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The Electrophysiology of Rabbit Descending Colon

II. Current-Voltage Relations of the Apical Membrane, the Basolateral Membrane, and the Parallel Pathways

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Summary. In this paper we employ the data described in the previous paper (I) to derive the current-voltage ($I-V$) relations of the basolateral membrane, the amiloride-insensitive "leak" pathway across the apical membrane, and the parallel pathways across rabbit descending colon. The results indicate that:

a) The resistance of the basolateral membrane is independent of the electrical potential difference across that barrier over the range −8 to 67 mV and averaged 195 Ωcm^2. The electromotive force across this barrier averaged 50 mV under control conditions and 48 mV in the presence of amiloride. The origin of this difference is discussed.

b) The resistance of the parallel pathways averaged 351 Ωcm^2 and was independent of the transepithelial electrical potential difference over the range −170 to +90 mV. The conductance of these pathways can be reasonably well accounted for by the partial ionic conductances of Na, K and Cl reported previously.

c) The resistance of the amiloride-insensitive pathway across the apical membrane averaged 1667 Ωcm^2 and the electromotive force across this pathway averaged −51 mV. These values are in excellent agreement with those determined by others. The ionic nature of this "leak" pathway remains to be elucidated.

Key words colon · apical membrane · basolateral membrane · parallel pathways · current-voltage relations · electrical circuit model

Introduction

In the preceding paper (Thompson, Suzuki & Schultz, 1982), a method was described for determining the "instantaneous" $I-V$ relations of rabbit descending colon employing transepithelial and intracellular measurements. The data were interpreted employing an equivalent electrical circuit model that includes an amiloride-insensitive conductance pathway across the apical membrane in parallel with the amiloride-sensitive Na entry mechanism. The $I-V$ relation of the amiloride-sensitive Na entry pathway across the apical membrane was derived and was shown to conform closely with the Goldman-Hodgkin-Katz constant-field flux equation over a wide range.

In this paper (II), we extend this analysis to cover the $I-V$ relations of the basolateral membrane, the amiloride-insensitive apical "leak" pathway, and the "lumped" conductive pathways in parallel with the amiloride-sensitive cells ("parallel pathways"), thereby providing a complete description of the electrophysiological properties of the barriers to ion flow across this epithelium.

In addition, we provide evidence supporting the assumptions employed in the previous paper to derive the Na current across the apical membrane (I^m_{Na}) as a function of the transapical electrical potential difference (ψ^{mc}).

Materials and Methods

The methods employed are described in detail in the preceding paper (Thompson et al., 1982) which will be henceforth referred to as (I). Data reduction and display were carried out using a minicomputer (LSI 11/03, Digital Equipment Corp.) which interfaced with a graphics terminal (Hewlett Packard 2647 A) and a four-pen $X-Y$ plotter (Hewlett Packard 9872 A). The notation and definitions that will be employed are given in (I).

Results and Interpretation

The $I-V$ Relation of the Basolateral Membrane.

The $I-V$ relation of the basolateral membrane was obtained using the following approach:

The total current across the tissue at any value of ψ^{ms}, $(I^{ms})_{\psi^{ms}}$, is the sum of a parallel pathways current, I^p and a transcellular current, I^c. Assuming that amiloride does not affect R^p (or r^p) within the time-frame of these studies, $(\Delta I^{ms})_{\psi^{ms}}=(I^c-I^{c'})=(\Delta I^c)_{\psi^{ms}}$. Justification for this assumption was provided in the previous paper. Now, according to the

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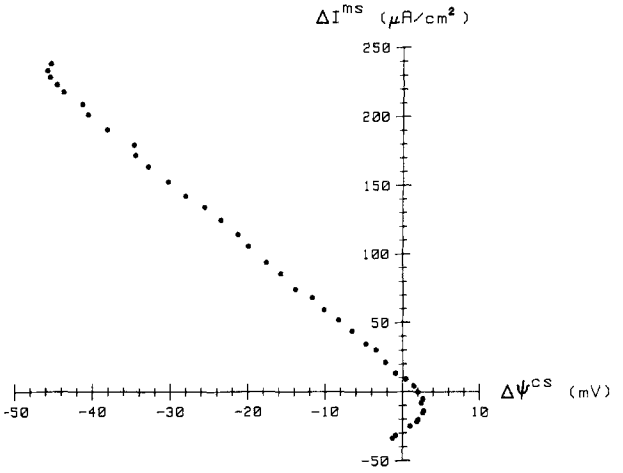

