

Mechanism of Intestinal Secretion

Effect of Serotonin on Rabbit Ileal Crypt and Villus Cells

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Abstract

To determine the mechanism of action of an intestinal secretagogue, serotonin, we have isolated crypt and villus cells and demonstrated Na:H and Cl:HCO₃ exchange activity using the intracellular pH-sensitive fluorescent dye, 2,7-bis (carboxyethyl)-5,6-carboxy-fluorescein. Serotonin alkalized both crypt and villus cells. Alkalinization in villus cells was HCO₃ dependent and Na independent. In contrast, alkalinization in crypt cells was HCO₃ independent and Na dependent. In villus cells, recovery from an alkaline load induced by Cl removal, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid or propionate pulse, known to occur via the Cl:HCO₃ exchange, is inhibited by serotonin. In contrast, in crypt cells, recovery from an acid load induced by Na removal, amiloride and NH₄Cl pulse, known to occur via Na:H exchange, is stimulated by serotonin. These data suggest that serotonin is inhibiting Cl:HCO₃ exchange in villus cells and stimulating Na:H exchange in crypt cells. These effects of serotonin would be expected to inhibit coupled Na and Cl absorption by villus cells and stimulate HCO₃ secretion by crypt cells in the intact ileum. (*J. Clin. Invest.* 1991; 87:743-746.) Key words: intestinal secretion • regulation of secretion • intracellular pH • bicarbonate secretion • intestinal secretagogues

Introduction

We have recently demonstrated differences in the distribution of transporters between villus and crypt cells in rabbit ileum (1). Villus cells contained Na-glucose and Na-alanine cotransporters and Na:H and Cl:HCO₃ exchangers on the brush border membrane (BBM)¹, whereas crypt cells had only a Cl:HCO₃ exchanger (Fig. 1). Both cells have a Na:H exchanger on the basolateral membrane (BLM). These results suggest that crypt cells cannot absorb NaCl since this occurs in the ileum by

the simultaneous operation of Na:H and Cl:HCO₃ exchangers (2). Because of the vectorial arrangement of a Na:H exchanger on the BLM and a Cl:HCO₃ exchanger on the BBM, crypt cells may secrete HCO₃. These results are consistent with other evidence suggesting that villus cells are primarily absorptive and crypt cells are primarily secretory (3-6). Agents that regulate ileal ion transport may thus affect villus and crypt cells differently. To test this hypothesis, we have determined the effect of a secretagogue, serotonin, on isolated villus and crypt cells.

Methods

Villus and crypt cells were separated from rabbit ileum by a modification² of the calcium-chelation technique of Weiser (7) and maintained in short term culture. Using this method six fractions of cells were sequentially collected and fraction one was used as villus cells and six as crypt cells. Enzyme markers, morphology, and transporter specificity were used to assure good separation of crypt and villus cells. The cells were maintained in short term culture for up to 6-8 h. Viability was assessed by trypan blue exclusion, linear incorporation of leucine into protein, Na-stimulated glucose uptake (villus cells), and preserved cell structure by electron microscopy.² The presence of Na:H and Cl:HCO₃ exchange and their role in the regulation of intracellular pH has been demonstrated in both cell types.²

Intracellular pH measurements. The cells were loaded with 10 μ M of the acetoxymethyl ester of BCECF (2,7-bis [carboxyethyl]-5,6-carboxy-fluorescein) from a 10-mM stock in DMSO for 10 min at 37°C. A cover slip coated with subconfluent monolayer of cells was mounted in a thermostatically controlled cuvette (37°C) in a spectrofluorometer (LS-5; Perkin-Elmer Corp., Norwalk, CT) with constant perfusion to wash away any leaked dye. The dye was alternatively excited at 450 nm and 500 nm, and the fluorescence emission measured at 530 nm. The BCECF fluorescence excitation ratio was calibrated using the high K⁺/nigericin technique.² All experiments were performed in CO₂/HCO₃ or Na-Hepes(HCO₃-free) solutions. The standard CO₂/HCO₃ solution contained (in mM): NaCl, 115; NaHCO₃, 25; K₂HPO₄, 2.4; KH₂PO₄, 0.4; MgCl₂, 1.25; CaCl₂, 1.25; and gased with 5% CO₂, 95% O₂, pH 7.4, at 37°C. The Na-Hepes solution contained (in mM): NaCl, 130; KCl, 4.5; KH₂PO₄, 1.2; MgSO₄, 1; CaCl₂, 1.25; Hepes, 20; and gased with 100% O₂, pH 7.4 at 37°C. Chemicals: BCECF-AM was purchased from Molecular Probes Inc., Junction City, OR; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid and serotonin were purchased from Sigma Chemical Co., St. Louis, MO.

