J. Physiol (1984), **346**, *pp.* 61–71
With 2 text-figures
Printed in Great Britain

AMILORIDE SENSITIVE AND INSENSITIVE SODIUM PATHWAYS AND THE CELLULAR SODIUM TRANSPORT POOL OF COLONIC EPITHELIUM IN RATS

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(*Received 6 April 1983*)

SUMMARY

1. Methods for measurement of the epithelial Na transport pool (Na_t) using epithelial scrapings and for analysing the transepithelial ionic fluxes of rat distal colon *in vivo* into transcellular and paracellular components have been used to study the amiloride sensitive (a.s.) and amiloride insensitive (a.i.) transcellular pathways in relation to variations of Na_t.

2. In the Na-replete normal rats, substitution of SO_4 for Cl in the lumen approximately halved the Na transported by a.i. pathways and reduced Na_t by about 60%, but in the Na-depleted rats, substitution of SO_4 did not affect either the Na transported by a.s. pathways or Na_t.

3. The value of Na_t for normal rats, with 150 mM-NaCl in the lumen, was 6–7 nmol Na mg^{-1} dry weight (corresponding to about 2–3 mmol kg^{-1} cell water) and fell by about 60% when lumen Na concentration was reduced to 50 mM. Its turnover half-time was 0·6 min.

4. Na_t was about threefold greater in the Na-depleted than in the normal rats but became undetectable when amiloride was in the lumen. Amiloride did not affect Na_t in normal rats.

5. We conclude that the increased Na absorption in Na depletion depended on substitution of a.s. for a.i. apical membrane pathways allowing increased Na entry into the epithelial cells so expanding Na_t and stimulating the basolateral Na pumps.

INTRODUCTION

Epithelia are usually considered as either 'tight' or 'leaky'. Tight epithelia have a relatively high electrical resistance and possess an active Na transport pathway which is completely blocked by the pyrazine diuretic amiloride (Diamond, 1978). Leaky epithelia, on the other hand, have lower electrical resistance and are insensitive to amiloride. Colonic epithelium, a 'medium tight' epithelium, is remarkable in being sensitive or insensitive to amiloride in different circumstances. Various factors, including species variation, the segment of colon studied, and, in some species, age and hormonal action, have been found to exert important influences on this

characteristic (Frizzell, Koch & Schultz, 1976; Cremaschi, Ferguson, Hénin, James, Meyer & Smith, 1979; Thomas & Skadhauge, 1982). In the rat, for example when the animal has a high Na intake, the epithelium of the distal colon behaves like a leaky epithelium absorbing Na by a mechanism which is totally insensitive to blocking by amiloride. When, however, the animal is treated with aldosterone or is Na-depleted, the epithelium assumes the characteristics of a tight epithelium, with Na absorption becoming highly sensitive to inhibition by amiloride (Will, Lebowitz & Hopfer, 1980; Edmonds, 1981). Amiloride is only active when present in fluid within the lumen and its onset of action is rapid. On colonic epithelium, as in tight epithelia, amiloride is assumed to act on the apical plasma membrane as a specific inhibitor of passive Na entry.

The simplest hypothesis suggested by these observations is that the apical membrane of the epithelial cells of the distal colon contains either amiloride sensitive and/or insensitive pathways which control Na entry from the lumen into the cellular Na transport pool (Na_t). The exit step is presumed to be common to both means of entry probably by an Na–K-ATPase mechanism which can be inhibited by ouabain (discussed by Wrong, Edmonds & Chadwick, 1981). This scheme implies that the entry at the apical membrane has a crucial part in controlling the rate of absorption (Thompson, Suzuki & Schultz, 1981). Our aim in the present work was to determine transcellular and paracellular movements of Na and Cl in the distal colon by an *in vivo* method which involved minimal disturbance of the gut segment and its blood supply. To analyse the results, we have used the concept that, in the normally absorptive colon, there are three potential routes for Na movement from lumen into blood (Edmonds, 1981). One route is assumed to be entirely passive ionic diffusion by the paracellular pathway dependent on the transepithelial electrochemical gradient. The other two routes are both transcellular and distinguished by their pathway across the apical membrane. One of these is unaffected by amiloride (amiloride insensitive, a.i.) while the other is completely blocked by amiloride (amiloride sensitive, a.s.). The a.i. path is characteristic of the epithelium of rats on a liberal Na intake, while the a.s. path in this tissue appears only when Na intake is limited or when the epithelium is stimulated by aldosterone.