Fig. 1. $\Delta I^{ms}=(I^{ms}-I^{ms\prime})$ obtained from Fig. 3 of (I) is plotted as a function of $\Delta\psi^{cs}=(\psi^{cs}-\psi^{cs\prime})$ obtained from Fig. 6 of (I) where each parameter is determined at the same ψ^{ms}

equivalent electrical circuit model illustrated in Fig. 7 of (I)

$$I^c=G^sE^s-G^s\psi^{cs} \tag{1}$$

and

$$I^{c'}=G^{s'}E^{s'}-G^{s'}\psi^{cs'}. \tag{2}$$

At this point we make the assumption that amiloride does not affect the conductance of the basolateral membrane within 60–90 sec after introducing this agent into the mucosal solution, so that $G^s=G^{s'}$ at each ψ^{ms}. [It should be noted that this assumption implicitly assumes that the $I-V$ relation of the basolateral membrane is linear over the range $-200\text{ mV}\leqq\psi^{ms}\leqq110\text{ mV}$ since $\psi^{cs}\neq\psi^{cs'}$ in this range (Fig. 6 of (I)).] Subtracting Eq. (2) from Eq. (1) we obtain

$$(\Delta I^c)=(\Delta I^{ms})=G^s(\Delta E^s)-G^s(\Delta\psi^{cs}). \tag{3}$$

The data given in Figs. 3 and 6 of (I) provide us with the values of (ΔI^c) and $(\Delta\psi^{cs})$ at each value of ψ^{ms}; it is important to note that these are actually measured parameters and are not dependent on the above assumption. The values of (ΔI^c) and $(\Delta\psi^{cs})$ are plotted against each other in Fig. 1. Starting at the upper left and proceeding to the lower right these data points correspond to an ascending order of ψ^{ms} from -200 to $+200$ mV in 10-mV increments. The relation between (ΔI^c) and $(\Delta\psi^{cs})$ for this experiment is linear over a wide range of $\Delta\psi^{cs}$, and for all eight experiments the relation was linear over the range corresponding to $-140\leqq\psi^{ms}\leqq110$ mV or $-8\text{ mV}\leqq\psi^{cs}\leqq67$ mV. This finding strongly supports the notion that G^s, given by the negative slope of the line, is independent of ψ^{ms} and ψ^{cs} over a wide range and is not affected by amiloride. The average value of G^s for all eight experiments was $5.3\pm0.4\text{ mS/cm}^2$, corresponding to a value of $R^s=195\,\Omega\text{cm}^2$. This value is in reasonable agreement with those reported earlier for this epithelium by Schultz, Frizzell and Nellans (1977) and Wills, Lewis and Eaton (1979*b*) of 100 and $160\,\Omega\text{cm}^2$, respectively.

Two other points should be noted. First, according to Eq. (3), the nonzero intercept on the abscissa is a measure of ΔE^s which averaged -1.8 ± 0.8 mV; the significance of this finding will be discussed below. Second, the departure from linearity seen in Fig. 1 when $\Delta I^c<0$ was a consistent finding and occurs when ψ^{ms} exceeds 110 mV which corresponds to a value of $\psi^{cs}\geqq55$ mV.

