

Villus and Crypt Cell Composition in the Secreting Mouse Jejunum Measured with X-ray Microanalysis

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Abstract. The response of the villus and crypt cells of the mouse jejunum to secretagogues has been assessed through measurements of cellular composition with x-ray microanalysis. In nonstimulated tissues the Na concentration ($[Na]_c$) of the crypt cells was significantly less, and the K ($[K]_c$) and Cl ($[Cl]_c$) concentrations were significantly greater, than that of the villus cells. There was also a decreasing gradient of $[Na]_c$ and increasing gradient of $[K]_c$ from the villus tip to crypt base due to a greater number of cells with a high $[Na]_c$ and low $[K]_c$ in the upper regions of the villi. Theophylline (10 mmol L^{-1}) stimulated a sustained increase in bumetanide sensitive short circuit current (I_{sc}) and significantly decreased the $[Na]_c$ of the villus cells. Similar, but smaller changes were seen in the crypt cells. Changes in villus cell $[Na]_c$ reflected a reduction in the number of cells with a high $[Na]_c$. Inhibition of the apical Na/H exchanger (1 mmol L^{-1} amiloride) had little effect on basal I_{sc} and the subsequent addition of theophylline increased I_{sc} to a comparable extent as seen without amiloride. However, after amiloride treatment the only change in cellular composition was a reduction in the $[Cl]_c$ of both crypt and villus cells, suggesting that both regions are involved in the secretory response. These data suggest that the dominant response of the jejunum to secretagogues is an inhibition of Na absorption via Na/H exchange in the villi and the secretory response is distributed throughout the crypt/villus axis.

Key words: Electroneutral Na absorption — Na/H exchange — Cl secretion — Crypt/villus axis — X-ray microanalysis

Introduction

The jejunal epithelium is both structurally and functionally complex. It consists of at least 5 different cell types (Cheng & LeBlond, 1974) which are subdivided into two major regions, the villi and the crypts. Functionally, the jejunal epithelium actively absorbs Na and nutrients via Na-coupled solute transport (Sullivan & Field, 1991) and Na and HCO_3^- through the action of a Na/H exchanger (Turnberg et al., 1970b). K (Turnberg, 1971) and Cl absorption occur passively (Turnberg et al., 1970b). Alternatively, with the appropriate stimulus the jejunum secretes copious quantities of fluid driven by electrogenic Cl transport (Sullivan & Field, 1991). Secretory stimuli usually stimulate electrogenic Cl secretion and inhibit electroneutral Na absorption (Sullivan & Field, 1991). This dual action of secretagogues plays an important role in the conversion of the intestine from an absorptive to a secretory state. In many instances the dominant effect of secretagogues is a reduction in the mucosal to serosal fluxes of Na and Cl rather than an increase in the serosal to mucosal fluxes (Field, 1971).

Based upon a large amount of circumstantial evidence, it is generally argued that the absorptive processes of the small intestine are localized in the villi and the secretory mechanisms in the crypts (Sullivan & Field, 1991). The distribution of the Na-glucose cotransporter supports this functional subdivision of the crypt/villus axis, with cotransport proteins being found only in the villi, and in increasing quantities towards the tip of the villi (Hwang, Hirayama & Wright, 1991). However, while NHE-3, the Na/H exchanger responsible for sodium absorption by the intestinal epithelium (Bookstein et al., 1994; Hoogerwerf et al., 1996) is concentrated in the villi, it is also present, albeit at lower levels, in the crypt cells (Hoogerwerf et al., 1996). Similarly, CFTR,

the putative secretory Cl channel (Bear et al., 1992; Welsh et al., 1992), is present throughout the crypt/villus axis of the jejunum, although there are higher amounts in the crypt than the villi (Strong, Boehm & Collins, 1994). These direct measurements of the distribution of the relevant transport proteins suggest that the initial functional subdivision of the crypt/villus axis, while qualitatively correct in that the crypts have the larger amounts of the secretory proteins and the villi larger amounts of the absorptive proteins, was quantitatively incorrect in that the crypts have the capacity for absorption and the villi the capacity for secretion.

The objective of this study was to provide a functional description of the role of the crypt and villus cells of the mouse jejunum in the response to secretagogues, through measurements of the composition of the cells with x-ray microanalysis. The data obtained indicate that an important aspect of the effects of secretagogues is an inhibition of the absorptive fluxes in the jejunum. This involves the inhibition of a Na/H exchanger and occurs predominantly in the villus cells. In contrast it would appear that secretagogues stimulate Cl secretion in both crypt and villus cells.

Materials and Methods

Jejunal epithelium was obtained from male Swiss random mice (28–32 days old). The mice were killed by cervical dislocation and the entire small intestine removed. The jejunum was separated from the ileum and duodenum and rinsed with NaCl Ringer's, cut along the mesentery and glued to small plastic annuli (exposed surface area 0.7 cm²) with tissue adhesive (Histocryl, Braun Melsungen, Germany). The rings were then mounted in modified Ussing chambers and superfused with mammalian Ringer's solution maintained at 37°C.

Cl secretion was stimulated by the addition of 10 mmol L⁻¹ theophylline to the mucosal and serosal baths.

ELECTRICAL MEASUREMENTS

Tissues were short circuited at all times by a computer controlled (Apple IIe) automatic voltage clamp. The computer recorded the short circuit current (*I*_{sc}) at 20-sec intervals. Transepithelial resistance (*R*_t) was determined by imposing a potential difference (2 mV) for 1 sec across the tissue every 2 min, and dividing the applied potential difference by the recorded change in current at the end of the pulse.

TISSUE PREPARATION

After appropriate incubations, the rings and attached tissues were removed from the Ussing chambers and blotted with Whatman 542 filter paper on both the mucosal and serosal surface to remove excess solution. An external standard solution containing albumin (30%) was applied to the mucosal surface with a Pasteur pipette and the excess removed with filter paper. The plastic ring and attached tissue were

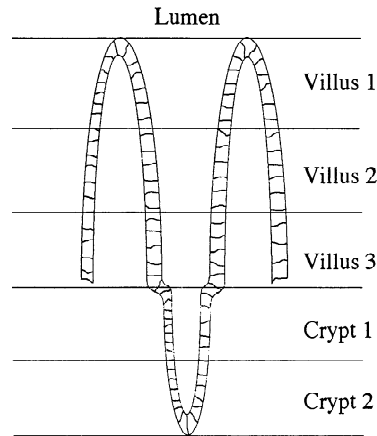


Fig. 1. Schematic diagram showing the 5 regions of the crypt/villus axis used for x-ray microanalysis. When only villus or crypt results are reported, measurements were taken throughout the villus or crypt regions.

then plunged into a propane-isopentane mixture at liquid nitrogen temperature. Time from removal of the tissue to freezing did not exceed 30 sec.

CRYOSECTIONING AND TISSUE ANALYSIS

Sections were cut at -80 to -90°C with a modified Cryocut I (Burlington Scientific Instruments) fitted to a Sorvall MBT-2 ultramicrotome. Sections were transferred to formvar coated 3 mm nickel slot grids which were then placed face down on formvar film on an aluminum holder. The sections were thus sandwiched between two films. At all times attempts were made to obtain sections which spanned the crypt/villus axis extending from the tip of the villi to the base of the crypts. This axis was then subdivided into five regions. The villi were divided into thirds and labeled villus 1, 2 and 3, extending from the villus tip to base. The crypts were divided in two sections; crypt 1 the upper region and crypt 2 the basal region (see Fig. 1). In all cases the cells analyzed were selected from the middle area of the 5 defined regions.

