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Na and Cl Transport Across the Isolated Turtle Colon: Parallel Pathways for Transmural Ion Movement

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Summary. Transmural fluxes of ^{3}H-mannitol and ^{22}Na or ^{36}Cl were measured simultaneously in portions of isolated turtle colon stripped of serosal musculature. The relationships between mannitol flux and the flux of Na or Cl are characteristic of simple diffusion and suggest that transmural mannitol flow is largely confined to a paracellular pathway where Na, Cl and mannitol move much as in free solution. The contribution of "edge damage" to the transmural mannitol flow appears to be minimal. Mucosal hyperosmolarity causes "blisters" in epithelial tight junctions and increases the diffusional permeability to Na and mannitol, suggesting that the rate-limiting barrier in the shunt path is the tight junction. If the total mucosa to serosa flux of Na is corrected for the portion traversing the shunt pathway it is apparent that changes in the short-circuit current are completely accounted for by the mucosa to serosal movement of Na through a cellular path. In addition, the serosa to mucosa flux of Na appears to be restricted to the shunt. These observations suggest that there is no appreciable "backflux" of Na through the active, cellular path. In the presence of 10^{-4} M amiloride the short-circuit current is markedly reduced and the mucosa to serosa Na flux is restricted to the shunt, so that the net Na flux is abolished. The small amiloride-insensitive short-circuit current is consistent with HCO_3 secretion. Mucosa to serosa and serosa to mucosa fluxes of Cl appear to be largely restricted to the paracellular shunt path and there is no evidence for any net flow of Cl under short-circuit conditions. The total tissue conductance can be described as the sum of three components: a shunt conductance which is linearly related to the transmural mannitol flow, an "active" conductance which is linearly related to the short-circuit current and a small residual conductance. The shunt conductance is attributable to the diffusive movements of Na and Cl through the paracellular path. Variations in the active Na transport from tissue to tissue are largely attributable to variations in the apparent conductance of the active Na transport path. The driving force for active Na transport can be described as an apparent emf of approximately 130 mV. These results suggest that transmural mannitol flux provides a quantitative estimate of the ion permeability and electrical conductance of a paracellular shunt path across the isolated turtle colon and thereby facilitates the study of the transport characteristics and electrical properties of cellular paths for transepithelial solute movement.

The simplest model which can account for the transport properties of an epithelial cell layer is one which allows for two parallel paths for transmural solute and water flow, one through the cells and another between

them. The transcellular path consists of at least two barriers in series, the membranes at the two borders of the cell layer. The paracellular path is a "shunt" whereby transmural flow circumvents the cells via tight junctions and lateral intercellular spaces. Several methods have been employed in an attempt to separate transepithelial flows into cellular and noncellular components. Frizzell, Koch and Schultz [10], Frizzell and Schultz [11] Nellans, Frizzell and Schultz [27] have used the response of diffusive ion flow to changes in transmural electrical potential to explore paracellular movements in the rabbit ileum and colon. Mandel and Curran [24] used urea flux in an attempt to mark the paracellular path in the frog skin. Moreno and Diamond [25, 26] have characterized the paracellular shunt in the gallbladder by means of transmural diffusion potentials.

The purpose of the present investigation was to explore the properties of the isolated colon of the freshwater turtle with the aim of developing a simple technique for separating transmural ion flow into cellular and noncellular components. We have attempted to discriminate between these two paths by measuring unidirectional ion fluxes simultaneously with that of ^{3}H-mannitol, a hydrophilic molecule which might be largely restricted to watery, noncellular regions. The results suggest that the transmural mannitol flow marks a paracellular path where Na and Cl move much as in free solution, so that for the turtle colon the transmural mannitol flow provides a quantitative estimate of the ion permeability and electrical conductance of the shunt and thereby facilitates the study of the transport characteristics and electrical properties of the cellular path.

Materials and Methods

Preparation of the Colonic Mucosa

Freshwater turtles, *Pseudemys scripta elegans*, (Mogul-Ed; Oshkosh, Wisconsin) were maintained unfed in fresh water from one to 10 days prior to an experiment. To remove the colon animals were doubly pithed and the plastron was separated from the remainder of the shell with the aid of an autopsy saw. The intestine was severed proximally at the ileo-ceacal valve and distally at the anus, and the ceacum and the colon were removed intact. The circular and longitudinal muscle layers were removed from the colon using a technique similar to that previously described for the toad colon [7]. The ceacal end of the tissue was tied onto a length of polyethylene tubing connected to a syringe and the colon was rinsed of its contents with chilled Ringer's solution. The anal end was then tied off and the colon was inflated with solution and maintained under moderate tension. A small cut was made through the muscle layers with a razor blade such that blunt-nosed scissors could be inserted between the muscle layers and the mucosa. The muscle layers were then removed from the entire colon by blunt dissection. This procedure has the advantage that during the dissection the mucosa is continually exposed to Ringer's solution and gross damage to the mucosa is evident due to fluid

