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# IONS AND WATER IN THE EPITHELIAL CELLS OF RABBIT DESCENDING COLON

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## SUMMARY

1. Isolated sheets of rabbit descending colon epithelial cells stripped from their underlying muscle coats were incubated in chambers at 37 °C with oxygenated media, and their non-inulin space water, sodium, potassium and chloride contents were subsequently determined.

2. With sodium Ringer bathing both surfaces, amiloride, $10^{-4}$ M, decreased non-inulin space sodium content by 76 mmol/kg dry wt. Ouabain, $10^{-3}$ M, caused loss of non-inulin space potassium which was not completely compensated for by uptake of sodium over 30 min incubation. Chloride and water, therefore, decreased. Amiloride, $10^{-4}$ M, inhibited but did not prevent this uptake of sodium after ouabain.

3. Tissues exposed to sodium-free choline Ringer rapidly exchanged non-inulin space sodium for choline and, more slowly, lost potassium, chloride and water. The equilibration of sodium in the non-inulin space when sodium Ringer was restored to the mucosal medium alone was largely amiloride-insensitive. For restoration of non-inulin space potassium to normal levels, sodium was required in the serosal but not the mucosal medium.

4. Neither the absence of glucose nor the absence of chloride from the mucosal medium affected the non-inulin space sodium content when sodium was restored to the mucosal medium bathing sodium-depleted tissues.

5. It is argued that, whereas non-inulin space potassium and water contents are synonymous with their cellular values, only about one third of non-inulin space sodium is cellular when sodium Ringer bathes both surfaces, and the concentration of the sodium within the cellular transport pool approximated 20 mmol/kg $H_2O$, consistent with estimates obtained from other techniques.

## INTRODUCTION

Many epithelia have, as one of their physiological properties, the ability to utilize metabolic energy to transport sodium from their luminal or mucosal surface to their serosal surface. Such active transepithelial transport of sodium has been extensively

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studied. But, whereas considerable information is now available about the effects of hormones, drugs and variations in medium composition on transepithelial sodium transport, there is comparatively little known about how these affect epithelial cellular composition.

The descending colon of the rabbit actively absorbs sodium and its transport properties have been well characterized in recent studies (Frizzell, Koch & Schultz, 1976; Frizzell & Schultz, 1978; Frizzell & Turnheim, 1978; Schultz, Frizzell & Nellans, 1977; Turnheim, Frizzell & Schultz, 1978; Wills, Lewis & Eaton, 1979; Yorio & Bentley, 1977). Though of relatively low transepithelial electrical resistance when compared, for example, to such 'tight' epithelia as amphibian skin and bladder, its transport properties (amiloride-sensitive sodium entry to cells from the lumen, absence of coupled glucose–sodium or chloride–sodium entry, responsiveness of sodium transport to aldosterone) place it within that category.

In the present studies, epithelial cell composition has been determined by conventional tissue analytical techniques (Macknight, 1980) using segments of rabbit colon epithelial cells from which the underlying muscle layers were removed by stripping with a glass slide (Schultz *et al.* 1977). This simple manoeuvre provides an epithelial cell layer, supported by thin strands of muscularis mucosae, which can be mounted in chambers and incubated *in vitro*. The results obtained are consistent with those described in epithelial cells from another 'tight' epithelium, the toad urinary bladder (Summarized by Macknight, 1980; and Macknight, DiBona & Leaf, 1980). Amiloride decreased the cellular content of sodium, with little effect on the cellular content of potassium. Ouabain caused loss of cellular potassium and uptake of sodium. As in toad urinary bladder, much of non-inulin space sodium appeared not to be within transporting epithelial cells.

## METHODS

*Media.* The media had the following composition (mmol/l); Bicarbonate-free Na Ringer: Na, 145; K, 5·4; Ca, 1·2; Mg, 1·2; Cl, 142; $HPO_4$, 2·4; $SO_4$, 1·2; $H_2PO_4$, 0·6; acetate, 5; glucose, 5; pH 7·4. Na Ringer: Na, 140; K, 5·4; Ca, 1·2; Mg, 1·2; Cl, 121; $HCO_3$, 21; $HPO_4$, 2·4; $H_2PO_4$, 0·6; $SO_4$, 1·2; glucose, 5; pH 7·4 when bubbled with 95 % $O_2$/5 % $CO_2$ at 37 °C.

In choline medium, choline chloride 119 mmol/l and choline bicarbonate 21 mmol/l replaced NaCl and $NaHCO_3$. Some media contained amiloride (a gift of Merck, Sharp and Dohme, New Zealand, Ltd.), and ouabain (Sigma Chemical Co., U.S.A.). [Methoxy-$^3$H]inulin and [carboxy-$^{14}$C]inulin, were obtained from New England Nuclear Corporation. Where media of identical composition bathed both surfaces, [$^{14}$C]inulin was employed. When media of different composition were used, the medium bathing the mucosal surface contained [$^3$H]inulin and the medium bathing the serosal surface contained [$^{14}$C]inulin. There was no measurable flux of these isotopes across the tissue.