With G^s and ΔE^s determined, then knowing *one* value of I^c, E^s can be calculated using Eq. (1) and $E^{s'}$ is then given by $E^s+\Delta E^s$. Clearly, when the tissue is short-circuited, I^{ms} is entirely transcellular (i.e., $I^p=0$) so that $I_{sc}={}_0I^c$. Inserting the value of I_{sc} and the corresponding value of ${}_0\psi^{cs}$ into Eq. (1) we obtain E^s. The average value of E^s in all eight experiments was 50 ± 1 mV which is in reasonable agreement with the value of 57 mV reported by Wills et al. (1979*b*). $E^{s'}$ averaged 48 ± 1 mV in good agreement with the value of 53 mV estimated by Schultz et al. (1977). Finally, as discussed below and by Schultz et al. (1977), since under short-circuit conditions in the presence of amiloride, $I^{c'}\cong0$ it follows from Eq. (2) that $E^{s'}\cong{}_0\psi^{cs'}$. The value of ${}_0\psi^{cs'}$ in these experiments averaged 49 ± 1 mV, in excellent agreement with the value of $E^{s'}$ determined, independently, from the graphic analysis described above. Thus, the interpretation of these data yields a self-consistent set of values that are also in good agreement with values previously reported for this tissue.

Finally, it should be recalled that one of the assumptions necessary to derive an expression for I^m_{Na} (Eq. (1) of (I)) is that G^s and E^s are not affected by amiloride. The data illustrated in Fig. 1 are consistent with the notion that G^s is not affected by amiloride and that, while E^s is reduced by amiloride, the reduction is less than 5%.

The $I-V$ Relations of the Transcellular Currents (I^c and $I^{c'}$)

Knowing G^s, E^s and $E^{s'}$, the transcellular currents under control conditions (I^c) and in the presence of amiloride ($I^{c'}$) can be calculated at every value of ψ^{cs} and $\psi^{cs'}$ over the range in which the relation between ΔI^c and $\Delta\psi^{cs}$ is linear using Eqs. (1) and (2). Further, since the corresponding values of ψ^{mc} and $\psi^{mc'}$ are known, it is a simple matter to construct

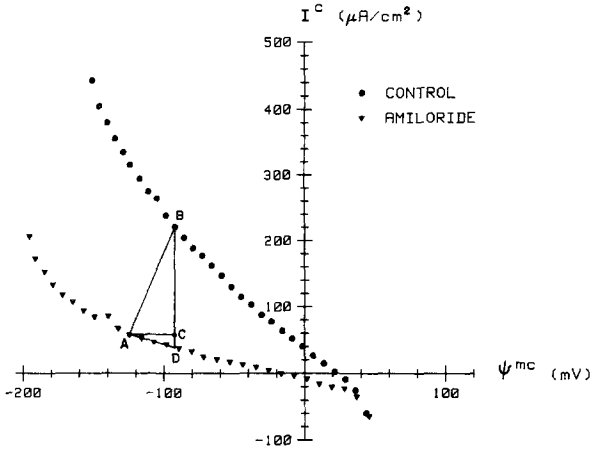

Fig. 2. $I-V$ relations of the apical membrane. The cellular component (I^c) of the transepithelial current is plotted as a function of ψ^{mc} in the absence (●) and in the presence of amiloride (▼). *See* the Appendix for the meaning of the lines connecting points *ABCD*

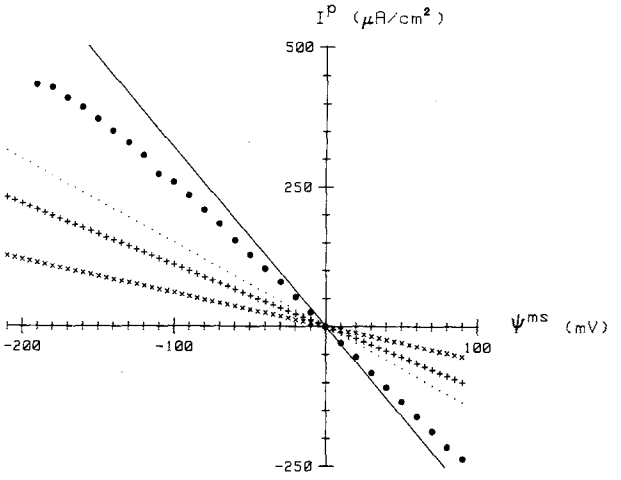

Fig. 3. $I-V$ relation of the parallel pathways. I^p (●) is calculated from $(I^p)_{\psi^{ms}}=(I^{ms}-I^c)_{\psi^{ms}}$ and is plotted as a function of ψ^{ms}. The other points represent the parallel currents due to Na (+). K (×) and Cl (·) determined from the data of Frizzell et al. (1976). The solid line is the sum of these ionic currents

the $I-V$ relations of the apical membrane under control conditions and in the presence of amiloride; the former represents the combined $I-V$ relation of the amiloride-sensitive and amiloride-insensitive pathways, whereas the latter represents the $I-V$ relation of the amiloride-insensitive pathway alone. Such a plot is illustrated in Fig. 2.