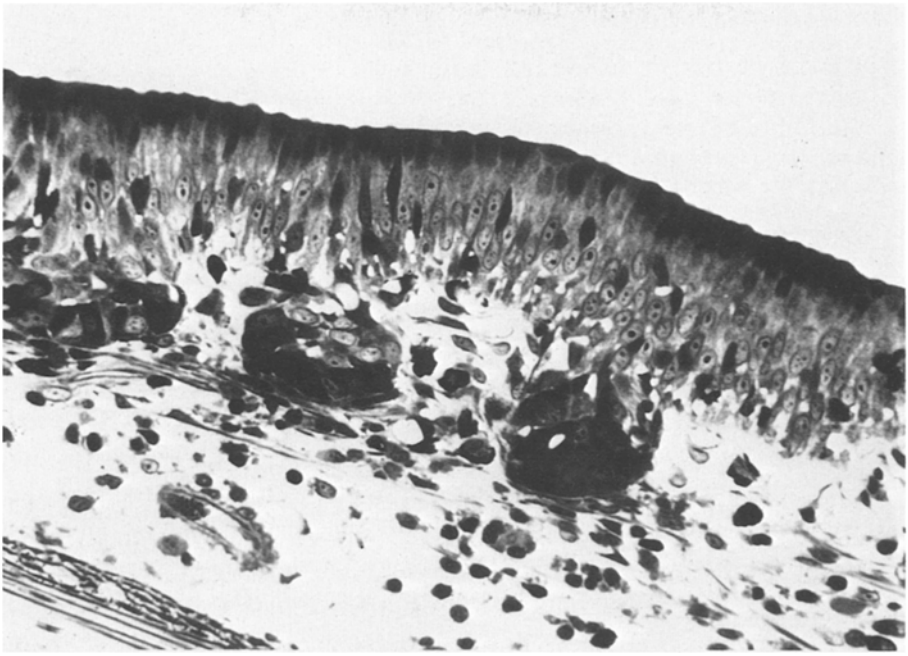

Fig. 1. Section of "stripped" turtle colon ($\sim 500\times$)

leakage from the resulting tube of mucosa. A light microscope section of tissue prepared in this manner is shown in Fig.1. The stripped colon is seen to consist of a single layer of cells on the mucosal side with a thin *muscularis mucosae* and a loose layer of connective tissue and possibly some glandular elements remaining on the serosal side.

Unidirectional Fluxes and Electrical Measurements

Portions of the stripped mucosa were mounted as flat sheets in lucite chambers similar to those described by Schultz and Zalusky [32]. The chambers were equipped with 4 Ringer's-agar bridges, two connected to calomel half-cells for the measurement of the transepithelial electrical potential difference (PD) and two connected to Ag-Ag Cl electrodes for passing current across the tissue. The area of exposed tissue was 1.08 cm^2. The transepithelial PD was maintained at zero mV by an automatic voltage clamp which compensates for the solution resistance between the PD sensing bridges and the tissue surface. The lucite chambers were connected to gas-lift circulators and both sides of the tissue were bathed by identical solutions containing (in mM): 112 Na Cl, 2.5 $KHCO_3$, 1.0 $CaCl_2$, 5.0 d-glucose, 2.5 Na-pyruvate and 5 d-mannitol. Each solution was stirred and oxygenated with air to yield a pH of 8.1 at 25 °C.

Unidirectional fluxes of Na or Cl were measured simultaneously with that of D-mannitol. Mucosal to serosal and serosal to mucosal fluxes were measured on paired tissues. In each case 20 μCi of ^{3}H-mannitol and either 4 μCi of ^{22}Na or 5 μCi of ^{36}Cl were added to one side of the tissue, the "hot side", and at least one hour was allowed to establish steady-state tracer flow. 1.0 ml samples were removed from the "cold side" at 30-min intervals thereafter and replaced in the chamber by 1 ml of cold Ringer's. The samples were placed directly into 10 ml of

Scintiverse (Fisher Scientific) and were subsequently assayed for ^{3}H and ^{22}Na or ^{36}Cl in a liquid scintillation spectrometer (Beckman). The short-circuit current (I_{sc}) and the open circuit PD were recorded at the time of each sample. The values of the electrical parameters presented in the text represent the mean values for a particular 30-min flux period. Tissue conductance was taken to be the ratio of I_{sc} to the open circuit PD under control conditions. In the presence of amiloride, due to the small values of I_{sc}, tissue conductance was assessed by measuring the current required to change the clamping potential by 10 mV.

Results

General Properties of the Isolated Mucosa

The results of experiments in which the flux of Na or Cl was measured simultaneously with mannitol are summarized in Table 1. In any one tissue the isotope fluxes and electrical parameters, after an initial period of equilibration, displayed great stability, varying less than 10 % over a period of 5 to 8 hours. The results indicate that the majority of the short-circuit current can be accounted for by net Na absorbtion and that under short-circuit conditions there is no net movement of Cl across the tissue. The short-circuit current, which ranged from 50 to 150 $\mu A/cm^2$, was reduced virtually instantaneously to values less than 10 $\mu A/cm^2$ by the addition of 10^{-4} M amiloride to the mucosal side of the tissue. Addition of amiloride to the serosal side had no effect. Table 2 shows the effect of amiloride on the ion fluxes and electrical properties of the colon. In this series of experiments fluxes and electrical parameters were measured during four control periods (before) and for five additional periods after the addition of 10^{-4} M amiloride to the mucosal side. The results from the 30-min period immediately following the addition of amiloride were discarded due to the possibility of nonsteady-state isotope flow. The results suggest that in the presence of amiloride the net Na flux is not distinguishable from zero and

Table 1. Transmural ion and mannitol fluxes across the isolated turtle colon

	I_{sc}	J_{ion}	J_{man} (μmole/cm^2-hr) $\times 10^{-2}$	G_T (mmho/cm^2)	n
	(μeq/cm^2-hr)				
Na(M→S)	2.62±0.24	2.83±0.24	0.97±0.06	1.80±0.11	13
Na(S→M)	2.86±0.28	0.60±0.08	1.48±0.17	2.26±0.15	13
Cl(M→S)	2.53±0.16	0.95±0.07	1.29±0.10	2.06±0.10	25
Cl(S→M)	2.75±0.15	0.95±0.06	1.28±0.07	2.03±0.07	25

All values $\bar{X} \pm SE$; n = number of tissues.