METHODS

Male albino rats weighing 350–450 g were fed on MRC 41B or, when Na intake was restricted, on a diet prepared mainly from glucose and rice. Rats described as Na-depleted had about 0·5–1·0 mmol Na (about 5–10 % of exchangeable body Na) removed by peritoneal dialysis with light ether anaesthesia and they were then given the rice diet for 5–7 days before experiment. The normal rats received NaCl 0·9 % solution to drink for 4 days preceding experiment to ensure a liberal Na intake. The Na-depleted rats had free access to water. Food was not removed until the morning of the experiment. Sodium pentobarbitone (about 6 mg 100 g^{-1} body wt. I.P.) was used for anaesthesia throughout.

Experimental procedures

Full details of the procedures used for studies on the distal colon have been described previously (Edmonds & Smith, 1979; Edmonds, 1981) and only a brief summary will be included here. Polythene cannulae containing heparinized saline were inserted into both external jugular veins. A segment of about 3 cm of descending colon was cannulated, with special care taken about the preservation of the blood supply. The abdomen was closed and body temperature maintained by a heater. An infusion pump was used to perfuse the lumen at a rate of 0·9 ml min^{-1} with solution warmed to 37 °C during measurement of the plasma-to-lumen flux (J_{sm}). All the effluent was

collected from the distal colon for subsequent analysis. Absorption (J_{net}) was measured over a brief period (10 min) during which perfusion was stopped. The lumen-to-plasma flux (J_{ms}) was calculated from the observed values of J_{sm} and J_{net}. The transepithelial p.d. was measured continuously during the experiments by means of bridges of 4% agar in 150 mM-NaCl, calomel half-cells and a high input impedance millivoltmeter. One bridge was inserted into the lumen through a side arm in the perfusion system; the other was placed on the peritoneal surface of the colon. Correction was made for small diffusion potential artifacts which were present if the perfusion solution and electrodes differed much in composition.

Epithelial scrapings

In one series of experiments, in order to assess the amount of Na entering the epithelial layer from the lumen, epithelial scrapings were prepared. This method seemed the most likely to prove satisfactory for the present type of preparation (MacKnight & Leaf, 1978). A measured amount of a chosen solution, usually 0·5 ml, to which ^{22}Na had been added to give specific activity of about 5 nCi ^{22}Na per micromole of Na, was introduced into the lumen to distend the cannulated segment. After 10 min the test solution was rinsed out using 300 mM-mannitol solution, 5 ml being passed through during 15 s and collected. The colon segment was then rapidly removed, opened and lightly blotted and pinned to a cork mat. The epithelium was scraped gently with a scalpel blade. Histological examination of pieces of colon showed that the superficial epithelial cells and possibly the superficial part of the crypts were removed by this means. Cytological examination of the scrapings showed sheets of uniform columnar epithelial cells. The small mass of epithelial cells obtained was immediately transferred to a disk of Whatman ashless filter paper and washed for 20 s with a further 5 ml of mannitol solution and the washings collected. The scrape was then transferred to a weighed disk of metal foil. After some practice, the whole procedure, from beginning the rinsing out of the test solution until the transfer of the scraping to the metal foil disk, was completed within 1·5 min. The scraping was dried, to obtain the dry weight and the dried material then extracted in 1 ml 100 mM-HNO_3 and subsequently diluted for radio-isotopic and chemical analysis. A few other experiments were done in which the lumen contained only 150 mM-NaCl solution, the scrapes being obtained 15 min after an intravenous injection of 10 μCi ^{22}Na in 0·5 ml 150 mM NaCl.

Chemicals and radio-isotopes

All solutions were prepared freshly for each experiment. Amiloride was added when indicated to give a concentration of 10^{-3} M which is adequate to block amiloride sensitive pathways even when the intraluminal Na concentration is 150 mM. It was freshly prepared in alkaline solution, neutralized and appropriately diluted. All solutions used were isotonic, mannitol being added where necessary. For measurement of J_{sm} for Na, about 300 nCi of ^{22}Na in 0·1 ml, was injected intravenously by one cannula as a rapid bolus injection about 1 h before the measurements. Blood samples for measurement of plasma radioactivity were taken from the other venous cannual. Na and K were measured by flame photometry and ^{22}Na by γ counting.

