Secretagogue response of goblet cells and columnar cells in human colonic crypts

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Halm, Dan R., and Susan Troutman Halm. Secretagogue response of goblet cells and columnar cells in human colonic crypts. Am. J. Physiol. Cell Physiol. 278: C212–C233, 2000.—Crypts of Lieberkühn were isolated from human colon, and differential interference contrast microscopy distinguished goblet and columnar cells. Activation with carbachol (CCh, 100 µM) or histamine (10 µM) released contents from goblet granules. Stimulation with prostaglandin E2 (PGE2, 5 µM) or adenosine (10 µM) did not release goblet granules but caused the apical margin of columnar cells to recede. Goblet volume was lost during stimulation with CCh or histamine (~160 fl/cell), but not with PGE2 or adenosine. Three-quarters of goblet cells were responsive to CCh but released only 30% of goblet volume. Half-time for goblet volume release was 3.7 min. PGE2 stimulated a prolonged fluid secretion that attained a rate of ~350 pl/min. Columnar cells lost ~50% of apical volume during maximal PGE2 stimulation, with a half-time of 3.3 min. In crypts from individuals with ulcerative colitis, goblet cells were hypersensitive to CCh for release of goblet volume. These results support separate regulation for mucus secretions from goblet cells and from columnar cells, with control mechanisms restricting total release of mucus stores.

THE COLONIC EPITHELIUM of mammals secretes fluid and mucus in response to various neurotransmitters and local mediators (9, 12, 13). Fluid secretion modifies luminal composition by increasing the aqueous volume and by adjusting concentrations of electrolytes such as K+ and H+. Low rates of fluid secretion could aid colonic motility and bacterial fermentation, whereas higher rates seen in pathophysiological states would serve to flush bacteria from the lumen. Mucus secretions contribute to the barrier function of the epithelium by protecting the epithelial cells from abrasion and also by slowing access of bacteria to those epithelial cells. Release of mucus from goblet cells generally is separate from sustained fluid secretion on the basis of sensitivity to specific secretagogues (35). A mucus substance distinct from goblet cell mucin is released from crypt columnar cells during stimulation of fluid secretion (14, 16). Control of secretion from goblet and columnar cells by separate secretagogues permits alteration of the relative proportion of mucus types released and the rate of fluid production.

Mucus secretion by goblet cells involves exocytotic release of granule contents at the apical membrane, as indicated by ultrastructural studies (51). Movement of granules within the cell is a microtubule-dependent process, and access of the granules to the apical membrane is restricted by the actin cytoskeleton. Further advances in examining dynamics of goblet cell secretion have been made using video-enhanced differential interference contrast (DIC) microscopy of living cells (4, 14, 20, 46, 52). Individual events of goblet granule release were visualized as changes in light intensity, indicating a rapid discharge (0.03–1.0 s) of mucus from granules (4, 20, 46). The time course of stimulation with mucus secretagogues consists of a rapid increase in the number of granule release events that reaches a maximum in 1–3 min and then subsides to a much slower sustained rate. Discharge of mucus from goblet cells in rabbit colonic crypts occurs via a similar exocytotic process (14, 52), and columnar cells also show stimulated emptying of contents from the apical pole of the cell (14). The results presented in this study were obtained with DIC microscopy of isolated human colonic crypts and indicate the presence of two morphologically distinguishable types of cells: goblet and columnar cells. Distinct secretagogues stimulated release of mucuslike material from apically stored granules in each of these cell types.

METHODS

Specimens of human colon were obtained from surgical resection material by a procedure approved by the Institutional Review Board (Ohio State University and Wright State University). After release by the pathologist, specimens were refrigerated in HEPES-buffered Ringer solution until they were picked up and transported to the laboratory (generally ~30 min). The epithelium was removed from underlying muscle by blunt dissection and placed in ice-cold standard Ringer solution. Colonic mucosal biopsies from cotton-top tamarins were obtained during semiannual endoscopic screening of a colony maintained by Dr. J. D. Wood at Ohio State University. This procedure was approved by the Institutional Laboratory Animal Care and Use Committee at Ohio State University. The tamarin biopsies were transported to the laboratory in ice-cold HEPES-buffered Ringer solution within 20 min.

Human colonic crypts were isolated from resection material of 17 patients (9 women and 8 men, 15 Caucasians and 2...
African-Americans; Ohio State University Hospitals) ranging in age from 35 to 69 yr. Resections were roughly divided between ascending or transverse sites (~55%) and descending or sigmoid sites (~45%); normal margins were released by the pathologist for experimental study. Resection had been performed for cancer (12 of 17 patients), diverticulitis (2 of 17 patients), and inflammatory bowel disease (IBD, 3 of 17 patients). All three patients with IBD were women; one had active ulcerative colitis, one had nonactive colitis (resected for metastatic cancer), and one had Crohn’s disease. Information on patient medications was not available for comparison with experimental results. Cotton-top tamarins are a species of New World monkey that develops symptoms resembling ulcerative colitis when in captivity (30). The tamarins supply colonic crypts for imaging (n = 4) were assessed for extent of disease by histological measures, including white blood cell infiltration: three had severe colitis, and one had moderate colitis (personal communication, K. S. Tefend and J. D. Wood). Differences among the human specimens were not readily discernible, except for IBD tissue, which could be distinguished by using objective measures presented in RESULTS. On the basis of this general similarity and the pathologist’s assessment of normal tissue margins, tissues from patients with cancer or diverticulitis were grouped together as normal.