There are several points worthy of note:

a) The $I-V$ relation of the amiloride-insensitive leak pathway across the apical membrane ($I^{c'}$ *vs.* $\psi^{mc'}$) appears to be nearly linear over the range $-130\,\text{mV}\leqq\psi^{mc}\leqq 30\,\text{mV}$ and the value of G_i^m $(=g_i^m)$ over this range for all experiments averaged 0.60 ± 0.01 mS/cm^2 (i.e., $R_i^m=1667\,\Omega\text{cm}^2$). The value of E_i^m (i.e., the value of $\psi^{mc'}$ when $I^{c'}=0$) was quite variable and averaged -51 ± 9 mV; this value is in reasonable agreement with the value of -57 mV estimated by Wills et al. (1979*b*) using a very different approach. The significance of this finding will be discussed below.

b) The $I-V$ relation for the total transcellular current under control conditions (I^c *vs.* ψ^{mc}) is decidedly curvilinear over most of the range. The value of ψ^{mc} at which $I^c=0$ (i.e., E^m) averaged -6 ± 8 mV. The average slope conductance of the apical membrane under short-circuit conditions (i.e., when $\psi^{mc}={}_0\psi^{mc}$) was 1.62 mS/cm^2, corresponding to a value of $r^m=636\,\Omega\text{cm}^2$. This value is in excellent agreement with that of 644 Ωcm^2 reported by Wills et al. (1979*b*).

Finally, it should be readily appreciated that if E_i^m is not significantly affected by amiloride the value of I_{Na}^m at any value of ψ^{mc} is given by

$$(I_{\text{Na}}^m)_{\psi^{mc}}=(I^c-I^{c'})_{\psi^{mc}} \tag{4}$$

and thus can be determined directly (graphically) from the data illustrated in Fig. 2; a formal derivation of Eq. (4) is given in the Appendix. Further, as also shown in the Appendix, the relation

$$(I_{\text{Na}}^m)_{\psi^{ms}}=(\Delta I^{ms})/f'=(\Delta I^c)/f' \tag{5}$$

which was derived from the equivalent electrical circuit model illustrated in Fig. 7 of (I) can be derived graphically from Fig. 2 over the range $-130\,\text{mV}\leqq\psi^{mc}\leqq+30\,\text{mV}$ where the relation between $I^{c'}$ and ψ^{mc} is linear (i.e., R_i^m is independent of ψ^{mc}).

The $I-V$ Relation of the Parallel Pathways

Inasmuch as $(I^p)_{\psi^{ms}}$ is given by $(I^{ms}-I^c)_{\psi^{ms}}$, the relation between I^p and ψ^{ms} can be readily determined from the data given in Fig. 2 and Figs. 3 and 6 of (I). Clearly, this is permissible only over the range of values of ψ^{ms} for which I^c may be calculated (i.e., the range of ψ^{ms} where the relation between (ΔI^c) and $(\Delta\psi^{cs})$ is linear). A typical result is illustrated in Fig. 3; clearly the $I-V$ relation of the parallel pathways is nearly linear over the range $-180\,\text{mV}\leqq\psi^{ms}\leqq 100\,\text{mV}$. It should be emphasized that while we have assumed, on reasonably strong grounds, that R^p is not affected by amiloride, we have not assumed that it is independent of ψ^{ms} (i.e., Ohmic). As will be discussed below, the finding of a linear relation between I^p and ψ^{ms} adds considerable support to the approach we have taken toward the interpretation of the experimental ("raw") data.

In these experiments R^p averaged 351 Ωcm^2, in excellent agreement with the value of 345 Ωcm^2 re-

ported by Schultz et al. (1977). Wills et al. (1979*b*) reported a value of 498 Ωcm^2; this difference may be due to differences in "edge damage."

