J. Membrane Biol. 27, 297–316 (1976)


# Ion Transport by Rabbit Colon

## I. Active and Passive Components

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Received 9 October 1975; Revised 21 January 1976

*Summary.* Descending rabbit colon, stripped of *muscularis externa*, absorbs Na and Cl under short-circuit conditions and exhibits a residual ion flux, consistent with $HCO_3$ secretion, whose magnitude is approximately equal to the rate of active Cl absorption. Net K transport was not observed under short-circuit conditions. The results of ion replacement studies and of treatment with ouabain or amiloride suggest that the short-circuit current $I_{sc}$ is determined solely by the rate of active Na transport and that the net movements of Cl and $HCO_3$ are mediated by a Na-independent, electrically-neutral, anion exchange process. Cyclic AMP stimulates an electrogenic Cl secretion, abolishes $HCO_3$ secretion but does not affect the rate of Na absorption under short-circuit conditions. Studies of the effect of transepithelial potential difference on the serosa-to-mucosa fluxes $J^i_{sm}$ of Na, K and Cl suggest that $J^{Na}_{sm}$, $J^{K}_{sm}$ and one-third of $J^{Cl}_{sm}$ may be attributed to ionic diffusion. The permeabilities of the passive conductance pathway(s) are such that $P_K:P_{Na}:P_{Cl}=1.0:0.07:0.11$. Electrolyte transport by *in vitro* rabbit colon closely resembles that reported from *in vivo* studies of mammalian colon and thus may serve as a useful model for the further study of colonic ion transport mechanisms.

Studies of electrolyte transport by mammalian colon have largely been restricted to *in vivo* preparations where electrochemical gradients cannot be readily manipulated. These studies have uniformly demonstrated that the colon absorbs Na and Cl and secretes K and $HCO_3$ when the lumen is exposed to protein-free electrolyte solution that resembles plasma [24, 30]. Under these conditions, a spontaneous electrical potential difference is observed with the lumen invariably electrically negative with respect to the plasma [24, 30]. These results suggest that the net movements of Na and $HCO_3$ are the results of active transport processes, whereas the nature of Cl and K transport cannot be inferred because their movements are in the direction of favorable electrical gradients.

Under *in vitro* conditions, electrochemical gradients across the colonic epithelium may be varied at will; however, only a few such studies have been reported and, unfortunately, these have yielded conflicting results. For example, Edmonds and Marriott [13] found that the rate of net Na transport across rat colon *in vivo* agreed with the short-circuit current

determined from separate *in vitro* studies, suggesting that the transport of Na alone accounts for the spontaneous electrical potential difference and short-circuit current across the tissue. In contrast, Binder and Rawlins [3] found that net Na absorption by rat colon *in vitro* markedly exceeded the simultaneously measured short-circuit current and concluded that the absorption of both Na and Cl by rat colon could be attributed to a neutral, coupled NaCl transport process. Thus, *in vitro* studies of ion transport by mammalian colon have not provided a consistent framework for the interpretation of results obtained *in vivo*. The present study is concerned with an analysis of Na, Cl and K movements across rabbit descending colon under short-circuit conditions; the passive fluxes of these ions across the epithelium were evaluated using a voltage-clamp procedure. The effects of ion replacement, ouabain, amiloride and cyclic AMP are also reported.

## Materials and Methods

Male, white rabbits (2–3 kg) were sacrificed with intravenous pentobarbital and a segment of descending colon, 10–15 cm in length, was removed. The tissue was opened along the mesenteric border to form a flat sheet, and rinsed free of intestinal contents with a normal 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; this solution had a pH of 7.4 at 37 °C when gassed with 95% $O_2$-5% $CO_2$. Na-free or Cl-free solutions were prepared by isosmotic replacement of NaCl with choline chloride or sodium isethionate; Cl-free solutions contained the sulfate salts of Mg and Ca. $HCO_3$-free solutions were prepared by isosmotic replacement of $NaHCO_3$ with $Na_2SO_4$ and mannitol.

A "partial mucosal strip" preparation (Fig. 1*b*) was obtained by removing the *muscularis externa* by blunt dissection. The whole-thickness tissue is shown in Fig. 1*a* for comparison.

Four segments of the "partial mucosal strip" were mounted in the short-circuit apparatus described by Schultz and Zalusky [28]. Bidirectional fluxes of $^{22}Na$, $^{36}Cl$ or $^{42}K$ were determined under short-circuit conditions using adjacent tissue segments from the same animal. Experiments were discarded if the electrical resistances of the four tissue segments differed by more than 25% or were less than 140 $\Omega\,cm^2$. Correction for fluid resistance was unnecessary since the fluid resistance measured in the absence of tissue was never greater than 5% of the total resistance determined with the tissue interposed between the electrodes.

It was apparent from our preliminary studies that there was some spontaneous variation in control values of $I_{sc}$ and net Na flux when different groups of animals were compared. Thus, in all cases, the effects of ion replacement, or agents added to the bathing media, were compared to control data obtained using tissue from the same animal.

Three types of experiments were performed. In the ion-replacement studies, bidirectional fluxes of Na or Cl were determined under control and experimental conditions using four adjacent tissue segments from the same animal. In the studies on the effects of ouabain, amiloride or cyclic AMP, bidirectional fluxes of both Na and Cl were determined on four adjacent segments during an initial control period. This was followed by an experimental period during which the agent was present in the bathing solutions. This procedure is justified by the finding that under control conditions in the presence of glucose (*see below*) ion fluxes are stable for 3–4 hours (Table 1). In both types of experiments, the tissues were continuously short-circuited except for brief periods, at 10-min intervals, when the open-circuit potential

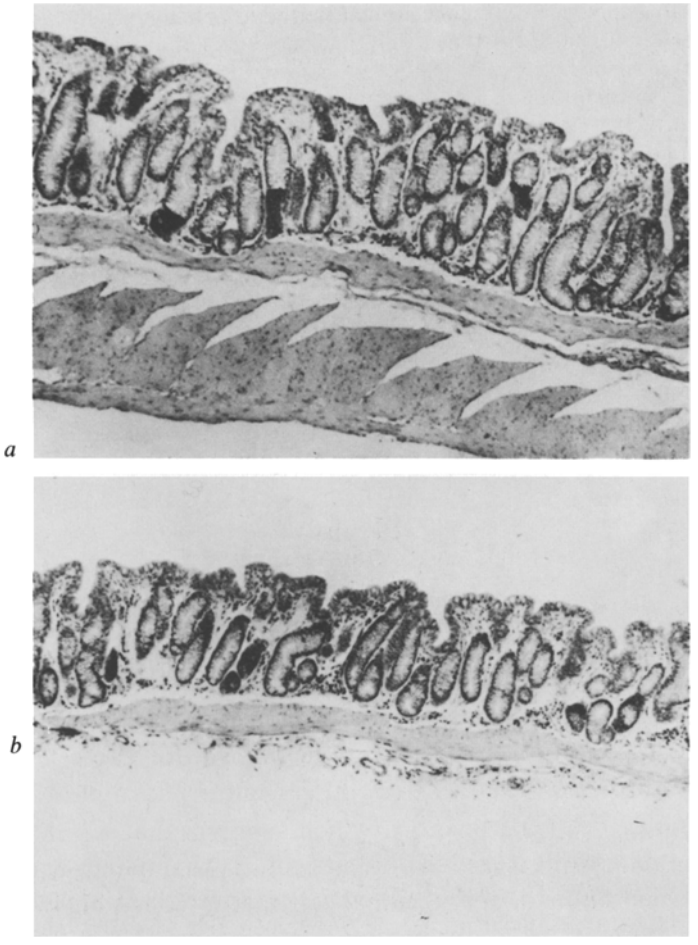


Fig. 1. Rabbit descending colon: (*a*) whole thickness tissue, (*b*) "partial mucosal strip" preparation, which retains the *muscularis mucosa*

