s'}_{Na}=E'_{Na}-\mathscr{E}^{s'}_{Na}. \tag{12A}$$

From considerations of a single-membrane model of a Na-transporting epithelium, Essig and Caplan (1968) have shown (their Eq. (18) but using our notation) that

$$E_{Na} = -(R_{r,Na}\mathscr{A}/R_r). \tag{13A}$$

Since $E_{Na} = E^m_{Na} + E^s_{Na}$ and $E^m_{Na} = \mathscr{E}^s_{Na}$, it follows that

$$E^s_{Na} = E_{Na} - \mathscr{E}^s_{Na}. \tag{14A}$$

Comparison of Eqs. (12A) and (14A) permit us to assess the assumption that E^s_{Na} is not affected by amiloride in the light of these theoretical considerations.

As noted above, the intracellular transport pools of Na in toad urinary bladder (Handler *et al.*, 1972; Macknight *et al.*, 1975), isolated frog skin (Dörge & Nagel, 1970; Nagel & Dörge, 1970), and rabbit colon (Frizzell, *in preparation*) are decreased in the presence of amiloride. This would lead to an increase in $\mathscr{E}^s_{Na}$. At the same time, the results of recent studies by Hong and Essig (1976) on toad bladder, and Larsen (1973) on toad skin suggest that E_{Na} (or, $-R_{r,Na}\mathscr{A}/R_r$) is significantly increased by amiloride. Thus it is entirely possible that the increase in E_{Na} completely (or almost completely) counterbalances the increase in $\mathscr{E}^s_{Na}$ so that E^s_{Na} is minimally affected by amiloride.

Finally, Eq. (9A) can be written in the form

$$I_{Na} = \alpha\mathscr{A} - \beta\mathscr{E}^s_{Na} - \beta\psi_{cs}$$

where $\alpha = R_{r,Na}/(R_{Na}R_r - R^2_{r,Na})$ and $\beta = R_r/(R_{Na}R_r - R^2_{r,Na})$. Thus, if $\mathscr{A}$ and $\mathscr{E}^s_{Na}$ are either independent of ψ_{cs} or linear functions of ψ_{cs} the analogue illustrated in Fig. 10 will display ohmic behavior (i.e., a linear $I-V$ relation).

It should be stressed that the model illustrated in Fig. 10 is entirely phenomenologic and simply describes coupling between the Na current across the baso-lateral membrane and an exergonic chemical reaction. It assumes only that this coupling can be described by a combination of linear relations and that Onsager's reciprocal relations are obeyed. Thus it treats the observable system as a black box without reference to underlying mechanism but at the same time may provide some insight into the relations among the "static head" emf of the pump (which is readily measurable) the affinity of the driving reaction and the chemical potential against which Na is pumped. However, clearly, additional studies are needed to establish the validity and usefulness of this analysis.

Appendix II

Under steady-state conditions we may write [*see* Eq. (4)]

$$\psi_{mc} = E_i^m - I_i R_i^m \tag{1B}$$

$$\psi_{cs} = E_i^s - I_i R_i^s \tag{2B}$$

for all ions i that traverse the transcellular pathway. Since $\psi_{ms} = \psi_{mc} + \psi_{cs}$

$$\psi_{ms} = E_i - I_i R_i \tag{3B}$$

or

$$I_i = (E_i/R_i) - (\psi_{ms}/R_i) \tag{4B}$$

where $E_i = E_i^m + E_i^s$ and $R_i = R_i^m + R_i^s$. For ion flows across the paracellular pathways we may write the lumped expression

$$I_L = (E_L/R_L) - (\psi_{ms}/R_L) \tag{5B}$$

where I_L is the *total* ionic current across the shunt pathway; R_L is the *total* resistance of the pathway and E_L is the electromotive force generated within the shunt due to transepithelial ionic asymmetries.

Thus, the total current across the tissue, I, is

$$I = \sum_i I_i + I_L = \sum_i (E_i/R_i) + (E_L/R_L) - \psi_{ms} \cdot \left\{ \sum_i (1/R_i) + (1/R_L) \right\} \tag{6B}$$

and, when, $I = 0$ (open-circuit condition)

$$\psi_{ms} = R_t \left\{ \sum_i (E_i/R_i) + E_L/R_L \right\} \tag{7B}$$

where, the total transepithelial resistance, R_t, is given by

$$(1/R_t) = \sum_i (1/R_i) + (1/R_L).$$

Eq. (7B) can be written

$$\psi_{ms} = R_t \left\{ (E_{\mathrm{Na}}/R_{\mathrm{Na}}) + \sum_j (E_j/R_j) + E_L/R_L \right\} \tag{8B}$$

where j represents all ions other than Na.

When the two bathing solutions have identical compositions so that $[\mathrm{Na}]_m = [\mathrm{Na}]_s$ and $[j]_m = [j]_s$ for all j, then $E_L \cong 0$ and $\psi_{ms} = R_t E_{\mathrm{Na}}/R_{\mathrm{Na}}$ when:

(a) The movement of j across both limiting membranes is strictly diffusional since under these conditions

$$E_j^m = (RT/\mathscr{F}) \ln([j]_m/[j]_c) = -E_j^s, \quad \text{so that } E_j = 0.$$

(b) The movement of j across *one* membrane is not strictly diffusional but the R_j of *the other* membrane is very large (approaches ∞), in which case E_j/R_j approaches zero (e.g., K, which appears to be actively transported into the cell across the baso-lateral membrane but the mucosal membrane is essentially impermeable to this ion).

(c) When there are, for example, two ions k and l distributed such that $(E_k/R_k) = -(E_l/R_l)$. From Eq. (8B) we see that these ions will not contribute to ψ_{ms} and from Eq. (4B) we see that under short-circuit conditions (when $\psi_{ms}=0$), $I_k = -I_l$ so that these currents cancel and do not contribute to I_{sc}.

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