Data presentation. Cell viability was assessed after each experiment by trypan blue dye exclusion. Only those experiments in which dye exclusion was > 85% were evaluated. Any given experiment and its control was performed on cells isolated from a single rabbit. A represen-

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1. Abbreviations used in this paper: BBM, brush border membrane; BCECF, 2,7-bis (carboxyethyl)-5,6-carboxy-fluorescein; BLM, basolateral membrane; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid.

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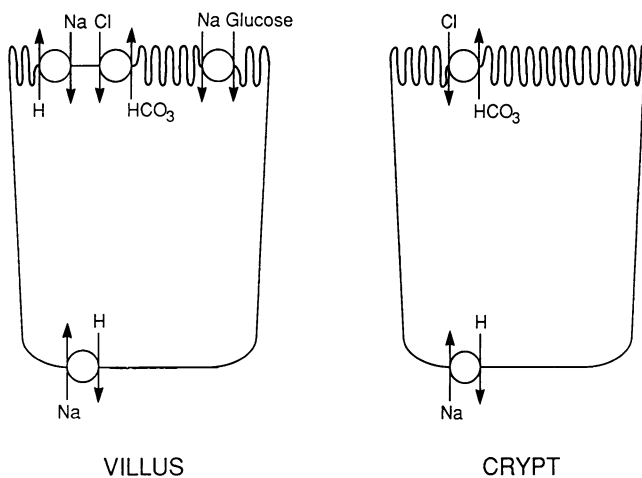


Figure 1. A model of crypt and villus cells demonstrating the distribution of some of the transporters.

tative example of each experiment, which was performed on four to six separate cell preparations from different animals, is shown. Paired Student's *t* test was used when statistical analyses were carried out as shown in Results.

Results

Fig. 2 *A* illustrates that 10 μ M serotonin causes a reversible alkalization of villus cells, but only in the presence of HCO_3^- . Baseline pH increased from 7.21 ± 0.01 to 7.28 ± 0.01 ($n = 6$, $P < 0.01$) in villus cells in the presence of HCO_3^- whereas there was no change in the absence of HCO_3^- . In contrast, serotonin causes an alkalization of crypt cells both in the presence and absence of HCO_3^- (Fig. 3 *A*). In the presence of HCO_3^- the pH change is from 7.29 ± 0.01 to 7.37 ± 0.01 and in the absence of HCO_3^- it is from 7.19 ± 0.01 to 7.27 ± 0.01 ($n = 5$, $P < 0.01$). Removal of Na, which acidifies both villus and crypt cells, probably by inhibiting Na:H exchange,² does not block serotonin-induced alkalization in villus cells (pH_i increases from

7.14 ± 0.01 to 7.21 ± 0.01 , $n = 4$, $P < 0.01$), suggesting a Na-independent process (Fig. 2 *B*); however, Na removal blocks the effect of serotonin in crypt cells (Fig. 3 *B*).