Two or more blocks from each piece of frozen tissue were cut and several sections from each block analyzed. The sections were transferred into a scanning electron microscope (JEOL JSM-840) with a hexland transfer device, pre-cooled with liquid nitrogen. The microscope stage was precooled (<-180°C). Sections were freeze dried by warming the stage.

ANALYSIS OF SECTIONS

Sections were imaged with a transmitted electron detector. Spectra were collected for 100 sec at 15 kV with a Tracor Northern X-ray 30 mm² detector connected to a Nucleus AD converter. The probe current (150–250 pA) was measured with a Faraday cup after each spectrum.

The response of the cells to secretagogues was quantified by changes in cellular ion concentration and content. To obtain measurements of cellular ion concentration spectra were collected from at least two relatively large areas of albumin above the cells. Data from these areas were then pooled to provide an average value and cellular composition (in mmol/kg wet weight) obtained by normalizing spectra to

the known concentrations in the peripheral albumin standard (Bowler, Purves & Macknight, 1991; Rick, Dorge & Thurau, 1982). To obtain estimates of ion content spectra were obtained from an area confined to the nucleus and all values normalized to the phosphorous signal (Bowler et al., 1996).

In the initial study of cellular composition under basal conditions tissues were obtained from 8 animals. In all subsequent experiments data were obtained from jejunal samples from 2–4 control animals and 2–4 experimental animals. All values are presented as mean \pm SE of the mean. Statistical significance was assessed by Student's *t* test or ANOVA with Fisher's post test.

SOLUTIONS

Solutions were prepared fresh as required from analytical grade reagents purchased from Sigma Chemicals (St. Louis, MO). The mammalian Ringer's had the following composition (in mmol L⁻¹): NaCl, 120; KCl, 5; CaCl₂, 1.25; MgSO₄, 1; NaHCO₃, 25; pH 7.4 Solutions added to the serosal chambers contained 10 mmol L⁻¹ glucose as a metabolic substrate and 10 mmol L⁻¹ mannitol was added to the mucosal solution to balance the osmolality. Also 100 μ mol L⁻¹ phloridzin was included in the mucosal bath to eliminate Na coupled glucose transport resulting from paracellular leak of glucose from the serosal solution (Pappenheimer, 1987). Mixing and maintenance of the solution pH was achieved by bubbling with 95%O₂/5%CO₂.

Stock solutions of bumetanide (a generous gift from Leo Pharmaceuticals) and phloridzin were prepared in ethanol and microlitre quantities added to the appropriate bath. Amiloride (Merck, Sharp and Dohme, New Zealand) was added directly to the appropriate bath as a solid to give final concentrations of 1 mmol L⁻¹. Theophylline was also added as a solid to give a final concentration of 10 mmol L⁻¹.

Results

MORPHOLOGY OF FREEZE DRIED SECTIONS

In general, sections could be cut from frozen, freeze dried jejunum that allowed discrimination between the villi and the crypts (Fig. 2A). Within the villi it was possible to identify well defined cytoplasmic and nuclear regions in the individual cells and a distinct brush border on their apical surface (Fig. 2B). Furthermore, although it was not possible to reliably identify all of the minor cell types of the villus (e.g., enteroendocrine cells), goblet cells, which constitute 4.7% of the total epithelial cells within the villi, could be easily distinguished from the enterocytes, which represents 95% of the epithelial cells of the villi (Cheng & LeBlond, 1974). Within the crypts identification of the various cellular types was more difficult. It was possible to discriminate the columnar cells, which represent 86% of the crypt epithelial cells (Cheng & LeBlond, 1974), from the goblet cells (6%) and Paneth cells (7.5%) which tend to be concentrated at the base of the crypts (Cheng, 1974) and contain large numbers of dark granules (Fig. 2A). The contents

of the columnar cells, however, were dominated by the nucleus and the cytoplasm was restricted to a small band at the margins of the cells (Fig. 2C).

At times cells were seen in the albumin layer with which the epithelia were coated immediately prior to freezing. These loose cells were presumably shed from the epithelium during preparation for freezing and may represent senescent cells (Lipkin, 1987).

INTERPRETATION OF DATA

The measurements in this study were restricted to the major epithelial cell type within the villi and crypts, the enterocytes and the undifferentiated columnar cells, respectively. Initially we attempted to obtain separate measurements of composition in both the cytoplasm and nucleus of all cells. However, because the contents of the undifferentiated columnar cells of the crypts were dominated by the nuclei, it was difficult to scan nuclear- and granular-free areas of the cytoplasm in these cells. Consequently, all measurements of cell composition were made in the nuclear region of the cells. This provides a distinct uniform intracellular region from which to sample composition and in other epithelial tissues it has been shown that for small diffusible ions such as Na, K and Cl the cytoplasm and nucleus behave as one distributional space (Rick et al., 1985).

In the estimation of cellular ion concentrations from external standards, it is assumed that the section is the same thickness at the sites of measurement of the external standards and the cells. If this is not the case, differences in thickness, and hence mass, between the two sites of measurement can result in incorrect estimates of ion concentration. This is generally minimized by measuring the external standard immediately adjacent to the measured cell. However, the geometry of the jejunal epithelium is such that the external albumin standard generally only diffused to the middle of the villi and never penetrated the crypt lumen (Fig. 2). Therefore, depending on the cell measured there could be appreciable distance between the external standard and the measured cell. To provide some indication of the possible effect of this, the results are presented both as a concentration, expressed in mmol/kg wet weight, and also as a content, obtained by normalizing the ion values to the phosphorous signal.

Phosphorous is a component of the non-diffusible proteins and therefore it is representative of the cellular mass, as is indicated by the relationship between cell mass and phosphorous concentration ([P]c) (Fig. 3). Thus variations in section thickness and cellular mass will be reflected in variations in phosphorous concentration. Therefore, phosphorous provides an internal control for the mass effects. Consistent with this is the