Table 2. Effect of amiloride on transmural fluxes

	I_{sc}	J_{ion}	J_{man}	G_T	n
	(μeq/cm^2-hr)		(μmole/cm^2-hr) $\times 10^{-2}$	(mmho/cm^2)	
before					
Na(M→S)	3.14±0.41	3.36±0.36	0.87±0.09	2.13±0.27	3
Na(S→M)	3.57±0.47	0.48±0.08	1.22±0.15	2.32±0.04	3
after					
Na(M→S)	0.19±0.03	0.52±0.02	1.19±0.11	1.51±0.17	3
Na(S→M)	0.23±0.09	0.65±0.13	1.60±0.30	1.71±0.08	3
before					
Cl(M→S)	2.84±0.51	0.75±0.12	0.89±0.06	1.82±0.14	6
Cl (S→M)	2.96±0.39	0.74±0.15	0.95±0.10	1.90±0.11	6
after					
Cl(M→S)	0.12±0.01	0.80±0.08	1.07±0.06	1.37±0.10	6
Cl(S→M)	0.16±0.03	0.78±0.07	1.13±0.07	1.32±0.11	6

$\bar{X} \pm SE$; n = number of tissues.

the short-circuit current is greatly reduced, though not abolished. Chloride fluxes appear to be unaffected by amiloride.

Sodium and Mannitol Fluxes

Fig. 2 shows the serosal to muscosal (S to M) flux of Na, J_{sm}^{Na}, under control conditions plotted versus the simultaneously measured S to M flux of mannitol, J_{sm}^{man}. In this plot and those which follow each point represents the mean of from four to eight 30-min flux periods for a single tissue. In all but a few instances the standard errors are less than the size of the dots. The plot shows a linear relation between the S to M flows of Na and mannitol with an intercept near the origin. The simplest interpretation of this result is that Na and mannitol traverse the same path from serosa to mucosa. Further support for this interpretation is provided by an analysis of the slope of this plot. If Na and mannitol move across the tissue by simple diffusion through an aqueous path, the permeabilities for each solute under short-circuit conditions would be given by:

$$P_i = f_i D_i / \Delta x$$

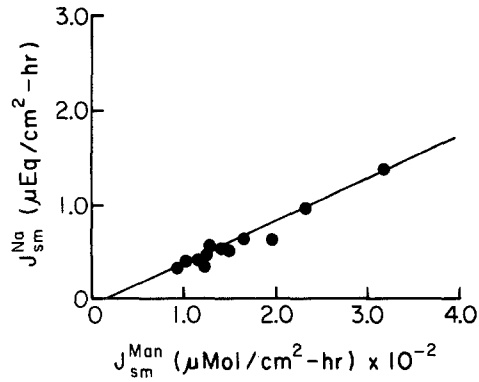

Fig. 2. J^{Na}_{sm} versus J^{man}_{sm} under control conditions. Each point represents one tissue

where P_i = permeability of i in cm/hr, D_i = the diffusion coefficient for i in the channel in cm^2/hr, Δx = the effective path length for diffusion across the tissue in cm, and f = fractional area available for diffusion. If Na and mannitol traverse the *same path* from S to M then the slope of a plot of J^{Na}_{sm} *vs.* J^{man}_{sm} is given by:

$$\text{slope} = D_{Na}[Na]/D_{man}[Man].$$

Values for $D_{Na^{22}}$ [28] and D_{man}[1] [19] in free solution yield a predicted slope of 0.44 whereas the slope calculated for the least-squares fit to the points is 0.47. This result strongly suggests that under the conditions of our experiments Na and mannitol cross the tissue from serosa to mucosa via a watery "leak" path where these solutes move much as in free solution.

If the S to M fluxes of Na and mannitol represent simple diffusional movement through a leak path then the M to S fluxes through this pathway should behave identically under short-circuit conditions. Fig. 3 shows a plot of J^{Na} versus J^{man} for experiments in which M to S and S to M fluxes were measured in paired tissues each treated with 10^{-4} M amiloride on the mucosal side. The points for both M to S and S to M fluxes can be described by a single straight line with an intercept near zero and a slope of 0.45, suggesting that the isolated stripped turtle colon is characterized by a watery, noncellular leak pathway where Na and mannitol move by simple diffusion.

1 The diffusion coefficient for ^{3}H-mannitol at 25 °C was computed from the value measured for ^{14}C-mannitol at 37 °C [19] using the temperature dependence of the viscosity of water and the Stokes-Einstein relation [28]. This value was in close agreement with that calculated assuming a Stokes radius of 3.6 Å [31].

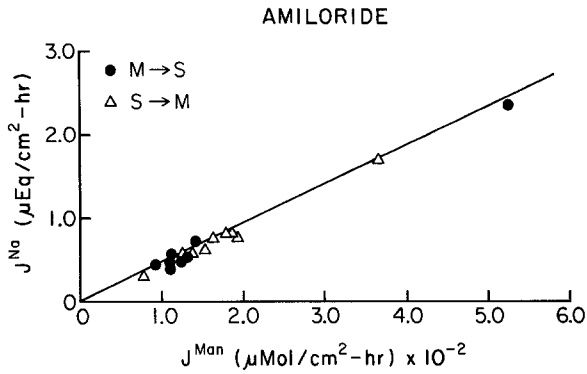

Fig. 3. J^{Na} versus J^{man} for M→S and S→M fluxes in the presence of amiloride

Sodium Flux through the "Active Path"

The serosal to mucosal movement of Na appears to be completely accounted for by that which moves through the leak path marked by mannitol, i.e.

$$J_{sm}^{Na} = \lambda J_{sm}^{man}$$

where

$$\lambda = \frac{D_{Na}[Na]}{D_{man}[Man]}.$$

The mucosal to serosal flow of Na through the *active* pathway should, therefore, be given by:

$$J_{active}^{Na} = J_{ms}^{Na} - \lambda J_{ms}^{man}.$$

Fig. 4 shows this quantity plotted versus I_{sc}. The least-squares line has a slope of 1.00 ± 0.03 suggesting the variation in I_{sc} from tissue to tissue is entirely attributable to changes in the active transport of Na through a cellular path. The intercept of 0.30 μeq/cm²-hr is in reasonable agreement with the average value of the amiloride-*insensitive* portion of the short-circuit current from Table 2 of about 0.20 μeq/cm²-hr.

