the estimates of ϵ_{nn} and ϵ_{pp} equally, so the changes in dilatation would correspond to equal contributions from these strain components. This is clearly not entirely the case as ϵ_{nn} and ϵ_{pp} are not well correlated for the most part (Fig. 1C). (ii) An obvious source of systematic error in determining dilatation is the effect of not taking proper account of the atmospheric variables: temperature, pressure, and water vapor pressure. To test this possibility, we assumed that the observed dilatation is a linear function of these three variables and determined this function by a least-squares procedure; we found that none of the coefficients were statistically significant.

13. Currently there is substantial disagreement between strain changes inferred from the two-color Geodimeter data set and those recorded with a Sacks-Evertson dilatometer at a depth of about 200 m within the northeast portion of the Pearblossom network [M. J. S. Johnston, I. S. Sacks, A. T. Linde, D. Myren, *Eos* **63**, 430 (1982)]. The fluctuations in dilatation recorded by the dilatometer are approximately an order of magnitude lower in amplitude than those inferred from the two-color Geodimeter data during corresponding time periods.

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Crypts Are the Site of Intestinal Fluid and Electrolyte Secretion

Abstract. *The site of adenosine 3',5'-monophosphate–mediated fluid and electrolyte secretion across mammalian large intestine was found to be the crypts of Lieberkühn by means of two techniques. First, the formation of fluid droplets was visualized on the oil-covered mucosal surface directly over crypt duct openings when secretion was stimulated. Second, microelectrode impalement of individual surface and crypt cells revealed that only the crypt cells produced a pattern of secretagogue-induced alterations in membrane potential and resistance that was characteristic of secretory epithelia.*

The large intestine of mammals displays morphologic heterogeneity, consisting of two distinct structural regions: the surface epithelium and the crypts (*1*). The surface epithelium is composed primarily of columnar epithelial cells, whereas the epithelial cells and goblet cells (in a ratio of approximately 3 to 1) of the crypts surround a central duct that opens onto the mucosal surface. The intestine also shows functional heterogeneity. Colonic mucosa absorbs salt and water by way of an electrogenic Na^+ transport process that is stimulated by aldosterone (*2*) and inhibited by amiloride (*3*). In contrast, salt and water secretion results from the activity of an electrogenic Cl^- transport process that is stimulated by adenosine 3',5'-monophosphate (cyclic AMP) or agents that increase cellular cyclic AMP content (for example, theophylline, prostaglandin E_2, cholera toxin, or vasoactive intestinal peptide) (*4*).

Studies of the electrophysiology of the surface cells of rabbit descending colon have disclosed the presence of electrogenic Na^+ absorption (*5*), but the site of electrogenic Cl^- secretion remains unresolved. Indirect evidence, derived primarily from studies of mammalian small intestine, suggests that the crypts are responsible for cyclic AMP–induced fluid and electrolyte secretion. This evidence includes the following. (i) Exposure of rabbit jejunum to hypertonic solutions damaged the villus cells and impaired glucose absorption but did not alter the secretory response to cholera toxin (*6*). (ii) Exposure of rabbit jejunum to cycloheximide elicited morphologic changes in the crypt cells and inhibited secretion without affecting glucose absorption or the morphology of the villus cells (*7*). (iii) Brief exposure of rat or hamster jejunum to cholera toxin increased the concentration of cyclic AMP in villus cells and inhibited electrolyte absorption, whereas a prolonged exposure to the toxin was required to increase the cyclic AMP content of crypts and elicit electrolyte secretion (*8*). (iv) Gallbladder and flounder intestine display electrolyte absorptive processes that are mechanistically similar to those of mammalian small intestine, but these tissues contain no crypts and do not secrete in response to cyclic AMP (*9*).

