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# Active Potassium Transport by Rabbit Descending Colon Epithelium

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**Summary.** Previous studies of rabbit descending colon have disagreed concerning potassium transport across this epithelium. Some authors reported active $K^+$ secretion under *in vitro* short-circuited conditions, while others suggested that $K^+$ transport occurs by passive diffusion through a highly potassium-selective paracellular route. For this reason, we re-examined potassium fluxes across the colon in the presence of specific and general metabolic inhibitors. In addition, electrochemical driving forces for potassium across the apical and basolateral membranes were measured using conventional and ion-sensitive microelectrodes. Under normal conditions a significant net $K^+$ secretion was observed ($J^K_{net} = -0.39 \pm 0.081$ $\mu$eq/cm$^2$hr) with $^{42}K$ fluxes, usually reaching steady-state within approximately 50 min following isotope addition. In colons treated with serosal addition of $10^{-4}$M ouabain, $J^K_{sm}$ was lowered by nearly 70% and $J^K_{ms}$ was elevated by approximately 50%. Thus a small but significant net absorption was present ($J^K_{net} = 0.12 \pm 0.027$ $\mu$eq/cm$^2$hr). Under control conditions, the net cellular electrochemical driving force for $K^+$ was 17 mV, favoring $K^+$ exit from the cell. Cell potential measurements indicated that potassium remained above equilibrium after ouabain, assuming that passive membrane permeabilities are not altered by this drug. Net $K^+$ fluxes were abolished by low temperature.

The results indicate that potassium transport by the colon may occur via transcellular mechanisms and is not solely restricted to a paracellular pathway. These findings are consistent with our previous electrical results which indicated a nonselective paracellular pathway. Thus potassium transport across the colon can be modeled as a paracellular shunt pathway in parallel with pump-leak systems on the apical and basolateral membranes.

**Key words** epithelium · potassium · transport · membrane potentials · intracellular $K^+$ activity · $^{42}K$ fluxes

## Introduction

Colon epithelium *in vivo* is the site of potassium secretion in a number of species including man (Edmonds, 1967; Hawker, Mashiter & Turnberg, 1978; Halm & Dawson, 1980). However, the mechanism of potassium transport across this epithelium has been disputed. In some studies potassium secretion was greater than expected from transepithelial electrical or chemical driving forces (*c.f.* Archampong, Harris & Clark, 1972; Yorio & Bentley, 1977; Powell, 1979; Kermode & Edmonds, 1980; Kliger, Binder, Bastl & Hayslett, 1981), suggesting active transport mechanisms. In contrast other investigations have shown evidence against active potassium transport. In a series of radioisotopic studies of the isolated rabbit descending colon epithelium, Frizzell, Schultz and co-workers (Frizzell, Koch & Schultz, 1976; Frizzell & Schultz, 1978; Frizzell & Turnheim, 1978) observed no net transport of potassium under *in vitro* short-circuited conditions. On the basis of these and $^{42}K$ uptake studies these authors also concluded that the apical membrane was essentially impermeable to potassium and that potassium transport occurred by diffusion through a highly potassium-selective paracellular pathway (*see also* Fromm & Schultz, 1980; Schultz, 1981).

Recent electrophysiological investigations of the rabbit descending colon, however, argue against a purely diffusional mechanism for potassium transport via a potassium-selective paracellular pathway. Wills et al. (1979*b*) found that replacement of mucosal $Na^+$ with $K^+$ did not significantly change the transepithelial potential as one might expect from the selectivity values previously reported (Frizzell et al., 1976). In addition, microelectrode measurements and equivalent circuit analysis of this epithelium indicated that the paracellular shunt was essentially nonselective since no significant electromotive force (emf) could be detected in the paracellular shunt pathway when a potassium gradient was applied across the epithelium. Furthermore, measurements of the resistance ratio (the ratio of apical membrane resistance to basolateral membrane resistance) revealed a significant conductance in the apical membrane after replacement of the mucosal NaCl bathing solution with $K_2SO_4$ Ringer's. Similar findings have been obtained in impedance studies of this epithelium (Clausen & Wills, 1981), suggesting that the apical membrane has an appreciable potassium conductance.

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Because of these discrepancies, the present paper re-examines potassium fluxes across the rabbit descending colon using the same type of preparation used in our previous microelectrode and impedance studies. It was expected that if potassium transport occurs strictly through a paracellular mechanism, then the fluxes of potassium under short-circuited conditions should be unaffected by manipulations which affect cellular potassium levels, given the absence of mechanical changes such as lateral intercellular space constriction. Conversely, if potassium transport occurs by an active mechanism then potassium secretion would most likely involve the Na—K ATPase on the basolateral membrane and should be inhibited by ouabain or general metabolic inhibitors such as cooling.

In the present experiments we determined $^{42}K$ fluxes under normal conditions, during low temperature, and after addition of $10^{-4}$ M ouabain to the serosal solution. In addition, we report membrane potentials and intracellular potassium activities for normal conditions and after ouabain inhibition of the Na—K pump.

