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CONTRIBUTION OF VILLUS AND INTERVILLUS EPITHELIUM TO INTESTINAL TRANSMURAL POTENTIAL DIFFERENCE AND RESPONSE TO THEOPHYLLINE AND SUGAR

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Summary

A chamber design is described which permits isolation of villus or intervillus epithelium from proximal segments of *Amphiuma* intestine and measurement of the transepithelial potential difference (ψ_{ms}) and short-circuit current (I_{sc}) produced by each. In media containing Cl^- and 10 mequiv./l HCO_3^- the villus generated a basal ψ_{ms} of 0.8 mV (serosa negative) and I_{sc} of 12 $\mu A/cm^2$ while the intervillus ψ_{ms} and I_{sc} were not different from zero. Acetazolamide altered the villus ψ_{ms} by 1.2 mV; the intervillus ψ_{ms} by only 0.3 mV. Transepithelial gradients of HCO_3^- appeared to generate diffusion potentials across the intervillus but not the villus epithelium. The actively transported sugar galactose elevated ψ_{ms} by 0.6 ± 0.1 mV in the intervillus epithelium and by 1.5 ± 0.2 mV in the villus epithelium for a response ratio (0.6/1.5) = 0.4. The response ratio for valine was 0.3. In contrast, the response ratios for theophylline (0.7) and cyclic AMP (0.7) were significantly higher. These observations indicate that the entire epithelium is responsive to theophylline and cyclic AMP while Na^+-dependent solute transport and the basal electrogenic ion transport processes are primarily functions of the cells lining the intestinal villus.

Introduction

There has been considerable controversy in the past regarding the source of the fluid and electrolyte secretion from the mucosa of the small intestine when exposed to cholera toxin, theophylline or other agents which elevate tissue

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cyclic AMP [1,2]. Some have argued that the secretions arise from the crypts [3—5] while others have argued that the villus [6,7] or the entire epithelium [8—12] participates in the secretory response. Nearly all of the electrical measurements performed in vitro to examine the electrolyte transport process in the secretory state have utilized the entire epithelium as a sheet. Under these circumstances the short-circuit current represents the net ion transport activity of both the villus and crypt epithelium. Therefore, this technique, used in this way, does not allow a differentiation between the ion transport processes occurring in the two portions of the epithelium. For this reason other measurements which can better localize the electrical response to secretory stimuli must be used. For example, Hirschorn and Frazier [9] utilizing the microelectrode technique reported that theophylline depolarizes the mucosal membrane of the intervillus cells of isolated rabbit ileum. Intervillus cells are not located in the crypts, per se, but rather are the epithelial cells lying between the villi. They found that theophylline had no effect on the villus cells except to increase the input resistance of the mucosal membrane. Other than their work no attempts have apparently been made to differentiate the electrical response of the various regions of the intestinal mucosa to secretory stimuli.

We have previously reported evidence derived from the short-circuiting technique that isolated segments of *Amphiuma* small intestine secrete HCO_3^- when exposed to theophylline [13,14]. This secretion is accompanied by an increase in the transmural potential in the same direction as that induced by actively transported sugars. In this report we describe a chamber design which permits isolation of villus or intervillus epithelia from *Amphiuma* intestine and measurement of the transepithelial potential difference and short-circuit current produced by each of these two regions. With this chamber we have been able to define the contribution of the villus and intervillus epithelium to ion transport observed in the whole mucosa and their respective roles in the electrogenic response to theophylline and cyclic AMP. In addition the response to the two solutes, galactose and valine, which stimulate Na^+ absorption were evaluated in each region. A preliminary report of some of this work was published previously [15].

Methods

Tissue chamber. Stripped segments of proximal small intestine from *Amphiuma* were clamped between halves of a lucite chamber diagrammed in Fig. 1. It was similar to the chambers used in earlier studies [13,14] but had a slit 1 mm wide and 7 mm long in place of the usual circular opening. Under 7—10 × magnification the intestine was stretched and oriented over the slit with the aid of pins on the face of the chamber. In this way 7.4 mm^2 of the intestinal mucosa, consisting of predominantly villus or intervillus epithelium, was isolated and exposed to the bathing solutions. On average the mounting procedure required 10 min. During this procedure the mucosa was frequently purged with oxygenated buffer. Appropriately placed bridges of saturated KCl in agar allowed measurement of the transvillus or transintervillus potential difference. For this reason the designation villus chamber is used to differentiate it from the whole tissue chamber used previously [13] that constrains a larger segment