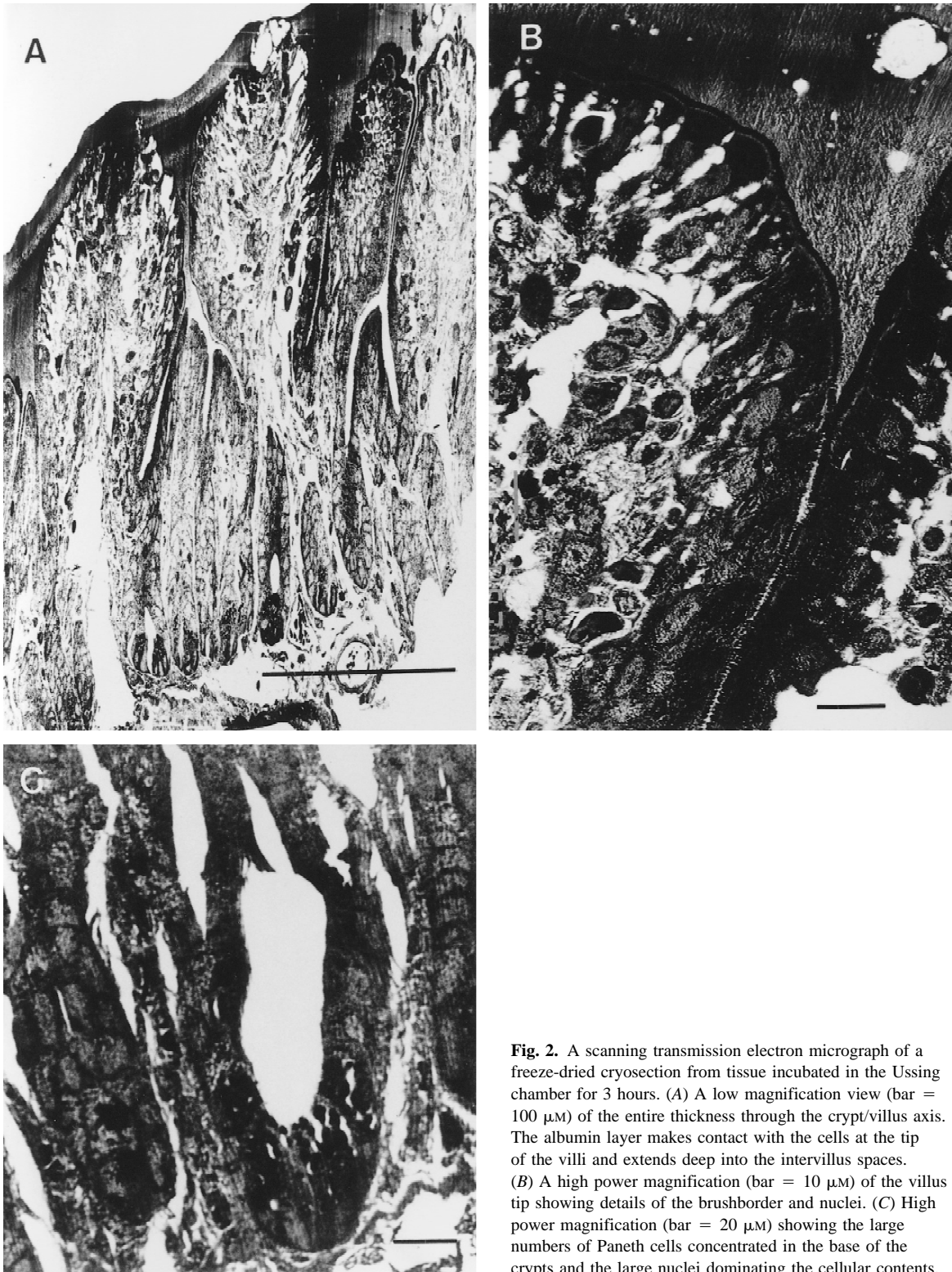


Fig. 2. A scanning transmission electron micrograph of a freeze-dried cryosection from tissue incubated in the Ussing chamber for 3 hours. (A) A low magnification view (bar = 100 μM) of the entire thickness through the crypt/villus axis. The albumin layer makes contact with the cells at the tip of the villi and extends deep into the intervillus spaces. (B) A high power magnification (bar = 10 μM) of the villus tip showing details of the brushborder and nuclei. (C) High power magnification (bar = 20 μM) showing the large numbers of Paneth cells concentrated in the base of the crypts and the large nuclei dominating the cellular contents.

close relationship between cell K concentration ($[\text{K}]_c$) and $[\text{P}]_c$ (Fig. 4A, $r = 0.69$) and cell Cl concentration ($[\text{Cl}]_c$) and $[\text{P}]_c$ (Fig. 4B, $r = 0.52$). The relationship between $[\text{P}]_c$ and cell Na concentration ($[\text{Na}]_c$) is less

clear (Fig. 4C). This most likely reflects the fact that cellular ion concentration is not only a function of mass of the section. Other factors, such as transport rate and cell type, will modify this relationship. With regards Na,

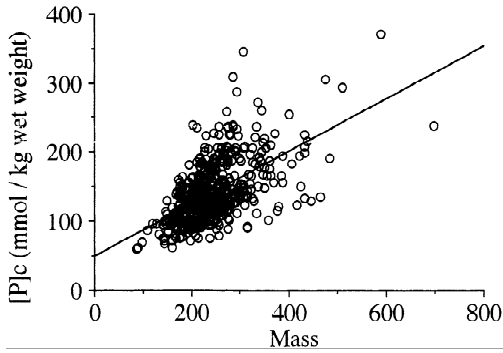


Fig. 3. Intracellular P concentration ([P]_c) as a function of cellular mass. Each point represents an individual measurement. Data from 4 animals and includes values from the crypt and villus regions. The linear regression line describes the function $[P]_c = 50 + 0.38 \text{ mass}$. The r value is 0.60.

in the jejunum it is the ion which is most profoundly effected by cell type and/or transport rate (*see* below). This normalization does have limitations, as the non-zero intercepts indicate the relationship between [P]_c and the other ions is more than a simple multiplicative factor.

COMPOSITION OF ENTEROCYTES AND UNDIFFERENTIATED COLUMNAR CELLS WITHIN THE VILLI AND CRYPTS OF UNSTIMULATED JEJUNUM

The composition of the jejunal enterocytes and undifferentiated columnar cells under basal conditions, expressed both as mmol/kg wet weight and normalized to the phosphorous signal, are summarized in Table 1. These values were obtained from 17 jejunal tissue samples from 8 mice. The average I_{sc} and R_t for these tissues immediately before freezing were $40 \pm 4 \mu\text{A}\cdot\text{cm}^{-2}$ and $39 \pm 3 \Omega\cdot\text{cm}^2$, respectively. Overall, the composition of these cells was comparable to that seen in other epithelia (Dörge et al., 1988; Halm & Rick, 1992; Rick et al., 1987), with a low $[\text{Na}]_c$ ($26.5 \pm 0.6 \text{ mmol/kg wet weight}$), high $[\text{K}]_c$ ($116.7 \pm 1.2 \text{ mmol/kg wet weight}$) and a relatively low $[\text{Cl}]_c$ ($21.8 \pm 0.3 \text{ mmol/kg wet weight}$).

Interestingly, there were significant differences in the composition of the crypt and villus cells. This was evident whether the data was expressed as mmol/kg wet weight or normalized to the phosphorous content. The mean $[\text{Na}]_c$ of the crypt cells ($17.2 \pm 0.5 \text{ mmol/kg wet weight}$) was approximately half that of the villus cells ($33.3 \pm 0.9 \text{ mmol/kg wet weight}$) ($P < 0.05$), while the mean $[\text{K}]_c$ of the crypt cells ($130.6 \pm 1.7 \text{ mmol/kg wet weight}$) was 22% greater ($P < 0.05$) than that of the villus cells (106.5 ± 1.4). The mean $[\text{Cl}]_c$ of the crypt cells ($23.3 \pm 0.3 \text{ mmol/kg wet weight}$) was slightly, although significantly ($P < 0.05$), greater than that of the villus cells ($20.8 \pm 0.41 \text{ mmol/kg wet weight}$).