Discussion

In the preceding section we analyzed the $I-V$ relations of the basolateral membrane, apical leak pathway, and the parallel (shunt) pathway in that order; this order was dictated by the order of data reduction. In this section we consider each of these barriers in turn, in the reverse order.

The Parallel Pathways

The $I-V$ relation of the parallel pathways was obtained by subtracting I^c, the *calculated* cellular component of I^{ms}, from the total transepithelial current (I^{ms}) at each ψ^{ms}, where the calculation of I^c was based on the specific assumptions that $R^p = R^{p'}$ and $R^s = R^{s'}$ at each ψ^{ms}. Thus the $I-V$ relation of the parallel pathways derived from these data is critically dependent on the validity of the assumptions and as such may be used to evaluate them. As illustrated in Fig. 3 the *calculated* $I-V$ relation of the parallel pathways is nearly linear over a wide range. Unfortunately, this $I-V$ relation could not be extended to values of $\psi^{ms} > 100$ mV because the calculation of I^p, as described above, is only valid over the range where the plot of ΔI^c *vs.* $\Delta\psi^{cs}$ is linear.

Frizzell, Koch and Schultz (1976) estimated the partial ionic conductances (G_i^p) of the parallel pathway to Na, K and Cl from the relations between the unidirectional fluxes of these ions from the serosal solution to the mucosal solution (measured using tracers) and ψ^{ms} over the range ± 50 mV, assuming that these flows are, for the most part, restricted to that route. Their results indicated that G_i^p is independent of ψ^{ms} over that range. Using the values of G_i^p reported by those investigators, the parallel ionic currents (I_i^p) attributable to the flow of each of these ions can be calculated at any value of ψ^{ms} from the relation

$$I_i^p = G_i^p \psi^{ms}. \tag{6}$$

In Fig. 3 the values of $I_{Na}^p(+)$, $I_{Cl}^p(\cdot)$ and $I_K^p(\times)$, calculated using Eq. (6) and the values for G_i^p given by Frizzell et al. (1976) are plotted as a function of ψ^{ms}. The solid line is the sum of these currents and provides a reasonable fit to the *derived* relation between I^p ($=\Sigma I_i^p$) and ψ^{ms}. This finding provides additional independent support to our approach toward data reduction.

The Amiloride-Insensitive Apical "Leak" Pathway

As discussed in (I), the inclusion of a "natural" amiloride-insensitive conductive pathway in the apical membrane was prompted by the finding that f' was significantly less than unity and that this could not be attributed to impalement damage of the apical membrane. The presence of such a pathway has been reported previously for rabbit urinary bladder (Lewis, Eaton & Diamond, 1976; Lewis, Eaton, Clausen & Diamond, 1977) and colon (Wills et al., 1979*b*) and for toad (Navarte & Finn, 1980; Palmer, Edelman & Lindemann, 1980) and *Necturus* (Frömter & Gebler, 1977) urinary bladder.

At present we cannot draw any firm conclusions regarding the ion(s) responsible for this conductive pathway. The polarity of E_i^m is consistent with an anion distribution where the cell activity is lower than that in the mucosal solution (e.g., Cl) and/or a cation distribution where the cell activity is higher than that in the mucosal solution (e.g., K). Navarte and Finn (1980) have concluded that the leak conductance in the apical membrane of toad urinary bladder is anionic. Fromm and Schultz (1981) have recently summarized the evidence against a significant K permeability of the apical membrane in rabbit colon.[1] One striking observation is that, unlike the values of E_{Na}^m and E^s, E_i^m was very variable. This suggests that E_i^m is the lumped Thévenin equivalent of a number of ions across the apical membrane and probably includes some variable contribution from E_{Na}^m inasmuch as the effect of amiloride may be incomplete.

The value of E_i^m averaged -51 mV in reasonable agreement with the value of -57 mV reported by Wills et al. (1976*b*). The value of G_i^m averaged 0.6 mS/cm^2 identical to that reported by Wills et al. (1979*b*). Since under short-circuit conditions ${}_0\psi^{mc}$ averaged -39 mV, the value of ${}_0I_i^m = G_i^m(E_i^m - {}_0\psi^{mc})$ is only -7 $\mu A/cm^2$ or -0.26 $\mu Eq/cm^2$ hr. These values are approximately one-tenth the value of I_{sc} or the rate of active Na transport by the tissue.