difference $\psi_{ms}$ was recorded. Tissue resistance was calculated from the ratio of open-circuit *PD* to short-circuit current $I_{sc}$ except in those instances where the $I_{sc}$ declined to low values (e.g., Na-free bathing media or ouabain). In these experiments, a brief (1 sec) pulse of direct current was passed across the tissue and the resulting *PD* increment was used to calculate the tissue resistance. Preliminary experiments indicated that the tissue behaved as an ohmic resistor over the range $\pm 50$ mV.

In a third series of experiments, the effect of $\psi_{ms}$ on the serosa-to-mucosa fluxes of Na, Cl and K was determined using the normal electrolyte solution. In these studies three consecutive unidirectional fluxes were determined with $\psi_{ms}$ clamped in random order at $\pm 50$ or 0 mV. In all experiments, the unidirectional flux represents the mean of 4–5 determinations at 10-min intervals, and all flux periods were preceded by a 20-min equilibration period which ensured the achievement of a steady-state.

Preliminary experiments using the normal electrolyte solution without added substrate indicated that the $I_{sc}$ steadily declined with time and after 1 hour was approximately 50% of that observed immediately after mounting. However, in the presence of 10 mM glucose, stable values of $I_{sc}$ and tissue resistance were maintained for at least four hours. Thus, in all studies reported, the electrolyte solutions routinely contained 10 mM glucose. A similar substrate requirement was reported by Parsons and Patterson [23] who found that maximal rates of fluid transport by everted sacs of rat colonic mucosa could be maintained for several hours if glucose or other substrates were included in the bathing media.

Amiloride was a generous gift of Merck, Sharpe and Dome, West Point, Pa. Ouabain and cyclic AMP were obtained from Sigma Chemical Co., $^{22}Na$ and $^{36}Cl$ from New England Nuclear and $^{42}K$ from ICN. Other chemicals were reagent grade. Results are expressed as the mean ± SEM based on the number of animals studied. Differences were analyzed using the Student $t$ test; a value of $P < 0.05$ was considered significant.

## Results

### *Transepithelial Ion Fluxes*

Steady-state unidirectional transmural Na, Cl and K fluxes across stripped rabbit colon under short-circuit conditions are given in Table 1. Under control conditions, as shown in the first row of Table 1, Na and Cl are actively absorbed. The short-circuit current ($I_{sc}$) is in close agreement with the rate of net Na transport, which suggests that Cl absorption is balanced by (an) unmeasured ion flux(es) of approximately equal magnitude. The orientation of this residual ion flux is consistent with $HCO_3$ (or OH) secretion, but the absorption of cation (e.g., H) cannot be ruled out. Tissue resistance $R_t$ under control conditions averaged 200–250 $\Omega$ cm$^2$, yielding a conductance $G_t$ of 4–5 mmhos/cm$^2$. This value is approximately sixfold lower than that observed in stripped rabbit ileum [15, 21], suggesting that, with respect to small intestine, the colonic epithelium is much more restrictive to passive ion movements. This conclusion is supported by the values obtained for unidirectional Na and Cl fluxes which are considerably lower than those observed in a similar preparation of rabbit ileum.

The second row of Table 1 gives the results of paired determinations of Na and K fluxes under control conditions. Again, there is good agreement between the net Na flux and short-circuit current. Net K transport across rabbit colon was not observed under short-circuit conditions. Therefore, calculation of the residual ion flux(es) was made using only the net fluxes of Na and Cl.

Table 1. Sodium, chloride and potassium fluxes across rabbit colon

| | $J_{ms}^{Na}$ | $J_{sm}^{Na}$ | $J_{net}^{Na}$ | $J_{ms}^{Cl}$ | $J_{sm}^{Cl}$ | $J_{net}^{Cl}$ | $I_{sc}$ | $J_r$ | $G_t$ |
|---|---|---|---|---|---|---|---|---|---|
| Control (16) | 4.4±0.4 | 1.4±0.1 | 3.0±0.3 | 5.6±0.5 | 4.0±0.2 | 1.5±0.4 | 2.8±0.1 | 1.4±0.5 | 5.2±0.1 |
| | | | | $J_{ms}^{K}$ | $J_{sm}^{K}$ | $J_{net}^{K}$ | | | |
| Control (9) | 4.2±0.3 | 1.3±0.2 | 2.9±0.4 | 0.57±0.06 | 0.52±0.08 | 0.05±0.09 | 2.7±0.3 | – | 4.1±0.2 |

All values are in μ Eq./cm², hr except $G_t$ which is in mmhos/cm². $J_r = I_{sc} - J_{net}^{Na} + J_{net}^{Cl}$, as explained in text. Numbers in parentheses are the numbers of animals studied. Ion fluxes were determined during a 3 hr incubation in the normal electrolyte solution.

Table 2. Effects of ion replacement on sodium and chloride fluxes across rabbit colon

| | $J_{ms}^{Na}$ | $J_{sm}^{Na}$ | $J_{net}^{Na}$ | $J_{ms}^{Cl}$ | $J_{sm}^{Cl}$ | $J_{net}^{Cl}$ | $I_{sc}$ | $J_r$ | $G_t$ |
|---|---|---|---|---|---|---|---|---|---|
| Control (5) | – | – | – | 5.3±0.5 | 4.3±0.5 | 1.0±0.1 | 2.7±0.4 | – | 5.7±0.3 |
| Na-Free | – | – | – | 6.6±0.5 | 5.3±0.6 | 1.3±0.3 | 0.0±0.0 | 1.3±0.3 | 4.4±0.2 |
| Control (8) | 3.2±0.4 | 1.4±0.2 | 1.8±0.3 | – | – | – | 1.5±0.2 | – | 4.3±0.2 |
| Cl-Free | 4.9±0.6 | 1.4±0.2 | 3.6±0.5 | – | – | – | 3.9±0.4 | 0.3±0.6 | 3.4±0.2 |
| Control (4, 6) | 5.0±0.4 | 1.1±0.1 | 3.9±0.3 | 7.1±0.3 | 5.4±0.3 | 1.7±0.3 | 3.7±0.3 | 1.6±0.5 | 4.4±0.2 |
| $HCO_3$-Free | 4.6±0.2 | 0.9±0.1 | 3.6±0.4 | 5.4±0.3 | 4.2±0.4 | 1.2±0.3 | 4.2±0.3 | 1.8±0.6 | 4.0±0.2 |

*See legend*, Table 1. In the $HCO_3$-free studies, Na fluxes were determined using paired tissues from 4 animals; Cl fluxes were determined on paired tissues from 6 animals.