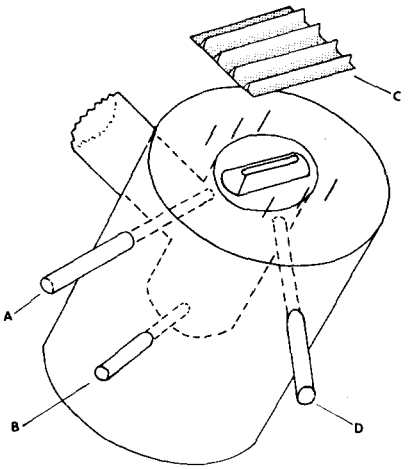

Fig. 1. Diagram representing the serosal half of the villus chamber used in the present study. The intestinal segment (C) was stripped of its muscle layers and stretched with the aid of pins on the chamber face to orient predominantly villus or intervillus epithelium (peaks or valleys, respectively) in the slit which was 1 mm × 7 mm. The tissue was then compressed by an identical (mucosal) half-chamber. Ports for potential sensing (A), current passing (B) and chamber perfusion (D) are shown.

of epithelium (31.5 mm^2) comprised of both villus and intervillus epithelia. In later experiments current-passing capabilities were designed into the villus chamber. Unless stated otherwise the short-circuit current was expressed per unit of chamber surface area. In a limited number of experiments adjacent pieces of tissue were introduced simultaneously into identical villus chambers. These are referred to as matched tissues. At the termination of the experiment the chambers were disassembled and the position of the tissue reexamined to insure that a clear separation of the mucosal areas was achieved. Transepithelial potential difference and short-circuit current were measured with an automatic voltage clamp device. Current-passing bridges were formed from the same media used to incubate the tissue.

Relative area estimate. The relative surface area of villus and intervillus epithelia was estimated from the ratio of dry weights of the tissue segments exposed in the chamber. After clamping villus or intervillus epithelium in the chamber the chambers were disassembled and the tissue stained with methylene blue. The segments were removed from the chamber, pinned and stretched on a wooden block and dried in the oven for 4—6 h. Upon cooling, the tissue outlined by the dye was cut away and weighed on a Mettler model B6 balance with an accuracy of 0.02 mg.

Bathing media. Most of these measurements employed a media containing in mequiv./l: 95 Na^+, 2.5 K^+, 0.9 Ca^{2+}, 1.0 Mg^{2+}, 88.8 Cl^-, 10.0 HCO_3^- and 20 mM mannitol and buffered to pH 7.1 by gassing with 95% O_2/5% CO_2. In some experiments a HCO_3^--free media was used in which chloride was used to replace bicarbonate. The media contained 5 mM tris(hydroxymethyl)aminomethane instead of phosphate and had a pH of 7.2. All added solutes were substituted on a equimolar basis for mannitol.

Statistical tests. Statistical tests of significance were conducted using Students *t*-test for paired comparisons. All errors are expressed as S.E.