Chloride and Mannitol Fluxes

In Fig. 5 values for Cl fluxes M to S and S to M are plotted versus the corresponding mannitol fluxes. Although there is a clear correlation between the Cl and mannitol movements there is more variability in the

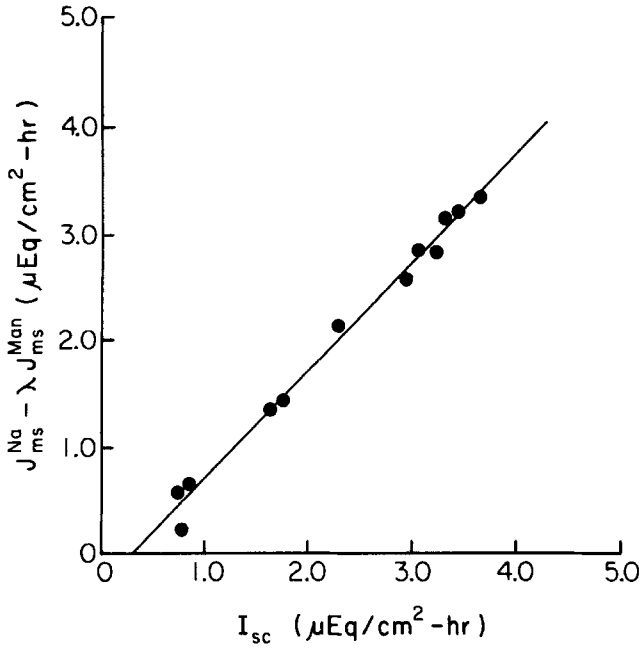

Fig. 4. Na flux through the active path plotted as a function of I_{sc}

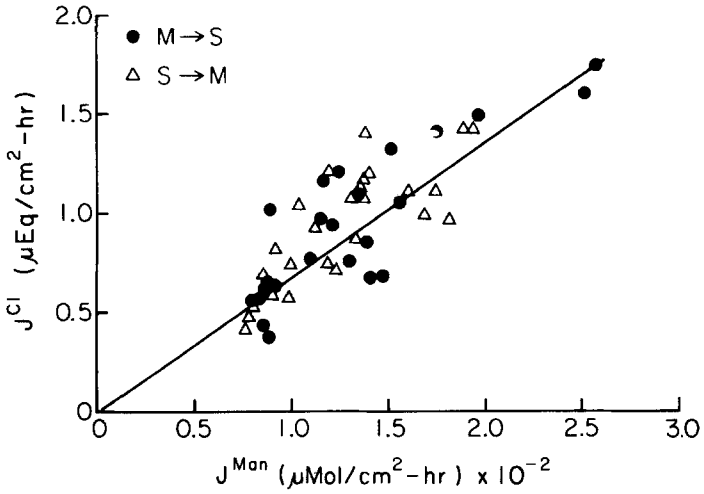

Fig. 5. J^{Cl} versus J^{man}; M→S and S→M fluxes plotted together

relation between J^{Cl} and J^{man} than was observed in the relation of J^{Na}_{sm} to J^{man}_{sm} (Fig. 2). Table 3 contains values for the slope, intercept and correlation coefficient of the least-squares regression lines for three groups of Cl and mannitol fluxes: the M to S fluxes, the S to M fluxes and the pooled data. The values obtained for the slope are virtually identical in each case and

Table 3. Linear regression coefficients (±SE) for J^{Cl} *vs.* J^{man}

Group	Slope	Intercept	r
M→S	0.58±0.40	0.19±0.60	0.80
S→M	0.66±0.22	0.10±0.30	0.79
all	0.61±0.28	0.16±0.60	0.80

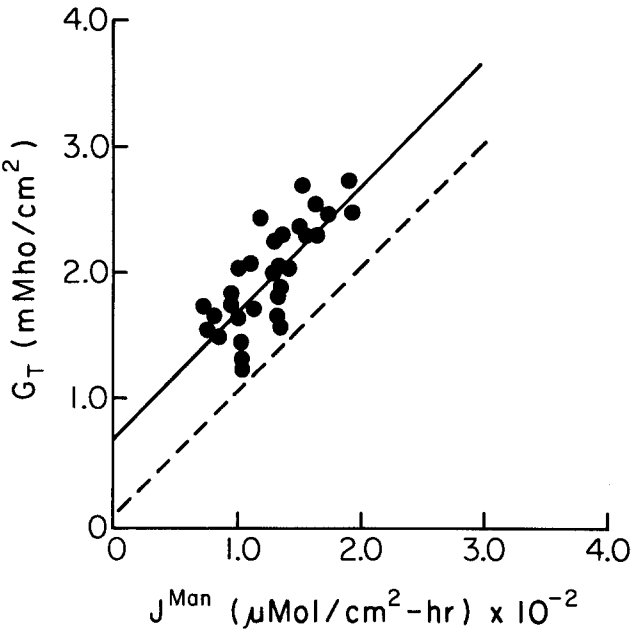

Fig. 6. Total tissue conductance G_T plotted as a function of mannitol flux

do not differ significantly from the value of 0.65 predicted from the free diffusion coefficient of ^{36}Cl at 25 °C [28]. Although the calculated intercepts for the three groups tend to be positive, due to the scatter of the points these values are not significantly different from zero.