Removal of Cl or addition of the anion exchange inhibitor DIDS, alkalinizes villus cells and prevents further alkalization by serotonin (Fig. 2, *C* and *D*). Recovery from the alkaline pH, when Cl is added or DIDS removed, is slower in the presence of serotonin (Fig. 2, *C* and *D*). In Fig. 2 *C* there is no difference between the pH_i of control and 5HT-treated cells (7.38 ± 0.02 vs. 7.38 ± 0.01) just before the readdition of Cl; however, the change in pH in 60 s (dpH/dt) after Cl readdition is significantly slower in the presence of 5HT (0.021 ± 0.001 vs. 0.065 ± 0.009 , $n = 4$, $P < 0.05$). Similarly, in Fig. 2 *D* there is no difference between pH_i of control and experiment cells (7.41 ± 0.01 vs. 7.41 ± 0.01) just before the removal of DIDS; however, just after DIDS removal dpH/dt is significantly slower in the presence of 5HT (0.040 ± 0.001 vs. 0.078 ± 0.004 , $n = 4$, $P < 0.01$). Further, when villus cells are alkalized by pulsing with Na-propionate the recovery from the alkaline load is slower in serotonin treated villus cells, an effect not seen in crypt cells (Fig. 4). In villus cells, from a maximum pH of 7.63 ± 0.01 in control cells and 7.63 ± 0.01 in 5HT cells, dpH/dt is 0.245 ± 0.008 in control and 0.111 ± 0.014 in 5HT cells ($n = 4$, $P < 0.01$). These results suggest that the alkalization induced by serotonin results from inhibition of $\text{Cl}:\text{HCO}_3^-$ exchange on the BBM of villus cells.

As stated previously, removal of Na blocks the serotonin-induced alkalization in crypt cells, suggesting a Na-dependent process (Fig. 3 *B*). Amiloride, a Na:H exchange inhibitor, also acidifies the crypt cell and blocks the effect of serotonin (Fig. 3 *C*), suggesting that the alkalization of crypt cells by serotonin results from stimulation of Na:H exchange. Supporting this possibility, the recovery from the relative acidity induced by Na removal or amiloride is faster in the presence of serotonin (Fig. 3 *B* and *C*). In Fig. 3 *B* there is no difference between pH_i of control and 5HT-treated cells (7.05 ± 0.03 vs. 7.05 ± 0.03) just before Na readdition; however, dpH/dt is significantly faster after Na readdition in the presence of 5HT (0.064 ± 0.003 vs. 0.127 ± 0.015 , $n = 4$, $P < 0.05$). Similarly, in