## Materials and Methods

Colons were obtained from white male New Zealand rabbits (2–3 kg). The animals were fed a normal diet (Purina) and given constant access to tap water. Following sacrifice of the rabbit with an intravenous injection of sodium pentobarbital, a section of descending colon was removed. Underlying muscle layers were dissected away using blunt dissection (Frizzell et al., 1976), and the tissue was mounted vertically between Ussing-type chambers that were modified to minimize edge damage (Lewis, Wills & Eaton, 1978). For isotope studies a four-port chamber was used. Bathing solutions consisted of (in mM): 136.2, $Na^+$; 7, $K^+$; 121, $Cl^-$; 2, $Ca^{2+}$; 1.2, $Mg^{2+}$; 25, $HCO_3^-$; 1.2, $H_2PO_4^-$; 1.2, $SO_4^{2-}$; and 11.1, glucose. Unless otherwise specified, all experiments were performed at 37 °C. Solution mixing, oxygenation, and pH regulation were maintained by continuous bubbling with a mixture of 95% $O_2$ and 5% $CO_2$. During cooling experiments the $CO_2$ content was decreased such that the pH was maintained at 7.4. Ouabain (Sigma) was dissolved into distilled water for a concentrated stock solution that was added to the bathing solution to a concentration of $10^{-4}$ M.

For radioisotopic flux determinations, four flux measurements were made in adjacent segments from the same tissue. Unidirectional fluxes from mucosa to serosa ($J_{ms}$) and from serosa to mucosa ($J_{sm}$) were determined in adjacent tissue pairs giving a total of two paired bi-directional fluxes per experiment. The exposed area of each segment was 1.27 $cm^2$ and the chamber volume was 3 ml. After a 20 to 30-min equilibration period in nonlabeled solutions, the bathing solutions were replaced and $^{42}K$-labeled solution (30 μCi; New England Nuclear) was added to one side. After a 20-min isotope equilibration period, samples were taken at 10-min intervals. Samples were counted on a gamma counter with appropriate corrections for decay, sampling period duration and background activity. To calculate specific activity, the $Na^+$ and $K^+$ concentrations of the labeled solutions were measured with a flame photometer.

Electrical measurements closely followed that of Wills et al. (1979*b*). Briefly, the transepithelial potential ($V_T$) was measured by Ag—AgCl wires placed close to and on either side of the epithelium. Current passing electrodes also consisted of Ag—AgCl wires placed at the rear of each chamber. The short-circuit current ($I_{sc}$) was defined as the amount of current required to reduce $V_T$ to 0 mV. Signals were measured with the aid of a storage oscilloscope (Tektronics, Model 5115) and a digital printer (Newport, Model 810). In microelectrode studies potentials were measured with a high impedance (WP Instruments, Model 750) electrometer and ion-sensitive microelectrode potentials were measured with an ultra-high impedance electrometer (WP Instruments Model F223A). These potentials were interfaced along with the transepithelial potential and current measurements to a 12 bit analog-to-digital converter and microprocessor computer system (North Star, Horizon II). This arrangement allowed measurement of all potentials to within ±0.1 mV. The transepithelial potential measurements were referenced to the serosal solution. All membrane potentials are reported with reference to the outside bathing solutions.

Tissues were continuously short-circuited except for brief periods (less than 30 sec) when the spontaneous open-circuit potential was measured. Series resistance was compensated for electronically using an automatic voltage clamp. Tissue resistance was calculated from $V_T$ and the short-circuit current using Ohm's law ($V_T/I_{sc}=R_T$) or by passing a current pulse across the epithelium ($\Delta V_T/\Delta I=R_T$). Similar to our previous electrical studies (Wills et al., 1979*b*) and the procedure of Frizzell et al. (1976), experiments were rejected if $R_T$ was below 150 Ω $cm^2$ or if $R_T$ differed by more than 25% between tissue segments.

### *Microelectrode Construction and Methods*

Microelectrodes were constructed from thin-walled borosilicate capillary tubing (Corning #7740). Tip resistances ranged from 12–30 MΩ when filled with 3 M KCl. Potassium-sensitive microelectrodes were fabricated in the same manner except that the microelectrodes were silanized before use. Silanization was achieved by heating the electrodes to 200 °C for 20 min then exposing them to *n*-trichlorobutrylchlorosilane vapors followed by baking for 10 min. After cooling, the tips were filled with potassium exchanger resin (Corning #477317; Medfield, Mass.) and the remainder of the shaft was filled with 0.5 M KCl. The microelectrodes were calibrated in a regime of pure and mixed salt solutions and in NaCl—$NaHCO_3$ Ringer's solution according to procedures described previously (Wills & Lewis, 1980; Wills et al., 1979*b*). Calibrations were repeated immediately before and after each use.

Intracellular $K^+$ activity ($a_iK$) was calculated as:

$$a_iK=(a_oK+K_{K/Na}\cdot a_oNa)\cdot\left[\exp\frac{nFz}{RT}(V_i-V_o-V_{bl})\right]-K_{K/Na}\cdot a_iNa \quad (1)$$

where $K_{K/Na}$ is selectivity for potassium over sodium, $a_oNa$ is the activity of Na in the extracellular fluid, $V_{bl}$ is the basolateral membrane potential, $V_i$ is the ion-sensitive potential, $n$ is a correction factor for nonideal slopes, $V_o$ is the microelectrode reading in the outside solution (arbitrarily adjusted to zero) and $R$, $T$, $z$, and $F$ have their usual meanings. Mean slope and selectivity for these electrodes averaged 59.7 ± 0.27 mV/decade and 41 ± 11.0:1, respectively. $a_iNa$ was unknown and assumed to be negligible for normal conditions. An intracellular $Na^+$ equal to the bulk bathing solution would introduce an error of less than 3 mM above the true value of $a_iK$.