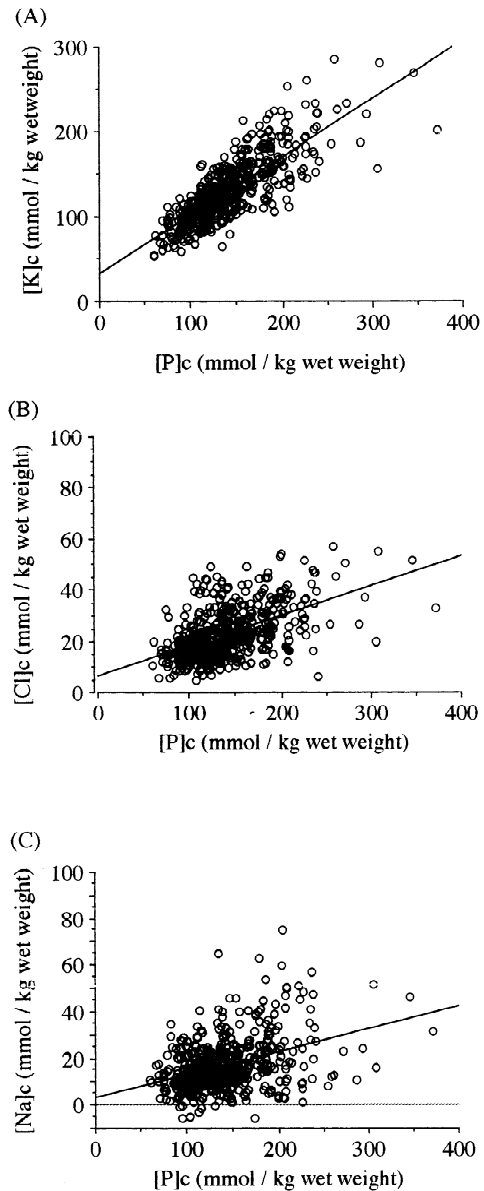


Fig. 4. Intracellular K ([K]_c), Cl ([Cl]_c) and Na ([Na]_c) concentration as a function of intracellular P concentration ([P]_c). Each point represents an individual measurement. Data from 4 animals. (A) $[\text{K}]_c = 33 + 0.689[\text{P}]_c$, $r = 0.69$. (B) $[\text{Cl}]_c$ as a function of $[\text{P}]_c$, $[\text{Cl}]_c = 6.7 + 0.117[\text{P}]_c$, $r = 0.52$. (C) $[\text{Na}]_c$ as a function of $[\text{P}]_c$, $[\text{Na}]_c = 3.3 + 0.092[\text{P}]_c$, $r = 0.32$.

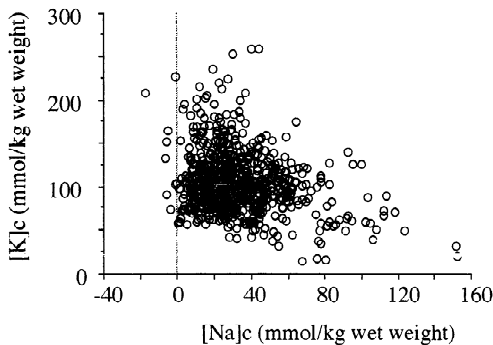
The difference in the $[\text{Na}]_c$ of the crypt and villus cells is associated with differences in the patterns of cell Na and K in the two regions. The villus cells had a much broader spread of $[\text{Na}]_c$ than the crypt cells, with a number of cells with a high $[\text{Na}]_c$ and low $[\text{K}]_c$ (Fig. 5).

More of these high Na, low K cells were found in the upper regions of the villi (Fig. 6, Villus 1 and Villus 2) than in the base of the villi (Fig. 6, Villus 3) or crypts (Fig. 6, Crypts 1 & 2) resulting in a significant decrease in the mean $[\text{Na}]_c$ from the villus tip to the crypt base

Table 1. Composition of mouse jejunal epithelial cells in non-stimulated tissues

	<i>n</i>	Na	K	Cl	P	Mass
		mmol/kg wet weight				g/kg
(A)						
All cells	1146	26.5 ± 0.6	116.7 ± 1.2	21.8 ± 0.3	141.3 ± 1.3	262 ± 2
Villus	658	33.3 ± 0.9	106.5 ± 1.4	20.8 ± 0.4	140.6 ± 1.7	276 ± 3
Crypt	488	17.2 ± 0.5*	130.6 ± 1.7*	23.3 ± 0.3*	142.2 ± 2.0	243 ± 3*
Loose cells	7	51.3 ± 5.0	64.2 ± 11.6	31.3 ± 3.0	90.5 ± 26.3	281 ± 6
(B)						
		Na/P	K/P	Cl/P		
All cells	1146	0.198 ± 0.005	0.840 ± 0.006	0.161 ± 0.002		
Villus	658	0.253 ± 0.007	0.768 ± 0.007	0.157 ± 0.003		
Crypt	488	0.123 ± 0.003*	0.937 ± 0.007*	0.168 ± 0.003*		
Loose cells	7	0.837 ± 0.181	0.735 ± 0.063	0.476 ± 0.108		

All measurements from the nuclear region. (A) Mean ionic concentration (mmol/kg wt weight). (B) Ionic content of cells. Na, K and Cl values normalized to the P content. Loose cells were located within the albumin standard applied to the tissue immediately before freezing. Values obtained for loose cells are not included in calculation of mean values for other groupings of cells. *n* = number of cells obtained from 17 jejunal samples from 8 mice. Crypt values labeled with an asterisk (*) are significantly different from villus ($P < 0.05$, ANOVA).

(A) Villus Cells

higher [Cl]c ($P < 0.05$) than the cells in any other region (Fig. 7C).

Also shown in Table 1 is the composition of the loose cells seen in the albumin layer. Only 7 cells were seen. These cells were swollen, as is evident from their low [P]c, and they had high [Na]c and [Cl]c and a reduced [K]c compared to cells within the epithelium.

STEADY-STATE Cl SECRETION AND THE COMPOSITION OF THE JEJUNAL EPITHELIAL CELLS

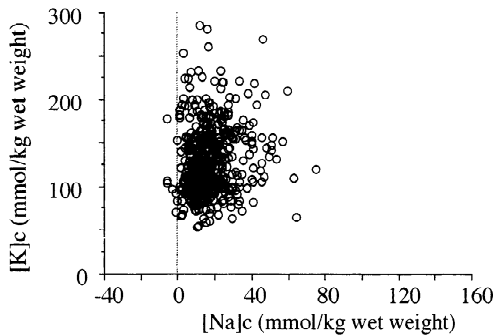
(B) Crypt Cells

Fig. 5. Intracellular K concentration ([K]c) as a function of intracellular Na concentration ([Na]c) for tissues under basal nonstimulated conditions. (A) Villus cells. (B) Crypt cells. Values from 17 pieces of tissue from 8 mice. Data summarized in Table 1.

(Fig. 7A). The reverse was true for potassium (Fig. 7B). In contrast there was little variation in the [Cl]c throughout the crypt/villus axis, with the exception of the cells at the base of the crypts (crypt 2) which had a significantly

In these experiments, cellular composition was measured either in tissues in which Cl secretion had been stimulated by theophylline (10 mmol L⁻¹ mucosal and serosal) or time matched controls. In general theophylline produced an initial peak increase in *I*_{sc} followed by a decline to a lower plateau value. The theophylline induced increase in *I*_{sc} was inhibited by bumetanide (*I*_{sc} before bumetanide 136 ± 13 μA.cm⁻² and after bumetanide 69 ± 8 μA.cm⁻², *n* = 11), consistent with a Cl secretory response (Fig. 8). Tissues used for analysis were frozen once the *I*_{sc} had reached a steady state value (20–30 min). The initial *I*_{sc} prior to theophylline was 26 ± 12 μA.cm⁻² (*n* = 3). This increased to a peak value of 162 ± 48 μA.cm⁻² after theophylline before declining to a steady state value of 95 ± 20 μA.cm⁻² immediately before freezing. In the controls the initial *I*_{sc} was 28 ± 12 μA.cm⁻², and this spontaneously increased throughout the course of the experiment to 54 ± 2 μA.cm⁻² (*n* = 3) immediately prior to freezing (Table 2).