Results

Differential response to galactose and theophylline

The small intestine of *Amphiuma* is characterized by villi which are not finger-like, as in mammalian intestine, but rather course longitudinally as a ridge with few villus to villus anastomoses. Applying lateral stretch to the tissue exposes long, thin areas of intervillus epithelium. This area is imbued with multicellular cell nests. The cell nests are a source of new cells which migrate to the overlying intervillus epithelium [16] *. In order to localize the electrical response to theophylline in the epithelium we have taken advantage of this peculiar anatomical feature of *Amphiuma* small intestine to make measurements of the electrical characteristics of the villus or intervillus epithelium in isolation. In the first series a stripped segment was placed in the chamber described above with either villus or intervillus epithelium oriented in the slit, while an adjacent segment of tissue was clamped into the whole tissue chamber. After incubating for 2 h in Cl^--based media with 10 mequiv./l HCO_3^- the effect of 10 mM galactose or 10 mM theophylline on the transepithelial potential (ψ_{ms}) was observed. 2 h later the response to both solutes presented simultaneously was observed. The average peak response of villus and intervillus epithelium to galactose and theophylline is tabulated in Table I and compared with the response in the companion whole mucosa. The mean response of the villus epithelium to galactose (series 1) was 1.5 ± 0.2 mV. The response of intervillus epithelium was 0.6 ± 0.1 mV and this was significantly smaller ($P < 0.01$). In contrast the responses of the villus epithelium and the intervillus epithelium to theophylline (series II) were 2.3 ± 0.3 mV and 1.6 ± 0.2 mV, respectively. These were not significantly different ($P > 0.05$). Expressed as a ratio of the response in the two epithelia (intervillus/villus) the results yield a response ratio of 0.40 for galactose and 0.69 for theophylline. These results indicate the villus epithelium responds well to both galactose and theophylline. On the other hand the intervillus epithelium, while responsive to theophylline, is poorly responsive to sugar. This fact is especially well illustrated in series I in which exposure of the intervillus epithelium to theophylline at 4 h produced an increase in ψ_{ms} exceeding that produced by galactose added previously. Thus theophylline is able to induce ion movements in both areas of the epithelium while galactose has a greater effect in the villus epithelium. These differences in response are not due to variations in response of unpaired tissues since, as seen in Table I, the whole tissue response measured on adjacent segments was nearly identical whether paired with villus or intervillus epithelium.

Table I also reveals that the response to either solute added alone is significantly greater than when added after addition of the other solute. For example, theophylline elevated ψ_{ms} of whole tissue by 3.2 mV when added first (series II) but only 2.1 mV when added after the tissue had been exposed to sugar (series I). This is a significant decrease ($P < 0.01$) but is not due to an

* *Amphiuma* intestine does not have true villi in the strict anatomical sense since the submucosa extends up into the intestinal folds. However, in view of the fact that the area between the folds contain cell nests which have a germinative role in the mucosal epithelium, we feel the fold and interfold areas are functionally equivalent to villus and crypt (or intervillus) areas. The data presented support this contention.

TABLE I

ELECTRICAL RESPONSE OF WHOLE MUCOSA, VILLUS AND INTERVILLUS EPITHELIUM TO ADDED SOLUTES

Values are mean ± S.E. (n = 4) and represent the peak response to the solute. n.s., not significantly different from zero at P = 0.05.

Series	Solute	Time of solute addition (h)	Whole mucosa (mV)	Villus (mV)	Whole mucosa (mV)	Intervillus (mV)	P
I	Galactose	2	2.0 ± 0.2	1.5 ± 0.2	2.1 ± 0.3	0.6 ± 0.1	<0.01
	Theophylline	4	2.1 ± 0.2	1.5 ± 0.3	2.1 ± 0.2	1.0 ± 0.1	n.s.
II	Theophylline	2	3.2 ± 0.2	2.3 ± 0.3	3.6 ± 0.6	1.6 ± 0.2	n.s.
	Galactose	4	1.3 ± 0.2	1.2 ± 0.2	1.5 ± 0.2	0.3 ± 0.1	<0.01

overall decline in tissue responsiveness to theophylline since in four tissues exposure to theophylline following more than 5 h incubation produces responses ($\Delta\psi_{ms}$ = 3.0 ± 0.1 mV) equal to that produced when the solute is added at 2 h. Thus the interaction evident in Table I suggests that there is a mechanism common to the electrogenic events produced by theophylline and galactose.

Differential response to cyclic AMP and valine

In a second series, adjacent segments of intestine were clamped into separate but identical villus chambers, one containing villus epithelium, the other intervillus epithelium. As seen in Fig. 2 the addition of valine (20 mM) after 4 h caused an immediate, large increase in ψ_{ms} of villus epithelium. The short-circuit current (I_{sc}) was also measured in this series and was similarly elevated (Fig. 2). In contrast to the villus epithelium the intervillus electrical properties