**Table 1.** Effects of ouabain on $K^+$ fluxes and electrical properties of the colon

| $\mu$eq/cm$^2$hr | Control | Ouabain | $\Delta$ Paired |
|---|---|---|---|
| $J^K_{ms}$ | 0.17 ± 0.034 | 0.26 ± 0.054[a] | 0.13 ± 0.035 |
| $J^K_{sm}$ | 0.54 ± 0.113 | 0.17 ± 0.036[a] | −0.34 ± 0.069 |
| $J^K_{net}$ | −0.39 ± 0.081 | 0.12 ± 0.027[a] | 0.46 ± 0.052 |
| $I_{sc}$ | 2.88 ± 0.191 | 0.45 ± 0.052[a] | −2.68 ± 0.026 |
| $G_T$ (mS/cm$^2$) | 3.40 ± 0.204 | 3.64 ± 0.275 | 0.61 ± 0.157 |
| $n$ | 23 | 21 | 13 |

$n$ = no. of tissues.
$\bar{x} \pm$ SEM
[a] $P < 0.005$.

The following results are expressed as means ± SEM based on the number of tissues studied. Differences between means were analyzed using the $t$-test for paired or independent means where appropriate.

## Results

In this section we first describe the effects of serosal ouabain on $^{42}K$ fluxes. We then report the effects of low temperature on net $K^+$ fluxes in normal and ouabain-treated colons. In the final section we present measurements of intracellular potassium activity and the electrochemical gradient for this ion across each of the cell membranes.

### *Time Course of $^{42}K$ Measurements*

Control colons showed a statistically significant secretion of potassium from serosa to mucosa ($J_{net} = -0.39 \pm 0.081$ $\mu$eq/cm$^2$hr; *see* Table 1). The mucosal-to-serosal flux ($J_{ms}$) was low and relatively constant during the first 30 to 50 min ($J_{ms} = 0.17 \pm 0.034$ $\mu$eq/cm$^2$hr). The serosal-to-mucosal $^{42}K$ flux, in contrast, showed a clear increase during the first 20–50 min following isotope addition and then reached a stable plateau value ($J_{sm} = 0.54 \pm 0.113$ $\mu$eq/cm$^2$hr). The initial increase in isotopic $J_{sm}$ reflects $^{42}K$ loading into the epithelium. A representative experiment is shown in Fig. 1.

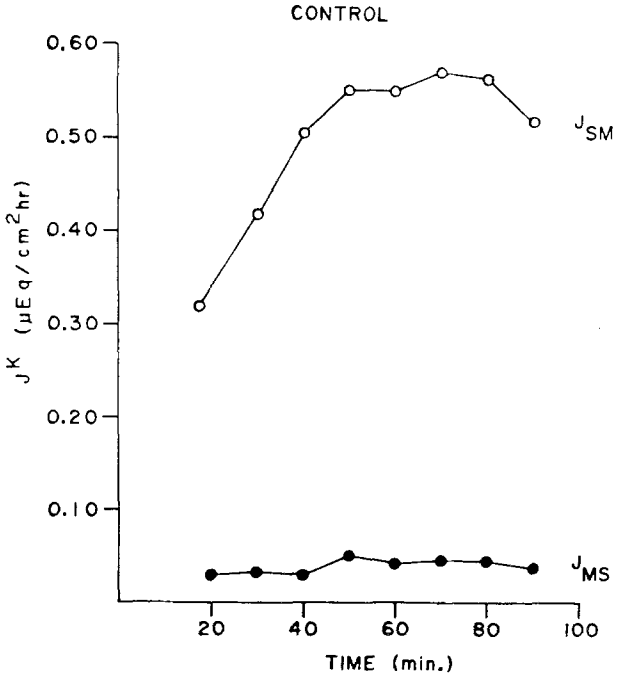


**Fig. 1.** Representative example of unidirectional $K^+$ fluxes from a control experiment. The colon was short circuited ($V_T = 0$) and isotope ($^{42}K$) was added at $t = 0$. ($t$ refers to time after isotope addition; samples at $t = 10$ were routinely eliminated from data analysis.) Note that in this experiment mucosal to serosal flux ($J^K_{ms}$) was relatively constant whereas the serosal to mucosal flux ($J^K_{sm}$) did not reach steady state until approximately 50 min after isotope addition. A statistically significant secretion ($J^K_{net}$) was observed throughout the experiment

### *Effects of Ouabain*

Colons treated with $10^{-4}$ M serosal ouabain showed the opposite pattern to that seen in control tissues. Ouabain resulted in a small but statistically significant net absorption of potassium ($J_{net} = 0.12 \pm 0.027$; *see* Table 1). $J_{sm}$ was significantly lower in ouabain-treated colons ($J_{sm} = 0.17 \pm 0.036$) as shown in Fig. 2 and Table 1. In these experiments, ouabain was added at the same time as the $^{42}K$-labeled solution. Both $J_{sm}$ and $J_{ms}$ reached stable values within approximately 30 min. In addition net absorption of $K^+$ was observed for periods of 1 hr or longer.