Calculations

The length of colon was measured after removal at the end of the experiment by mounting on a standard glass tube as used previously. The surface area of the epithelium was calculated on the basis of 1 cm length equivalent to 2·2 cm^2. The flux of Na into the lumen was calculated as previously described from the rate of entry of ^{22}Na into the perfusate and the mean plasma specific activities during the collection periods. The previous study of Edmonds & Smith (1979) showed that the flux J_{sm} evaluated by this technique indicated the plasma-to-lumen flux through the paracellular passive diffusion pathway. The two components of the lumen-to-plasma flux, namely J^P_{ms} (passive, paracellular component) and J^C_{ms} (transcellular component), were calculated as follows, First the passive flux from lumen-to-plasma, J^P_{ms} was estimated using the flux ratio equation thus:

$$J^P_{ms} = J_{sm}\, C_m/C_s \exp(\psi_{ms} F/RT),$$

where C_m and C_s were the concentrations in the lumen and plasma respectively, ψ_{ms} was the transepithelial p.d. and F, R and T have their usual meaning. The amount of Na or Cl which passed by the transcellular route J^C_{ms} was finally estimated from:

$$J_{ms} = J^P_{ms} + J^C_{ms}.$$

The cellular Na transport pool (Na_t) was calculated from:

$$(Na_t) = \frac{^{22}\text{Na content of scraping mg}^{-1}}{\text{mean specific activity of lumen solution}}.$$

The denominator value was derived from measurement of specific activity of the fresh solution and that collected after 10 min within the lumen. Results are given as means with standard errors of the mean unless otherwise indicated and statistical significance examined by Student's t test.

RESULTS

The epithelium of the distal colon of rats taking our standard diet and drinking water showed some, although small, sensitivity to amiloride. This appeared to depend on normal aldosterone secretion. Thus, if the rats were adrenalectomized (maintained on dexamethasone) or given a liberal salt intake, the colonic epithelium became insensitive to amiloride (Will *et al.* 1980; Edmonds, 1981). In the current work, we have therefore used rats on a liberal salt intake (saline to drink) to study the a.i. Na transport mechanism, confirming by preliminary measurements that the epithelium was, in fact, amiloride insensitive. Rats which have been Na-depleted have an epithelium completely sensitive to amiloride and so these animals were used for studies of the a.s. Na transport mechanism.

Transcellular Na fluxes

Effect of luminal Na concentration. The Na transepithelial fluxes were measured when either 150 mM-NaCl or 50 mM-NaCl were in the lumen, mannitol being added to preserve the isotonicity of the solutions. In both groups of rats, the flux J_{ms} was approximately halved by reducing the luminal Na concentration (Fig. 1). Calculation of the transcellular components showed that J^C_{ms} fell to 56 % and 51 % of the original values in the normal control and Na-depleted rats respectively. Thus, reduction of luminal Na concentration did not produce a proportionate fall in transcellular Na movement whether by the a.i. or the a.s. pathway.

Effect of substituting SO_4 for Cl in the lumen. The results obtained by Binder & Rawlins (1973) from studies on rat colon *in vitro* strongly suggested that Na absorption was linked in some way to Cl absorption. In our preparation we therefore examined the effect of substituting SO_4 for Cl on both the a.i. and the a.s. Na pathways. The results from the former, tested in normal rats, showed that substitution of SO_4 approximately halved the Na movement through the a.i. transcellular pathway (Fig. 1). However, although J^C_{ms} was reduced, in no experiment was it abolished. K secretion was not significantly affected by SO_4 substitution and the transepithelial p.d. showed only a small but not significant increase ($12 \pm 1{\cdot}1$–$14 \pm 0{\cdot}9$ mV, lumen negative). In contrast, absorption of Na by the a.s. pathway in the Na-depleted rats, clearly did not depend on the presence of Cl within the lumen, the value of J^C_{ms} being little influenced by substituting SO_4 for Cl (Fig. 1). The change of transepithelial p.d. was also greater, the p.d. rising significantly when SO_4 was substituted (from $42 \pm 4{\cdot}6$ to $53 \pm 4{\cdot}8$ mV; $P < 0{\cdot}05$) as did K secretion (from $22 \pm 1{\cdot}4$ to $31 \pm 1{\cdot}3$ nmol min^{-1} cm^{-2}; $P < 0{\cdot}005$).