In the present study, we used two direct techniques to identify the site of intestinal fluid and electrolyte secretion. We used rabbit descending colon in vitro as the experimental preparation because the absorptive and secretory processes have been well characterized and because the flat-surface epithelium and the crypts can be visualized separately. The tissue was stretched across a Lucite holding ring yielding an exposed tissue area of 1 cm^2 and mounted, mucosal surface up, on a microscope stage where it was examined with a Nikon phase-contrast microscope. The mucosal surface was dried and covered with oil while the serosal compartment was perfused with Ringer solution. As shown in Fig. 1A, the crypts and semitransparent surface epithelium can be readily distinguished.

Stimulation of secretion by addition of prostaglandin E_2 (PGE_2) to the serosal perfusate resulted in the appearance of small droplets of fluid on the mucosal surface (Fig. 1B). The droplets always appeared over the crypts. Fluid droplets could not be visualized over all crypts at all times. In many instances the secreted fluid spread over the surface epithelium rather than standing up in the oil as a readily visible bubble. Thus, bubbles that were initially visible because of the interface contrast between the oil and water phases would become invisible as fluid spread over the surface. This was especially true when secretion had pro-

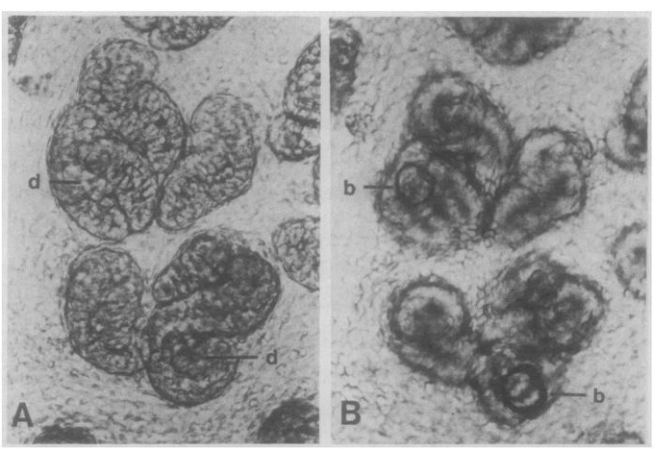

Fig. 1. Light micrograph of colonic mucosa, in vitro, looking down on the mucosal surface. The serosal surface was continuously perfused with Ringer solution containing (in millimolar concentrations): Na, 143; K, 5.4; Ca, 1.2; Mg, 1.2; Cl, 123.7; HCO_3, 24; HPO_4, 2.4; H_2PO_4, 0.6; and glucose, 10. This solution was aerated with 95 percent O_2 and 5 percent CO_2 and maintained at 37°C. The mucosal surface was blotted gently with tissue paper and covered with a thin layer of water-equilibrated paraffin oil (×175). (A) Nonstimulated tissue. The crypts, (*d*, crypt duct openings) can be seen lying beneath the semitransparent surface epithelium. (B) Same area of tissue 10 minutes after the addition of PGE_2 (10^{-6} *M*) to the serosal bathing solution. Note location of fluid bubbles (*b*) over the crypt duct openings. The epithelium now appears slightly blurred because the fluid droplets have been brought into focus.

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gressed to the stage where droplets from adjacent crypts (which seemed to lie in clusters) fused with one another.

The appearance of fluid over the crypt openings with subsequent wetting of the surface epithelium provides evidence against the notion that fluid was actually secreted by the surface cells and collected over the crypts. Droplets of fluid deposited directly on the surface epithelium with a micropipette, either before or after addition of PGE_2, remained stationary and did not spread to the crypt openings. In addition, when drying of the surface was incomplete, small fluid droplets were trapped on the surface beneath the oil; these did not change in size or migrate to the crypt openings before or during stimulation with PGE_2. There appeared to be a low rate of crypt secretion in nonstimulated tissues, but it is not clear whether this results from mucus or fluid production.