Tissue Conductance

Fig. 6 shows the total tissue conductance G_T, from the Na and Cl flux experiments plotted versus the mannitol flux. The form of this plot suggests that the total conductance consists of at least two parts. One portion of the conductance is highly correlated with the transmural mannitol flux and thus may represent the conductance of the noncellular

leak path marked by mannitol. The remaining portion of the conductance would presumably represent the conductance of the cellular path. The least-squares fit to these points has a slope of 1.01 ± 0.09 with an intercept of 0.80 ± 0.12. A similar plot of G_T versus J^{man} for tissues treated with amiloride can be described by a line with a slope identical to that for the control points but having an intercept of 0.15 mmho/cm^2, and is indicated by the dotted line on Fig. 6. The effect of amiloride is to reduce the intercept of a plot of G_T versus J^{man}, leaving the slope unchanged. Amiloride specifically inhibits the entry of Na across the apical cell membrane of the turtle colon [33] and is thus expected to reduce or abolish the conductance of the Na active transport path. This behavior, therefore, suggests that the portion of G_T correlated with J^{man} represents the leak conductance while the intercept is the average value of the conductance of the cellular path. The observation that amiloride does not reduce the intercept to zero is consonant with the existence of a small, amiloride-insensitive I_{sc} (see Fig. 4 and Discussion).

The total conductance of the leak path should be approximated by the sum of the partial conductances of Na and Cl, i.e.

$$G_L \simeq G_L^{Na} + G_L^{Cl}$$

where G_L is the total conductance of the leak path and G_L^{Na} and G_L^{Cl} are the partial conductances of Na and Cl in the leak path. Under short-circuit conditions the partial ionic conductance in mmho/cm^2 for an ion which moves by simple diffusion is *numerically* equal to the unidirectional flux of that ion in units of μeq/cm^2-hr. The relations previously obtained between the flows of Na and Cl and mannitol, therefore, suggest that the expression for G_L can be rewritten as:

$$G_L \simeq 0.47\, J_{man} + 0.65\, J_{man}$$
$$G_L \simeq 1.12\, J_{man}.$$

The least-squares line which describes the G_T *vs.* J_{man} plot has a slope of 1.0 a value in reasonable agreement with that predicted by unidirectional flux data.

The Anatomical Site of the Leak Path

The leak pathway across the isolated turtle colon as defined by transmural mannitol flow has the characteristics of a watery route for transmural diffusion where Na, Cl and mannitol move much as in free solution. The

stripped portions of colon employed in this study were mounted by compression between two halves of a lucite flux chamber with an edge-to-surface ratio of about 4. Clearly, it is of interest to determine if these experiments reveal the properties of the paracellular shunt pathway (i.e. tight junctions and lateral intercellular spaces) which is characteristic of epithelial tissues [2, 8, 12, 13, 22, 26, 35, 36] or rather if the leak path defined by mannitol represents a spurious shunt around the "damaged edge" of the tissue [14].

In an attempt to evaluate the possible role of edge damage in transmural mannitol flow we have conducted experiments with portions of colon mounted without compression using a modification of the technique of Helman and Miller [14]. A segment of colon (Area ≈ 1 cm^2) is glued on the serosal side to a rubber gasket using a cyanocrylate tissue adhesive (Eastman) and is gently sealed on the mucosal side with silicone vacuum grease. The use of a similar technique with the frog skin appears to virtually eliminate "edge effects" in this preparation [14]. The average values for the total tissue conductance and I_{sc} for 100 tissues mounted in this manner were 1.9 ± 0.1 and 78.7 ± 4.3 respectively, and were not significantly different from those presented in Table 1 obtained in the standard lucite chamber. Serosa to mucosa Na and mannitol fluxes measured using "glued" preparations yielded average values not different from those in Table 1 and exhibited an identical relation, characteristic of simple diffusion, as did tissues mounted in conventional chambers (Dawson, *unpublished observations*). These experiments suggest that if edge damage does make a significant contribution to the overall "shunt permeability" of the isolated turtle colon, then the effects are not eliminated even by the most delicate treatment of the tissue.

The overall resistance of an isolated epithelium to transmural solute diffusion via paracellular shunt paths consists of at least three barriers in series: the "tight junctions" between adjacent epithelial cells, the lateral intercellular spaces, and a serosal unstirred layer. In the case of the turtle colon this submucosal space can be as thick as 350–400 μ. Since the physical properties of these barriers are essentially unknown, the question arises as to which constitutes the rate-limiting step for transmural diffusion.

Recently it has been shown that it is possible to modify the properties of the tight junctions of the toad urinary bladder in a relatively predictable fashion by increasing the osmolarity of the mucosal medium [8]. This treatment results in "blistering" and deformation of tight junctions and a consequent increase in tranepithelial conductance [8], and solute permeability [23]. We have carried out similar experiments on the isolated

Table 4. Effect of mucosal hyperosmolarity on Na and mannitol fluxes in the presence of amiloride

	G_T (mmho/cm^2)	J_{sm}^{man} (μmole/cm^2-hr) $\times 10^{-2}$	J_{sm}^{Na} (μeq/cm^2-hr)	J_{sm}^{Na}/J_{sm}^{man}	n
Before	1.26±0.33	1.04±0.27	0.47±0.12	0.44±0.01	6
After	1.94±0.31	1.73±0.27	0.86±0.13	0.49±0.01	6

$\bar{X}\pm$SE; n=number of tissues.

In each experiment fluxes were measured during three 30-min "control" periods. Sufficient mannitol was then added to the mucosal bathing solution to raise the osmolarity by approximately 250 mosmol, and sampling was continued for five additional 30-min periods. Fluxes from the period immediately following the addition of mannitol were discarded.

turtle colon by measuring serosa to mucosa fluxes of Na and mannitol before and after making the mucosal medium hypertonic by the addition of mannitol. Amiloride (10^{-4} M) was also present to prevent any S to M flow of Na through the active path. Table 4 shows the results of experiments in which the osmolarity of the mucosal medium was increased by approximately 250 mosmol by the addition of mannitol. Clearly, mucosal hyperosmolarity results in a marked increase in tissue conductance and in the S to M fluxes of Na and mannitol. Note, however, that the ratio J_{sm}^{Na}/J_{sm}^{man}, is close to the value of 0.44 predicted on the basis of simple diffusion. Tissues subjected to mucosal hyperosmolarity were prepared for electron microscopy according to the techniques of DiBona and Civan [8]. Tissues were fixed after the conductance showed at least a 30 % increase over control levels. All tissues examined showed evidence of blistering and deformation of tight junctions identical to that reported by DiBona and Civan. Junctional blistering was absent in control tissues. This result suggests that the tight junctions are the rate-limiting barrier to paracellular solute diffusion under normal circumstances.