*Ion Replacement Studies*

The results of paired studies evaluating the effects of Na-free media on transmural Cl fluxes and the electrical parameters are presented in Table 2. The short-circuit current was abolished in Na-free solutions again suggesting that the $I_{sc}$ is determined only by the rate of active Na absorption. Net Cl transport was unaffected when both mucosal and serosal solutions were rendered Na-free. These findings require that the observed rate of Cl absorption be balanced by (a) residual ion flux(es) of equal magnitude. Both unidirectional Cl fluxes showed some tendency to increase with Na replacement, although the changes were not statistically significant. Tissue conductance decreased by $1.3 \pm 0.3$ mmhos/cm$^2$ when Na was replaced with choline.

The effect of Cl-free bathing solutions on transmural Na fluxes is given in the third and fourth rows of Table 2. Replacement of Cl with isethionate resulted in a marked increase in both net Na absorption and the $I_{sc}$; the increase in $J_{net}^{Na}$ was due to an increase in $J_{ms}^{Na}$ alone. We are unable, at present, to offer an explanation for the stimulation of Na absorption in the presence of isethionate, but it should be noted that the control fluxes were lower than usual. In the absence of Cl, the rate of net Na absorption adequately accounted for the $I_{sc}$ so that a significant residual current was not observed. Thus, the residual ion flux appears to depend upon the presence of Cl or on Cl absorption. Tissue conductance decreased significantly by $0.9 \pm 0.3$ mmhos/cm$^2$ when Cl was replaced with isethionate.

Transmural fluxes of Na and Cl in control and $HCO_3$-free media are given in the lower portion of Table 2. Sodium and Cl fluxes were obtained using tissue from different animals; however, in this case the values for $I_{sc}$ and $G_t$ from the control groups differed by less than 10% so that the results were combined for convenience of presentation. Replacement of $HCO_3$ with sulfate and mannitol had no effect on Na or Cl absorption. As above, net Na absorption and the $I_{sc}$ did not differ significantly, and the residual ion flux calculated from these data did not differ significantly from the control value or from the observed rate of Cl absorption.

*Effects of Ouabain and Amiloride*

Addition of ouabain ($10^{-4}$ M) to both mucosal and serosal solutions following a control period resulted in a slow decline in $I_{sc}$ which reached a value not markedly different from zero by 20 min. As shown in Table 3,

Table 3. Effects of ouabain and amiloride on sodium, chloride and potassium fluxes across rabbit colon

| | $J_{ms}^{Na}$ | $J_{sm}^{Na}$ | $J_{net}^{Na}$ | $J_{ms}^{Cl}$ | $J_{sm}^{Cl}$ | $J_{net}^{Cl}$ | $I_{sc}$ | $J_r$ | $G_t$ |
|---|---|---|---|---|---|---|---|---|---|
| Control (6) | 3.9±0.5 | 1.5±0.2 | 2.4±0.4 | 5.6±0.9 | 4.1±0.5 | 1.5±0.4 | 2.2±0.2 | 1.3±0.6 | 4.4±0.2 |
| Ouabain $10^{-4}$ M | 2.4±0.3 | 2.0±0.2 | 0.4±0.2 | 7.3±1.2 | 5.9±0.7 | 1.4±0.6 | 0.3±0.0 | 1.2±0.6 | 4.4±0.2 |
| Control (4) | – | – | – | 5.5±0.8 | 3.7±0.3 | 1.8±0.5 | 2.1±0.2 | – | 3.6±0.2 |
| Amiloride $10^{-4}$ M | – | – | – | 5.9±0.7 | 3.8±0.3 | 2.1±0.5 | 0.5±0.1 | – | 3.9±0.2 |
| | | | | $J_{ms}^{K}$ | $J_{sm}^{K}$ | $J_{net}^{K}$ | | | |
| Control (9) | 4.0±0.3 | 0.9±0.1 | 3.1+0.3 | 0.49±0.05 | 0.45±0.04 | 0.04±0.08 | 3.3±0.3 | – | 4.2±0.1 |
| Amiloride $10^{-4}$ M | 1.6±0.2 | 1.1±0.1 | 0.5±0.1 | 0.55±0.06 | 0.53±0.06 | 0.02±0.11 | 0.3±0.1 | – | 3.7±0.2 |

*See legend*, Table 1. Fluxes in the presence of inhibitor were determined following an initial control period and, in the ouabain studies, a 30 min preincubation with the glycoside.

Table 4. Effects of cyclic AMP on sodium and chloride fluxes across rabbit colon

| | $J_{ms}^{Na}$ | $J_{sm}^{Na}$ | $J_{net}^{Na}$ | $J_{ms}^{Cl}$ | $J_{sm}^{Cl}$ | $J_{net}^{Cl}$ | $I_{sc}$ | $J_r$ | $G_t$ |
|---|---|---|---|---|---|---|---|---|---|
| Control (7) | 3.1±0.4 | 1.5±0.2 | 1.6±0.4 | 5.5±0.2 | 4.3±0.2 | 1.2±0.2 | 1.6±0.2 | 1.2±0.3 | 4.5±0.2 |
| Cyclic AMP 7.5 mM | 4.2±0.4 | 2.3±0.3 | 1.9±0.3 | 5.0±0.4 | 6.4±0.5 | −1.4±0.4 | 3.8±0.2 | 0.5±0.5 | 6.3±0.4 |

*See legend*, Table 1. Fluxes in the presence of cyclic AMP were determined following an initial control period and a 20 min preincubation in the presence of the cyclic nucleotide.

active Na absorption was essentially abolished in the presence of ouabain and agreed favorably with the $I_{sc}$ both in the presence and absence of ouabain. The inhibition of $J_{net}^{Na}$ is entirely attributable to a decrease in $J_{ms}^{Na}$ with no significant change in $J_{sm}^{Na}$. Chloride absorption and the residual ion flux were not significantly affected by ouabain and did not differ significantly from one another under both control and experimental conditions. The bidirectional fluxes of Cl show a tendency to increase in the presence of ouabain as was previously noted in tissues exposed to Na-free bathing solutions, but again, the apparent changes in Cl fluxes were not statistically significant.