*Procedure.* Adult rabbits of a New Zealand white strain were stunned, and a 15–20 cm length of descending colon was removed beginning distally at the rectum. The segment of colon was immediately placed in ice-cold bicarbonate-free sodium Ringer where it remained until being opened, washed free of its contents and stripped.

Stripping was performed in ice cold bicarbonate-free sodium Ringer using a glass slide. The epithelial cells with their underlying muscularis mucosae were readily separated from the remaining supporting tissues without visible tearing or damage, in all but a few colons. Any damaged tissues were discarded.

In initial experiments, stripped segments were simply transferred to fresh sodium Ringer containing bicarbonate and incubated at 37 °C with 95 % $O_2$/5 % $CO_2$ bubbled through the medium. Data on the constancy of the inulin space were obtained in this way.

For the definitive experiments in which one surface only of the tissue was to be exposed to sodium-free media or to hormones or drugs, stripped preparations were mounted in simple chambers in which they were supported on their serosal surface by nylon mesh. The rectangular chambers exposed 7·5 $cm^2$ of tissue to the bathing media. All incubations were carried out under open-circuit conditions, since in many experiments solutions of different compositions were used to bathe the two tissue surfaces. Under such conditions diffusion potentials are generated across the tissue and short-circuit current cannot be equated with active sodium transport.

Incubations were performed at 37 °C with 95 % $O_2$/5 % $CO_2$ bubbled continuously through the media bathing both surfaces of the preparations. In all experiments sodium Ringer bathed both surfaces initially for 30–40 min. Then either fresh sodium Ringer or sodium-free choline medium was substituted as detailed in the Tables. When sodium-free medium replaced sodium Ringer, chambers were first washed through three times over 1 min and the medium was replaced with fresh choline medium after 5 min. Flame photometry revealed that medium sodium was less than 0·2 mmol/l with choline medium bathing both surfaces after 30 min incubation. During incubations, transepithelial potential differences were monitored periodically via 3 M-KCl–agar bridges and calomel electrodes.

At the end of the incubation the chambers were rapidly drained and opened, and the epithelial cell preparation was cut from the chamber together with its supporting nylon mesh. This mesh served to hold the tissue as a flat sheet during the initial blotting. Tissue was blotted on Whatman no. 541 filter paper, the nylon mesh being removed before the final blotting. In all but one experiment, where tissue was cut into five pieces, tissue was then cut into two and each half was analysed separately. This provided about 10 mg dry weight of tissue for analysis in each weighing tube.

*Analyses.* Tissue water content was determined by loss of weight after drying for at least 2 h in a hot air oven at 105 °C (Little, 1964). Ions and inulin were extracted from dried tissue overnight in 0·1 M-$HNO_3$ (Little, 1964; McIver & Macknight, 1974). The adequacy of these drying and extraction procedures were confirmed for this tissue in prelimary experiments. Sodium and potassium in the acid extracts were measured with an EEL flame photometer and chloride determined by the method of Cotlove, Trantham & Bowman (1958) using an Aminco Chloride Titrator. Radioactivity in medium and tissue extracts was determined by liquid scintillation counting in a three channel Packard TriCarb Liquid Scintillation counter using 2 ml samples in 17 ml Triton X-100-toluene fluor. Medium osmolalities were checked by freezing point depression on an Advanced Instrument Co. osmometer, model 3 W.

*Presentation of results.* Tissue water and ion contents have been corrected for the space occupied by inulin. Non-inulin space water contents are expressed in kg $H_2O$/kg tissue dry wt. and ion contents in mmol/kg tissue dry wt. The extent to which these non-inulin space values represent cellular composition is discussed later.

The values quoted in the text and Tables are the mean ± S.E. of mean of the number of individual samples analysed, shown in parentheses in the Tables. The statistical significance of differences between groups has been determined using Student's *t* test.

## RESULTS

Initial experiments were performed to determine the time required for equilibration of inulin in the stripped colon preparation. Within 15 min, the space (as a percentage of tissue water) had reached a value which remained relatively constant for 60–90 min as did the derived non-inulin space water content. Both [$^3$H] and [$^{14}$C]inulin occupied the same space in the stripped colon preparation as they do in other epithelial preparations (McIver & Macknight, 1974). Thus the subsequent use of these isotopes as markers for mucosal and serosal inulin spaces in the same piece of tissue is justified.