In a second group of tissues, fluxes were determined in the same tissues before and after ouabain treatment. A representative experiment is shown in Fig. 3 which shows the time course of ouabain effects on the fluxes. Note that $J_{ms}$ increases almost immediately, whereas $J_{sm}$ takes nearly 30 min to reach a new steady-state value. These changes are summarized in Table 1 as "$\Delta$ paired" values ($n = 13$). The striking effects of ouabain illustrated in Fig. 3 include: (i) the decrease in $J_{sm}$ ($\Delta J_{sm} = -0.34 \pm 0.069$), (ii) the reversal of the direction of net $K^+$ transport and (iii) the sustained elevation of $J_{ms}$ ($\Delta J_{ms} = 0.13 \pm 0.35$). As indicated in this figure, $J_{ms}$ before ouabain was not significantly different from $J_{sm}$ after ouabain. In both control and ouabain-treated colons the net transport of potassium occurred in the absence of an apparent net electrochemical gradient for this ion since the tissue was short-circuited and

**Table 2.** Effects of temperature on potassium fluxes

| $\mu$eq/cm$^2$ hr | Control | | | Ouabain | | |
|---|---|---|---|---|---|---|
| | $J^K_{ms}$ | $J^K_{sm}$ | $J^K_{net}$ | $J^K_{ms}$ | $J^K_{sm}$ | $J^K_{net}$ |
| 37 °C | 0.24±0.182 | 0.80±0.110 | −0.57±0.055 | 0.22±0.010 | 0.07±0.005 | 0.15±0.008 |
| 12 °C | 0.19±0.146 | 0.18±0.055[a] | 0.09±0.158[a] | 0.08±0.014[a] | 0.05±0.005 | 0.03±0.007[a] |
| $\Delta$ Paired | −0.05±0.041 | −0.62±0.051[a] | −0.66±0.093[a] | −0.14±0.004[a] | −0.02±0.001 | −0.12±0.005[a] |

$\bar{x}$±SEM
[a] $P<0.05$.

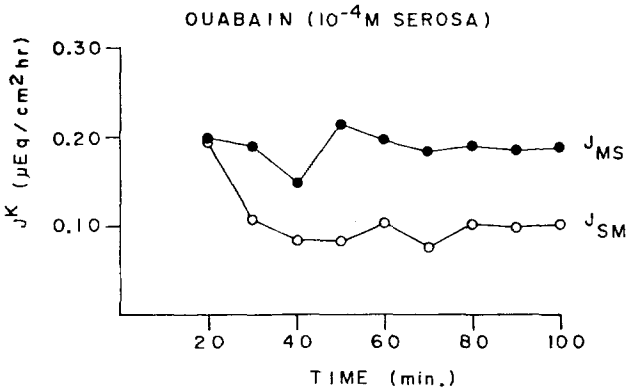


**Fig. 2.** Example of potassium fluxes for a ouabain-treated colon. The procedure was the same as in Fig. 1 except that $10^{-4}$ M ouabain was added to the serosal solution at $t=0$. $J^K_{sm}$ was significantly lower than in controls, and a small, statistically significant elevation occurred in $J^K_{ms}$. Within 30 min following ouabain addition a steady state was achieved with net active absorption

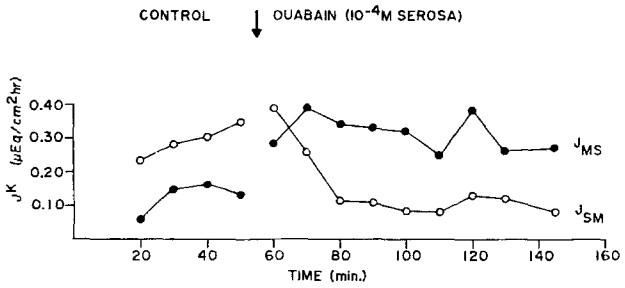


**Fig. 3.** A single experiment showing potassium fluxes before and after ouabain from the same tissue. $10^{-4}$ M ouabain was added to the serosal solution at $t=55$. In this experiment pre-ouabain fluxes were not permitted to reach steady state. Following ouabain addition steady-state values were achieved within 30 min, again showing net absorption in the absence of chemical or electrical driving forces

bathed on both sides with the same Ringer's solution.

Addition of ouabain to both the mucosal and serosal solutions or subsequent addition of mucosal ouabain to serosally ouabain-treated colons produced similar effects to serosal addition alone ($J^K_{ms}$ $=0.213\pm0.033$ $\mu$eq/cm$^2$hr, $J^K_{sm}=0.091\pm0.029$ $\mu$eq/cm$^2$hr, $n=3$; NS).