Addition of amiloride to the mucosal solution alone resulted in a rapid decline of $I_{sc}$ toward zero. Amiloride added to the serosal solution had no effect on $I_{sc}$. Fig. 2 shows a typical result of the effect of amiloride on the transepithelial *PD*, $\psi_{ms}$, which was 20–25 mV in this preparation prior to the addition of amiloride. The deflections in $\psi_{ms}(\Delta\psi_{ms})$ are the results of passing repetitive, square-wave pulses of direct current (50 μA) of 0.5 sec duration across the tissue. Prior to the addition of amiloride, tissue resistance was 290 Ω cm$^2$, and the short-circuit current $I_{sc}$ was 80 μA/cm$^2$. The $\psi_{ms}$ reached zero 45 sec after amiloride was added to the mucosal solution and the deflections in $\psi_{ms}(\Delta\psi'_{ms})$ indicate that during the same period, tissue resistance had increased to 400 Ω cm$^2$. In some instances, the transepithelial *PD* reversed polarity but, on the average, the final value achieved was slightly greater than zero ($2 \pm 1$ mV).

The results given in the third and fourth rows of Table 3 indicate that chloride transport was not affected by the presence of amiloride. In this

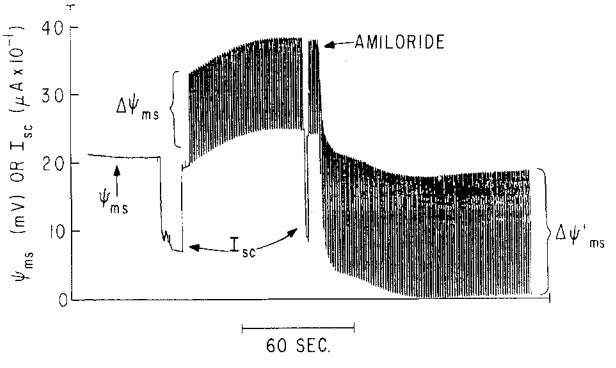


Fig. 2. Time-course of the effect of amiloride on $\psi_{ms}$. $I_{sc}$ is the short-circuit current obtained using an automatic voltage clamp, $\Delta\psi_{ms}$ and $\Delta\psi'_{ms}$ represent the change in $\psi_{ms}$ due to passing repetitive, square wave pulses of direct current (50 μA) across the tissue before and after the addition of amiloride (*arrow*) to the mucosal solution alone

series of experiments, bidirectional Na fluxes were not determined so that a reliable value for $J_r$ cannot be calculated. However, the fact that significant Cl absorption was observed under conditions where the $I_{sc}$ and Na absorption *(see below)* were essentially abolished again indicates that a significant residual ion flux persists in the presence of amiloride whose magnitude must be approximately equal to the rate of active Cl absorption. These findings closely resemble those obtained using Na-free media and ouabain and support the conclusion that the active movements of Cl and the unidentified ion(s) are independent of Na transport.

The lower portion of Table 3 gives the results of paired determinations of Na and K fluxes in the presence and absence of amiloride. Active Na absorption and the $I_{sc}$ were markedly inhibited by addition of $10^{-4}$ M amiloride to the mucosal solution. The decrease in net Na flux is due to a decrease in $J_{ms}^{Na}$ alone. As noted previously, net K transport across rabbit colon was not observed under short-circuit conditions, and as shown by the data of Table 3, amiloride had no effect on the bidirectional K fluxes. In this series of experiments, tissue conductance was significantly decreased by amiloride in agreement with the results shown in Fig. 2. This decrease in tissue conductance was not apparent in the studies of the effect of amiloride on Cl fluxes; however, in many instances the effect of amiloride on tissue conductance appears to be characterized by a rapid decline followed by a more prolonged return toward control values. Thus, simply averaging tissue conductance over the entire flux period, as in Table 3, will obscure this time-dependent behavior *(see below)*.

### *Effects of Cyclic AMP*

Addition of cyclic AMP (7.5 mM) to both mucosal and serosal bathing media increased the $I_{sc}$ 2.5-fold. The rise in short-circuit current was more prolonged than that observed in rabbit ileum [14] and was not complete until 15–25 min after addition of the cyclic nucleotide. During this period tissue conductance increased 41 %. As shown in Table 4 cyclic AMP produced a discrepancy between the rate of active Na absorption and the $I_{sc}$. Sodium transport was unaffected, and the increase in short-circuit current was associated with a reversal of the direction of net Cl movement from absorption to secretion. In the presence of cyclic AMP, the sum of net Na and Cl fluxes, $3.3 \pm 0.5$ μEq/cm$^2$ hr, did not differ significantly from the simultaneously measured $I_{sc}$ of $3.8 \pm 0.2$ μEq/cm$^2$ hr indicating that Cl secretion contributes directly to the short-circuit current in the presence

of cyclic AMP. The residual ion flux, calculated from these data, does not differ significantly from zero.

### *Effect of $\psi_{ms}$ on Serosa-to-Mucosa Ion Fluxes*

We have previously demonstrated that [18]

$$J^i_{sm} = {}_{0d}J^i_{sm}[\xi/(\exp \xi) - 1] + {}_{m}J^i_{sm}$$

where $J^i_{sm}$ is the total serosa-to-mucosa flux of an ion $i$ in the presence of any $\psi_{ms}$; ${}_{0d}J^i_{sm}$ is the diffusional flux of $i$ from serosa-to-mucosa under short-

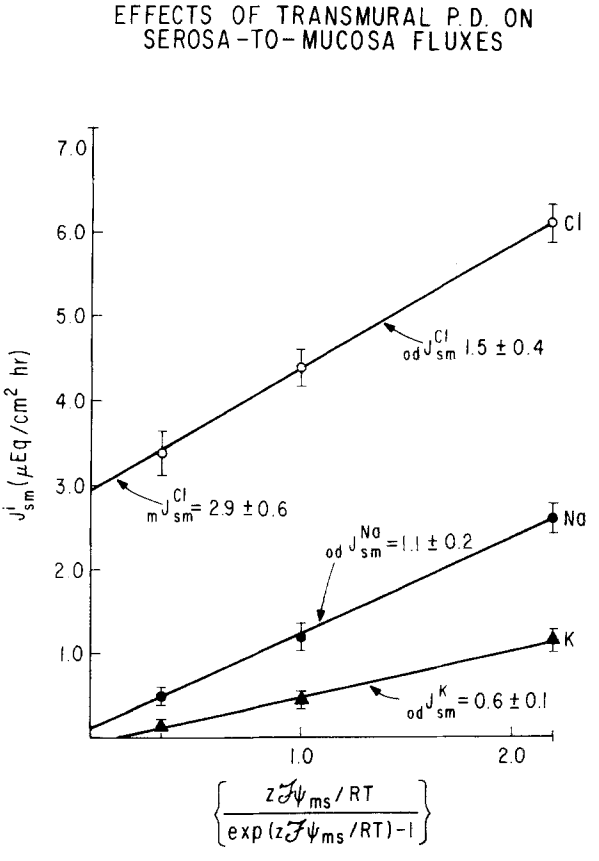


Fig. 3. Serosa-to-mucosal fluxes of Na, K and Cl as a function of $\psi_{ms}$. ${}_{m}J^i_{sm}$ represents the $\psi_{ms}$-independent component of $J^i_{sm}$ and ${}_{0d}J^i_{sm}$ represents the diffusional, $\psi_{ms}$-dependent component of $J^i_{sm}$ under short-circuit conditions. Tissue conductances at the three values of $\psi_{ms}$ did not differ significantly ($\psi_{ms} = 50$ mV, $G_t = 4.3 \pm 0.2$ mmhos/cm$^2$; $\psi_{ms} = 0$, $G_t = 4.1 \pm 0.2$; $\psi_{ms} = -50$, $G_t = 4.2 \pm 0.2$; n = 9)