In rabbit colon, sodium enters the epithelial cells from the lumen across the apical cellular membrane predominantly through an amiloride-sensitive pathway (Frizzell & Turnheim, 1978). One would, therefore, predict that amiloride at a concentration sufficient to abolish transepithelial sodium transport ($10^{-4}$ M, Frizzell *et al.* 1976; Frizzell & Turnheim, 1978) should decrease epithelial cell sodium content. Addition

of amiloride, $10^{-4}$ M final concentration, to the mucosal medium rapidly abolished transepithelial potential difference. Occasionally, as noted by Frizzell *et al.* (1976), a small reversal of the polarity of the transepithelial potential difference was observed. Exposure of the mucosal surface to amiloride resulted in a highly statistically significant decrease in non-inulin space sodium content. (Table 1). This decrease was accompanied by statistically significant decreases in non-inulin space

TABLE 1. Effects of amiloride and of ouabain on non-inulin space composition of rabbit colon epithelial preparations

| | | Non-inulin space | | | |
|---|---|---|---|---|---|
| | Inulin space (% tissue $H_2O$) | $H_2O$ (kg/kg dry wt.) | Na (mmol/kg dry wt.) | K (mmol/kg dry wt.) | Cl (mmol/kg dry wt.) |
| Na Ringer (44) | 43·7±1·5 | 3·29±0·10 | 229±8 | 404±7 | 253±9 |
| Amiloride (45) | 38·7±1·9 | 2·82±0·07 | 153±7 | 425±4 | 186±7 |
| Ouabain (45) | 41·7±1·3 | 2·86±0·09 | 400±11 | 171±4 | 205±9 |
| Amiloride and ouabain (45) | 39·4±1·3 | 2·55±0·05 | 346±7 | 187±4 | 181±7 |
| Difference between Na Ringer and amiloride | | 0·47 | 76 | 21 | 67 |
| *P* | | < 0·001 | < 0·001 | < 0·01 | < 0·001 |
| Difference between Na Ringer and ouabain | | 0·42 | 171 | 232 | 48 |
| *P* | | < 0·001 | < 0·001 | < 0·001 | < 0·001 |
| Difference between Na Ringer and amiloride and ouabain | | 0·74 | 117 | 216 | 72 |
| *P* | | < 0·001 | < 0·001 | < 0·001 | < 0·001 |
| Difference between ouabain and amiloride and ouabain | | 0·32 | 54 | 17 | 24 |
| *P* | | < 0·005 | < 0·001 | < 0·005 | < 0·05 |

Data from colons of thirteen rabbits. Segments of stripped colon were mounted in chambers and incubated under open-circuit conditions at 37 °C. After initial incubation for 30 min with Na Ringer, tissues were then exposed for 30 min either to Na Ringer, or to Na Ringer containing amiloride, $10^{-4}$ M, mucosa, ouabain, $10^{-3}$ M, serosa or to both inhibitors.

water and chloride contents to an extent appropriate to the decreased sodium content. There was also a small, though significant, increase in cellular potassium content, and the derived potassium concentration rose from 123 mmol/kg non-inulin space water to 151 mmol/kg non-inulin space water. These changes in non-inulin space composition after amiloride cannot be accounted for by any effect of amiloride on inulin space (Table 1). Indeed, inulin space as a percentage of tissue water actually decreased slightly after amiloride.

Transepithelial sodium transport requires that sodium entering the cells from the luminal solution should be extruded from the cells to the interstitial fluid, a process

believed to be mediated through the activity of the cardiac glycoside-sensitive enzyme Na-K-ATPase. With sodium Ringer bathing the serosa, addition of ouabain, $10^{-3}$ M, which blocks transepithelial sodium transport by rabbit colon completely (Frizzell *et al.* 1976), resulted in a large loss of cellular potassium (232 mmol/kg dry wt.) with a gain of somewhat less sodium by the non-inulin space (171 mmol/kg dry wt.). This decrease of 61 mmol/kg dry wt. in cation was associated with a decrease of chloride of 48 mmol/kg dry wt. and of water of 0·42 kg/kg dry wt. (Table 1), changes expected

TABLE 2. Effects of amiloride and of restoration of mucosal sodium to sodium-depleted isolated rabbit colon epithelial preparations

| | | Non-inulin space | | | |
|---|---|---|---|---|---|
| | Inulin space (% tissue $H_2O$) | $H_2O$ (kg/kg dry wt.) | Na (mmol/kg dry wt.) | K (mmol/kg dry wt.) | Cl (mmol/kg dry wt.) |
| Choline medium (46) | 44·8 ± 1·4 | 1·68 ± 0·05 | 17 ± 1 | 265 ± 3 | 91 ± 5 |
| Na Ringer mucosa (39) | 42·8 ± 1·3 | 1·98 ± 0·05 | 129 ± 4 | 311 ± 5 | 122 ± 5 |
| Na Ringer and amiloride mucosa (39) | 44·3 ± 1·2 | 1·83 ± 0·06 | 112 ± 5 | 300 ± 4 | 107 ± 6 |
| Difference between choline medium and Na Ringer mucosa | 2·0 | 0·30 | 112 | 46 | 31 |
| *P* | > 0·30 | < 0·001 | < 0·001 | < 0·001 | < 0·001 |
| Difference between Na Ringer and amiloride | 1·5 | 0·15 | 16 | 11 | 15 |
| *P* | > 0·40 | > 0·05 | < 0·02 | > 0·05 | > 0·05 |