### *Effects of Low Temperature*

Because of the unexpected finding of net $K^+$ transport in both secretory and absorptive directions and the absence of a known specific inhibitor for $K^+$ absorption, fluxes were also determined in the presence of a more general yet reversible inhibitor of transport processes, low temperature. In this case tissues were cooled from 37 to 12 °C. The results of three such experiments are summarized in Table 2[1]. A representative flux experiment on segments from the same colon is shown in Fig. 4. In control tissues, cooling suppressed $J_{sm}$ ($\Delta J_{sm}=-0.62\pm0.051$) while $J_{ms}$ was unchanged. Conversely, in ouabain-treated colons, cooling caused a significant suppression of $J_{ms}$ ($\Delta J_{ms}=-0.14\pm0.004$) while $J_{sm}$ was not affected. There was no significant net transport of $K^+$ for either group during cooling. With respect to electrical parameters, $V_T$ and $I_{sc}$ were significantly suppressed by cooling in control tissues ($\Delta V_T=28$ $\pm10.8$ mV; $\Delta I_{sc}=62\pm21.1$ $\mu$A/cm$^2$; *see* Table 3). These results were qualitatively similar to the effects of ouabain. In ouabain-treated tissues, $V_T$ was slight-

[1] The control and ouabain experiments summarized in Tables 2 and 3 were paired experiments performed on tissues from the same animals. At the time that $^{42}$K-labeled solutions were introduced, ouabain was added to one pair of ports in the 4-port Ussing chamber. The remaining pair of ports was untreated. Two pairs of bi-directional fluxes, one for the control and one for the ouabain-treated condition, were then determined simultaneously. Because of the low sample size and larger variability in the control condition (due to large bi-directional fluxes in one control experiment), the mean values for control fluxes were larger than in Table 1. For the same reason, $J_{ms}$ values at 37 °C were not different for control and ouabain conditions in Table 2.

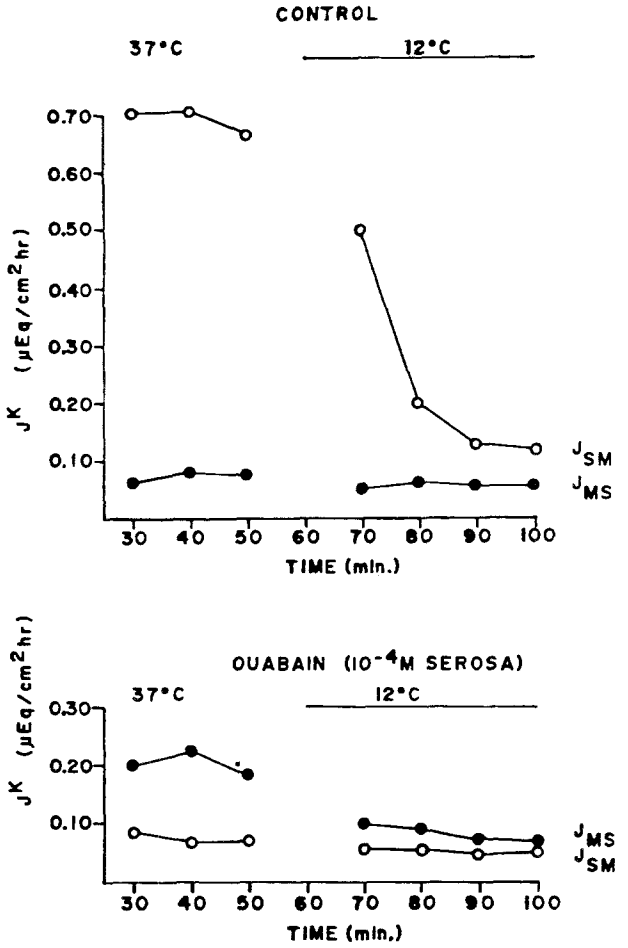


**Fig. 4.** An example of effects of lowered temperature on potassium fluxes in control (top graph) and ouabain-treated colons (bottom graph). Tissue segments were from the same animal, and the experiments were conducted simultaneously. Isotope was added at $t=0$. In the lower graph $10^{-4}$ M serosal ouabain was also added at this time. At $t=60$ the tissues were cooled to approximately 12 °C. In control tissues $J^K_{sm}$ decreased, similar to the effects of ouabain. However, unlike ouabain-treated colons, $J^K_{ms}$ remained unchanged. In contrast, the ouabain-treated colons showed the opposite pattern: $J^K_{ms}$ decreased while $J^K_{sm}$ was unaltered. After cooling, fluxes reached a new steady state in both conditions within 30–40 min. No significant net fluxes were found during cooling in this time period

ly reduced by cooling ($\Delta V_T = 1 \pm 0.5$ mV) and $I_{sc}$ was lower than at 37 °C ($\Delta I_{sc} = 8 \pm 2.8$ μA/cm²). Rewarming the tissues returned the flux and electrical measurements to approximately 80% of their initial values.

### *Microelectrode Results*

Table 4 presents the measured membrane potentials and calculated ion activities before and after ouabain addition ($10^{-4}$ M serosa). Before ouabain addition, the apical membrane potential averaged $-20 \pm 3.8$ mV (cell interior negative) and the basolateral membrane potential ($V_{bl}$) was $-63 \pm 3.3$ mV (cell interior negative). The averaged intracellular $K^+$ activity ($a_i K$) calculated from the $K^+$-sensitive microelectrodes was $73 \pm 6.1$ mM ($n=8$ animals; 3–6 readings per animal). The calculated equilibrium potential for potassium ($E_K$) was $-69 \pm 2.1$ mV. The electrochemical driving force ($\Delta\mu_K/F$) for this ion under open-circuited conditions was 49 mV for the apical membrane and 6 mV for the basolateral membrane, with both gradients favorable for $K^+$ exit from the cell. Under short-circuited conditions the apical and basolateral membrane potentials were both equal to $-56 \pm 3.1$ mV ($V^c_{I_{sc}}$ cell interior negative). Thus under short-circuited conditions $\Delta\mu_K/F$ was $17 \pm 2.8$ mV, again favoring $K^+$ exit across each membrane.