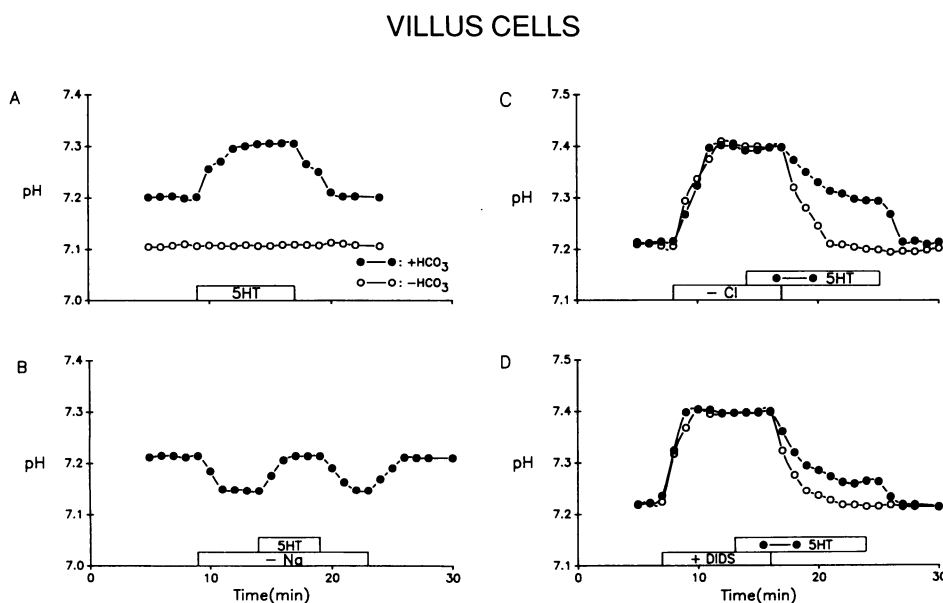


Figure 2. Effect of 10 μ M serotonin on villus cells. (*A*) Cells were perfused with HCO_3^- -containing (closed circles) or HCO_3^- -free (open circles) solutions (see below) containing 10 μ M serotonin during the period shown. (*B*) Effect of serotonin in Na-free solution in villus cells. Na removal was accomplished by substituting choline for Na in the standard $\text{CO}_2/\text{HCO}_3^-$ solution. (*C*) and (*D*) Effect of serotonin on villus cells in Cl-free and DIDS-containing (1 mM) solutions. Cl removal was accomplished by substitution with gluconate in the standard $\text{CO}_2/\text{HCO}_3^-$ solution.

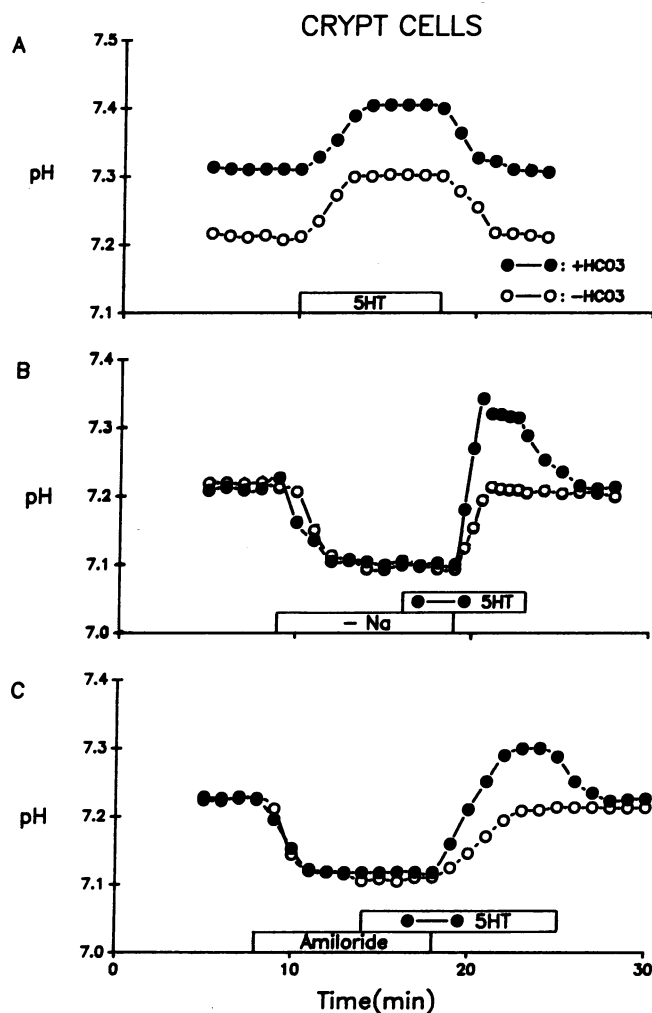


Figure 3. Effect of 10 μ M serotonin on crypt cells. (A) Cells were perfused with HCO₃-containing (closed circles) or HCO₃-free (open circles) solutions containing 10 μ M serotonin during the period shown. (B) and (C) Effect of serotonin in Na-free and amiloride-containing (1 mM) solutions in crypt cells. Na removal was accomplished by substituting choline for Na.

Fig. 3 C there is no difference between the pH_i of control and 5HT-treated cells (7.09 ± 0.02 vs. 7.10 ± 0.02) just before amiloride removal; however, dpH/dt after amiloride removal is significantly faster in the presence of 5HT (0.022 ± 0.003 vs. 0.041 ± 0.002 , $n = 4$, $P < 0.05$). Finally, when crypt cells are acid-loaded by pulsing with NH₄Cl, recovery from the acid load is faster in the presence of serotonin in crypt cells, but not villus cells (Fig. 5). In crypt cells, from a nadir pH of 6.59 ± 0.04 in control cells and 6.59 ± 0.03 in experiment cells, dpH/dt is 0.239 ± 0.014 in control and 0.393 ± 0.017 in 5HT cells ($n = 4$, $P < 0.05$).