Data from colons of fourteen to seventeen rabbits. Segments of stripped colon were mounted in chambers and incubated under open-circuit conditions at 37 °C. Initially Na Ringer bathed both surfaces. After 30 min chambers were washed with choline medium and tissues incubated for 30 min under Na-free conditions. Some tissues were taken for analysis, the remainder were exposed to either Na Ringer or Na Ringer and amiloride, $10^{-4}$ M, mucosa, choline medium serosa, for 15–30 min before analysis.

if the cells lost potassium more rapidly than they gained sodium over the 30 min incubation with ouabain. When tissues were exposed to amiloride in the mucosal medium as well as to ouabain in the serosal medium, the loss of cellular potassium was little affected but cells gained less sodium. As required for electroneutrality and osmotic equilibrium, non-inulin space chloride and water contents were comparably decreased (Table 1).

Though, over-all, amiloride had a highly significant effect on non-inulin space sodium content, only about one third of the total non-inulin space sodium was affected (Table 1). A similar result in toad urinary bladder epithelial cells seems to reflect the presence of a large component of serosal sodium which, though unaccounted for by the inulin space, lies outside of the epithelial cells (Macknight *et al.* 1980). Therefore, tissues were depleted of sodium before being exposed only on their mucosal surfaces to sodium Ringer with or without amiloride (Table 2). Substitution of choline for sodium in the media resulted in a rapid loss of tissue sodium. In initial

experiments, analysed in Table 3, it was found that sodium had fallen to its 30 min value of $17 \pm 1$ mmol/kg dry wt. within 5–10 min and that little further change occurred over 60 min of incubation. But tissues lost not only sodium; they also lost potassium and chloride though the medium concentrations of potassium chloride were unchanged when compared with sodium Ringer. Unlike the rapid sodium loss, the loss of potassium was a gradual and progressive process over 30 min and was paralleled by losses of cellular chloride and of cellular water. Because of uncertainty

TABLE 3. Derived non-inulin space composition of isolated rabbit colon epithelial preparations exposed to sodium-free choline medium

| | Non-inulin space | | | |
|---|---|---|---|---|
| | $H_2O$ (kg/kg dry wt.) | Na (mmol/kg dry wt.) | K (mmol/kg dry wt.) | Cl (mmol/kg dry wt.) |
| Na Ringer (44) | 3·29 | 229 | 404 | 253 |
| Choline medium | | | | |
| 2 min (6) | 2·70 | 67 | 364 | 234 |
| 5 min (6) | 2·31 | 24 | 342 | 174 |
| 10 min (6) | 2·21 | 17 | 311 | 155 |
| 30 min (46) | 1·68 | 17 | 265 | 91 |
| 60 min (16) | 1·77 | 12 | 252 | 102 |

Data from colons of three or more rabbits. Values for Na Ringer from Table 1 and for 30 min choline medium from Table 2. Data for 2, 5 and 10 min choline medium calculated from tissue compositions using an inulin space of 44 % tissue water. Data for 60 min choline medium calculated from measured inulin spaces.

as to the rate at which inulin would equilibrate as tissue water changed, no attempt was made to measure inulin spaces directly over the first minutes after exposure of tissues to choline medium. However, the constancy of the values of extracellular spaces in Tables 1 and 2 justifies the assumption that the space remained relatively constant as a percentage of tissue water and allows estimation of the non-inulin space compositions for the 2, 5 and 10 min exposures to choline medium as provided in Table 3.

It thus appears that two distinct processes followed the replacement of medium sodium by choline. First, there was a rapid exchange of non-inulin space sodium for medium choline largely completed within the first 2 min with relatively little loss of cellular potassium or of non-inulin space water or chloride. Secondly, there was a continuing gradual loss of about 40 % of cellular potassium accompanied by losses of non-inulin space chloride and water over the first 30 min.

Even after 60 min incubation in sodium-free choline medium, there was a small residual amount of sodium in the non-inulin space. Whether this represented sodium within the epithelial cells, within the relatively few smooth muscle cells of the muscularis mucosae, or in some other compartment, remains to be shown.