Following ouabain addition, $a_i K$ and the membrane potentials fell rapidly, reaching a new steady-state level within 20–30 min. Intracellular $K^+$ was reduced by approximately 85% from 73 mM to 11 $\pm 1.6$ mM. The basolateral membrane potential was decreased by approximately 80%, falling to an average value of $-14 \pm 1.0$ mV. The apical membrane potential was diminished by 11 mV with a post-

**Table 3.** Effects of temperature on the electrical properties of the colon

| | Control | | Ouabain | |
|---|---|---|---|---|
| | $V_T$ (mV) | $I_{sc}$ (μA/cm²) | $V_T$ (mV) | $I_{sc}$ (μA/cm²) |
| 37 °C | −29 ±10.4 | 70 ±19.6 | −4 ±0.9 | 16 ±2.2 |
| 12 °C | − 1.0[a] ± 1.2 | 8[a] ± 2.9 | −3[a] ±0.4 | 9[a] ±1.6 |
| Δ Paired | 28 ±10.8 | −62 ±21.1 | 1[a] ±0.5 | −8 ±2.7 |
| | $n=3$ | | | |

[a] $P<0.05$.

**Table 4.** Summary of conventional and $K^+$-sensitive intracellular recordings

| | $V_a^*$ (mV) | $V_{bl}^*$ (mV) | $V^{c*}_{I_{sc}}$ (mV) | $a_i^K$ (mM) | $E_K$ (mV) | $\Delta\tilde{\mu}_K/F$ (mV) |
|---|---|---|---|---|---|---|
| Control | −20 ±3.8 | −63 ±3.3 | −56 ±3.1 | 73 ±6.1 | −69 ±2.1 | 17 ±2.8 |
| $n=8$ | | | | | | |
| Ouabain | −9[a] ±1.4 | −14[a] ±1.0 | −13[a] ±2.6 | 11[a] ±1.6 | −18[a] ±3.8 | 7.0[a] ±3.8 |
| $n=6$ | | | | | | |

* Cell interior negative.
[a] $P<0.005$.

ouabain value of $-9 \pm 1.4$ mV. After ouabain the calculated $E_K$ was $-18 \pm 3.8$ mV and $\Delta\tilde{\mu}_K/F$ (for short-circuited conditions) was $7.0 \pm 3.8$ mV, favorable for $K^+$ exit. However, a portion of the measured $a_iK$ will reflect increased $a_iNa^+$. Therefore these values represent a maximum estimate for $E_K$ and $\Delta\tilde{\mu}_K/F$. This problem will be addressed in the discussion.

## Discussion

The present results indicate that potassium transport by the rabbit descending colon can occur by transcellular mechanisms in addition to passive diffusional mechanisms as previously proposed (Frizzell et al., 1976; Schultz, 1981). In the present experiments net potassium transport was observed in the absence of net electrical or chemical gradients across the epithelium. During control conditions the direction of the net transport was secretion from the serosal side to the lumen. However, after serosal addition of $10^{-4}$ M ouabain the direction of $K^+$ transport was reversed and net absorption was observed. A clear finding was the effect of ouabain on unidirectional fluxes. The serosa-to-mucosa flux ($J_{sm}$) was suppressed by nearly 70%, and a small but statistically significant increase occurred in the mucosa-to-serosal flux ($J_{ms}$). The time course of these changes were similar to the effects of the drug on cell membrane potentials and intracellular potassium activity ($a_iK$). Within 20–30 min $a_iK$ decreased by at least 85% and the intracellular potentials similarly decreased to new plateau values ($\Delta V^c_{Isc} \sim 43$ mV).

The finding of net absorption after ouabain, to our knowledge, is a new observation in the physiology of the rabbit colon. Such a result indicates that potassium transport by the colon may involve two possibly separated transport systems: a net secretory system and a net absorptive one. Both of these systems were suppressed by low temperature. Therefore the activity of these systems appear to be linked either directly or indirectly to the metabolic activity of the cell.

The finding of net potassium secretion, in contrast, is not new in studies of ion transport across the *in vitro* rabbit descending colon. The first report of net $K^+$ secretion under short-circuited conditions was by Yorio and Bentley (1977). In addition, we note that since our initial presentation of these results (Wills & Biagi, 1980), McCabe, Cooke and Sullivan (1981) have also reported preliminary evidence for net potassium transport in the rabbit colon.