Observations on epithelial scrapings

The object of these experiments was to estimate the Na content in the cellular Na transport pool (Na_t) particularly in relationship to changes in composition of the lumen solution. The test solution remained in the lumen for 10 min before the tissue was removed, allowing a preliminary measurement of absorption rate and ensuring

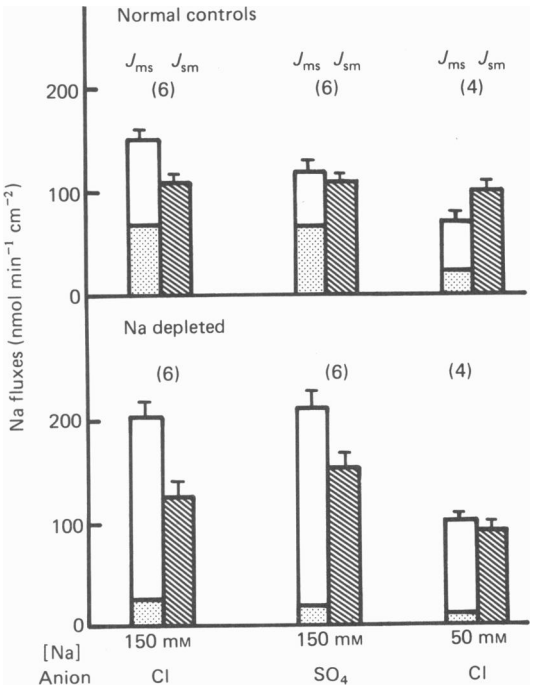

Fig. 1. Unidirectional Na fluxes in the distal colon when the lumen contained 150 mM- or 50 mM-NaCl or 75 mM-Na_2SO_4 with mannitol added for isotonity. The J_{ms} flux is shown divided into its estimated components: the stippled area represents the passive paracellular component (J^P_{ms}), the open area, the transcellular component (J^C_{ms}). The value of J^P_{ms} is much reduced in the Na-depleted rats as there is a greater opposing electrical gradient. The value of n for both figures is in parentheses.

that a steady state with respect to epithelial Na had been reached. We confirmed that this was so in a series of preliminary studies which showed that ^{22}Na content per milligramme dry weight was similar whether a 10 or a 20 min exposure period was used. The epithelium which had been scraped off and spread out onto filter paper was first rinsed with cold mannitol solution in an attempt to remove any extracellular Na. This rinsing process removed $32 \pm 12\%$ ($\pm$S.D.) of the ^{22}Na and 33% (62 ± 11 ($\pm$S.D.) nmol mg^{-1}) of the Na present in the scraping. The amount of ^{22}Na remaining in the epithelium together with the specific activity of the Na in the lumen were used to calculate the value of Na_t. The initial measurements showed that the amount of ^{22}Na remaining in the scraping was very small and therefore relatively high Na specific activity in the lumen solution was essential to obtain accurate estimates.

Na transport pool in normal rats. When the lumen contained 150 mM-NaCl the value

of Na_t was only about 4 % of the total Na measured in the mannitol-washed epithelial scrapings (Table 1). Reducing the NaCl concentration in the lumen to 50 mM caused a highly significant reduction of Na_t ($P < 0.01$) but no detectable change in the total epithelial Na.

As shown above, when SO_4 was substituted for Cl in the normal rats, the transcellular Na flux was approximately halved (Fig. 1). Estimation of the Na

TABLE 1. Total epithelial Na and K, and Na in the transport pool (Na_t) measured in epithelial scrapings from groups of normal Na-replete rats in which the lumen had contained various solutions including ^{22}Na

Lumen Na (mM)	Lumen Anion present	n	Transport pool (Na_t)	Total epithelial Na	Total epithelial K
150	Cl	10	4·9±0·3	116±9	279±8
50	Cl	5	2·2±0·7	118±5	271±9
150	SO_4	4	2·1±0·4	130±11	280±20

The results are expressed in nmol mg^{-1} dry weight±S.E. of the mean. n in Tables and Figures refers to the number of rats.