[1] Wills and Biagi (1980) have observed K secretion by short-circuited rabbit colon indicating that the permeability of the apical membrane of this epithelium to K may vary under different conditions. To examine the possibility that an apical K conductance is responsible for or contributes to G_i^m, several experiments were carried out to determine the effect of $(K)_m$ on ψ^{mc}. The results indicated that raising $(K)_m$ from 4.2 to 54 mM by replacement of choline at constant $[Na]_m$ had no significant effect on ψ^{mc}; these findings are consistent with the notion that under the conditions of these studies G_K^m is negligible.

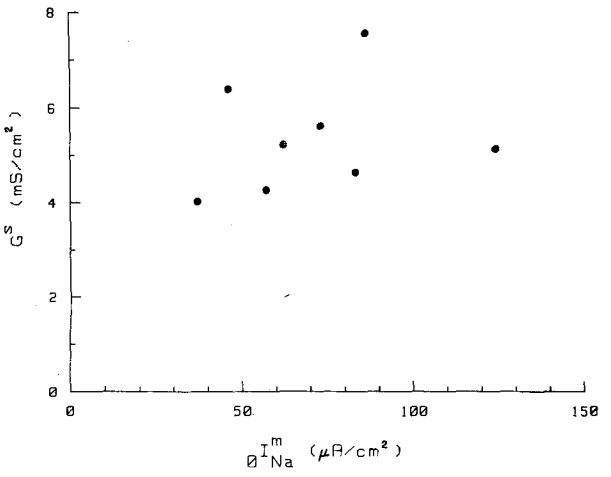

Fig. 4. The conductance of the basolateral membrane (G^s) is plotted against the spontaneous rate of Na transport ($_0I^m_{Na}$) obtained under steady-state short-circuit conditions

The Properties of the Basolateral Membrane

The data illustrated in Fig. 1 are consistent with the notion that G^s is independent of ψ^{cs} over the range $-8\,\text{mV} \leqq \psi^{cs} \leqq \sim 55\,\text{mV}$ and averaged 5.3 mS/cm^2. Wills, Eaton, Lewis and Ifshin (1979*a*) examined the $I-V$ relations of this barrier after "functionally" eliminating the apical membrane with nystatin; the mucosal solution was a K_2SO_4-saline designed to mimic the intracellular K activity. They reported that the $I-V$ relation could be fit by the constant-field equation involving finite permeabilities to K, Na and Cl; the "reversal potential" (the value of ψ^{cs} when $I^s=0$) was found to be 50 mV. Figure 1 of their paper indicates that the $I-V$ relation of this barrier is essentially linear over the range $+60 > \psi^{cs} > 0$ (no data are presented for the range $\psi^{cs} < 0$ but, if the relation continues to conform to the GHK equation, it must remain nearly linear). A significant departure from linearity was observed only when $\psi^{cs} > +60$ mV. Thus the present results are consistent with the findings of Wills et al. (1979*a*).

In these experiments, E^s averaged 50 mV and fell to 48 mV in the presence of amiloride. It is difficult at present to interpret these findings. Evidence has been presented that the Na-K exchange pump at the basolateral membrane is rheogenic (Wills et al., 1979*b*). If so, E^s must be comprised of a contribution from the pump as well as the electromotive forces resulting from the asymmetric distribution of *all* permeant species across that barrier. The contribution from the pump may be estimated assuming a 3Na:2K stoichiometry which appears to be the case for a number of nonepithelial cells (Glynn & Karlish, 1975); Nielsen (1979) has presented evidence for a 3Na:2K stoichiometry in frog skin and a similar stoichiometry has been suggested for rabbit urinary bladder (Lewis & Wills, 1981) and turtle colon (Kirk, Halm & Dawson, 1980). Given this assumption, the fact that the I_{sc} in these studies averaged 60 μA/cm^2 infers that the "pump current," I^s_p, was 20 μA/cm^2. As discussed by Schultz (1980), the contribution of the pump current to E^s is simply $I^s_p R^s = 3.8$ mV. In this respect it is of interest that in the presence of amiloride, E^s declined by 2 mV. However, inasmuch as amiloride leads to a hyperpolarization of the cell interior by approximately 10 mV, it is possible that this is accompanied by a redistribution of ions which would affect E^s. Thus, although the finding that $\Delta E^s = 2$ mV is in reasonable agreement with what would be expected from the elimination of the pump current, this value could also be influenced by other factors.