Frizzell et al. (1976), using a similar preparation to that used in the present study, found no net transport of potassium under *in vitro* short-circuited conditions. The reasons for this apparent discrepancy in results are not at this time clear. There is no apparent discrepancy concerning transepithelial electrical properties such as conductance. It is obvious, however, that the mucosal membrane functions differently in the two experimental groups. While some subtle, yet critical, difference in experimental technique may exist, it seems more reasonable to expect that the metabolic state of the animal (e.g., diet or hormonal levels) may underlie this important difference. The relative activities of the secretory and reabsorptive systems, demonstrated in the present results, must reflect Na—K ATPase activity as well as apical and basolateral potassium conductances. Any or all of these factors may be under metabolic control. In this sense the present results raise several new issues concerning the regulation of potassium transport by the colon. In the following sections we address specific aspects of these issues and integrate these findings with our previous electrophysiological data from this epithelium.

### *Paracellular Potassium Transport*

According to our previous microelectrode determinations of the equivalent circuit parameters of the descending colon, the paracellular pathway has a conductance roughly equivalent to the resistance of the transcellular pathway (Wills et al., 1979*b*). Consequently, the role of paracellular potassium movements cannot be neglected when the magnitude of unidirectional transepithelial fluxes is considered. One can *estimate* the magnitude of paracellular $K^+$ movements relative to transcellular transport from the serosa-to-mucosa unidirectional fluxes before and after ouabain. To do so, it must be assumed that flux before ouabain represents both cellular and paracellular fluxes and that the flux after ouabain is restricted to the paracellular route. This ratio suggests that paracellular flux represents 30% of the total serosal to mucosal flux under control conditions.

Is the paracellular pathway highly potassium-selective as generally believed? Our previous electrophysiological investigations did not support this hypothesis. Replacement of mucosal $Na^+$ by $K^+$ did not produce a large change in the transepithelial potential ($\sim 50$ mV) as one might expect from the selectivity values previously reported for this pathway by Frizzell et al. (1976). In addition, using microelectrode measurements to perform an equivalent

circuit analysis of this epithelium, a significant paracellular emf could not be detected under conditions of a transepithelial potassium gradient similar to that described above (Wills et al., 1979*b*). This observation finds support in the present data as well. By estimating an apparent "$P_K$" for the colon as $P_K = J_{sm}/[K]$, we calculate a $P_K$ before ouabain of 0.08 cm/hr. This value represents a $P_K$ for both cellular and paracellular fluxes. After ouabain, $P_K$ is reduced to 0.02 cm/hr, a value which comparable to the $P_{Na}$ and $P_{Cl}$ values reported for the paracellular route by Frizzell et al. (1976). Thus the results of previous and present experiments are consistent with a nonselective paracellular pathway.

### *$K^+$ Secretion: Implications for Apical Membrane Properties*

On the basis of these results and in view of our previous electrical studies, it is conceivable that the net $K^+$ secretion observed under short-circuited conditions may be accounted for by an uptake step via the Na—K ATPase in the basolateral membrane and passive exit across the apical membrane. The feasibility of such a system requires: (i) that intracellular potassium be above equilibrium and (ii) that the apical membrane possesses a significant potassium permeability. With respect to apical membrane properties, previous electrical experiments indicated that a potassium conductance may be present in this membrane. For example, microelectrode measurements performed in the presence of mucosal amiloride by Wills et al. (1979*b*) revealed an appreciable leak conductance in the apical membrane of the rabbit descending colon. While the ionic selectivity of this leak pathway was not fully characterized, similar measurements performed after replacement of the mucosal NaCl Ringer's solution with $K_2SO_4$ Ringer's indicated that at least part of this leak was due to a potassium conductance. Similar results supporting an apical membrane potassium conductance have been found using impedance analysis (Clausen & Wills, 1981) and, more recently, fluctuation analysis (Zeiske, Wills & Van Driessche, 1981). The latter authors found that manipulations of the potassium driving force across the apical membrane of the rabbit colon were associated with changes in the current fluctuations across the epithelium. These findings taken together support the notion that potassium can cross the apical membrane by conductive channels.

By using the present data it is possible to estimate the necessary magnitude for such a conductive $K^+$ pathway in the apical membrane. Given the hypothesis that the measured net $K^+$ secretion reflects movement across the apical membrane, then by dividing by Faraday's constant, one can convert the measured net $K^+$ flux into a current ($I_K = \mu A/cm^2$). The $K^+$ conductance is then calculated from $I_K$ and $\Delta\tilde{\mu}_K/F$, using Ohm's law. In this manner, the apical membrane conductance was estimated as 610 $\mu S/cm^2$. Thus a purely conductive mechanism for potassium transport would require a minimum apical membrane potassium conductance of approximately 610 $\mu S/cm^2$ to support the measured flux. Conversely, the apical membrane potassium resistance would be approximately 1.6 $k\Omega cm^2$.

In the impedance analysis studies (Clausen & Wills, 1981), the potassium resistance of the apical membrane was estimated as approximately 3.7 $k\Omega cm^2$. Given the relatively small sample size of their study, the agreement between the two estimates is reasonable. However, more experiments will be needed to determine whether all of the net flux is accounted for by conductive mechanisms or whether a portion of this flux may be electrically silent.