Discussion

We have previously demonstrated differences in the distribution of transporters in ileal crypt and villus cells (1) (Fig. 1). Na:H and Cl:HCO₃ exchangers are present on the BBM of villus cells, whereas only Cl:HCO₃ exchange is present on the BBM of crypt cells. Na:H exchange is present on the BLM of

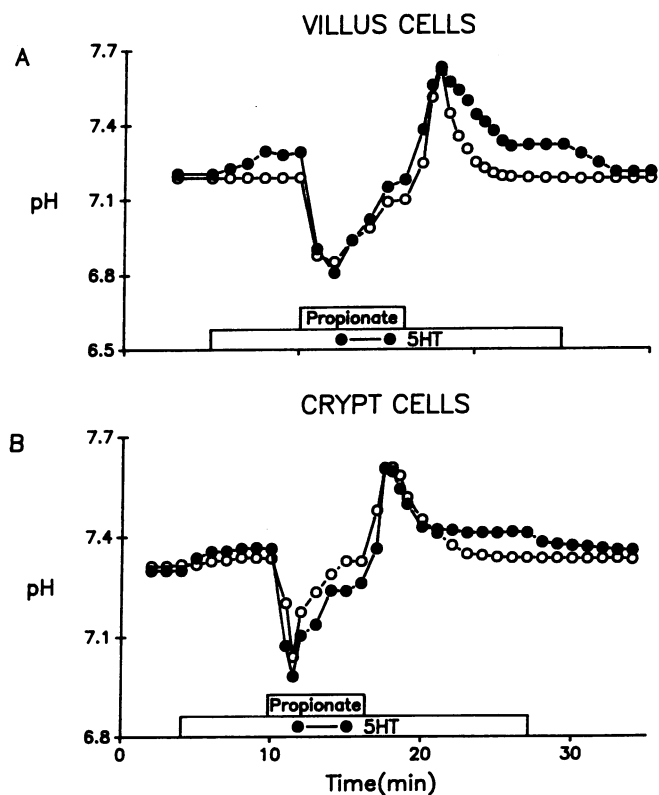


Figure 4. Effect of serotonin on recovery from an alkaline load in villus (A) and crypt (B) cells. The cells are first perfused with the standard CO₂/HCO₃ solution then with 50 mM Na-propionate replacing 50 mM of NaCl, in the presence (closed circles) or absence (open circles) of 10 μ M serotonin during the time period shown.

both cell types. This vectorial arrangement of transporters suggests that villus cells absorb NaCl and crypt cells secrete HCO₃ (1, 2). Our current studies indicate that serotonin, a neurohumoral agent present in the ileum, has different effects on villus and crypt cells. It induces alkalization in both cell types, but this appears to result from different mechanisms. In villus cells it is a HCO₃-dependent, Na-independent process characterized by a slow recovery from an alkaline load, suggesting inhibition of Cl:HCO₃ exchange. The alkalization induced by Cl removal or DIDS (Fig. 2) suggests operation of the Cl:HCO₃ exchanger in the basal state and is consistent with serotonin causing a similar inhibition of Cl:HCO₃ exchange in villus cells.

The alkalization of crypt cells by serotonin, on the other hand, is HCO₃ independent, Na dependent, and amiloride sensitive, all suggesting stimulation of Na:H exchange, which is further supported by the serotonin-induced accelerated recovery from an acid load induced by NH₄Cl, Na removal, or amiloride.

It has been demonstrated in intact rabbit ileum, in vitro, using the Ussing chamber technique, that serotonin blocks NaCl absorption (8). Our finding that serotonin inhibits Cl:HCO₃ exchange in villus cells provides an explanation for this inhibition, since in rabbit ileum Na:Cl absorption occurs by dual operation of Na:H and Cl:HCO₃ exchange (2). Donowitz et al. have presented evidence that serotonin's effect in rabbit ileum is mediated by an increase in intracellular Ca that activates protein kinase C (8, 9). Donowitz et al. have also