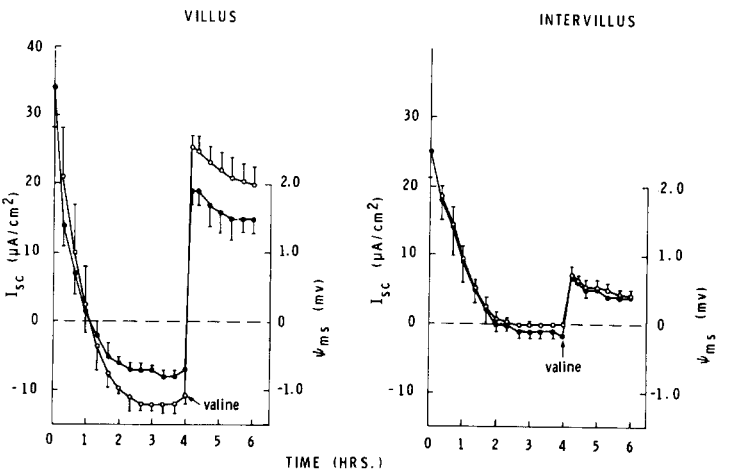

Fig. 2. Time course of the villus and intervillus potential (●) and short-circuit current (○) before and after addition of 20 mM valine in paired segments from three animals. HCO_3^- was present in the media at 10 mequiv./l.

were only slightly altered by valine (Fig. 2). Comparing the epithelia, the response was significantly larger in the villus epithelium ($P < 0.02$). The ratio of the potential response to valine for the two epithelia was 0.32, nearly that observed for galactose.

Using an identical protocol the response to exogenous cyclic AMP (7.5 mM) was examined in the two epithelia. Like theophylline the effect on ψ_{ms} was not significantly different for the two epithelia ($P > 0.15$) averaging 2.3 ± 0.6 mV in the villus and 1.6 ± 0.3 mV in the intervillus. However, in this series the I_{sc} response to cyclic AMP was significantly greater for villus than intervillus epithelia ($P < 0.02$). Nevertheless the response ratio for cyclic AMP (0.7) relative to that for valine further illustrates that the intervillus epithelium is more responsive to cyclic AMP than to the actively transported amino acid valine. Taken together these results indicate that the cells that comprise the intervillus epithelium are not primarily engaged in sodium-dependent transport of valine or galactose but contain ion transport mechanisms sensitive to stimulants of intestinal HCO_3^- secretion.

A complicating factor in the comparison of the response of villus and intervillus epithelia to solutes is the difference in the surface area of the two regions. The intervillus epithelium is flat and its area is closely approximated by the area of the slit opening of the chamber. In contrast the villus epithelium rises above the surface and its surface area exceeds that of the slit. Therefore the density of the I_{sc} reported in Figs. 2 and 3 is an overestimate for the villus. The difference in surface area was approximated from the weights of the individual epithelia. The villus was found to have a surface area 1.9 times greater than the intervillus by this approach. This probably represents an overestimate since a large percentage of the villus weight must be non-cellular lamina propria. Using this revised estimate of surface area the I_{sc} response ratio (intervillus/villus) for

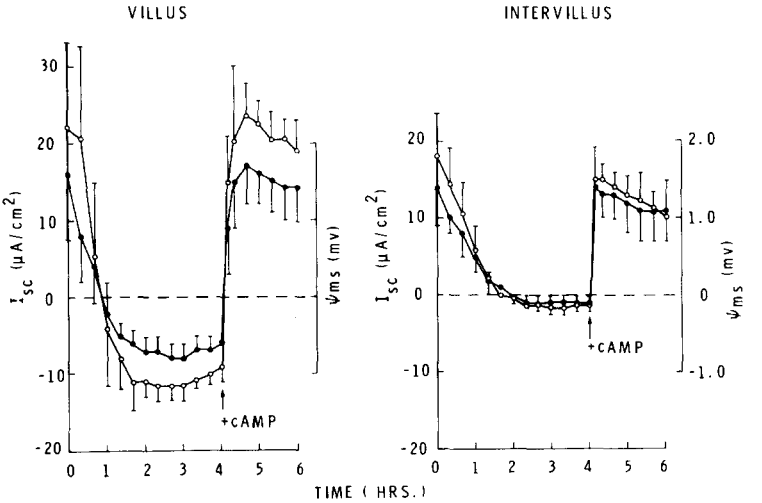

Fig. 3. Time course of the villus and intervillus potential (•) and short-circuit current (○) before and after addition of 7.5 mM cyclic AMP in paired segments from three animals. HCO_3^- was present in the media at 10 mequiv./l.

valine increased from 0.2 to 0.4 while that for cyclic AMP increased from 0.5 to 1.0. The corrected values correspond well with the ψ_{ms} response ratios for valine (0.3) and cyclic AMP (0.7). Thus even if differences in the surface area of the epithelia are considered the results still indicate that both regions of the mucosa respond to cyclic AMP while the intervillus responds poorly to valine.