Table 5. Passive conductances across rabbit colon

| Ion (mM) | ${}_pG_i$ (mmhos/cm$^2$) | $P_i$ (cm/hr) |
|---|---|---|
| Na (140) | $1.1 \pm 0.2$ | $0.008 \pm 0.002$ |
| Cl (124) | $1.5 \pm 0.4$ | $0.012 \pm 0.003$ |
| K (5.4) | $0.6 \pm 0.1$ | $0.12 \pm 0.02$ |

The partial ionic conductance ${}_pG_i$ is numerically equal to the diffusional unidirectional flux under short-circuit conditions ${}_{0d}J^i_{sm}$, *see* Fig. 3. Ionic permeability, $P_i = {}_pG_i/[i]$. The relative permeabilities are $P_K : P_{Na} : P_{Cl} = 1.0 : 0.07 : 0.11$. The free solution mobility ratios are $\lambda_K : \lambda_{Na} : \lambda_{Cl} = 1.0 : 0.7 : 1.0$.

circuit conditions; ${}_mJ^i_{sm}$ is a nondiffusional, *PD*-independent component of $J^i_{sm}$; and $\xi = z_i \mathcal{F} \psi_{ms}/RT$. This relation was evaluated for the serosa-to-mucosa fluxes of Na, K and Cl at three values of $\psi_{ms}$ ($\pm 50$ mV and 0 mV) as described above. A plot of $J^i_{sm}$ as a function of $[\xi/(\exp \xi) - 1]$ should yield a straight line having a slope of ${}_{0d}J^i_{sm}$ and an intercept on the ordinate of ${}_mJ^i_{sm}$. Fig. 3 presents the results of these studies. The lines were drawn from a least-squares regression of the individual data points whose mean $\pm$ SEM are given in the figure. A significant linear relation was obtained for each ion ($r > 0.90$). Table 5 gives the values of ${}_{0d}J^i_{sm}$ derived from the regression analysis. For both Na and K, the values obtained for the *PD*-independent component of serosa-to-mucosa flux, ${}_mJ^i_{sm}$, do not differ significantly from zero, suggesting that the entire serosa-to-mucosa fluxes of Na and K can be attributed to simple diffusion. Since ${}_0J^K_{sm} = {}_0J^K_{ms}$ it follows that the bidirectional transepithelial fluxes of K are strictly diffusional. On the other hand, a significant *PD*-independent component of serosa-to-mucosa Cl flux is observed; only one-third of $J^{Cl}_{sm}$ may be attributed to ionic diffusion.

## Discussion

### *Sodium and Potassium Transport*

Isolated rabbit colon actively absorbs Na under short-circuit conditions at a rate which does not differ significantly from the simultaneously measured $I_{sc}$. Moreover, in the absence of Na, the short-circuit current is abolished. These findings strongly suggest that active Na transport is the major determinant of the transepithelial potential difference and short-circuit current in this tissue. This conclusion is in agreement with the re-

sults of a number of studies employing *in vivo* and *in vitro* preparations of mammalian and amphibian colon. Thus, Curran and Schwartz [9] reported that the ratio of bidirectional Na fluxes across perfused rat colon significantly exceeded that predicted from the Ussing flux-ratio equation and demonstrated that Na could be absorbed from lumen to plasma against both concentration and electrical potential differences. Edmonds and Marriott [13] examined the potential difference and short-circuit current across rat colon *in vitro* and found general agreement between the observed $I_{sc}$ and the rate of Na absorption determined from *in vivo* studies. In addition, isolated guinea pig [31], frog [8] and toad [7] colon actively absorb Na at rates that are in good agreement with the short-circuit currents.

The equality between active Na absorption and the $I_{sc}$ in rabbit colon is supported by a number of additional observations: (a) removal of Cl or $HCO_3$ from the bathing media does not affect the $I_{sc}$ or $J_{net}^{Na}$ suggesting that Na transport is independent of anion transport mechanisms. (b) Ouabain and amiloride abolish the $PD$, $I_{sc}$ and active Na absorption without affecting active Cl absorption. Thus, the rate of active Na transport appears to be the *sole* determinant of the short-circuit current and transepithelial potential difference across this tissue.

There is no evidence for active, transepithelial K transport by isolated rabbit colon; bidirectional K fluxes do not differ under short-circuit conditions. Yet, the results of *in vivo* studies have uniformly demonstrated K secretion under open-circuit conditions [24, 30]. In view of the magnitude and polarity of the transepithelial potential difference observed in these and other studies, the present results suggest that the factors responsible for K secretion, under open-circuit conditions, are the presence of a favorable electrical driving force coupled with a relatively high diffusional permeability for K *(see below)*. In a study of ion transport across segments of human colonic mucosa under short-circuit conditions, Archampong *et al.* [1] observed net K appearance in the mucosal solution when assayed by flame photometry. The authors conclude, however, that this apparent K secretion resulted from progressive release of K from cells deteriorating during the period of study.

### *Chloride-Bicarbonate Exchange*

Under a variety of experimental conditions, rabbit colon actively absorbs Cl at a rate of 1–2 $\mu Eq/cm^2$, hr. Since Na transport appears to

account for the short-circuit current, the rate of Cl absorption must be balanced by a residual (or unmeasured) ion flux of approximately equal magnitude. The orientation of this residual ion flux is consistent with $HCO_3$ secretion (OH secretion or H absorption). This conclusion is in agreement with the results of a number of *in vivo* studies employing perfused loops of mammalian large intestine [10, 11, 22, 25]. Chloride absorption and $HCO_3$ secretion are routinely observed using direct chemical assay techniques for these ions, and several investigators have implicated a $Cl-HCO_3$ exchange process to account, at least in part, for net Cl absorption by *in vivo* colon [11, 22, 26]. This conclusion stems primarily from the observation that $HCO_3$ secretion is dependent upon the presence of Cl in the luminal perfusion solution.

The results of the present study are consistent with the presence of a Cl-$HCO_3$ exchange mechanism in rabbit colon. Replacement of Cl in the bathing media with isethionate (Table 2) abolished the residual ion flux, without affecting the equality between Na absorption and the short-circuit current. When $J_{net}^{Na}$ and $I_{sc}$ are abolished or markedly reduced (Na-free media, ouabain, amiloride), Cl absorption is not significantly affected and is balanced, in each instance, by a residual ion flux of approximately equal magnitude. Thus, the net absorption of Cl by rabbit colon is an electrically-neutral transport process which, in all likelihood, is mediated by a $Cl-HCO_3$ exchange mechanism.