TABLE 2. Measurements on epithelial scrapings as in Table 1 but from Na-depleted rats. Results are expressed in nmol mg^{-1} dry weight±S.E. of the mean

Lumen Na (mM)	Lumen Anion present	n	Transport pool (Na_t)	Total epithelial Na	Total epithelial K
150	Cl	4	15·4±4·2	119±6	277±12
150 +amiloride	Cl	4	0	125±9	278±11
50	Cl	4	4·3±1·6	118±8	271±12
150	SO_4	4	12·1±3·7	126±11	280±14

transport pool under these conditions showed that the pool had decreased considerably ($P < 0.01$), averaging, when SO_4 was present, about 40 % of that found when Cl was the accompanying anion. The addition of 10^{-3} M-amiloride did not significantly alter Na_t in the normal rats. The total amount of Na present in the epithelial scrapings in all instances considerably exceeded that in the Na transport pool. The amount of K mg^{-1} dry weight was similar in all of the studies and agrees reasonably well with that obtained in previous work (Edmonds & Smith, 1979).

Intravenous ^{22}Na and the total epithelial Na. The considerable difference between the total epithelial Na measured chemically and the Na transport pool was a constant feature of these experiments. In an attempt to determine the nature of this apparent Na excess, several further experiments were carried out in which an intravenous bolus of ^{22}Na of about 10 μCi was given. The lumen of the experimental segment of colon contained 150 mM-NaCl and after 15 min the segment was removed and the scraping prepared in the usual way. The specific activity of the total epithelial Na was compared with that of the plasma. In all three experiments, the former significantly exceeded the latter, the total epithelial Na count rate averaging 3·7±0·3 compared

with the plasma Na count rate of $2{\cdot}3 \pm 0{\cdot}2$ counts 1000 s^{-1} $nmol^{-1}$ of Na. The rapid exchange of the intravenously injected ^{22}Na with the total epithelial Na strongly suggested that the latter is located outside the cells.

Na turnover in the epithelial cells and the estimated value of Na_t. The expected turnover rate can be estimated from Na_t and the calculated value of the Na

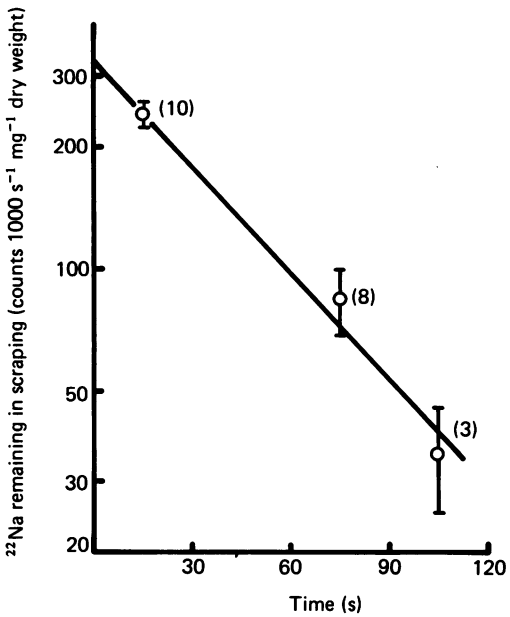

Fig. 2. Rate of elution of ^{22}Na from the epithelium of rat colon. Preliminary 10 min exposure to ^{22}Na in the lumen was followed by a 15 s mannitol rinse with or without a preceding 60 or 90 s rinse with non-radioactive 150 mM-NaCl. Zero time refers to the beginning of the rinse when ^{22}Na was rapidly rinsed out of the lumen. The observations are plotted against the time of removal of the tissue from the animal. The intraluminal ^{22}Na-containing solution had a count rate of approximately 50 counts per 1000 s and for comparison all the results were normalized to precisely this.

transcellular flux, J^C_{ms}, on the assumption that all this Na passes through the Na transport pool and is actively transported out of the cells through the basolateral membranes. For the calculation for the normal rats, we take a value of 5·3 mg dry weight per centimetre^2 of epithelium (based on mean of three estimates) and evaluate Na_t cm^{-2}. When 150 mM-NaCl was in the lumen, we therefore assign a value for Na_t of 25 nmol cm^{-2} and for J^C_{ms} an average value of 80 nmol min cm^{-2} (Fig. 1). Thus, with a simple, single intracellular compartment, a turnover half-time of about 0·2–0·3 min is expected.