Basal electrical characteristics

Of equal importance and significance is the observation of a clear difference in the basal electrical characteristics of the two regions. As seen in Figs. 1 and 2 although electrical stability was slow to develop in both areas, as noted previously in whole mucosa [13], after 4 h only the villus epithelium generated a spontaneous potential, serosa negative to mucosa. This is the polarity observed in whole tissue [13]. For the two series ψ_{ms} averaged −0.7 mV after 4 h; the I_{sc} averaged 9.9 μA/cm^2. The ψ_{ms} and I_{sc} developed by the intervillus epithelium were not significantly different from zero ($P > 0.20$). Confirmation of this difference between the two regions was also evident from their relative responses to acetazolamide (10^{-4} M) in a separate series. We previously demonstrated that the basal, serosa-negative potential in whole mucosa is rapidly and completely inhibited by acetazolamide [13]. In villus segments not exposed to theophylline, acetazolamide changed ψ_{ms} by 1.2 ± 0.3 mV ($n = 4$) in 25 mequiv./l HCO_3^- media but altered the intervillus ψ_{ms} of paired adjacent segments by only 0.3 ± 0.1 mV.

Relative resistances of villus and intervillus epithelia

The resistance of both regions of the mucosa was determined by measuring the voltage response to small, stepwise increments in applied current maximizing at 4 μA. The response was corrected for the resistance of the bathing media and was linear. The tissue resistance was identical in the two regions averaging $74.9 \pm 5.8\ \Omega \cdot \text{cm}^2$ in the villus and $76.2 \pm 4.5\ \Omega \cdot \text{cm}^2$ in the intervillus of adjacent segments from five animals. If, as estimated above, the area of villus epithelium exposed in the chamber exceeds that of the intervillus by a factor of 1.9 then the true resistance of the villus is much higher. Viewed in this way the intervillus epithelium appears to be a pathway of lower electrical resistance. However, the validation of this interpretation must await a more definitive measure of villus epithelial resistance.

Unilateral HCO_3^- replacement

It was reported previously that the serosa-negative ψ_{ms} observed in whole mucosa only develops in a media buffered with HCO_3^-/CO_2 [13]. When media HCO_3^- was replaced unilaterally in order to determine whether the ψ_{ms} was dependent on mucosal or serosal HCO_3^- it appeared to produce diffusion potentials for HCO_3^-. Furthermore, the response to theophylline appeared more dependent on serosal HCO_3^-. In order to localize these responses villus and intervillus epithelia were exposed on one side to media containing 10 mequiv./l HCO_3^- gassed with 95% O_2/5% CO_2. HCO_3^--free media was employed on the opposite side and gassed with 100% O_2. A typical result for intervillus epithelium is seen in Fig. 4. In Table II, which shows the mean electrical response for both regions, it is seen that the villus epithelium did not develop a signifi-

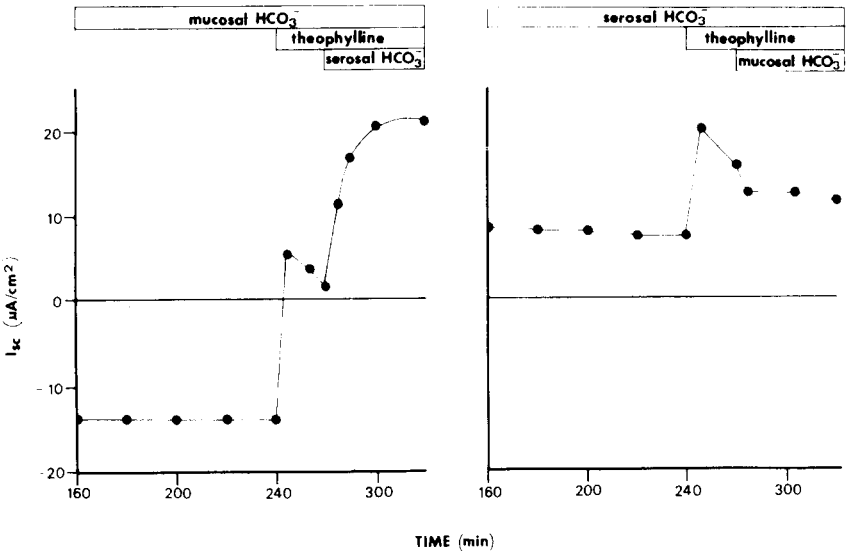

Fig. 4. Time course of typical electrical response of paired intervillus epithelia to exposure to mucosal (left) or serosal (right) HCO_3^- (10 mequiv./l). The tissues were subsequently exposed to 10 mM theophylline followed by exposure to HCO_3^- on the side formerly lacking this ion.