Chloride absorption by rabbit colon was not significantly affected by removal of $HCO_3$ (and $CO_2$) from the bathing media (Table 2). The magnitude of the residual ion flux in the absence of $HCO_3$ did not differ from that observed under control conditions. Similar findings have been reported by Field and collaborators [12, 14, 16] for $HCO_3$ secretion by rabbit ileum where $HCO_3$ appearance was determined by chemical assay techniques. These findings suggest that secreted $HCO_3$ may be derived from cellular $CO_2$ metabolism rather than from the serosal solution. A similar conclusion was reached by Carlinsky and Lew [4] in their studies of $HCO_3$ secretion by *in vitro* toad colon. Presumably, cellular $HCO_3$ production is catalyzed from $CO_2$ by carbonic anhydrase and thus made available for secretion from cell-to-mucosal solution in exchange for mucosal Cl. This scheme for cellular $HCO_3$ production is also consistent with the relatively high levels of carbonic anhydrase in rat and guinea pig colon reported by Carter and Parsons [5], and with the findings of Phillips and Schmalz [26] and Parsons [22] that the carbonic anhydrase inhibitor, acetazolamide, markedly reduces both Cl absorption and $HCO_3$ secretion by rat colon.

Binder and Rawlins [3] have reported evidence for coupling between the absorption of Na and Cl. Addition of glucose to the solutions bathing isolated rat colon markedly stimulated the absorption of both Na and Cl with no change in the short-circuit current. Replacement of either Na with choline or Cl with isethionate prevented the increases in Cl or Na absorption elicited by glucose. These results are consistent with a direct interaction of Na and Cl with a transport mechanism for both ions. These findings are difficult to reconcile with the studies of Curran and Schwarz [9] and Edmonds and Marriott [13] on *in vivo* rat colon discussed previously. Thus, for the case of rat colon, the ionic basis of the short-circuit current and transepithelial *PD* remains to be resolved. However, for rabbit colon the present evidence strongly implicates net Na transport as the sole determinant of the short-circuit current while chloride appears to be absorbed by a Na-independent anion exchange process.

### *Effect of Cyclic AMP*

In the presence of cyclic AMP, net Cl movement is converted from a neutral absorptive process to an electrogenic secretory process. In addition, the residual ion flux is abolished so that the neutral anion exchange mechanism which normally results in active Cl absorption appears to be interrupted. Thus, in the presence of cyclic AMP the net fluxes of both Na and Cl account for the enhanced $I_{sc}$ (Table 4).

The effects of cyclic AMP on electrolyte transport by several epithelia have now been investigated and Table 6 illustrates certain relationships which have emerged from these studies. In rabbit gallbladder, Na and Cl absorption are obligatorily coupled by virtue of a neutral NaCl influx mechanism located at the mucosal membrane [17]. This NaCl influx process is inhibited by cyclic AMP so that the effect of this agent on the

Table 6. Relation between presence of coupled NaCl transport mechanisms and effects of cyclic AMP in various epithelia

| Epithelium | Rabbit Gall-bladder | Rabbit Ileum | Rat Colon | Rabbit Colon | Cornea | Seminal Vesicle |
|---|---|---|---|---|---|---|
| Increased $I_{sc}$ | 0 | + | + | + | + | + |
| Cl secretion elicited | 0 | + | + | + | + | + |
| Na absorption decreased | + | + | + | 0 | 0 | 0 |
| Presence of coupled NaCl transport | + | + | + | 0 | 0 | 0 |

gallbladder is an inhibition of *neutral* NaCl *absorption* [17]. The small transepithelial electrical potential difference across the gallbladder is not influenced by the cyclic nucleotide, and Cl secretion is not observed [17]. In contrast, cyclic AMP elicits Cl secretion *and* stimulates the short-circuit current across rabbit colon, frog and rabbit cornea [33], and guinea pig seminal vesicle [19]. However, in these epithelia there is no evidence that Na and Cl absorption are coupled and there is, likewise, no effect of cyclic AMP on Na absorption. Rabbit ileum and rat colon appear to lie between these two extremes sharing characteristics in common with both groups. In rabbit ileum approximately 50 % of active Na absorption is coupled to the simultaneous absorption of Cl [21] by means of a neutral influx mechanism at the mucosal membrane that is inhibited by cyclic AMP [20]. As discussed previously, Binder and Rawlins [3] have presented evidence suggesting that Na and Cl absorption by rat colon are coupled. In these tissues, cyclic AMP increases the $I_{sc}$, abolishes Na absorption and promotes Cl secretion [2, 14, 21]. In rabbit ileum, Na absorption is abolished[1] since the coupled NaCl influx process is inhibited by cyclic AMP.

In all instances, the increase in short-circuit current is associated with Cl secretion which appears to be electrogenic, and, in every case, Na absorption is affected only in those tissues which possess a coupled transport process for NaCl. It is of interest in this regard that some controversy has developed regarding the nature of the Cl secretion elicited by cyclic AMP in rabbit ileum, i.e., neutral *vs.* electrogenic. The results of this survey suggest that the effect of cyclic AMP on Cl transport by rabbit ileum may involve both an inhibition of neutral, Na-coupled, Cl absorption and stimulation of electrogenic Cl secretion. Indeed, the primary effect of cyclic AMP appears to be on Cl transport mechanisms in various epithelia. In tissues where the transport of other ions is coupled to the movement of Cl, their movements are also affected. Thus, in rabbit colon Cl absorption appears to be coupled to the simultaneous secretion of $HCO_3$ and the effect of cyclic AMP is not only to elicit Cl secretion but also to abolish the neutral exchange of Cl for $HCO_3$.

### *Active and Passive Conductance Pathways*

Total tissue conductance is the sum of conductances due to active, current-generating ion transport processes ($_aG_i$) and that due to diffusional

1 Sheerin and Field [29] have recently reported that elevation of the $HCO_3$ concentration or pH of the serosal solution results in Na secretion by rabbit ileum in the presence of cyclic AMP.

ion movements across the tissue (${}_pG_i$). Thus,

$$G_t = \sum {}_aG_i + \sum {}_pG_i.$$

Analysis of the passive components of unidirectional Na and K fluxes across rabbit colon suggests that the entire serosa-to-mucosa fluxes of these ions can be attributed to diffusional movements (Fig. 3, Table 5). In contrast, diffusional movement of Cl accounts for only one-third of the total serosa-to-mucosa Cl flux under short-circuit conditions, the remaining two-thirds being independent of the transepithelial *PD*. This relatively large non-diffusional Cl movement could represent Cl−Cl exchange as was suggested by Cooperstein and Hogben [8] for frog colon.