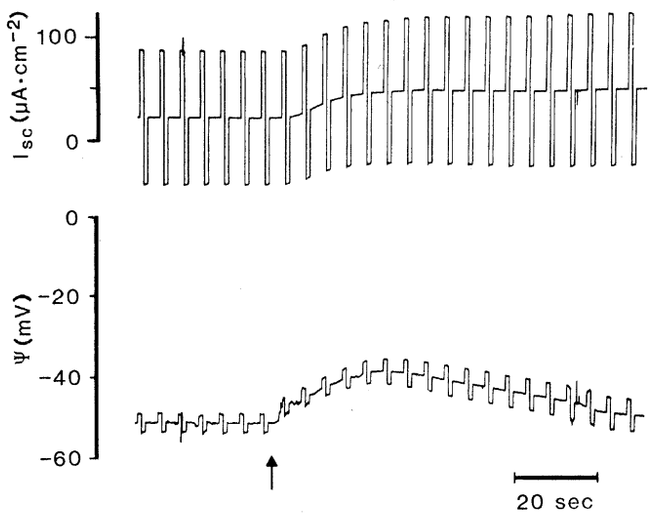

Fig. 2. Record obtained during microelectrode impalement of a crypt cell under short-circuit conditions [for details of the method, see Table 1 legend and (*11*)]. The I_{sc} (upper trace) is interrupted by bipolar, transepithelial current pulses sufficient in magnitude to intermittently clamp the transepithelial electrical potential difference at ±10 mV. Thus, the width of these current pulses is inversely related to the transepithelial resistance, R_t. The lower record provides the electrical potential difference across the basolateral membrane, ψ, of an impaled crypt cell, and the concurrent deflections in ψ due to current pulsing. The increase induced by PGE_2, $10^{-6}M$ serosal solution (arrow), results from a redistribution of resistance in the limiting cell membrane, as discussed in the text.

Furosemide has been shown to inhibit Cl^- secretion in a variety of tissues, including rabbit descending colon (*4*). Addition of furosemide ($10^{-3}M$) to the serosal perfusate prevented formation of the PGE_2-induced fluid droplets. Thus, direct visualization of fluid droplet formation, stimulated by PGE_2 and inhibited by furosemide, indicates that the crypts are the site of cyclic AMP–mediated fluid secretion by colonic mucosa. The mucus-producing goblet cells of the crypts discharge in response to parasympathetic stimuli, but not in response to cyclic AMP, theophylline, or PGE_2 (*1, 10*). Therefore, the PGE_2-induced emergence of fluid from the crypts probably results from the activity of the crypt epithelial cells and is not complicated by mucus production.

Microelectrode techniques (*11*) were used to determine the site of electrolyte secretion by measuring the electrical responses of individual surface and crypt cells to PGE_2. We found (Fig. 2, upper trace) that PGE_2 increased electrogenic Cl^- secretion across the colonic mucosa as reflected by increased short-circuit current (I_{sc}) and decreased transepithelial resistance (R_t) (see legend to Fig. 2). At the same time, the transmembrane electrical potential difference (ψ) of an impaled crypt cell depolarized (Fig. 2, lower trace).

Table 1 provides a summary of these studies. In response to PGE_2, the depolarization of ψ and the decrease in fractional resistance, f_r, are only observed in impaled crypt cells, not in surface cells. The f_r represents the relative contribution of the apical membrane to the total, transcellular resistance, $R_a/(R_a + R_b)$, where R_a and R_b are the resistances of the apical and basolateral membranes, respectively. Concomitant decreases in R_t and crypt cell f_r suggest that PGE_2 decreases apical membrane resistance, R_a. This electrical response to secretagogue has been observed in two other Cl^--secreting tissues, tracheal epithelium (*11*) and cornea (*12*), and has been shown to result from a cyclic AMP–induced increase in apical membrane Cl^- permeability.

