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INTESTINAL ELECTROGENIC HCO_3^- ABSORPTION LOCALIZED TO VILLUS EPITHELIUM

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Isolated segments of jejunum from *Amphiuma* absorb HCO_3^- electrogenically by a process sensitive to acetazolamide. Using a tissue chamber of special design which permits isolation of villus or intervillus epithelium the transepithelial electrical potential (ψ_{ms}) of each region was measured. The serosa-negative ψ_{ms} generated by the villus epithelium was more negative than that of the intervillus and exhibited greater sensitivity to acetazolamide. Both regions were responsive to other agents which alter epithelial ion transport. The results indicate that intestinal HCO_3^- absorption is localized predominantly within cells lining the villus epithelium.

HCO_3^- is absorbed actively in the jejunum of *Amphiuma* [1] as well as in the rat [2–5] and the human [6]. In *Amphiuma* the absorption of HCO_3^- by in vitro segments is electrogenic and is blocked by various metabolic inhibitors including acetazolamide, the inhibitor of carbonic anhydrase [1]. Previously we reported on the use of an Ussing -type tissue chamber of special design (the villus chamber) to isolate and characterize the transport properties of anatomically distinct regions of *Amphiuma* intestinal mucosa, namely the villus (or folds) and intervillus epithelium [7]. It was shown that the processes of Cl^- absorption and sugar-stimulated Na^+ absorption, both electrogenic in *Amphiuma*, occurred predominantly in the villi. In contrast electrogenic HCO_3^- secretion (from serosa to mucosa) induced by theophylline occurred in both the villus and intervillus epithelium. In this report evidence derived from the villus chamber is presented that the opposite process, HCO_3^- absorption (from mucosa to serosa), is largely restricted to the villus mucosa.

Two adjacent segments of *Amphiuma* jejunum were stripped of their smooth muscle layers [8]. Under 10× magnification each segment was stretched and oriented over the slit opening in the villus chamber to isolate predominantly villus mucosa in one chamber and intervillus mucosa in the other. Segments were bathed in a medium containing in mmol/l: 95 Na^+ 2.5 K^+, 0.9 Ca^{2+}, 1.0 Mg^{2+}, 25 HCO_3^-, 37 $SO_4^=$ and 55 mannitol buffered to pH 7.4 by gassing with 95% O_2/5% CO_2. 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 (ψ_{ms}) was measured as reported previously [7].

Intact jejunal segments of *Amphiuma* intestine incubated in Cl^--free media containing HCO_3^- generate a serosa-negative electrical potential due to electrogenic HCO_3^- absorption [1]. Using the villus chamber it is seen in Fig. 1 that the ψ_{ms} generated across the villus epithelium was negative after 4 h while ψ_{ms} of the intervillus epithelium from an adjacent segment of jejunum of the same animal was near zero. The average villus ψ_{ms} in five animals (Table I) was significantly more negative than the intervillus ψ_{ms} ($P < 0.02$). As also shown in Fig. 1 addition to the media of the

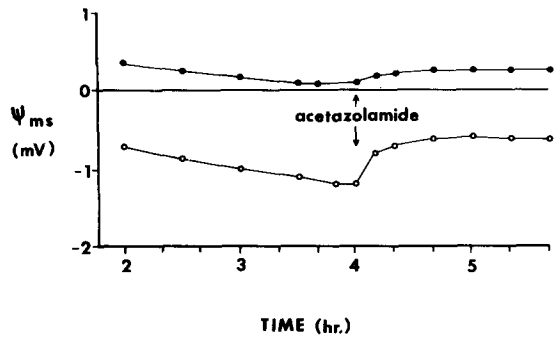

Fig. 1. The transepithelial electrical potential generated across the villus epithelium (○———○) and of the intervillus epithelium (●———●). Effect of acetazolamide addition.

TABLE I

STEADY-STATE TRANSMURAL POTENTIAL AND RESPONSE TO ACETAZOLAMIDE IN VILLUS AND INTERVILLUS EPITHELIA

Values are mean±S.E., of steady-state transepithelial electrical potential in mV in paired tissues from five animals. The *P* value was derived from statistical comparison of the electrical potentials in villus vs. intervillus epithelium using Student's *t*-test.