The Apparent Driving Force for Active Na Transport

The relation between total tissue conductance and transmural mannitol flux suggests that the mannitol flux for any one tissue provides an estimate of the conductance of the leak path. Thus for any tissue the apparent conductance of the cellular path G_c, may be obtained from the relation:

$$G_c = G_T - 1.0\, J_{\text{man}}$$

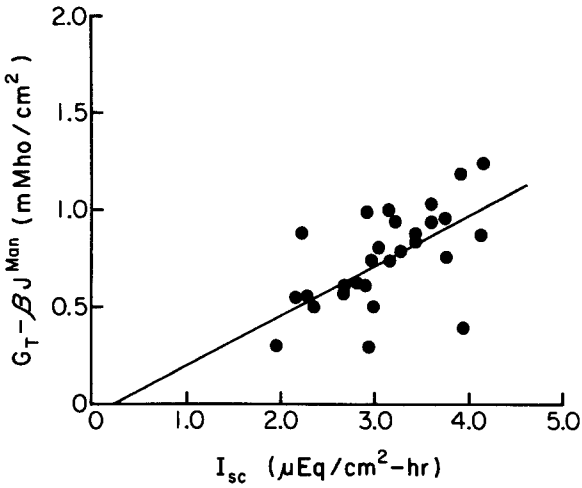

Fig. 7. The apparent cellular conductance of the colon-plotted versus I_{sc}

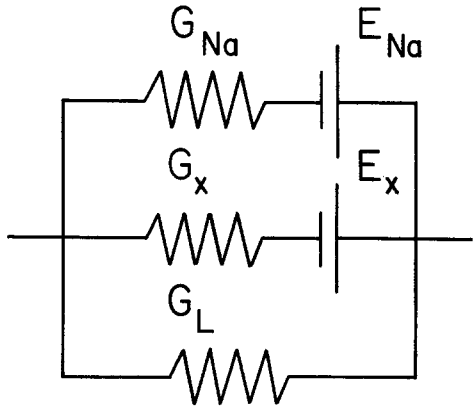

Fig. 8. An equivalent circuit representation of ion transport by the turtle colon

where G_c is the conductance of the cellular path in mmho/cm^2. Fig. 7 shows values for G_c, the apparent conductance of the cellular path, calculated using the above equation and plotted versus I_{sc}. The plot indicates a linear relation between G_c and I_{sc}. The possible significance of this linear relation can be seen from an analysis of a simple, parallel path equivalent circuit, which for the turtle colon, must consist of at least three branches as shown in Fig. 8. G_{Na} and E_{Na} represent, respectively, the apparent conductance and the apparent emf of the active Na transport path and G_L represents the conductance of the leak path. Recalling that amiloride reduces active Na transport to zero but does *not* abolish I_{sc}, we insert a third branch characterized by an apparent emf and conductance E_x and G_x

which are taken to represent the amiloride-*insensitive* portion of I_{sc}. The results shown in Fig. 4 suggest that variations in I_{sc} from tissue to tissue in the absence of amiloride can be attributed solely to variations in net Na absorbtion. According to the equivalent circuit of Fig. 8 the I_{sc} due to Na transport is given by:

$$I_{sc}^{Na} = G_{Na} E_{Na}.$$

Thus, if changes in I_{sc}^{Na} are due predominantly to changes in the apparent conductance of the active path G_{Na}, rather than to changes in the apparent emf E_{Na}, then G_{Na} should be linearly related to I_{sc}^{Na}, and the total *cellular* conductance G_c will be given by:

$$G_c = G_x + (1/E_{Na}) I_{sc}^{Na}.$$

The linear relation between G_c and I_{sc} (Fig. 7) suggests that differences in I_{sc} from tissue to tissue may be attributed largely to differences in the apparent conductance of the active path. The intercept of the plot of G_c versus I_{sc} (Fig.7) is not significantly different from zero. If G_x is taken to be a constant, however, the above relation for G_c predicts a positive intercept of this plot. A comparison of the average total cellular conductance (*see below*) with the amiloride-sensitive portion of this conductance suggests that G_x must be of the order of 0.15 to 0.25 mmho/cm^2 and would thus be easily obscured by the scatter in the G_c *vs.* I_{sc} plot. The average apparent emf of the active Na tranport path E_{Na} may be calculated from the slope of the G_c *vs.* I_{sc} plot to be about 125 mV.

An independent estimate of E_{Na} can be obtained from the changes in conductance and short-circuit current produced by amiloride. Amiloride (10^{-4} M) appears to completely block Na entry across the apical cell membrane of the turtle colon [33]. If we assume that amiloride reduces G_{Na} to zero as indicated in Fig. 5, but does not affect the conductance of the leak path, then G_{Na} is given by:

$$G_{Na} = G_T^b - G_T^a$$

where G_T^b and G_T^a are the total tissue conductances before and after amiloride. The value of E_{Na} may then be calculated from the amiloride-induced change in the I_{sc}, i.e.

$$E_{Na} = \Delta I_{sc}/G_{Na}.$$

Values for the relevant parameters for 25 experiments in which I_{sc} and G_T were measured before and after amiloride (10^{-4} M) are shown in Table 5.