The diffusional unidirectional flux of an ion under short-circuit conditions ${}_{0d}J^i_{sm}$ expressed in μEq/cm²hr is numerically equal to its partial ionic conductance due to diffusion through passive conductance pathways ${}_pG_i$ expressed in mmhos/cm² [18]. The sum of the partial ionic conductances of Na, Cl and K as shown in Table 5 is 3.2 mmhos/cm². The tissue conductance $G_t$ in these studies averaged 4.2 mmhos/cm² so that the diffusional movements of Na, Cl and K account for 76 % of the total tissue conductance. As shown in Table 2, replacement of $HCO_3$ in the bathing media with $SO_4$ reduced $G_t$ by approximately 0.4 mmhos/cm². If this value represents the diffusional $HCO_3$ conductance, we may estimate that approximately 85 % of the conductance of the tissue is attributable to ionic diffusion.

The remaining 15 % (0.6 mmhos/cm²) of tissue conductance appears to be due to active, current-generating ion transport ${}_aG_i$. This value is in good agreement with the reduction in $G_t$ which accompanies inhibition of active Na absorption by amiloride (Fig. 2 and *unpublished observations*). The observation that active Na transport is the only transport process which contributes to the $I_{sc}$ under control conditions suggests that the active absorption of Na accounts for approximately 0.6 mmhos/cm² of the total tissue conductance in these experiments. These findings support the conclusion that the active movements of ions other than Na across the tissue represent nonconductive or neutral transport processes.

Replacement of an ion in the bathing media with an impermeant substitute should result in a decrease in tissue conductance equal to ${}_{0d}J^i_{sm}$ if the net movement of that ion does not contribute to the $I_{sc}$. Substitution of choline for Na results in a decrease in tissue conductance of 1.3 ± 0.3 mmhos/cm² (Table 2). However, as discussed above, the active and passive components of Na conductance are 0.6 and 1.1 mmhos/cm²,

respectively, suggesting that Na replacement should have decreased tissue conductance by 1.7 mmhos/cm$^2$. The finding that the decrease in tissue conductance is somewhat less than that expected may indicate that choline is not a strictly impermeant substitute for Na. However, as shown by the data of Table 3, the decrease in tissue conductance expected from inhibition of active Na absorption with ouabain is not observed, and, during *prolonged* exposure to amiloride, tissue conductance may not differ significantly from prior control values. Recently, Chen and Walser [6] reported a 67 % decrease in tissue conductance of toad urinary bladder in the presence of ouabain. They attributed this ouabain-sensitive conductance to active Na transport since passive Na fluxes were not affected by the glycoside. Since active Na conductance in rabbit colon appears to account for only 15 % of total tissue conductance, it is clear that a small increase in passive conductance of this epithelium could easily offset the decline expected from abolition of ${}_aG_{Na}$. Thus, it is possible that ouabain, Na-free media and prolonged exposure to amiloride cause a nonspecific increase in passive conductance which obscures the transport-related conductance change in rabbit colon. The apparent increases in bidirectional Cl fluxes in the presence of Na-free media and in serosa-to-mucosa Na flux in the presence of ouabain are consistent with this conclusion. Thus, the *rapid* decline in tissue conductance following addition of amiloride (Fig. 2) appears to provide the most reliable estimate of active Na conductance since possible nonspecific effects due to the inhibition of active Na absorption are probably insignificant at this time.

Replacement of Cl with isethionate decreased total conductance by 0.9 $\pm$ 0.3 mmhos/cm$^2$ (Table 2) which is in reasonable agreement with the passive Cl conductance of 1.5 $\pm$ 0.4 mmhos/cm$^2$ (Table 5). In several preliminary experiments, sulfate was employed as a substitute for Cl and tissue conductance decreased 1.2 $\pm$ 0.2 mmhos/cm$^2$ (n = 6) compared to control values. Thus, isethionate and sulfate appear to be relatively impermeant substitutes for Cl.

In the presence of cyclc AMP, active Cl secretion contributes to the $I_{sc}$ so that the increase in tissue conductance following addition of cyclic AMP may represent the addition of an active conductance component due to Cl secretion. However, the increase in total conductance is also paralleled by a trend toward increased bidirectional fluxes of Na (with no change in net Na flux). The changes in tissue conductance and $J_{sm}^{Na}$ are 41 and 51 %, respectively. This finding, together with the observation that the entire serosa-to-mucosa Na flux appears to be strictly diffusional, suggests that at least a fraction of the elevated tissue conductance in the

presence of cyclic AMP is due to an increase in the passive conductance to ions. If this is correct, then it is likely that the increase in $J_{sm}^{Cl}$ with cyclic AMP is, in part, due to an increase in passive permeability of the tissue.

At present we cannot identify the anatomic counterparts of the passive conductance pathways across rabbit colon.[2] However, there is reason to believe that the diffusional movements of K are restricted to a paracellular pathway. If, in rabbit colon, high cellular K concentrations are maintained by an active K uptake mechanism restricted to the basolateral membranes, as appears to be the case for other epithelia [28, 32], then a significant transcellular K conductance should lead to active K secretion. Net K transport was not observed under short-circuit conditions; however, when when amphotericin B is added to the mucosal solution alone, active K secretion is observed *(unpublished observations)*. Thus, under control conditions, the bidirectional fluxes of K across rabbit colon, which are diffusional, appear to be excluded from the transcellular pathway by virtue of a low mucosal membrane permeability to K.

If this is true, then some features of the ion selectivity of the paracellular pathway can be inferred from these studies. As shown in Table 5, ${}_{0d}J_{sm}^{i}/[i]$ (where $[i]$ is the ion concentration in the bathing media) gives the transepithelial permeability to diffusional ion flow $P_i$. These values were calculated for Na, Cl and K and are given in the last column of Table 5. The values for $P_{Na}$ and $P_{Cl}$ represent the upper limit for permeation through the paracellular pathway since a transcellular passive conductance for these ions cannot be excluded. Thus, the paracellular pathway appears to favor the diffusion of K over Cl since the *upper limit* on Cl permeability via this route is tenfold less than the K permeability ($P_{Cl}/P_K = 0.11$), whereas the free-solution mobilities of these ions are roughly equal. Similarly, the *upper limit* on the Na permeability of the K conductance pathway ($P_{Na}/P_K = 0.07$) is tenfold lower than that expected from their free-solution mobility ratio ($\lambda_{Na}/\lambda_K = 0.7$). This suggests that there is a marked steric restriction on the movement of Na compared to K via the paracellular conductance pathway. An interesting finding which emerges from these calculations is that $P_{Na}/P_{Cl}$ (0.64) is the same as the ratio of their mobilities in free solution ($\lambda_{Na}/\lambda_{Cl} = 0.7$). This may suggest that, rather than restricting the diffusional

2 In general, a strictly linear relation between $J_{sm}^{i}$ and $[\xi/(\exp \xi) - 1]$ (Fig. 3) implies diffusion through a paracellular pathway. However, under some circumstances apparent linearity (within experimental error) could be observed over the range $\pm 50$ mV even though diffusion is transcellular (Schultz and Frizzell, *unpublished observations*). The good agreement between ${}_pG_i$ calculated from Fig. 3 and that observed when ions are replaced with impermeant species supports our analyses and suggests that most of ${}_{0d}J_{sm}^{i}$ is paracellular.

flows of Na and Cl, there may be some property of the passive conductance pathway(s) that markedly augments the diffusion of K. Indeed this finding may implicate more than one pathway for the movement of K across rabbit colon, one of which may behave as a highly K selective shunt, while the ionic permeabilities of the other pathway may allow ions to diffuse at rates in accordance with their free-solution mobilities. Clearly, one candidate for the latter pathway is a damaged edge of tissue lying at the circumference of the chamber aperture. In any event, these data indicate that the passive conductance pathway(s) across rabbit colon restrict the passive backflux of the actively transported ions, Na and Cl, while permitting the passive secretion of K down a favorable electrical gradient under open-circuit conditions.