The restoration of sodium to the mucosal medium after 30 min incubation in sodium-free medium resulted in a large increase in non-inulin space sodium with considerably smaller changes in potassium, chloride and water (Table 2). Of this

increase, only 14% was sensitive to amiloride. Again, as in the experiments in which choline medium replaced sodium Ringer, it seems that most of the change in non-inulin space sodium was accounted for by sodium–choline exchange without change in either the chloride content or the osmolality of that portion of the non-inulin space in which this exchange occurred.

Though it was possible, therefore, to demonstrate a small, though statistically significant, decrease in non-inulin space sodium after amiloride in these experiments,

TABLE 4. Effects of restoration of sodium to sodium-depleted isolated rabbit colon epithelial preparations

| | Non-inulin space | | | |
|---|---|---|---|---|
| | $H_2O$ (kg/kg dry wt.) | Na (mmol/kg dry wt.) | K (mmol/kg dry wt.) | Cl (mmol/kg dry wt.) |
| Na Ringer m and s throughout (8) | 2·10±0·14 | 163±12 | 372±13 | 154±15 |
| Na Ringer m and s 15 min (10) | 2·39±0·04 | 126±5 | 399±8 | 174±5 |
| Na Ringer m, choline medium, s 15 min (10) | 2·00±0·10 | 154±9 | 307±7 | 112±8 |
| Choline medium m, Na Ringer, s 15 min (10) | 2·38±0·12 | 50±15 | 381±10 | 166±9 |

Data from colons of five rabbits. Segments of stripped colon were mounted in chambers and incubated under open-circuit conditions at 37 °C. Initially Na Ringer bathed both surfaces. In each experiment, one tissue was exposed to Na Ringer throughout. For the remainder, chambers were washed with choline medium after 30 min and tissues incubated for 30 min under Na-free conditions. Na Ringer was then restored to either the mucosal (m) or serosal (s) surface or to both (m and s) for 15 min.

the amiloride-insensitive portion of the sodium gained was large and, at 112 mmol/kg dry wt., amounted to about two thirds of the total non-inulin space sodium after amiloride of 153 mmol/kg dry wt. (Table 1). Thus, unlike the findings in epithelial cells of toad urinary bladder (Macknight, Civan & Leaf, 1975*a*), most of the non-inulin space sodium appeared to be of mucosal, not serosal origin. This was confirmed (Table 4) by restoring sodium to either the mucosal, or serosal medium alone or to both media. In this set of experiments, non-inulin space water and ions were somewhat lower than the average values observed over a much larger series of experiments. Nevertheless, it is apparent that replacement of sodium in the serosal medium alone resulted in a much lower non-inulin space sodium content (50±15 mmol/kg dry wt.) than replacement either in both media or in only the mucosal medium.

Additionally, it was found that the presence of sodium in the serosal medium, but not in the mucosal medium, was essential for the complete re-accumulation of the cellular potassium lost by tissues during incubation in choline medium (Table 4). This unexpected observation was confirmed in an additional series of experiments. It was also found that the re-accumulation of potassium was not reduced with sodium Ringer serosa, and amiloride $10^{-4}$ M in choline medium mucosa. Thus diffusion of sodium from the serosal medium through the paracellular pathway with uptake across the

apical cellular membrane was excluded as a possible contributor to the observed potassium re-accumulation. It is concluded, therefore, that sodium in the serosal but not in the muscosal medium is essential for the re-accumulation of potassium to normal levels. However, in as much as ouabain, $10^{-3}$ M, in serosal choline medium further decreased potassium re-accumulation with sodium Ringer mucosa (non-inulin space potassium content $288 \pm 8$ mmol/kg dry wt. in the absence of, and $234 \pm 4$ mmol/kg dry wt. in the presence of ouabain for 15 min, $n = 6$), some ouabain-sensitive potassium uptake must have continued with sodium available only from the mucosal medium.

Finally, two series of experiments were performed to confirm that neither glucose nor chloride in the mucosal medium stimulated sodium entry to the tissues. No change in potential difference was observed in the rabbit colon preparations when 5 mM-glucose was added to the mucosal medium, and chemical analysis after sodium-depleted tissues were exposed for 15 min to sodium Ringer with or without 5 mM-glucose revealed no difference in non-inulin space sodium ($113 \pm 6$, $106 \pm 14$ mmol/kg dry wt. respectively, $n = 4$, $P > 0{\cdot}60$).

Unlike coupled sodium–glucose entry (Rose & Schultz, 1971; Schultz & Curran, 1970), coupled sodium–chloride entry to cells is electrically silent and cannot, therefore, be tested for by measurements of transepithelial potential difference. To exclude the possibility of coupled sodium–chloride cotransport in our preparations, sodium-depleted tissues were exposed for 15 min on their mucosal surface to either normal sodium Ringer or Ringer in which NaCl has been isosmotically replaced with Na gluconate and the sulphate salt of Ca used in place of $CaCl_2$ (the serosa was bathed by choline medium throughout). No significant differences in non-inulin space sodium were detected (values were $107 \pm 7$ and $92 \pm 8$ respectively, $n = 12$, $P > 0{\cdot}20$). It was concluded, therefore, that the amiloride-insensitive non-inulin space sodium of mucosal origin reflected neither coupled sodium–glucose nor coupled sodium–chloride entry to the cells across the apical membrane.