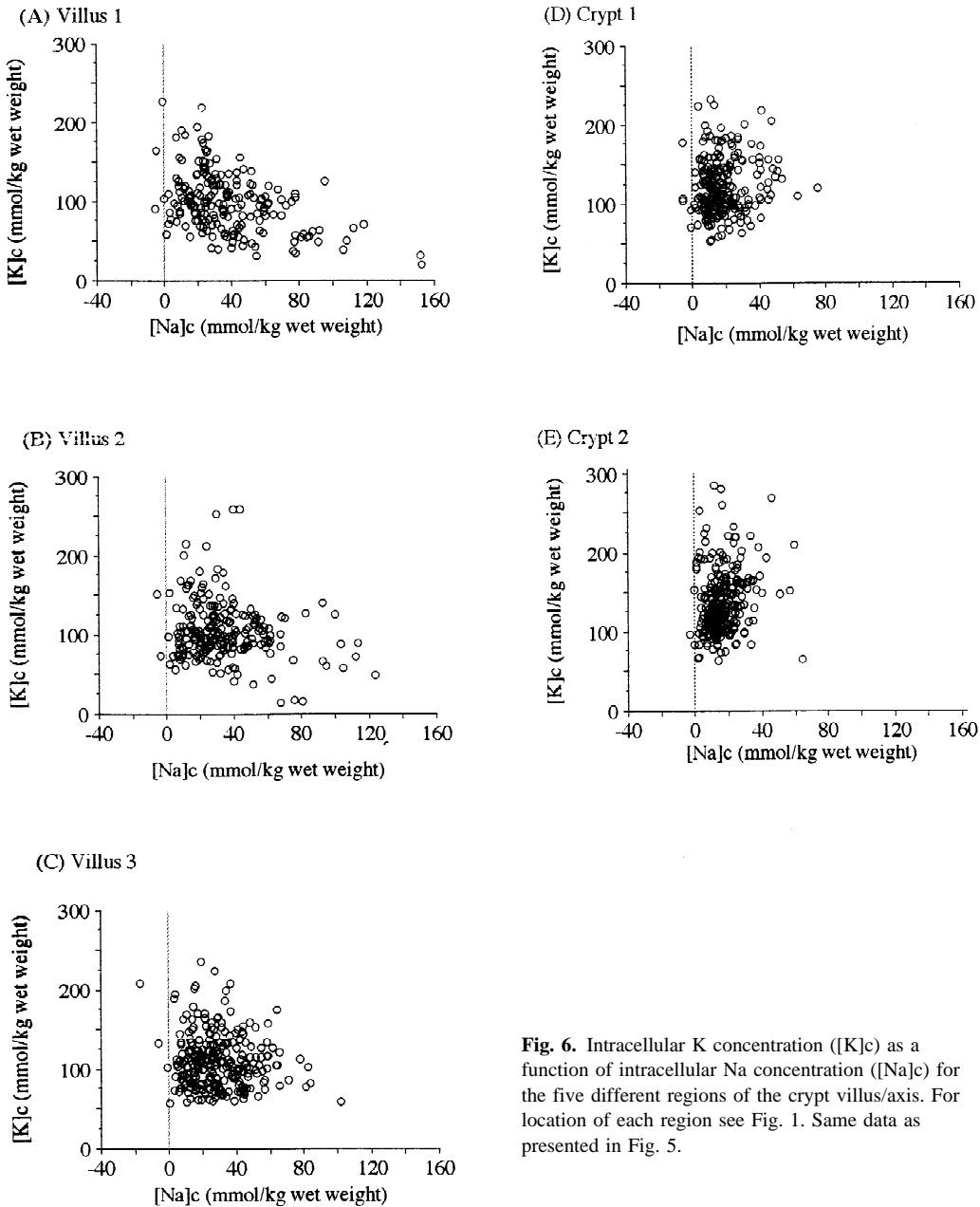


Fig. 6. Intracellular K concentration ([K]c) as a function of intracellular Na concentration ([Na]c) for the five different regions of the crypt villus/axis. For location of each region see Fig. 1. Same data as presented in Fig. 5.

The most significant change in composition associated with the stimulation of Cl secretion was a marked reduction in the [Na]c of both the villus and crypt cells. Within the villi, the [Na]c fell from 37.7 ± 2.6 to 20.3 ± 1.3 mmol/kg wet weight, whereas there was a smaller, but significant decrease in [Na]c of the crypt cells from 26.3 ± 1.9 to 16.4 ± 1.4 mmol/kg wet weight.

The basis for this change in [Na]c is apparent from a consideration of the Na distributions of the cells. In the villus cells of the control tissues there were a number of cells which had high [Na]c as is evident from the broad range of [Na]c (Fig. 9A). Following stimulation of Cl

secretion the number of villus cells with a high [Na]c decreased markedly (Fig. 9C). In contrast, in the crypt cells, while there was a fall in the mean [Na]c with the stimulation of Cl secretion, the change was much smaller (Table 3). This reflects the fact that in the nonstimulated crypt cells there were very few cells with a high [Na]c (Fig. 9B), although the stimulation of Cl secretion did result in a reduction in the number of high Na cells (Fig. 9D).

Interpretation of the changes in the [K]c and [Cl]c following stimulation of secretion is complicated by the significant differences in [P]c between the two groups of

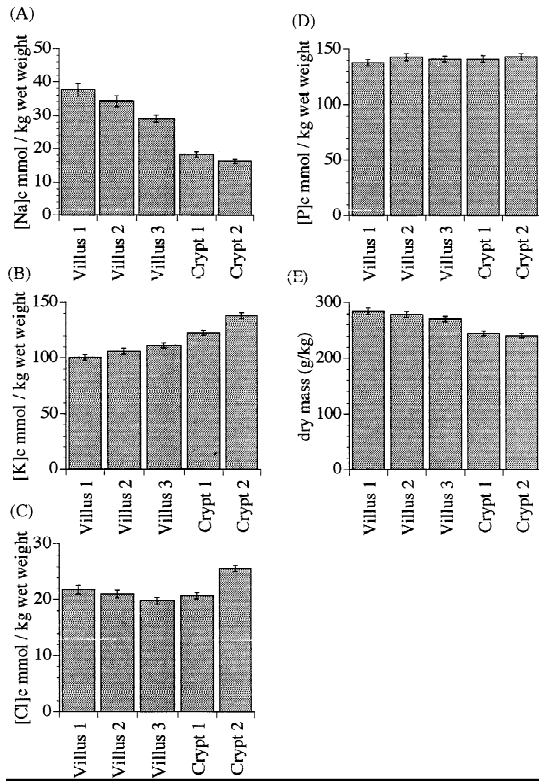


Fig. 7. Cellular ion concentration of jejunal epithelial cells located in various regions throughout the crypt villus axis. (A) Intracellular Na concentration ([Na]c); (B) Intracellular K concentration ([K]c); (C) Intracellular Cl concentration ([Cl]c). (D) Intracellular phosphorous concentration ([P]c). (E) Mass. For location of each region see Fig. 1.