The authors gratefully acknowledge the expert technical assistance of Mr. Dennis Clayton. This study was supported by Public Health Service Research Grants from the National Institute of Arthritis, Metabolism and Digestive Diseases (AM 18199 and AM 16275) and the American Heart Association (75620).

## References

1. Archampong, E.Q., Harris, J., Clark, C.G. 1972. The absorption and secretion of water and electrolytes across the healthy and the diseased human colonic mucosa measured *in vitro*. *Gut* **13**:880
2. Binder, H.J., Rawlins, C.L. 1973. Effect of conjugated dihydroxy bile salts on electrolyte transport in rat colon. *J. Clin. Invest.* **52**:1460
3. Binder, H.J., Rawlins, C.L. 1973. Electrolyte transport across isolated large intestinal mucosa. *Am. J. Physiol.* **225**:1232
4. Carlinsky, N.J., Lew, V.L. 1970. Bicarbonate secretion and non Na component of the short-circuit current in the isolated colonic mocosa of *Bufo arenarum*. *J. Physiol. London* **206**:529
5. Carter, M.J., Parsons, D.S. 1971. The isoenzymes of carbonic anhydrase: tissue, subcellular distribution and functional significance with particular reference to the intestinal tract. *J. Physiol. London* **215**:71
6. Chen, J.S., Walser, M. 1975. Sodium fluxes through the active transport pathway in toad bladder. *J. Membrane Biol.* **21**:87
7. Cofre, G., Crabbe, J. 1967. Active sodium transport by the colon of *Bufo marinus*: Stimulation by aldosterone and antidiuretic hormone. *J. Physiol. London* **188**:177
8. Cooperstein, I.L., Hogben, C.A.M. 1959. Ionic transfer across isolated frog large intestine. *J. Gen. Physiol.* **42**:461
9. Curran, P.F., Schwartz, G.F. 1960. Na, Cl, and water transport by rat colon. *J. Gen. Physiol.* **43**:555
10. D'Agostino, A., Leadbetter, W.F., Schwartz, W.B. 1953. Alterations in the ionic composition of isotonic saline solution instilled into the colon. *J. Clin. Invest.* **32**:444

11. Devroede, G.J., Phillips, S.F. 1969. Conservation of sodium, chloride, and water by the human colon. *Gastroenterology* **56**:101
12. Dietz, J., Field, M. 1973. Ion transport in rabbit ileal mucosa. IV Bicarbonate secretion. *Am. J. Physiol.* **225**:858
13. Edmonds, C.J., Marriott, J. 1968. Electrical potential and short-circuit current of an *in vitro* preparation of rat colon mucosa. *J. Physiol. London* **194**:479
14. Field, M. 1971. Ion transport in rabbit ileal mucosa. II. Effects of cyclic 3′, 5′-AMP. *Am. J. Physiol.* **221**:992
15. Field, M., Fromm, D., McColl, I. 1971. Ion transport in rabbit ileal mucosa. I. Na and Cl fluxes and short-circuit current. *Am. J. Physiol.* **220**:1388
16. Field, M., McColl, I. 1973. Ion transport in rabbit ileal mucosa. III. Effects of catecholamines. *Am. J. Physiol.* **225**:852
17. Frizzell, R.A., Dugas, M.C., Schultz, S.G. 1975. Sodium chloride transport by rabbit gallbladder: Direct evidence for a coupled NaCl influx process. *J. Gen. Physiol.* **65**:769
18. Frizzell, R.A., Schultz, S.G. 1972. Ionic conductances of extracellular shunt pathway in rabbit ileum: Influence of shunt on transmural sodium transport and electrical potential differences. *J. Gen. Physiol.* **59**:318
19. Levine, N., Rinaldo, J.E., Schultz, S.G. 1975. Ion transport by guinea pig seminal vesicle. *J. Physiol. London* **246**:197
20. Nellans, H.N., Frizzell, R.A., Schultz, S.G. 1973. Coupled sodium-chloride influx across the brush border of rabbit ileum. *Am. J. Physiol.* **225**:467
21. Nellans, H.N., Frizzell, R.A., Schultz, S.G. 1974. Brush border processes and transepithelial Na and Cl transport by rabbit ileum. *Am. J. Physiol.* **226**:1131
22. Parsons, D.S. 1956. The absorption of bicarbonate-saline solutions by the small intestine and colon of the white rat. *Q. J. Exp. Physiol.* **41**:410
23. Parsons, D.S., Paterson, C.R. 1965. Fluid and solute transport across rat colonic mucosa. *Q. J. Exp. Physiol.* **50**:220
24. Phillips, S.F. 1969. Absorption and secretion by the colon. *Gastroenterology* **56**:966
25. Phillips, S.F., Code, C.F. 1966. Sorption of potassium in the small and large intestine. *Am. J. Physiol.* **211**:607
26. Phillips, S.F., Schmalz, P.F. 1970. Bicarbonate secretion by the rat colon: effect of intraluminal chloride and acetazolamide. *Proc. Soc. Exp. Biol. Med.* **135**:116
27. Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1974. Ion transport by mammalian small intestine. *Annu. Rev. Physiol.* **36**:51
28. Schultz, S.G., Zalusky, R. 1964. Ion transport in isolated rabbit ileum: I. Short-circuit current and Na fluxes. *J. Gen. Physiol.* **47**:567
29. Sheerin, H.E., Field, M. 1975. Ileal $HCO_3$ secretion: Relationship to Na and Cl transport and effect of theophylline. *Am. J. Physiol.* **228**:1065
30. Turnberg, L.A. 1970. Electrolyte absorption from the colon. *Gut* **11**:1049
31. Ussing, H.H., Anderson, B. 1955. The relation between solvent drag and the active transport of ions. *Proc. 3rd Int. Cong. Biochem.* (Brussells), p. 434
32. Ussing, H.H., Erlij, D., Lassen, U. 1974. Transport pathways in biological membranes. *Annu. Rev. Physiol.* **36**:17
33. Zadunaisky, J.A., Lande, M.A., Chalfie, M., Neufeld, A.H. 1973. Ion pumps in the cornea and their stimulation by epinephrine and cyclic AMP. *Exp. Eye Res.* **15**:577