## DISCUSSION

These results show that in a preparation of rabbit colon epithelial cells separated from the underlying mass of smooth muscle and supporting tissues, it is possible to demonstrate significant changes in non-inulin space composition in tissues exposed to amiloride, ouabain, and to sodium-free choline medium. These changes must reflect to a great extent changes in epithelial cell composition for, in light microscopic sections of stripped colon, it was apparent that epithelial cells were the dominant cellular component, with the smooth muscle cells of the muscularis mucosae contributing perhaps 5 % to the total. Therefore, in the discussion which follows, data will be interpreted in terms of epithelial composition and the small background contribution of other cellular elements ignored.

To what extent is the non-inulin space composition presented in the Results synonymous with cellular content? With potassium there is no problem, for the extracellular space concentration of this ion is so low that extracellular potassium contributes only 5–15 mmol/kg dry wt. to total tissue potassium of about 400 mmol/kg dry wt. Thus non-inulin space potassium content must be virtually

identical with cellular potassium content. With sodium, chloride and water, however, the extracellular space contributes appreciably to the tissue content.

The adequacy of inulin as a marker for extracellular water is best assessed by estimating cellular potassium concentration from cellular potassium content and non-inulin space water content, for imprecision in the measurement of the extracellular space will affect the water content but not the potassium content. From Table 1, the derived cellular potassium concentration with sodium Ringer bathing both surfaces, is 123 mmol/kg non-inulin space water, a value typical of mammalian cells in general. Assuming an activity coefficient for potassium of 0·76 within cellular water, this value corresponds to a potassium activity of 93 mmol/kg $H_2O$, a value comparable to potassium activities measured in epithelia with potassium-selective micro-electrodes (for example, Garcia-Diaz & Armstrong, 1980; Wills *et al.* 1979). Therefore, as judged by cellular potassium, non-inulin space water provides a good estimate of cellular water content in this and in other epithelia (Macknight, 1980).

However, the non-inulin space sodium (229 mmol/kg wt., 70 mmol/kg cell water, Table 1), as in other epithelia (Macknight *et al.* 1980), is greater than estimates of the sodium transport pool in colonic cells involved in mucosal-to-serosal transepithelial sodium transport. For example, Schultz *et al.* (1977) predicted a pool of 14 mM from electrophysiological considerations, a value consistent with recent data from other transporting epithelia, obtained from electron microprobe analysis (Rick, Dörge, Macknight, Leaf & Thurau, 1978*a*; Rick, Dörge, Von Arnim & Thurau, 1978*b*) and sodium-selective micro-electrodes (Garcia-Diaz & Armstrong, 1980; Graf & Giebisch, 1979; O'Doherty, Garcia-Diaz & Armstrong, 1979; Wills & Lewis, 1980). Furthermore, after amiloride at a concentration which abolishes both short-circuit current and the related influx of sodium from the mucosal medium to the epithelial cells (Frizzell & Turnheim, 1978), the sodium content decreased only by about one-third. In other 'tight' epithelia, cell sodium after amiloride has been found to fall to very low values (no more than 2–3 mmol/kg dry wt.: Macknight, Civan & Leaf, 1975*a*; Rick *et al.* 1978*b*).

Three possibilities need consideration. First, much of the sodium might occupy an extracellular space inaccessible to inulin. Though other tracers have been used to estimate extracellular spaces in a variety of tissues, none has proved more suitable than inulin for studies in which epithelia are incubated for more than a few minutes (McIver & Macknight, 1974; Hughes, 1980). Smaller molecular weight solutes enter the epithelia more rapidly, but equilibrate through much of the total tissue water. Furthermore, they may also diffuse across the epithelium through the paracellular pathways (for example, mannitol in turtle colon: Dawson, 1977), and are, therefore, unsuitable for studies in which tissues are bathed with media of different compositions on each surface. In any event, an additional extracellular space inaccessible to inulin could not contain an isosmotic extracellular fluid. To account for the sodium not lost from the cells after amiloride (153 mmol/kg dry wt., Table 1), an extra 1·09 kg $H_2O$/kg dry wt. would have to be extracellular, (requiring an extracellular space of 62·3% tissue water). Intracellular water would then be 2·20 kg/dry wt. and cellular potassim concentration 184 mmol/kg water, an unreasonably high value.