Given that at least a portion of the net $K^+$ secretion may involve conductive channels in the apical membrane, how will potassium exit across this membrane be influenced by the transepithelial potential? As indicated in Table 4, under open-circuited conditions in the presence of a lumen-negative transepithelial potential, $V_a$ averaged $-20$ mV and had a mean value of $-56$ mV under short-circuited conditions ($V_T = 0$ mV). Thus the net driving force for potassium exit was lower under short-circuited conditions ($\Delta\tilde{\mu}_K/F = 17$ mV) than during open-circuit ($\Delta\tilde{\mu}_K/F = 49$ mV). Thus $K^+$ exit across the apical membrane will be greater in the presence of large lumen-negative transepithelial potentials and will behave in a similar manner to paracellular $K^+$ movements in response to transepithelial electrical gradients. Again the magnitude of fluxes through these two pathways will depend on their relative $K^+$ conductances and net potassium driving forces.

### *Implications of Intracellular $K^+$ Activity Levels for Transcellular Transport*

A second aspect of the present study that is relevant to our understanding of net $K^+$ secretion or absorption concerns intracellular potassium actively levels before and after ouabain addition. In the present study, as originally reported by Wills et al. (1979*b*), intracellular potassium activity ($a_i K$) was above equilibrium. Inhibition of the Na—K ATPase by ouabain significantly reduced intracellular potassium levels. Therefore the Na—K ATPase appears to play

a significant role in maintaining intracellular potassium activity levels. However, it is unknown whether the Na—K ATPase is solely responsible for this function. It is difficult to assess whether intracellular potassium is maintained above equilibrium after ouabain without precise information concerning the level of intracellular $Na^+$ under these conditions. Nonetheless, if $a_iK$ truly remains above equilibrium as indicated by the data in Table 4 then a second uphill entry mechanism may be present. A clear finding in the present study was that the basolateral membrane potential was not totally abolished by ouabain ($V_{bl}$ after ouabain $= -14$ mV). The basolateral membrane has a high potassium conductance and is normally highly selective for potassium over $Na^+$ or $Cl^-$ ($P_{Na}/P_K$ and $P_{Cl}/P_K \sim 0.05$; Wills et al., 1979*a*, *b*). Assuming that these selective permeabilities are not changed after ouabain as reported by Lewis and Wills (1981) for the rabbit urinary bladder, then the presence of a measurable basolateral membrane potential in ouabain-treated colons may also indicate that potassium is above equilibrium under these conditions. Further evidence for a second active uptake system comes from preliminary experiments (Wills, 1981) in which 5 mM $BaCl_2$ was added to the serosal solution of ouabain-treated colons in order to reduce passive efflux out of the cell. Within 10 min following $Ba^{2+}$ addition, a significant increase in $a_iK$ could be measured using ion-sensitive microelectrodes. $V_{bl}$ did not increase enough to account for this change in $a_iK$. Thus the increase is not due to passive redistribution but rather reflects an active uptake mechanism.

### *Net $K^+$ Absorption: Implications for the Apical Membrane*

If $a_iK$ remains above equilibrium as suggested by the above arguments, then it is probable that net $K^+$ absorption requires an active uptake of potassium at the apical border with a diffusional exit step across the basolateral membrane. At this point it is too early to anticipate the mechanism of this net absorptive system. However, it is unlikely that this system is a ouabain-sensitive Na—K ATPase situated in the apical membrane, as suggested by the results of Husted and Steinmetz (1980) for $K^+$ absorption in the turtle bladder. Luminally applied ouabain in our experiments had virtually no effect on the net $K^+$ absorption. It is conceivable that this absorptive system might be coupled to the transport of other ions such as $Cl^-$ or $H^+$. We note that a ouabain-insensitive, $K^+$-activated enzyme believed to be similar to the gastric mucosa H—K ATPase has been recently identified in isolated brush-border preparations of rabbit descending colon (Gustin, Goodman & Rasmussen, 1980; Gustin and Goodman, 1982). However, the relationship between this enzyme and the net $K^+$ transport systems which we have observed remains for future study.

It is difficult at this time to speculate whether or not the $K^+$ absorption, seen after ouabain, is important under more physiological conditions. The potential and/or concentration dependence of the system are unknown. In the present study $J_{ms}$ before ouabain was essentially equal to $J_{sm}$ after ouabain. It is possible that these fluxes may represent paracellular $K^+$ movements. If so, the increase in $J_{ms}$ may indicate that the absorptive system is not effective under control conditions but is activated by some aspects of the ouabain condition. A complete understanding of this system will clearly require further experiments.

### *Conclusions*

In summary, the present results support our previous electrical findings and suggest that, in addition to paracellular movement, potassium can also be transported by transcellular mechanisms. Moreover, measurement of bi-directional $K^+$ fluxes after ouabain addition to the serosal solution revealed the presence of a previously undescribed net $K^+$ absorption. These results suggest that $K^+$ transport in the colon is mediated at least in part by two active transport systems: a secretory system and an absorptive one. It is possible that these absorptive and secretory systems may work together to regulate intracellular potassium activity levels. Similar models for potassium transport have been previously proposed for the renal distal tubule (*cf.* Giebisch, 1979). In such models both the apical and basolateral membranes contain potassium pump-leak systems. Our measurements in colon also suggest the existence of pump-leak mechanisms in the apical and basolateral membranes. Thus the regulation of potassium transport in the colon may be more complex than previously believed.

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