Table 5. Effect of amiloride on G_T and I_{sc}

	I_{sc} ($\mu A/cm^2$)	G_T (mmho/cm^2)	E_{Na} (mV)
Before	76.3±4.3	2.0±0.2	
After	4.2±0.4	1.4±0.2	
Δ	72.1±4.2	0.6±0.04	128.9±7.9

$n=25$; $\bar{X}\pm$SE.

Table 6. Average values for equivalent circuit parameters characteristic of the isolated turtle colon bathed on both sides by identical solutions containing 114 mM Na

$G_T=2.02\pm0.05$ mmho/cm^2	$E_{Na}=125$ mV
$G_L=1.28\pm0.05$ mmho/cm^2	$G_{Na}=0.60\pm0.04$ mmho/cm^2
$G_c=0.74\pm0.03$ mmho/cm^2	$G_x=0.14\pm0.1$ mmho/cm^2

The calculated value for E_{Na} of 129 mV is in excellent agreement with that obtained from the slope of the G_c *vs.* I_{sc} plot.

Values obtained for G_T and J^{man} from transmural flux experiments permit a calculation of the average values of the cellular and noncellular portions of the conductance. Experiments with amiloride yield average values for the portion of I_{sc} due to active Na transport I_{sc}^{Na}, and for the conductance of the active Na transport path G_{Na}. These values, along with the calculated value for G_x, are shown in Table 6 and constitute a general description of the electrical properties of the "average" turtle colon.

Discussion

Transmural Na and Cl Movements

These results suggest that transmural solute flows across the isolated, stripped turtle colon may traverse at least two parallel pathways. One path; shared by mannitol, Na and Cl, exhibits properties consistent with a simple barrier to transepithelial diffusion; while the second path, from which mannitol appears to be excluded, is the route of active Na absorbtion. The properties of the pathway marked by mannitol are predictable on the basis of the free solution behavior of Na, Cl and mannitol. Thus, this pathway does not discriminate among these solutes on the basis of their charge or size to an extent greater than an equivalent layer of water. This behavior

is difficult to reconcile with the expected properties of cell membranes; rather it suggests that the pathway marked by mannitol is one which circumvents the cells at least in part through the tight junctions and lateral intercellular spaces which are characteristic of epithelial tissues. Although the present experiments do not rule out a contribution of "edge damage" to transmural mannitol flows, it is clear that techniques which have proven successful in reducing edge damage effects in frog skin [14] do not significantly alter the behavior of the "mannitol pathway" across the turtle colon.

If the observed mannitol flows represent movement through the "tight junctions" and lateral intercellular spaces then these results suggest that this path has the properties of a free solution shunt for the solutes examined. Furthermore, the effects of mucosal hypertonicity on the structure and permeability properties of the tissues indicate that the major barrier to solute movement through the shunt pathway is the so-called "tight junction". That solutes of the size of Na, Cl and mannitol might enter "tight junctions" in an "unrestricted" fashion is in accord with the observations of DiBona and Civan [8] who suggest that molecules as large as raffinose (radius = 6 Å, ref. [8]) can enter the tight junctions of the toad urinary bladder. It is of interest in this regard that the apparent relative shunt permeabilities Na and Cl in two other relatively "tight" epithelia, the frog skin [23] and the rabbit colon [10], appear to be in accord with free solution mobility ratios. This is in contrast to observations on two leaky epithelia, the rabbit gallbladder [26] and the rabbit ileum [11, 30] which indicate that the shunt path in these tissues is cation selective, i.e. $P_{Na} > P_{Cl}$.

Na Fluxes and the Source of the Short-Circuit Current

The serosa to mucosa flow of Na across the isolated turtle colon appears to be largely restricted to the shunt pathway defined by the mannitol flux. The consistent relation between the movements of Na and mannitol in the shunt suggests that the mucosa to serosa mannitol flow may be used as a convenient means of separating the mucosa to serosa Na flux into cellular and noncellular components. The relation of the cellular component of the mucosa to serosa Na flux defined in this manner to the I_{sc} indicates that variations in I_{sc} from tissue to tissue are virtually entirely attributable to differences in active Na transport. Furthermore, the *net* flow of Na across the tissue is, for practical purposes, equal to the *unidirectional* flow of Na through the cells. This behavior has implications with regard to the "back-flux" of Na through the active path which will be discussed below, but it

also suggests that there is relatively little *passive*, transmural, movement of Na through cellular paths. Thus, although the colon may be expected to contain more than one cell type, significant transmural Na movement appears to occur through only two paths, one noncellular and passive, and another cellular and active. Amiloride abolishes net Na transport by eliminating the cellular component of the mucosa to serosa Na flow, and in the presence of amiloride M to S and S to M fluxes of this ion appear to be restricted to the shunt path. The results of this study are in accord with previous studies of isolated colons of amphibians [5, 6, 7, 20] and mammals [1, 10] which indicate that the primary source of I_{sc} in these tissues is active Na absorbtion.

It is of interest to note from Fig. 4 that, although *changes* in active Na absorption appear to be solely responsible for *changes* in I_{sc}, the nonzero intercept of this plot suggests that a small component of active ion transport will remain in the absence of active Na movement. The existence of a non-Na portion of I_{sc} is also suggested by the observation that amiloride reduces net Na transport to zero although a significant I_{sc} remains. The lack of complete identity between the total I_{sc} and net Na absorption has been a consistent observation studies of the amphibian colon [3, 5, 6, 7, 20]. Carlinsky and Lew [3] suggested that the nonsodium portion of the I_{sc} in the colon of *Bufo arenarum* was due to HCO_3 secretion from an intracellular compartment. The direction of the residual I_{sc} in the turtle colon is consistent with a small component of HCO_3 secretion although clearly H absorption or OH secretion would produce similar effects. In preliminary experiments (Dawson, *unpublished observations*) we have found that the amiloride-insensitive component of I_{sc} persists when the colon is bathed by solutions in which all Cl has been replaced by isethionate or SO_4. In addition, the amiloride-insensitive I_{sc} is stimulated by raising the ambient HCO_3 concentration from 2.5 to 25 mM. These observations suggest that the amiloride-insensitive I_{sc} may be attributable to a rheogenic HCO_3 or H transport process. In contrast, Frizzell, Koch and Schultz [10] have obtained evidence in the rabbit colon for an *electrically neutral* secretion of HCO_3 in exchange for Cl.