The finding reported in (I) that the basolateral membrane is depolarized and that R^s is significantly reduced when the serosal solution contains 86 mM K (activity) is consistent with the notion that this barrier is predominantly K-selective as concluded by Wills et al. (1979*a*, *b*). The intracellular K activity in rabbit colon determined using K-selective microelectrodes is approximately 76 mM (Wills et al., 1979*b*; M.E. Duffey and S.G. Schultz, *unpublished observations*). Thus, $E^s_K (= (RT/\mathcal{F}) \ln[(K)_c/(K)_s]) = 78$ mV. This value is significantly greater than the value of $(E^s - \Delta E^s) = 48$ mV, indicating that E^s_K must be "shunted" by other permeant ions; the full identity of these ions and their relative permeabilities remain to be established.[2]

Finally, as discussed by Schultz (1981), for a number of epithelial tissues such as frog skin, *Necturus* urinary bladder (Frömter & Gebler, 1977) and *Necturus* small intestine (Gunter-Smith, Grasset & Schultz, 1982), there appears to be a direct relation between the rate of active Na transport and the conductance of the basolateral membrane (similar to that illustrated for the apical membrane in Fig. 11 of the preceding paper). In Fig. 4, G^s is plotted *vs.* $_0I^m_{Na}$ for all eight experiments on rabbit colon. Clearly, in this tissue G^s is independent of the rate of Na transport, $_0I^m_{Na}$; similar results have been reported for rabbit urinary bladder (Lewis et al., 1976). This finding is consistent with the notion that over the four-fold range of spontaneous variation in I_{sc} the basola-

[2] In their experiments on nystatin-treated segments of rabbit descending colon, Wills et al. (1979*b*) assumed that the basolateral membrane is only permeable to Na, K and Cl and estimated the permeabilities to these ions from the best fit of the constant-field flux equation to the experimental data. The ranges of their estimates (in our notation) are: $P^s_K = 1.4\text{–}11.6 \times 10^{-5}$ cm/sec; $P^s_{Na}/P^s_K = 0.05\text{–}0.09$ and $P^s_{Cl}/P^s_K = 0.01\text{–}0.06$.

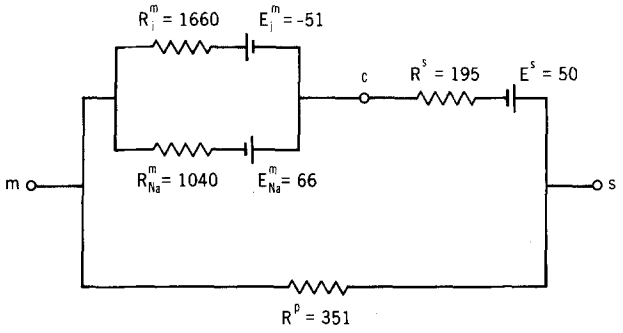

Fig. 5. Electrical equivalent circuit model for rabbit colon. The R's are the *chord* resistances and the E's are the reversal potentials for each pathway when the tissue is under steady-state short-circuit conditions

teral pump behaves like a constant current source whose activity does not contribute to G^s. However, clearly, additional studies are needed to clarify the electrophysiologic behavior of the pump and its contribution to R^s and E^s.

Equivalent Electrical Circuit Model of Rabbit Colon

In this series of papers (I and II) we have developed an analysis of I-V relations in rabbit descending colon based on the equivalent electrical circuit model shown in Fig. 7 of (I). In Fig. 5 we again illustrate this model and include the mean values we have determined for each parameter under short-circuit conditions. Clearly the circuit in Fig. 5 is not the only possible choice for an equivalent electrical circuit model; rather, it is the simplest circuit model consistent with our data. Alternative models have been proposed by Wills et al. (1979*b*) which include additional amiloride-insensitive components in parallel with the above model. Analysis of our data in terms of the more complex circuit models would require additional measurements and assumptions. However, it is important to note that the $I-V$ relation of the apical Na entry pathway would remain unchanged as would the value we determine for R^s of the Na-transporting cells.