In a series of experiments, we then measured the turnover rate directly by rinsing out the ^{22}Na with non-radioactive 150 mM-NaCl for either 60 or 90 s followed by the 15 s mannitol rinse and preparation of the scraping in the usual way. The results (Fig. 2) did show that a rapid removal of ^{22}Na from the epithelium took place, although the kinetics with $t_{\frac{1}{2}}$ of 0·6 min were not quite as fast as expected. Extrapolating back to zero time, gave a value for Na_t which should correspond to that when ^{22}Na was in

the lumen and so be a true estimate of the pool size. From our data, this was 6–7 nmol Na mg^{-1} dry weight.

Na transport pool in Na-depleted rats. The results of these studies, summarized in Table 2, showed that there were no significant changes in the total epithelial Na and K either between the various conditions examined or between the normal and the Na-depleted rats. In contrast the value of Na_t showed considerable variation with the various experiments. Thus the addition of 10^{-3} M-amiloride to the lumen solution completely eliminated the epithelial Na transport pool in the Na-depleted rats. The substitution of SO_4 for Cl was associated with a small average reduction but this was not statistically significant (in the normal rats, this manoeuvre produced a substantial reduction of the pool). On the other hand, reducing the Na concentration in the lumen to 50 mM produced a fall in Na_t to less than one-third of its value when 150 mM solution was in the lumen. Finally, comparison of the data in Tables 1 and 2 demonstrates the difference between the Na transport pool in normal and Na-depleted states. Both with 50 and 150 mM-NaCl in the lumen, the value of Na_t was two- to threefold greater in the Na-depleted rats. When SO_4 was the anion in the lumen, the difference was even more evident, with the Na transport pool being nearly six times greater in the epithelium of Na-depleted animals.

DISCUSSION

Our results showed that the amount of Na in the epithelial transport pool of the colon cells, even when 150 mM-NaCl was in the lumen and when a considerable transcellular Na flux was occurring, was very small, amounting in the normal rats to about 2% of the K present. These measurements seemed very likely to reflect the true intracellular Na transport pool as they were consistent and changes were produced by various experimental procedures, as for example by Na-depletion, amiloride and SO_4. It is likely that the method works especially well for *in vivo* preparations as the transported ^{22}Na suffers little arrest in the submucosa which is richly perfused with capillaries. The cell water content could not be determined directly in our studies but can be deduced from the K measurements. Taking cell K concentration as 125 mmol kg^{-1} cell water (see MacKnight *et al.* 1982), we estimate cell water in the epithelia as 12 μl cm^2 and thus Na_t in the normal rats (150 mM-NaCl in the lumen) was 3 mmol kg^{-1} water.

Such a low concentration suggests that the Na transport pool may be located in a segregated Na compartment. Recently, an ultrastructural system has been described in salt and water transporting epithelia (Møllgård & Rostgaard, 1978; Eldrup, Frederiksen, Møllgård & Rostgaard, 1982). A distinct cellular compartment appeared to be formed of a tubulo-cisternal endoplasmic reticulum which made contact both with the apical and basolateral membranes. Such a system could explain the apparent small capacity, fast turnover Na transport pool demonstrated in our current epithelial studies.

As the observed Na transport pool was so small relative to the transcellular Na flux, the fast turnover rate was anticipated. The pool size was considerably smaller, and turnover kinetics considerably faster, than suggested by MacKnight, Mason, Rose & Sherman (1982) from the results of studies on rabbit distal colon. They found

a transport pool of 20 mmol kg^{-1} water and turnover rate of once in 6 min. The difference may be due to species or to method, as their preparation was *in vitro*.