Previous studies (*5*) have shown that the surface epithelial cells are responsible for electrogenic NA^+ absorption. Mucosal application of the diuretic amiloride (which inhibits transepithelial Na^+ transport by decreasing apical membrane Na^+ permeability) hyperpolarizes ψ and increases f_r of surface epithelial cells (*5*). As shown in Table 1, addition of amilor-

Table 1. Response of crypt and surface cells to stimulation of transepithelial Cl^- secretion with PGE_2 or to inhibition of transepithelial Na^+ absorption with amiloride. I_{sc} and R_t are transepithelial parameters that reflect the response of the total tissue to PGE_2 or amiloride, whereas ψ and f_r (see text) are recorded only from impaled crypt or surface cells, as designated above. Microelectrode tips were advanced into crypt cells across their basolateral membranes and into surface cells across their apical membranes. The crypt cells were impaled under a Leitz TS stereomicroscope (×160) and impalement was verified by two methods. First, dimpling of the crypt was often observed during puncture. Second, transient voltage deflections were recorded as the electrode tip crossed the remnants of muscularis mucosa at the basal surface. If a stable cell recording was not obtained within 200 μm after passing the muscularis, the electrode was withdrawn completely from the tissue and the process repeated. The total tissue thickness is approximately 500 μm, so that these recordings can only be obtained from cells comprising the crypt. These studies were conducted under short-circuit conditions, so that the apical and basolateral membrane potentials are equal, that is, a single ψ value is given. All impalements were maintained for at least 30 seconds before, and 30 to 90 seconds after the addition of PGE_2 or amiloride. Results are means (± standard error) for n impalements of N tissues as follows: PGE_2 on crypt cells ($N = 9$, $N = 6$); PGE_2 on surface cells ($N = 27$, $N = 5$); amiloride on crypt cells ($N = 3$, $N = 2$). Values were taken during the steady state. The I_{sc} across rabbit colon under control conditions reflects the rate of electrogenic Na^+ absorption, whereas the PGE_2-induced increment in I_{sc} is a measure of Cl^- secretion rate (*3, 4*). Thus, the difference in I_{sc} under control conditions obtained in these groups of tissues, and noted previously (*3*), results from variations in the spontaneous rate of Na^+ absorption.

Treatment	I_{sc} (μA · cm^{-2})	R_t (ohm · cm^2)	ψ (mV)	f_r
		Crypt cells		
Control	26 ± 5	149 ± 8	50 ± 3	0.77 ± 0.05
PGE_2	46 ± 5*	135 ± 6*	42 ± 3*	0.68 ± 0.05*
		Surface cells		
Control	102 ± 25	164 ± 30	44 ± 2	0.58 ± 0.05
PGE_2	140 ± 28*	120 ± 18*	43 ± 2	0.58 ± 0.03
		Crypt cells		
Control	78 ± 6	201 ± 28	49 ± 6	0.72 ± 0.02
Amiloride	30 ± 17	240 ± 53	50 ± 5	0.73 ± 0.02

*Significant difference from paired control value ($P < .05$).

ide ($10^{-4}M$) to the mucosal bathing solution decreased the I_{sc}, reflecting decreased Na^+ absorption, but did not alter ψ or f_r of crypt cells. This finding suggests that the crypt cells are not involved in amiloride-sensitive electrogenic Na^+ absorption.

Our results indicate that cells of the colonic crypts and not the surface epithelium are responsible for cyclic AMP–mediated electrogenic Cl^- secretion. These results, plus those of previous studies (*5*), also indicate that surface epithelial cells and not crypt cells are responsible for electrogenic Na^+ absorption. Thus, the structural heterogeneity of the colon is paralleled by a functional heterogeneity: the surface epithelium absorbs and the crypts secrete.