	Villus	Intervillus	*P*
Control	−0.7±0.1	−0.1±0.1	<0.02
+Acetazolamide (10^{-4} M)	−0.0±0.2	0.2±0.1	
Δ	0.7±0.1	0.2±0.1	<0.01

carbonic anhydrase inhibitor acetazolamide (0.1 mM), which reduces electrogenic HCO_3^- absorption [1], produced a rapid reduction of ψ_{ms} in the villus epithelium but had a much smaller effect in the intervillus region. In Table I the increase in ψ_{ms} in the villus epithelium after acetazolamide addition was significantly greater than that in the intervillus mucosa ($P < 0.01$).

For comparison the response of villus and intervillus epithelia to galactose and theophylline is seen in Table IIA. In eight tissues the average response to mucosal addition of the sugar galactose (55 mM) was 2.3 ± 0.3 mV in the villus and 1.4 ± 0.2 mV in the intervillus. The difference in response (0.9 ± 0.2 mV) was highly significant ($P < 0.01$) indicating that galactose-stimulated Na^+ absorption exists predominantly in the villus. In contrast the electrical response upon exposure to theophylline (10 mM) after galactose was smaller and not statistically different in the two epithelia ($P > 0.05$). This electrical effect is due to stimulation of electrogenic HCO_3^- secretion, a process which is independent of HCO_3^- absorption [9,10]. The equal responsiveness of the two regions to theophylline was verified in a second series of three animals in which the two regions were not pre-exposed to galactose. As seen in Table IIB theophylline produced larger but still equal ($P > 0.05$) changes in the ψ_{ms} in both regions. These results with galactose and theophylline are important for the present study because they show that the failure of the intervillus mucosa to develop a significant basal ψ_{ms} (Table I) is not due to

TABLE II

ELECTRICAL RESPONSE OF VILLUS AND INTERVILLUS EPITHELIA TO ADDED SOLUTES

Values are mean±S.E. and are the maximal stimulation of ψ_{ms} (in mV) after addition of the solute. *n* is the number of paired jejunal segments examined. n.s., not significantly different from zero at $P = 0.05$. The media mannitol was replaced mole for mole with galactose or theophylline to maintain osmolarity.

Series	*n*	+Galactose (55 mM)		+Theophylline (10 mM)	
		Villus	Intervillus	Villus	Intervillus
A	8	2.3±0.3	1.4±0.2 ($P<0.01$)	0.5±0.1 (n.s.)	0.7±0.1
B	3	–	–	2.4±0.1 (n.s.)	2.2±0.1

a loss of viability since the intervillus region responded electrically to an identical degree as the villus mucosa when exposed to theophylline. Of secondary importance the results of Table II confirm our earlier observations [7] that (1) galactose-induced Na^+ absorption is most prominent in the villus epithelium, (2) theophylline-induced HCO_3^- secretion occurs in both regions and (3) pre-exposure to galactose reduces the response to subsequent addition of theophylline. It is concluded that jejunal HCO_3^- absorption is largely a function of the villus absorptive cells.

Amphiuma small intestine is capable of both HCO_3^- absorption and secretion. HCO_3^- secretion is divisible into Cl^- and Na^+-dependent components [11]. The Cl^--dependent component of HCO_3^- secretion is probably localized in the villus mucosa since electrogenic Cl^- absorption, which is linked with HCO_3^- secretion, occurs predominantly in the villus mucosa as determined previously with the villus chamber [7]. In contrast the Na^+-dependent component of HCO_3^- secretion, the only component stimulated by cyclic AMP or theophylline [12], is distributed throughout the mucosa (Ref. 7 and Table II). In this study the process of HCO_3^- absorption (or H^+ secretion) is seen to be localized predominantly in the villus mucosa. Since the epithelial cells lining the villus are older, having migrated from the cell nests within the intervillus region [13], the absorption of HCO_3^-, like active sugar and Cl^- absorption, is a function that apparently develops with maturation of the cells. It remains to be determined whether those villus cells which are active in HCO_3^- absorption are also responsible for Cl^- absorption and Cl^--dependent HCO_3^- secretion.

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