The total Na content of the epithelial scraping was much greater than the amount of Na_t. Relating total Na to water content, we estimate 54 mmol kg^{-1} water. The latter is similar to that we obtained previously when we employed the inulin method to estimate extracellular space (Edmonds & Nielsen, 1968). MacKnight *et al.* (1982) also found a relatively large amount of Na in the epithelium of rabbit colon which was not extracellular as judged by inulin distribution. Our present studies with intravenously injected ^{22}Na did show, however, that this Na was labelled rapidly with the radio-isotope and so did not appear to be intracellular. Moreover, *in vitro* measurements have indicated that the basolateral membranes of the epithelium of colon are relatively impermeable to Na (Frizzell, Koch & Schultz, 1976; Wills, Eaton, Lewis & Ifshin, 1979) so also suggesting that a large intracellular Na pool (although segregated from the Na transport pool) is most unlikely. At present we have no definite evidence as to the location of this excess Na but we can conclude that it exchanges rapidly with plasma Na and is very unlikely therefore to be intracellular.

In several respects, the a.i. and a.s. apical pathways, as exemplified in the normal and the Na-depleted rats, can be contrasted. There is first the effect of amiloride itself. In the current experiments, amiloride had no effect on Na_t in the normal rats, which is as expected since Na absorption itself is not influenced (Will, Lebowitz & Hopfer, 1980; Edmonds, 1981). In the Na-depleted rats, on the other hand, Na_t became undetectable when amiloride was in the lumen, a finding wholly consistent with its ability to block Na absorption completely in these animals. Second, there was a striking difference in the effect of substituting SO_4 for Cl in the lumen solution. In the Na-depleted rats, no significant change either in the transcellular Na flux, J^C_{ms}, or in Na_t was demonstrable whereas in the normals a marked reduction in both was found. These findings are consistent with the notion that a.i. pathways probably involve some form of complex ionic interaction at the plasma membrane (Binder & Rawlins, 1973; Argenzio & Whipp, 1979; Edmonds, 1982) while the a.s. pathways are probably simple diffusion channels (Lindemann & van Driessche, 1977; Zeiske, Wills & van Driessche, 1982).

Finally a considerable difference in the size of the Na transport pool was observed between the normal and the Na-depleted rats when a similar Na concentration was in the lumen. The increased transcellular Na flux of the Na-depleted rats was accompanied by an approximately threefold rise in Na_t, a change of similar magnitude to that observed in the cells of rabbit urinary bladder by ion-selective electrode (Eaton, 1981). In both normal and Na-depleted rats, increasing the luminal Na concentration increased J^C_{ms} and Na_t. Together these findings strongly suggest that the amount of Na in the transport pool determines the amount of Na transported out of the cells. It is relevant here to note that in previous studies (Thompson & Edmonds, 1974) we were unable to detect any difference in ouabain-sensitive Na–K-ATPase between scrapes taken from normal and Na-depleted rats, indicating that pump density was similar in the two states.

The general consistency of the observations persuades us that the methods allow a reasonably accurate assessment of the epithelial Na transport pool in relation to the transcellular Na flows in the epithelium of colon. The results together indicate

that the Na transport pool contains variable amounts of Na and that the properties of the apical plasma membrane and the composition of the solution in the lumen dominate the pool size. The amount of Na in the pool then appears to determine the amount of Na pumped out across the basolateral membranes. The substitution of a.s. for a.i. pathways in the apical membrane in Na-depleted animals (a consequence largely of prolonged aldosterone stimulation) facilitates access of Na to the pool from the lumen. Our results show that this change leads to an enlarged Na transport pool for any given intraluminal Na concentration and so is responsible for the increased Na absorption characteristic of the Na-depleted animal.

We are grateful to Dr E. A. Hudson for cytological examinations.

REFERENCES

ARGENZIO, R. A. & WHIPP, S. C. (1979). Inter-relationship of sodium, chloride, bicarbonate and acetate transport by the colon of the pig. *J. Physiol.* **295**, 365–381.

BINDER, H. J. & RAWLINS, C. L. (1973). Electrolyte transport across isolated large intestinal mucosa. *Am. J. Physiol.* **225**, 1232–1239.