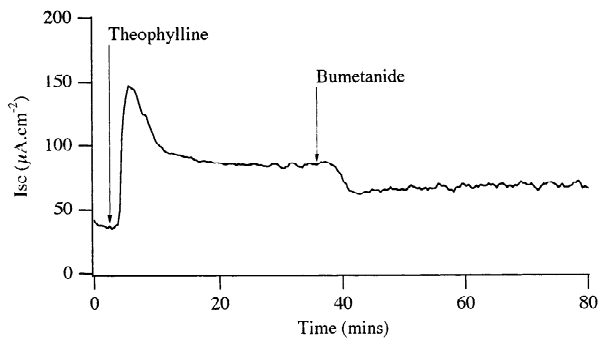


Fig. 8. A representative example of the effects of 10 mmol L⁻¹ mucosal and serosal theophylline on the *I*_{sc} generated by the mouse jejunal epithelium and the subsequent effects of 10 $\mu\text{mol L}^{-1}$ serosal bumetanide.

tissues (Table 3). However, consideration of the cellular ion contents, expressed as the ion/phosphorous ratio, provide some indication of the effect of secretagogues on these ions. Consistent with the effects of secretagogues on [Na]c, there was a fall in the Na content of both the villus and crypt cells. There was also an increase in the

Table 2. Variation in short circuit current (*I*_{sc}) in response to theophylline (10 mmol L⁻¹ mucosal and serosal) in the absence or presence of 1 mmol L⁻¹ mucosal amiloride.

	(n)	<i>I</i> _{sc} ($\mu\text{A}\cdot\text{cm}^{-2}$)		
		Initial	Peak	Steady state
No Amiloride				
Unstimulated controls	3	28 ± 12		54 ± 2
Stimulated tissues	3	26 ± 12	162 ± 48	95 ± 20*
1 mM Mucosal amiloride				
Unstimulated controls	4	20 ± 8		37 ± 7
Stimulated tissues	4	18 ± 7	118 ± 21	86 ± 10*

Values labeled with an asterisk (*) are significantly different to their respective controls (Unpaired *t*-test. *P* < 0.05).

K content and Cl content of the villus and crypt cells in the stimulated tissues (Table 3).

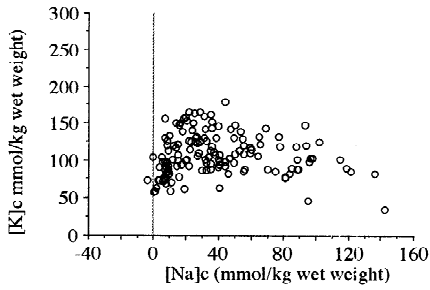
EFFECT OF AMILORIDE ON CHANGES IN CELLULAR COMPOSITION ASSOCIATED WITH Cl SECRETION

The increase in *I*_{sc} following theophylline is consistent with the stimulation of Cl secretion. Thus it might be expected that those cells involved in the secretory response would have an increased Na concentration due to increased influx of Na into the cells via the NaK2Cl cotransporter. Indeed, in the frog cornea (Rick et al., 1987), the stimulation of Cl secretion results in a significant increase in [Na]c with little change in [Cl]c. However, the jejunum also actively absorbs Na (Powell, 1985) and, in the absence of mucosal organic solutes such as glucose and amino acids, the dominant mechanism of Na absorption is Na/H exchange (Gunter & Wright, 1983; Turnberg et al., 1970a) which is inhibited by theophylline and other agents which stimulate Cl secretion (Nellans, Frizzell & Schultz, 1973). It is possible, therefore, that an inhibition of Na/H exchange was responsible for the reduced [Na]c of the epithelial cells following the stimulation of Cl secretion. To test this possibility, tissues were incubated with 1 mmol L⁻¹ mucosal amiloride, a concentration which is known to inhibit intestinal Na/H exchange (Knickelbein, Aronson & Dobbins, 1988), for 30 min before Cl secretion was stimulated with theophylline. Once the *I*_{sc} had reached a steady state the tissues were frozen for analysis. In time matched controls the tissues were incubated with amiloride, but Cl secretion was not stimulated.

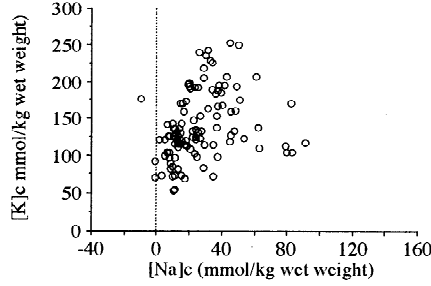
When compared to previous experiments amiloride had no effect on the basal *I*_{sc} or the response to theophylline (Table 2). However, it appeared to prevent the secretagogue induced falls in [Na]c of the villus and crypt cells (Table 4) and the distributions of [Na]c and [K]c of the crypt and villus cells were very similar and

Unstimulated tissues

(A) Villus cells

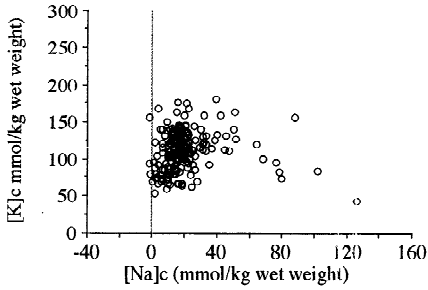


(B) Crypt cells



Stimulated tissues

(C) Villus cells



(D) Crypt cells

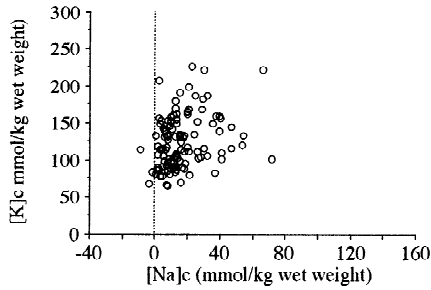


Fig. 9. Intracellular K concentration ($[K]_c$) as a function of intracellular Na concentration ($[Na]_c$) for control tissues and tissues stimulated to secrete with 10 mmol L^{-1} serosal and mucosal theophylline. (A) Control values from villus region of unstimulated tissues. (B) Control values from crypt region of unstimulated tissues. (C) Values from villus region of secreting tissues. (D) Values from crypt region of secreting tissues. Values from at least 3 animals. Data summarized in Table 3.

Table 3. Comparison of the effects of stimulation of Cl secretion (10 mmol L^{-1} mucosal and serosal theophylline) on the composition of cells within the crypts and villi of the mouse jejunal epithelium.

	Villus		Crypt	
	Control ($n = 180$)	Exp. ($n = 149$)	Control ($n = 113$)	Exp. ($n = 100$)
Na	37.7 ± 2.6	$20.3 \pm 1.3^*$	26.3 ± 1.9	$16.4 \pm 1.4^*$
K	107.5 ± 2.3	110.6 ± 2.0	140.3 ± 4.4	$124.0 \pm 3.4^*$
Cl	14.3 ± 0.6	16.0 ± 0.6	24.7 ± 1.1	24.2 ± 1.7
P	158.0 ± 3.8	$134.3 \pm 2.8^*$	167.1 ± 5.3	$132.7 \pm 3.5^*$
dw (g/kg)	252 ± 4	252 ± 6	222 ± 8	237 ± 5
Na/P	0.221 ± 0.013	$0.151 \pm 0.009^*$	0.154 ± 0.010	$0.125 \pm 0.010^*$
K/P	0.705 ± 0.013	$0.848 \pm 0.012^*$	0.856 ± 0.016	$0.943 \pm 0.017^*$
Cl/P	0.093 ± 0.003	$0.122 \pm 0.004^*$	0.150 ± 0.005	$0.180 \pm 0.011^*$

n = number of cells. *Significantly different to control value ($P < 0.05$, ANOVA).

essentially unchanged by the addition of the secretagogues (Fig. 10).