cant short circuit when HCO_3^- was present in either the mucosal or serosal compartment alone. In contrast, this maneuver generated electrical potentials across the intervillus epithelium suggestive of HCO_3^- diffusion potentials (Fig. 4 and Table II) as observed in whole mucosa [13]. Although the potential generated in the intervillus in the presence of serosal HCO_3^- was not significant the observations can be explained most simply by the presence in the intervillus epithelium of passive conductive pathways for HCO_3^-.

The addition of theophylline in the presence of a HCO_3^- gradient increased the short-circuit current similarly in both areas of the intestinal epithelium

TABLE II

INFLUENCE OF UNILATERAL HCO_3^- EXPOSURE ON I_{sc} AND RESPONSE TO THEOPHYLLINE IN VILLUS AND INTERVILLUS EPITHELIA

The steady-state short-circuit current (I_{sc}) was measured in paired segments of villus and intervillus epithelium in the presence of mucosal or serosal HCO_3^- alone (i.e. basal I_{sc}), following addition of theophylline (10 mM) and then on exposure to HCO_3^- on the side formerly lacking this ion. n, the number of segments examined.

	Villus I_{sc} (μA/cm²)	n	Intervillus I_{sc} (μA/cm²)	n
Musocal HCO_3^-	−0.3 ± 0.9	6	−6.0 ± 2.2 *	5
+ Theophylline	9.5 ± 2.7 **	6	3.8 ± 0.8 **	5
+ Serosal HCO_3^-	15.5 ± 1.9 **	3	14.6 ± 1.9 **	5
Serosal HCO_3^-	2.4 ± 1.5	6	2.8 ± 1.5	5
+ Theophylline	15.7 ± 2.2 **	6	11.2 ± 2.5 **	5
+ Mucosal HCO_3^-	14.8 ± 1.6	3	7.0 ± 0.9 **	5

* A basal I_{sc} significantly different from zero at $P = 0.05$.
** A change in I_{sc} significantly different from zero at $P = 0.05$.

(Table II) as reported for whole tissue [13]. Subsequent introduction of HCO_3^- on the side previously HCO_3^--free produced a further elevation of the current only in the segment previously exposed to mucosal HCO_3^- (Fig. 4, Table II). There was no enhancing effect of mucosal HCO_3^- on the segment preexposed to serosal HCO_3^-. Thus the conclusion in whole tissue that serosal HCO_3^- is most important in the response to theophylline [13] can be seen to apply as well to both regions of the intestinal epithelium.

The electrical responses following theophylline addition were similar in both intervillus epithelium, which exhibited HCO_3^- diffusion potentials and villus epithelium, which did not (Table II). This suggests that theophylline eliminated the diffusion potential for HCO_3^- in the intervillus. This conclusion has some support in the literature. Moore et al. [17] have reported that the diffusion potentials induced across dog ileum when mannitol was infused into the lumen were eliminated by cholera toxin. Also, Powell [18] found that the passive permeability to Cl^- relative to Na^+ and K^+ was increased in rabbit ileal mucosa following exposure to theophylline or cholera toxin and suggested that the permselectivity of paracellular routes of ion permeation was altered.

Discussion

The purpose of this study was to localize to the villus or intervillus epithelium the electrical response to theophylline and actively transported solutes observed in whole tissue. The villus chamber utilized here permitted a successful separation of the two mucosal regions as evidenced by their differential responsiveness to the added solutes. The electrical response to actively transported sugars and amino acids arose chiefly in the villus epithelium indicating this as the principal site for the cotransport of these solutes with Na^+. This finding is in keeping with the studies of Kinter and Wilson [19] who localized sugar and amino acid transport to the cells lining the villus of the hamster small intestine using autoradiographic methods. In addition, efforts to demonstrate enzyme distribution at various levels of the villus have indicated that disaccharidases and dipeptidases predominate in the villus cells [20,21].