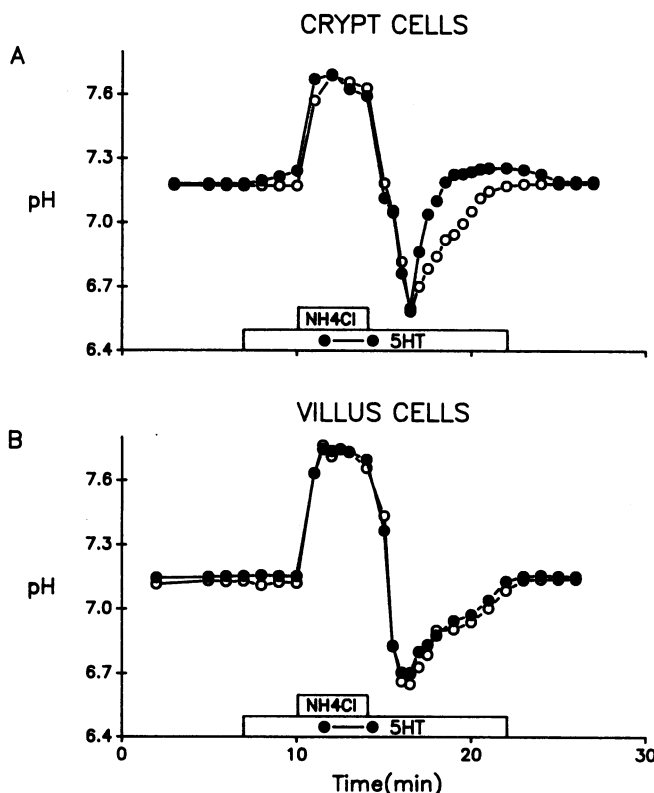


Figure 5. Effect of serotonin on recovery from an acid load in crypt (A) and villus (B) cells. The cells are first perfused with the standard Na-Hepes solution then with 30 mM NH_4Cl replacing 30 mM of NaCl, in the presence (closed circles) or in the absence (open circles) of 10 μM serotonin during the time period shown.

recently presented data indicating that protein kinase C activation inhibits Na:H exchange in rabbit ileal BBM vesicles (10). This last observation is clearly not in agreement with our observation of no effect of serotonin on Na:H exchange in villus cells and stimulation of Na:H exchange in crypt cells. There is no obvious explanation for this discrepancy; however, (a) serotonin has not been directly shown to activate protein kinase C in ileum; (b) differences in technique (brush border vesicles versus isolated cells) may be important; and (c) we may have missed a subtle inhibition of Na:H exchange, masked by a greater inhibition of Cl:HCO_3 exchange (11).

Hirose and Chang have presented evidence that serotonin inhibits Na:H exchange in chicken enterocytes (12). In this study, however, no attempt was made to distinguish between jejunum and ileum or crypt and villus cells. Further, there are no studies on the effect of serotonin on electrolyte transport in chicken intestine (as opposed to rabbit ileum), thus correlation between isolated cell and intact tissue studies is not possible.

Stimulation of Na:H exchange in crypt cells would be expected to produce OH or HCO_3 secretion. This has not been demonstrated with serotonin in intact ileum *in vitro*. An interesting and as yet unexplained phenomenon is that no secretagogues have been shown to stimulate HCO_3 secretion *in vitro* in ileum, though this clearly has been demonstrated *in vivo* in humans and animals with cholera enterotoxin (13, 14). Stimulation of Na:H exchange on the BLM of crypt cells is thus a

possible explanation for the bicarbonate secretion observed in diarrheal diseases. Secretagogues, including serotonin, stimulate Cl secretion, probably by inserting or activating a Cl channel in the BBM of crypt cells (15). This Cl channel would facilitate HCO_3 secretion via Cl:HCO_3 exchange by enabling Cl to recycle across the BBM.

Our results not only provide insight into the mechanism of action of serotonin, they further demonstrate the different transport characteristics of villus and crypt cells in the ileum and emphasize the need to separate these two cell populations when determining the regulation of electrolyte transport by neurohumoral agents.