Chloride Fluxes

Chloride fluxes across the isolated turtle colon appear to be largely confined to the shunt pathway defined by mannitol and these experiments provide no evidence for any *net* flow of Cl under short-circuit conditions.

Amiloride has no discernable effect on unidirectional Cl fluxes indicating that Cl movements are largely unrelated to net Na transport under short-circuit conditions. The analysis of the movements of Cl across the turtle colon is complicated by the great variability in the relation between the fluxes of Cl and mannitol. One simple explanation for this behavior is that there is a small component of Cl movement through the cells but this component varies greatly from one preparation to another. With regard to a possible cellular component of the transmural Cl flux it is of interest that Cooperstein and Hogben [6], in their study of the bullfrog colon, found no net Cl movement, yet they observed that the Cl flux ratio at a transmural PD of 45 mV deviated appreciably from that expected from simple diffusion. In addition, their data indicate that the ratio, J_{sm}^{Cl}/J_{sm}^{Na}, is significantly *higher* than that predicted on the basis of the free solution mobilities of the ions. Thus if, as in the present study, J_{sm}^{Na} for the bullfrog colon is confined to a noncellular leak path where Na and Cl move as if in free solution, this elevated ratio may also be indicative of a cellular component of the Cl flow. In their recent study of the rabbit colon Frizzell et al. [10] showed that this preparation actively absorbs Cl but the net Cl transport appeared to be independent of active Na transport. Binder and Rawlins [1], however, found evidence for coupling of active Na and Cl movements across the rat colon.

Electrical Properties of the Isolated Colon

The electrical properties of the isolated turtle colon appear to be adequately described by an equivalent circuit consisting of at least three branches as indicated in Fig. 8. The conductance of the leak path G_L is quantitatively related to the transmural mannitol flux, and can be completely accounted for by the diffusive movements of Na and Cl. Thus, G_L appears to be a measure of the dissipative properties of a pathway for diffusive ion flow across the tissue.

The consistent relation between the conductance of the shunt path and transmural mannitol flow permits a calculation of the cellular component of the total tissue conductance, and facilitates a comparison of the cellular conductance with the rate of active Na transport. These experiments suggest that the properties of the cellular pathway for active Na transport can be characterized by an apparent conductance G_{Na} and an apparent emf, E_{Na}. Although the physical significance of these equivalent circuit parameters is not clear at present [9, 16, 17, 18], it is to be expected that

G_{Na} reflects the dissipative properties of the active transport path whereas E_{Na} is a measure of the effective "driving force" for active Na transport. If variations in I_{sc} from tissue to tissue are examined within this framework it appears that the changes in net Na transport may be largely attributed to changes in G_{Na}, the apparent conductance. The apparent emf, E_{Na}, appears to remain relatively constant. Similarly, in the rabbit urinary bladder [21] variations in net Na transport are highly correlated with tissue conductance. The estimated value for the apparent emf of the active Na transport system in the turtle colon of about 125 mV is similar to values for E_{Na} determined for the toad urinary bladder [37] and the rabbit colon (Frizzell + Schultz, *personal communication*).

Na "Backflux" through the Active Path

In the present experiments the serosa to mucosa flux of Na appeared to be restricted to the shunt pathway defined by mannitol. In addition, the slope of a plot of the *cellular portion* of J_{ms}^{Na} versus I_{sc} was not different from 1.0 (Fig. 4), i.e. the mucosa to serosa unidirectional flux of Na through the cells was apparently *equal* to the *net* Na flux. Thus, within the accuracy of these flux measurements it is not possible to detect any "backflux" of Na through the active, cellular path. Previous studies on Na transport by other isolated epithelia have produced similar results. Civan [4], for instance, concluded from current-voltage relations that the active pathway in the toad urinary bladder possessed rectifying properties, and exhibited a near infinite resistance to Na movement from serosa to mucosa. Similarly, Saito, Lief and Essig [29] were unable to detect any isotopic "backflux" through the active path in the toad bladder; the serosa to mucosa flow of Na appeared to be restricted to an extracellular route. Nellans, Frizzell and Schultz [27] and Frizzell *et al.* [10] found that the total serosa to mucosa flow of Na in the rabbit ileum and the rabbit colon could be attributed to simple diffusion.

The inability to detect an isotopic backflux through the "Na pump" pathway is, perhaps, not surprising in view of the apparent emf of the active path. Ussing and Zehran [34] suggested that the Na flux ratio in frog skin provided a measure of an effective driving force for active transport, E_{Na}. Subsequent studies by Helman, O'Neil and Fisher [15], using frog skins in which edge damage was reduced, have shown a good correspondence between E_{Na} as determined by the flux ratio and that determined from equivalent circuit analysis. Thus, we may obtain an estimate for the turtle

colon of the Na flux ratio in the *active pathway* from the value of E_{Na} derived from equivalent circuit analysis. The flux ratio in the active pathway under short-circuit conditions is given by:

$$J^0_{ms}/J^0_{sm} = \exp\{zFE^0_{Na}/RT\}$$

where J^0_{ms}, J^0_{sm} and E^0_{Na} represent the values of the Na fluxes and the apparent emf under short-circuit conditions and z, F, R, and T have their usual significance. A value for E_{Na} of 125 mV yields a Na flux ratio of about 122. Thus for an M to S flow through the active path of 3 μeq/cm^2-hr the expected backflux would be less than 0.03 μeq/cm^2-hr, well within the noise of the Na flux determinations in this study.

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