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Appendix

The $I-V$ relation of the amiloride-sensitive Na entry step, I_{Na}^m *vs.* ψ^{mc} (Fig. 8 of (I)) as well as Eq. (1) of (I) may be determined directly by graphical analysis of Fig. 2. Referring to Fig. 7 of (I) the following relations describe I_{Na}^m and I_i^m at any given value of ψ^{mc}:

$$I_{Na}^m = G_{Na}^m(E_{Na}^m - \psi^{mc}) \tag{A1}$$

$$I_i^m = G_i^m(E_i^m - \psi^{mc}) \tag{A2}$$

so that

$$I^c = I_{Na}^m + I_i^m = G_{Na}^m E_{Na}^m + G_i^m E_i^m - (G_{Na}^m + G_i^m)\psi^{mc}. \tag{A3}$$

The transcellular current in the presence of amiloride, $I^{c'}$, at that value of ψ^{mc} is given by:

$$I^{c'} = I_i^{m'} = G_i^{m'}(E_i^{m'} - \psi^{mc}). \tag{A4}$$

Thus, if E_i^m and G_i^m are not affected by amiloride (i.e., $E_i^m = E_i^{m'}$ and $G_i^m = G_i^{m'}$) it follows that:

$$(I^c - I^{c'})_{\psi^{mc}} = (G_{Na}^m E_{Na}^m - G_{Na}^m \psi^{mc})_{\psi^{mc}} = (I_{Na}^m)_{\psi^{mc}}. \tag{A5}$$

Consequently, the relation I_{Na}^m *vs.* ψ^{mc} may be generated by plotting the difference $(I^c - I^{c'})_{\psi^{mc}}$ indicated by segment $\overline{BD}$ in Fig. 2 against the corresponding value of ψ^{mc}.

An analytic expression for I_{Na}^m may be obtained by considering the segment $\overline{AB}$ which connects the values of I^c and $I^{c'}$ calculated for the same value of ψ^{ms}; hence, the length $\|\overline{BC}\|$ is $(\Delta I^c)_{\psi^{ms}} = (\Delta I^{ms})_{\psi^{ms}}$. Clearly, then, segment $\|\overline{CD}\|$ is the amount by which the amiloride-blockable current ($\|\overline{BD}\|$) exceeds the change in transepithelial current ($\|\overline{BC}\|$). The magnitude $\|\overline{CD}\|$ can be calculated from the length $\|\overline{AC}\|$, which is $(\Delta\psi^{mc})_{\psi^{ms}}$, and the slope of $\overline{AD}$, which over the linear range of $I^{c'}$ *vs.* ψ^{mc} is $g_i^m = G_i^m$. Thus we may write

$$I_{Na}^m = \|\overline{BC}\| + \|\overline{CD}\| = (\Delta I^{ms})_{\psi^{ms}} + g_i^m(\Delta\psi^{mc})_{\psi^{ms}}. \tag{A6}$$

Further, since $(\Delta\psi^{mc})_{\psi^{ms}} = -(\Delta\psi^{cs})_{\psi^{ms}}$ we may write using Eq. (3) of (II) that

$$I_{Na}^m = (\Delta I^{ms})_{\psi^{ms}} + g_i^m(\Delta I^{ms})_{\psi^{ms}}/g^s - g_i^m \Delta E^s. \tag{A7}$$

Since g_i^m and ΔE^s are both small (*see* Results), the last term in Eq. (A7) may be reasonably neglected so that

$$I_{Na}^m \cong (\Delta I^{ms})_{\psi^{ms}}(1 + g_i^m/g^s)$$
$$\cong (\Delta I^{ms})_{\psi^{ms}}/f' \tag{A8}$$

where $f' = 1/(1 + g_i^m/g^s)$ is the fractional resistance in the presence of amiloride.

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