Acknowledgments

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References

- Knickelbein, R. G., P. S. Aronson, and J. W. Dobbins. 1988. Membrane distribution of sodium-hydrogen and chloride-bicarbonate exchangers in crypt and villus cell membranes from rabbit ileum. *J. Clin. Invest.* 82:2158-2163.
- Knickelbein, R. G., P. S. Aronson, and J. W. Dobbins. 1985. Sodium and chloride transport across rabbit ileal brush border. II. Evidence for Cl:HCO_3 exchange and mechanism of coupling. *Am. J. Physiol.* 249:G236-245.
- Welsh, M. J., P. L. Smith, M. Fromm, and R. A. Frizzell. 1982. Crypts are the site of intestinal fluid and electrolyte secretion. *Science (Wash. DC)*. 218:1219-1221.
- Kerzner, B., M. H. Kelly, D. G. Gall, D. G. Butler, and J. R. Hamilton. 1977. Transmissible gastroenteritis: sodium transport and the intestinal epithelium during the course of viral enteritis. *Gastroenterology*. 72:457-461.
- MacLeod, R. J., and J. R. Hamilton. 1987. Absence of a cAMP-mediated antiabsorptive effect in an undifferentiated jejunal epithelium. *Am. J. Physiol.* 252:G776-G782.
- Whipp, S. C., H. W. Moon, L. J. Kemeny, and R. A. Argenzio. 1985. Effect of virus-induced destruction of villus epithelium on intestinal secretion induced by heat-stable *Escherichia coli* enterotoxins and prostaglandin E1 in swine. *Am. J. Vet. Res.* 46:637-642.
- Weiser, M. M. 1973. Intestinal epithelial cell surface membrane glycoprotein synthesis. I. An indicator of cellular differentiation. *J. Biol. Chem.* 248:2536-2541.
- Donowitz, M., N. Asarkof, and G. Pike. 1980. Calcium dependence of serotonin-induced changes in rabbit ileal electrolyte transport. *J. Clin. Invest.* 66:341-352.
- Donowitz, M., M. E. Cohen, M. Gould, and G. W. G. Sharp. 1989. Elevated intracellular Ca^{2+} acts through protein kinase C to regulate rabbit ileal NaCl absorption. *J. Clin. Invest.* 83:1953-1962.
- Donowitz, M., M. E. Cohen, J. Wesolek, J. McCullen, R. P. Rood, and G. W. G. Sharp. 1989. Ca^{2+} inhibits rabbit ileal brush border Na^+/H^+ exchange by translocation of protein kinase C (PKC). *Gastroenterology*. 96:A127.
- Rodd, R. P., E. Emmer, J. Wesolek, J. McCullen, Z. Husain, M. E. Cohen, R. S. Braitwaite, H. Murer, G. W. Sharp, and M. Donowitz. 1988. Regulation of the rabbit ileal brush-border Na^+/H^+ exchanger by an ATP-requiring Ca^{2+} /calmodulin-mediated process. *J. Clin. Invest.* 82: 1091-1097.
- Hirose, R., and E. B. Chang. 1988. Effect of serotonin on Na^+-H^+ exchange and intracellular calcium in isolated chicken enterocytes. *Am. J. Physiol.* 254:G891-G897.
- Hubel, K. A. 1974. The mechanism of bicarbonate secretion in rabbit ileum exposed to cholera toxin. *J. Clin. Invest.* 53:964-970.
- Banwell, J. G., N. G. Pierce, R. C. Mitra, K. L. Brigham, G. J. Caranasos, R. I. Keimowitz, D. S. Fedson, J. Thomas, S. L. Gorbach, R. B. Sack, and A. Mondal. 1970. Intestinal fluid and electrolyte transport in human colon. *J. Clin. Invest.* 49:183-195.
- Dharmasathaphorn, K., K. G. Mandel, H. Mausi, and J. A. McRoberts. 1985. Vasoactive intestinal polypeptide-induced chloride secretion by a colonic epithelial cell line. Direct participation of a basolaterally localized Na^+ , K^+ , Cl^- cotransport system. *J. Clin. Invest.* 75: 462-471.