Interestingly, a comparison of the $[Na]_c$ of the amiloride treated tissues to the overall control values in Table 1, suggests that amiloride, like theophylline, induced a significant ($P < 0.05$) fall in the villus $[Na]_c$, (Control and amiloride treated tissues, $[Na]_c = 33 \pm 0.9$ and $22.3 \pm 1.4 \text{ mmol/kg wet weight}$, respectively)

although there was no apparent change in the $[Na]_c$ of the crypt cells.

Significantly, following treatment with amiloride there was a secretagogue induced fall in the mean $[Cl]_c$ of both the villus and crypt cells. The $[Cl]_c$ of the villus cells fell from 21.7 ± 1.2 to $16.5 \pm 0.6 \text{ mmol/kg wet weight}$ ($P < 0.05$) while the $[Cl]_c$ of the crypt cells fell from 26.3 ± 1.6 to 22.0 ± 1.1 ($P < 0.05$) (Table 4).

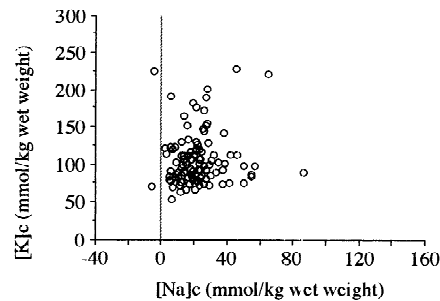
Table 4. Variation in composition of mouse jejunal epithelial cells following the stimulation of Cl secretion (10 mmol L⁻¹ mucosal and serosal theophylline) in the presence of 1 mmol L⁻¹ mucosal amiloride.

	Villus		Crypt	
	Control (<i>n</i> = 105)	Exp. (<i>n</i> = 122)	Control (<i>n</i> = 91)	Exp. (<i>n</i> = 92)
Na	22.3 ± 1.4	21.1 ± 1.3	20.8 ± 1.5	18.1 ± 1.2
K	107.8 ± 3.5	114.7 ± 2.7	123.8 ± 4.2	124.7 ± 3.2
Cl	21.7 ± 1.2	16.5 ± 0.6*	26.3 ± 1.6	22.0 ± 1.1*
P	133.2 ± 4.0	146.2 ± 3.8*	144.7 ± 4.9	145.4 ± 4.3
dw (g/kg)	245 ± 6	224 ± 5*	221 ± 5	203 ± 5*
Na/P	0.173 ± 0.011	0.148 ± 0.010	0.141 ± 0.009	0.127 ± 0.008
K/P	0.819 ± 0.016	0.804 ± 0.014	0.871 ± 0.015	0.873 ± 0.013
Cl/P	0.166 ± 0.008	0.119 ± 0.005*	0.185 ± 0.011	0.151 ± 0.006*

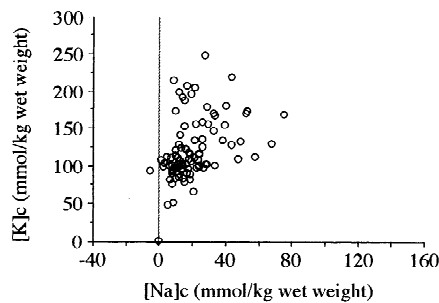
n = number of cells. *Significantly different to control value (*P* < 0.05, ANOVA).

Unstimulated Tissues

(A) Villus cells

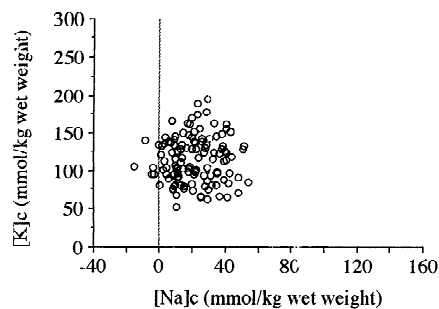


(B) Crypt cells



Stimulated Tissues

(C) Villus cells



(D) Crypt cells

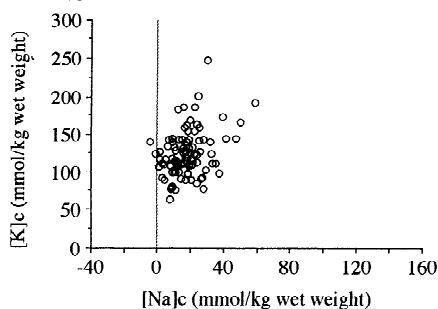


Fig. 10. Intracellular K concentration ([K]c) as a function of intracellular Na concentration ([Na]c) for tissues exposed to amiloride prior to stimulation of secretion with 10 mmol L⁻¹ serosal and mucosal theophylline. (A) Control values from villus region of unstimulated tissues. (B) Control values from crypt region of unstimulated tissues. (C) Values from villus region of secreting tissues. (D) Values from crypt region of secreting tissues. Values from at least two animals. Data summarized in Table 4.

Similar changes in cellular composition following treatment with amiloride and subsequent stimulation of Cl secretion are evident if the ion contents are considered. The Na content of villus cells is significantly less (*P* < 0.05) in tissues treated with amiloride (Na/P = 0.173 ± 0.011) compared to the untreated tissues (0.253 ± 0.007). The subsequent stimulation of Cl secretion did not result in further changes in the Na content of either

the villus or crypt cells (Table 4) but there was a significant reduction in the Cl content of both the villus and crypt cells (Table 4).

Discussion

For some time it has been argued that the absorptive and secretory mechanisms of the intestine are located in dif-

ferent cell types within the epithelium. The evidence for this is, in the main, circumstantial (*see* Sullivan & Field, 1991) and there is considerable data suggesting that, in the small intestine at least, this separation of absorptive and secretory mechanisms is not as clear cut as initially proposed (de Jonge, 1975; Stewart & Turnberg, 1989). The primary objective of this study was to provide a functional description of the response of the crypt and villus cells of the jejunal epithelium to secretagogues. The results obtained through measurement of the cellular composition with x-ray microanalysis suggest that the dominant response of the jejunal epithelial cells to secretagogues was an inhibition of Na absorption via the Na/H exchanger. This response was localized mainly to the villus cells. Changes in cellular composition associated with the stimulation of Cl secretion were less apparent and appeared to involve both the villus and crypt cells.

CELLULAR COMPOSITION WITHIN THE CRYPT/VILLUS AXIS UNDER BASAL CONDITIONS

We only measured the composition of the undifferentiated columnar cells in the crypts and enterocytes in the villi as these two cell types represent more than 85% of the total cells in the crypt and villi and thus are most likely the cells responsible for fluid and electrolyte transport in the two regions. It is unlikely that the results are contaminated by measurements of other cell type. It was reasonably easy to exclude goblet and Paneth cells and, while it was not possible to identify enteroendocrine cells, they represent only 0.4% of the total cells within the epithelium (Cheng, 1974).