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Nature's Ballistic Missile

Abstract. *The parasitic fungus* Haptoglossa mirabilis *infects its rotifer host by means of a gun-shaped attack cell. The anterior end of the cell is elongated to form a barrel; the wall at the mouth is invaginated deep into the cell to form a bore. A walled chamber at the base of the bore houses a complex, missile-like attack apparatus. The projectile is fired from the gun cell at high speed to accomplish initial penetration of the host.*

The endoparasitic fungus *Haptoglossa mirabilis* (Eumycota) infects its Adineta (Rotifera) host by means of a gun-like injection cell. When a moving rotifer strikes this cell, a missile is shot through the host's cuticle, a hypodermic-like structure is inserted into the body, and a walled sporidium is pumped in to initiate infection (*1*). The sporidium grows into a large, cylindrical thallus, which at maturity produces several evacuation tubes through which biflagellate zoospores escape to the exterior. After a swarming period, each zoospore produces a spherical cyst that germinates almost immediately to produce a "gun" cell. The mature cell with attached cyst (Fig. 1) is shaped like a miniature cannon and is anchored to the substrate by an adhesive pad in such a way that the "barrel" is raised at an angle to the substrate to facilitate host encounters. Only a fraction of a second elapses between contact with the host and release of the infective sporidium (*2*).

Barron (*1*) suggested that the mechanism of attack in *Haptoglossa* might be similar to that described for the unrelated, plant-parasitic *Polymyxa betae* Keskin (*3*) and *Plasmodiophora brassicae* Wor. (*4*) of the Plasmodiophorales (Myxomycota). In the Plasmodiophorales an electron-dense, bullet-like structure ("stachel") forms within a tubular cavity ("rohr") inside the zoospore cyst. The rohr possesses a short, narrow tail ("schlauch"). At the moment of attack, the rohr is evaginated to form a bulbous structure (adhesorium), which attaches to the host. The stachel passes down the rohr and penetrates the host wall. The schlauch evaginates through the hole in the wall and an amoeboid infection unit is injected into a host cell. In *P. brassicae* it takes about 1 minute to produce the adhesorium, penetrate the wall, and infect the host.

Fig. 1. Light micrograph of a cluster of five gun cells of *Haptoglossa mirabilis*. The four peripheral cells are in a mature but unfired state. The central cell has fired but failed to penetrate a rotifer; the sporidium (*s*) is still attached to the empty gun cell by the hypodermic-like tube (×3300).

Even though *H. mirabilis* and the Plasmodiophorales may not be closely related, their mode of attack is very similar. However, the gun cell of *H. mirabilis* is more sophisticated in structure and design than the encysted zoospore of the Plasmodiophorales. We report here preliminary ultrastructural observations of the gun cell, with special reference to the unique structure of the missile itself.

Methods for recovering and culturing *Adineta* rotifers and *H. mirabilis* have been outlined elsewhere (*5*). The rotifers were used as bait to recover the fungus from greenhouse soil and to maintain the fungus in nonaxenic culture. A petri dish containing 2 percent water agar to which gun cells had attached themselves was rinsed gently in running tap water to remove surface debris. The washed plate was then flooded with fixative solution containing 3 percent glutaraldehyde and 1.5 percent acrolein in $0.07M$ phosphate buffer (*p*H 6.8). The fluid was drawn off slowly with a Pasteur pipette and the dish containing the parasite was overlaid with lukewarm water agar. Small blocks of agar (1 by 1 mm) with the gun cells sandwiched between them were cut out and placed in fresh fixative solution for 4 to 5 hours. The material was postfixed in 1 percent OsO_4 made up in the same buffer, dehydrated through a graded acetone series, and embedded in Spurr's plastic. Sections were cut with diamond knives, stained with 4 percent uranyl acetate in absolute methanol and Reynold's lead citrate, and viewed with a Phillips 200A or a JEOL JEM 100 CX electron microscope operating at 60 kV.

Figure 1 is a light micrograph of a cluster of five gun cells of *H. mirabilis*. The four peripheral cells are in a mature but unfired state. In these the protoplasm is concentrated in the tapering forward section (barrel) and the swollen

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