In contrast to the differential response to the actively absorbed solutes galactose and valine, the large response of both areas of the epithelium to theophylline or cyclic AMP demonstrates that the entire mucosal surface responds with increased ion transport when exposed to agents which elevate cellular levels of cyclic AMP. In earlier reports we have presented evidence that the whole mucosa secretes HCO_3^- electrogenically when exposed to theophylline [13]. At present there is no reason to believe that the ion transport process stimulated by this agent is different in the two regions. Thus the present study in conjunction with earlier measurements in whole tissue indicates that immature intervillus cells and mature villus cells alike secrete HCO_3^- (or absorb H^+) when their levels of cyclic AMP are elevated.

The numerous attempts at differentiating the role of villus and crypt cell function during experimentally induced intestinal secretion have been reviewed by Field [22] and, more recently, Schwartz et al. [11], who have detailed the shortcomings of some of these studies. Several studies have offered evidence that both villus and crypt cells respond to secretory stimuli [8—12], while in

other studies either villus [6,23] or crypt cells [3—5] were implicated in the secretory response. In a study most related to this one, Hirschorn and Frazier [9] using microelectrodes reported that theophylline caused depolarization of the mucosal membrane of intervillus cells and a reduction of membrane resistance of villus cells in rabbit ileum. Unfortunately, the instability of the membrane potential measurements they reported, their low absolute value and their insensitivity to actively transported sugars contrasts sharply with more recent reports [23,25—27].

Two studies have served to exclude a role for the villus epithelium in the secretory response. These have involved the use of cycloheximide [23] or hypertonic solutions [5] to inhibit the function of crypt and villus cells, respectively. Although the findings are consistent with a primary role of the crypts in secretion the use of both agents has been criticized for their lack of sensitivity (e.g. Ref. 11). More importantly, Lee and Silverberg [6] were able to directly demonstrate a reduction in fluid pressure in the central lacteal of the villus in dogs exposed to cholera toxin, in strong support of a role of the villus in the response to secretory stimulants. The data presented in this report are a direct, unambiguous measure of the ion transport response to secretory stimuli and furnish strong support for an effect of such stimuli on the entire epithelium.

It was surprising to find an electrical interaction between galactose and theophylline in view of the reported independence of secretion induced by prostaglandins, cholera toxin or theophylline and glucose-induced absorption [28—30]. However, there are previous reports that theophylline or aminophylline alter sugar transport [31,32] or amino acid transport [33]. Since actively transported sugars and amino acids depolarize the mucosal membrane of several amphibian and mammalian intestinal preparations [24—27] while increasing ψ_{ms} it is possible that theophylline, which also increases ψ_{ms}, depolarizes the mucosal membrane as well. The electrical interaction may be related then to the extent to which the membrane can be depolarized by these synergists.

Of considerable significance to intestinal electrophysiology is the demonstration in this study that in bicarbonate media the basal electrical characteristics of the intestinal mucosa are generated solely by the cells in the villus. Recent evidence indicates that the basal serosa-negative I_{sc} is due to net Cl^- absorption (White, J.F., unpublished observations). This absorptive capacity therefore appears to be confined to the villus epithelium. In agreement with this idea the density of I_{sc} reported here for the villus epithelium (12 $\mu A/cm^2$) is nearly twice that reported previously for the whole mucosa [13]. Contrasting with the villus epithelium the intervillus epithelium did not develop a significant transepithelial potential. This was not due to excessive damage to the tissue as a result of the clamping procedure since it exhibited relatively large electrical responses to theophylline and cyclic AMP. For *Amphiuma* then the basal ψ_{ms} in HCO_3^- buffer is not dominated by crypt cell ion transport processes as suggested for rat jejunum [34,35] but is due completely to villus cell processes.

In these studies we have succeeded in differentiating some of the ion transport properties of the two areas of the intestinal epithelium. Since the intervillus cells eventually migrate to the tip of the villus [16] then the overall view which arises is one in which immature intervillus cells, having a poor ability to transport sugars or amino acids and not contributing to the basal electrical

characteristics of the tissue are still capable of HCO_3^- secretion in response to theophylline. As the cells migrate up the villus they gain the additional capacity to actively transport sugars and amino acids and absorb Cl^- electrogenically.

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