CREMASCHI, D., FERGUSON, D. R., HÉNIN, J. P. S., JAMES, P. S., MEYER, G. & SMITH, M. W. (1979). Post-natal development of amiloride sensitive sodium transport in pig distal colon. *J. Physiol.* **292**, 481–494.

DIAMOND, J. M. (1978). Channels in epithelial membranes and junctions. *Fedn Proc.* **37**, 2639–2644.

EATON, D. C. (1981). Intracellular sodium ion activity and sodium transport in rabbit urinary bladder. *J. Physiol.* **316**, 527–544.

EDMONDS, C. J. (1981). Amiloride sensitivity of the transepithelial electrical potential and of sodium and potassium transport in rat distal colon *in vivo*. *J. Physiol.* **313**, 547–559.

EDMONDS, C. J. (1982). Anion transport in the colon. *Phil. Trans. R. Soc. B* **299**, 575–584.

EDMONDS, C. J. & NIELSEN, O. E. (1968). Transmembrane electrical potential differences and ionic composition of mucosal cells of rat colon. *Acta physiol. scand.* **72**, 338–349.

EDMONDS, C. J. & SMITH, T. (1979). Epithelial transport pathways of rat colon determined *in vivo* by impulse response analysis. *J. Physiol.* **296**, 471–485.

ELDRUP, E., FREDERIKSEN, O., MØLLGÅRD, K. & ROSTGAARD, J. (1982). Effects of a small serosal hydrostatic pressure on sodium and water transport and morphology in rabbit gall bladder. *J. Physiol.* **331**, 67–85.

FRIZZELL, R. A., KOCH, M. J. & SCHULTZ, S. G. (1976). Ion transport by rabbit colon. I. Active and passive components. *J. Membrane Biol.* **27**, 297–316.

LINDEMANN, B. & VAN DRIESSCHE, W. (1977). Sodium-specific membrane channels of frog skin are pores: current fluctuations reveal high turnover. *Science, N.Y.* **195**, 292–294.

MACKNIGHT, A. D. C. & LEAF, A. (1978). The sodium transport pool. *Am. J. Physiol.* **234**, F1–9.

MACKNIGHT, A. D. C., MASON, D. R., ROSE, R. C. & SHERMAN, B. (1982). Ions and water in the epithelial cells of rabbit descending colon. *J. Physiol.* **333**, 111–123.

MØLLGÅRD, K. & ROSTGAARD, J. (1978). Morphological aspects of some sodium transporting epithelia suggesting a transcellular pathway via elements of the endoplasmic reticulum. *J. Membrane Biol.* **42**, 71–89.

THOMAS, D. H. & SKADHAUGE, E. (1982). Regulation of electrolyte transport in lower intestine of birds. In *Electrolyte and Water Transport across Gastrointestinal Epithelia*, ed. CASE, R. M., GARNER, A., TURNBERG, L. A. & YOUNG, J. A., pp. 295–302. New York: Raven Press.

THOMPSON, B. D. & EDMONDS, C. J. (1974). Aldosterone, sodium depletion and hypothyroidism on the ATPase activity of rat colonic epithelium. *J. Endocr.* **62**, 489–496.

THOMPSON, S. M., SUZUKI, Y. & SCHULTZ, S. G. (1981). Current–voltage properties of active sodium transport pathway across rabbit colon. In *Ion Transport by Epithelia*, ed. SCHULTZ, S. G. New York: Raven Press.

WILL, P. C., LEBOWITZ, J. L. & HOPFER, U. (1980). Induction of amiloride-sensitive sodium transport in the rat colon by mineralocorticoids. *Am. J. Physiol.* **238**, F261–268.

WILLS, N. K., EATON, D. C., LEWIS, S. A. & IFSHIN, M. S. (1979). Current–voltage relationship of basolateral membrane of tight epithelium. *J. Membrane Biol.* **45**, 81–108.

WRONG, O. W., EDMONDS, C. J. & CHADWICK, V. S. (1981). *The Large Intestine*, pp. 113–121. Lancaster: MTP Press Ltd.

ZEISKE, W., WILLS, N. K. & VAN DRIESSCHE, W. (1982) Na^+ channels and amiloride-induced noise in the mammalian colon epithelium *Biochim. biophys. Acta* **688**, 201–210.