In general the [Na]c and [K]c concentrations of the jejunal epithelial cells are comparable to those reported for other epithelia (Dörge et al., 1988; Halm & Rick, 1992; Rick et al., 1987). However, the [Cl]c is somewhat lower than that seen in other secreting epithelia. Estimates of Cl activity by correcting for dry weight and assuming an intracellular activity coefficient similar to the external environment (≈ 0.75) yields a value of 21 mmol L⁻¹ in the villi and 23 mmol L⁻¹ in the crypts. Measurements with intracellular microelectrodes gave a comparable value in surface cells of the rabbit colonic epithelium (Wills, 1985) which are thought to be absorptive (Welsh et al., 1982). In contrast the Cl activity in secreting epithelia is generally higher ranging from 37 to 48 mmol L⁻¹ (Welsh, 1983; Greger et al., 1984; Shorofsky, Field & Fozzard, 1984; Halm & Rick, 1992).

There was a distinct gradient in mean [Na]c and [K]c throughout the crypt/villus axis. The mean [Na]c increased along the crypt to the villus tip, whereas the mean [K]c decreased. These gradients in ion concentration resulted in marked differences in the [Na]c and [K]c of the crypt and villus cells. These differences were eliminated following incubation of the tissues with mu-

cosal amiloride or following the stimulation of Cl secretion. Both amiloride (Knickelbein et al., 1988) and secretagogues (Clarke & Harline, 1996) inhibit the apical Na/H exchanger involved in electroneutral Na absorption by the small intestine. Thus it would appear that this gradient in ion concentrations is due in the main to differential expression or activity of the Na/H exchanger along the crypt/villus axis. Certainly it is well established there are much higher levels of Na/H exchanger activity in the villus cells than the crypt cells (Knickelbein et al., 1988; Sundaram, Knickelbein & Dobbins, 1991).

A second factor contributing to this gradient may reflect changes in cellular composition associated with maturation and death of the epithelial cells. In the jejunum the epithelial cells are derived from a mitotically active region near the base of the crypt and migrate towards the villus tip where they are shed 2–3 days later (Gordon, 1989). In general, associated with senescence of epithelial cells is a marked increase in Na and Cl content and fall in K content, (Bowler et al., 1991; Rick et al., 1978). We identified a small number of cells in the albumin standard applied to the tissues before freezing. These cells had a high [Na]c and [Cl]c and low [K]c and are presumably representative of cells shed into the lumen. As there are likely to be more cells approaching senescence near the tip of the villus, inclusion of these cells in the measurement may contribute to the observed gradients in [Na]c and [K]c.

The other major ion involved in fluid and electrolyte transport by the intestine, Cl, showed little variation in concentration throughout the crypt/villus axis, other than a higher concentration in the cells within the base of the crypts. The reason for this is unknown. Care was taken to ensure all measurements were made from undifferentiated columnar cells.

CHANGE IN CELLULAR COMPOSITION IN RESPONSE TO SECRETAGOGUES

The increase in *I*_{sc} seen following the addition of theophylline to the bathing medium is consistent with the stimulation of Cl secretion (Grubb, 1995; Clarke & Harline, 1996). This stimulation of Cl secretion will modify the influx and efflux of Na ions in the secretory cells and, in other secreting epithelia, this results in increased cellular Na (Rick et al., 1985; Rick et al., 1987; Halm & Rick, 1992). In the jejunum, however, the major effect of secretagogues upon epithelial cell composition was a decrease in [Na]c. This decrease in [Na]c is consistent with the inhibition of the apical Na/H exchanger by the secretagogue (Clarke & Harline, 1996). This conclusion is supported by the observation that prior exposure to amiloride prevented further reductions in [Na]c by secretagogues.

Interestingly, in the presence of amiloride, which

inhibits Na/H exchange activity and so limits changes in composition to those associated with the stimulation of Cl secretion, there were less marked changes in cellular composition associated with the stimulation of Cl secretion (Table 4). There was a small, but significant decrease in [Cl]c, which has been seen in other secretory epithelia (Greger et al., 1984; Shorofsky et al., 1984; Halm & Rick, 1992). However, the [Na]c did not change. It seems paradoxical that variations in the rate of Na entry into the cells via the Na/H exchanger are associated with changes in [Na]c, whereas changes in the rate of Cl secretion, which will involve increased influx of Na into the cell via the NaK2Cl cotransporter, are not. This may reflect differences in the mechanisms involved in the coordination of the influx and efflux rates between electroneutral Na absorption and electrogenic Cl secretion. Potentially, changes in [Na]c may play an important role in linking changes in apical Na entry and basolateral Na exit via the Na/K ATPase and associated changes in basolateral K conductance in the absorptive process, perhaps through its effects on cellular pH (Harvey & Ehrenfeld, 1988). In contrast, other control mechanisms, such as changes in intracellular Ca or other second messengers, may coordinate the changes in apical Cl conductance and basolateral K conductance and Na/K-ATPase activity associated with changes in the secretory rate, without appreciable changes in cellular composition.

LOCATION OF ABSORPTIVE AND SECRETORY PROCESSES

The gradient in [Na]c through the crypt/villus axis suggests that the Na/H exchangers involved in the electroneutral absorption of Na by the jejunum are localized to the villi. This is consistent with early attempts to demonstrate functional distribution of the Na/H exchanger in intestinal tissue by sequential isolation of cells throughout the crypt/villus axis (Knickelbein et al., 1988; Sundaram et al., 1991). However, the [Na]c in the control tissues is only a measure of the relative distribution/activity of the Na/H exchangers in the different cells and the reduction of [Na]c in the crypt and villus cells in response to secretagogues suggests that the Na/H exchanger may be present in the apical membrane of both cell types. This is supported by immunocytochemical localization of NHE-3, the intestinal apical Na/H exchanger, to the apical membrane of both villus and crypt cells of the jejunum and ileum (Hoogerwerf et al., 1996). Furthermore, Knickelbein et al. (1988) demonstrated low levels of Na/H activity within the crypt fraction of the rabbit ileum, but discounted this as contamination by villus cells.

It proved more difficult to localize the secretory cell within the crypt/villus axis. Other than a higher [Cl]c in the cells at the base of the crypts, there was no significant

trends in [Cl]c in the different cell types in the non-secreting tissues. Furthermore, the only change in cellular composition consistent with the stimulation of Cl secretion was a fall in [Cl]c of both the villus and crypt cells when secretion was stimulated after inhibition of the apical Na/H exchanger with amiloride (Table 4). These changes in cellular [Cl]c presumably reflect alterations in the rates of Cl entry and exit from cells which respond to the secretagogues. Similar small changes have been reported in other secretory epithelia (Greger et al., 1984; Shorofsky et al., 1984) and supports the notion that both the crypts and villi secrete (de Jonge, 1975; Stewart & Turnberg, 1989).

In conclusion, there is a marked gradient in [Na]c and [K]c throughout the crypt/villus axis of the mouse jejunum due, in the main, to an apparent variable expression/activity of an apical Na/H exchanger. The most significant changes in cellular composition in response to secretagogues were consistent with the inhibition of an apical Na/H exchanger. While it appeared there were greater levels of exchanger activity in the villi, the crypts may also be capable of electroneutral Na absorption. In contrast, changes in cellular composition associated with the stimulation of Cl secretion were only apparent after inhibition of the apical Na/H exchanger with amiloride. Then there was a significant decrease in [Cl]c of both the villi and crypts following the stimulation of secretion, suggesting a secretory role for both the villi and crypts.

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