The second possibility, that there are extracellular regions of sodium concentration greater than the bulk medium concentration used in calculating the extracellular content of sodium (for example, possible hyperconcentration of sodium in the lateral intercellular spaces) is also untenable. The average extracellular water content in the tissues incubated with sodium Ringer (Table 1) was 2·55 kg $H_2O$/kg dry wt. and tissue sodium averaged 586 mmol/kg dry wt. To reduce calculated cellular sodium even to 100 mmol/kg dry wt. would require that sodium throughout the whole inulin space water be at a concentration of 191 mmol/l, 51 mmol/l greater than the bulk medium concentration. Similarly, after amiloride, (when any lateral space hyperosomolality resulting from

transepithelial sodium transport should be abolished), an average inulin space sodium concentration of 220 mmol/l is required to reduce cellular sodium to 10 mmol/kg dry wt.

Thirdly, since neither failure of inulin to equilibrate through the extracellular water, nor a restricted extracellular hyperconcentration of sodium provide plausible explanations for the magnitude of the non-inulin space sodium, could cellular heterogeneity account for the result? Colonic epithelium comprises not only cells involved in amiloride-sensitive mucosal-to-serosal transport of sodium, but also mucus-secreting Goblet cells and cells, probably in the bases of the crypts, which secrete rather than absorb sodium chloride (Welsh & Frizzell, 1980). This possibility, however, cannot reconcile the sodium data with cellular sodium contents and concentrations reported in other 'tight' epithelia, and estimated for rabbit colon, which approximate 10–20 mmol/kg water. Given that the solid mass is similar, and assuming that all non-inulin space ions and water are distributed equally between cell types, then as judged by non-inulin space sodium contents before and after amiloride (Table 1), only about one-third of every kilogram dry weight of cells would be involved in amiloride-sensitive sodium transport. With water content divided in the same proportions, both transporting and non-transporting cells would contain sodium at a concentration of about 70 mmol/kg water. To decrease sodium concentration in the transporting cells to 20 mmol/kg water or so, the total non-inulin space water content must be confined to the transporting cells, clearly an impossibility. In additon, after amiloride, non-inulin space water decreased by 0·47 kg/kg dry wt. (Table 1). This water must have been lost from amiloride-sensitive cells whose water content would be reduced from 1·10 to 0·63 kg in each kilogram dry weight if these cells comprised only one-third of epithelial cell mass. Since potassium content did not decrease, potassium concentration in these cells would have increased from 123 to 214 mmol/kg $H_2O$, a value far greater than has been measured in any epithelium. Therefore, though different epithelial cell types are found within the colonic epithelium, and these must contain some cellular sodium, the possibility that all the non-inulin space sodium is cellular with only one-third of the cells engaged in active sodium absorption, is untenable. Instead, this analysis suggests that the majority of the cells are transporting sodium via an amiloride-sensitive pathway, and that other cell types must have relatively low cellular sodiums comparable to those of the absorbing cells, as has been demonstrated by electron microprobe analysis of amphibian epithelia (Rick *et al.* 1978*a*, *b*).

These considerations, therefore, render unlikely the possibilities that inulin-inaccessible extracellular water, hyperconcentrations of extracellular sodium in extracellular fluid, or cellular heterogeneity can explain the sodium data. As in other epithelia there is at present no established explanation. In contrast to some other epithelia (for example, toad urinary bladder: Macknight *et al.* 1975*a*) where most of the amiloride-sensitive non-inulin space sodium is associated with the basolateral surface, about two-thirds of this sodium was associated with the mucosal surface (Tables 2 and 4). It remains possible that the mucopolysaccharides which overlie the apical plasma membranes and occupy the crypts may preferentially adsorb sodium thereby serving to maintain a relatively constant activity of sodium in the immediate vicinity of the apical plasma membranes. Such a mechanism could facilitate sodium absorption from solutions of low sodium concentration.

With much of the non-inulin space sodium not within the epithelial cells involved in active sodium absorption, it is difficult to estimate precisely the cellular sodium transport pool. In as much as the cellular content decreased on average by 76 mmol/kg dry wt. after amiloride (Table 1), a loss accompanied by appropriate decreases in chloride and water contents, cellular sodium content must be at least this great when sodium Ringer bathes both surfaces of the cells. Sodium concentration would then approximate 20 mmol/kg water, a value very similar to that measured by electron microprobe analysis in epithelial cells of toad urinary bladder (Rick *et al.* 1978*a*), frog skin (Rick *et al.* 1978*b*) and distal tubular cells of rat kidney (Beck, Bauer, Bauer, Mason, Dörge, Rick & Thurau, 1980), all of which are 'tight' epithelia.

Comparison of these results with those reported by Turnheim *et al.* (1978) for a similar preparation of rabbit descending colon reveals close agreement with the data obtained in sodium Ringer (0·55 mmol/cm$^2$ or 220 mmol/kg dry wt., using our approximate tissue weight/cm$^2$ of 2·5 mg, compared with 229 ± 8, Table 1), with sodium-free mucosal solution (60 mmol/kg dry wt. compared with 50 ± 15, Table 4,) and with sodium-free serosal solution (160 mmol/kg dry wt. compared with 129 ± 4, Table 2). However, these workers do not present data for tissues exposed to amiloride; they equate the non-inulin space sodium of mucosal origin to cellular sodium. As shown (Table 2), most of this sodium is unaffected by amiloride and, as argued above, it seems that cellular sodium approximates 70 mmol/kg dry wt. rather than the 160 mmol/kg dry wt. assumed by Turnheim *et al.* (1978) to be cellular. Our analysis does not, therefore, support their notion of a cellular compartment in parallel with an active transport pathway of much smaller capacity, both of which are sensitive to amiloride. Instead we postulate a single transport pool with a sodium concentration of about 20 mmol/kg $H_2O$, which, with the transport rates reported by Turnheim *et al.* (1978) would give a turnover rate for the cellular sodium pool of about 10 times/h or once every 6 min.

Analysis of the effects of ouabain (Table 1) reveals that, as predicted, there was a marked loss of cellular potassium. This was accompanied by an uptake of sodium to the cells which was, however, insufficient over the 30 min time course of the experiments to balance completely the potassium loss. Therefore, the cells shrank, losing chloride and water (Table 1). The sodium gained by the cells was derived partly from the mucosal medium, for cells gained less sodium with amiloride and ouabain. However, a considerable fraction of the sodium gained was derived from the serosal medium (as in toad urinary bladder, Macknight *et al.* 1975*b*). This does not indicate, however, that the apical and basolateral membrane permeabilities to sodium are normally similar, for, in a variety of 'tight' epithelia, as cellular sodium increases, apical membrane sodium permeability decreases (Erlij & Smith, 1973; Lewis, Eaton & Diamond, 1976; MacRobbie & Ussing, 1961; Turnheim *et al.* 1978). The shrinkage of rabbit colon cells exposed to ouabain is not inconsistent with the simple view that cellular volume is regulated by the conventional sodium pump (Macknight & Leaf, 1977). A greater membrane permeability to potassium than to sodium, with resultant loss initially of some cellular potassium with chloride and water rather than only in exchange for medium sodium would cause such shrinkage. Though cellular swelling must inevitably follow inhibition of the pump, such swelling may be a slow process.

The cell shrinkage with incubation in sodium-free choline medium (Tables 2 and 3) and the requirements of serosal rather than mucosal medium sodium for restoration of cellular potassium and water content to more normal values (Table 4) deserve mention. Complete substitution of choline for sodium in the medium bathing both surfaces results in loss of cellular potassium in other epithelia also (for example, mammalian kidney slices: Hughes & Macknight, 1977; toad urinary bladder epithelial cells: Hughes, 1980). This loss would be consistent with inhibition of the Na-K-ATPase in the absence of cellular sodium for, even if some choline enters the cells, choline cannot substitute for sodium as an activator of the enzyme (Hokin & Dahl, 1972; Skou, 1975). The losses of chloride and water to an extent comparable to the loss of potassium, are as expected if the basolateral membranes are relatively impermeable

to choline over the 30 min incubation. With the tissues again bathed on both surfaces by sodium Ringer, cellular potassium had recovered to control values within 15 min, with an uptake of chloride and of water (Table 4). The same recovery was seen with sodium Ringer only in the serosal surface, but not with sodium Ringer only on the mucosal surface. A major determinant of the activity of the sodium–potassium pump in epithelial cells is the concentration of cellular sodium (Jørgensen, 1980). Since sodium enters the cells predominantly across the apical membrane in 'tight' epithelia, one would anticipate that restoration of cellular potassium would have been enhanced by mucosal rather than by serosal sodium. Yet the fact that cellular potassium content is unaffected (and concentration actually higher) when apical sodium entry is blocked by amiloride (Table 1) in tissues bathed on both surfaces by sodium Ringer indicates that serosal sodium contributes to the maintenance of a normal cellular potassium. A small leakage of sodium from serosal medium to cells might suffice to activate the Na-K-ATPase sufficiently to prevent cellular potassium from falling after amiloride. Whether such a backflux would be sufficient to enable potassium to be re-accumulated to a greater extent than would increased cellular sodium with sodium Ringer mucosa, is questionable. For, if sodium concentration in the micro-environment of the basolateral membrane Na-K-ATPase were determined more by sodium backflux from the serosal medium than by cytoplasmic sodium concentration, would the rate of transepithelial sodium transport vary with mucosal medium sodium concentration (Leaf, 1965; Kirschner, 1955; Ussing, 1949)?

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