Individual colonic crypts were dissected from the colonic epithelium with use of fine forceps on the stage of a dissecting microscope (×40) in HEPES-buffered Ringer solution with 5% serum albumin added. The standard mammalian Ringer solution contained (in mM) 145 Na+ 145 , 5 K+, 2 Ca2+, 1.2 Mg2+, 125 Cl−, 25 HCO3−, 4 PO4 3−, and 10 glycine. Solutions were continuously gassed with 95% O2-5% CO2, which maintained solution pH at 7.4. HEPES-buffered Ringer solution contained 10 mM HEPES with HCO3− replaced by Cl−, and the pH was titrated to 7.4.

Crypts were imaged in a chamber mounted on the stage of an inverted microscope (Zeiss Axiovert); the bottom of the chamber was formed by a no. 1 coverslip (14). Isolated crypts were held by glass pipettes or adhered by a coat of polylysine to the chamber bottom coverslip. The glass pipettes were made to accommodate the relatively short length of colonic crypts and were manipulated with a system from Vestastra Scientific (Vestavia Hills, AL). For luminal perfusion, the blind end of the crypt was cut off with a sharpened needle before transfer to the imaging chamber. Luminal perfusate was HEPES-buffered Ringer solution. Luminal perfusion rate was estimated as 1–5 nl/min on the basis of perfusion pressure, pipette dimensions, and previous measurements with renal tubules (45). The bath was continuously perfused with standard Ringer solution with use of a peristaltic pump, and effluent was removed by suction tube; the solution was bubbled with 95% O2-5% CO2 and warmed by passage through a heated water jacket to maintain bath temperature at 37°C. Bath solution flow was 3 ml/min (~8 chamber vol/min).

Crypt images were formed with DIC optics: a × 40 oil immersion (1.4 NA) lens and a nonimmersion (0.65 NA) condenser (6). A video camera (model CCD300E, Videscope International) was used to record images to videotape (Sony) and to computer disk with use of the Image-1 system (Universal Imaging). A × 2 coupling lens (Diagnostic Instruments) was used to fill the camera field.

Image analysis. Morphometric measurements of recorded crypt images were performed with Image-1 software. Quantification of volume was obtained with focus adjusted to the midline of the crypt, because this plane of section allowed ready measurement of crypt and lumen diameter as well as a complete profile of the epithelium from base to apex. Crypt diameter was taken at the base of epithelial cells on opposite sides of the crypt (inside the pericryptal sheath); lumen diameter was taken at the apexes of opposing cells. A consistent group of cells was monitored in successive images by marking the boundaries between distinct, identified cells near the lateral margins of the image frame. Diameters were measured at nine evenly spaced points along the crypt segment defined by the length markers. Segment length was determined at cell apex, cell base, and midway along cell height for both epithelial margins (6 values). Crypt (Vcr) and luminal volumes (Vlu) (23) were calculated on the basis of a piecewise cylindrical model with use of average crypt diameter (D), average lumen diameter (d), average section length (L), and standard deviations (σD and σd) of these values (Eq. 1)

\[ \text{V}_{\text{cr}} = \frac{\pi}{4} L (D^2 + \sigma_D^2) \]

\[ \text{V}_{\text{lu}} = \frac{\pi}{4} L (d^2 + \sigma_d^2) \]

This calculation assumes that cells seen in the plane of focus are representative of all cells in that annulus of the crypt. Epithelial volume was calculated as the difference between crypt and luminal volume. All volumes were normalized to epithelial volume obtained in the initial control image for each experiment to adjust for differences in defined segment lengths (cell number) between crypts. Generally, 300–400 crypt epithelial cells were represented by epithelial volume in each experiment. Repeated measures on the same image indicated that reproducibility was ±0.1 µm for diameter measurements and ±0.5 µm for length measurements. Propagation of these error estimates for volume calculations (Eq. 1) gave an error estimate for relative epithelial volume of ±0.6%.

Volume of the zone containing apical granules (Vag) was calculated (similar to Eq. 1) by using the diameter of this zone (Dg), measured from the base of goblet granule masses in cells on opposite sides of the lumen (Vag = Vcr − Vlu), where Vg is goblet region volume; this volume includes apices of columnar cells, which often contain granules (21, 40, 48). Normalization to control epithelial volume gave the relative volume fraction for this apical zone. Error propagation provides an error estimate for relative apical zone volume of ±3%. Contribution of goblet granule masses to apical zone volume was estimated by a point-counting technique (58). A square array of points (2-µm image spacing) was overlaid (skewed from the crypt axis) on crypt images, resulting in ~500 points over the apical zone. The ratio of points within goblets to all points in the apical zone was the fraction of volume contributed by goblets. This estimate requires two corrections to reflect the actual volume distribution: section thickness and crypt geometry. The highly refractile goblet granules were readily recognized in images, but the contribution of goblet granules is overestimated, because granules can be seen throughout the depth of focus. The depth of focus expected for video imaging with this lens-condenser combination, 0.7 µm (18), can be used to calculate a correction of 0.88 (58). The other bias in the measurement underestimates the contribution of goblets, because longitudinal crypt midline sections were used, rather than random sections. Goblet fractional areas measured by point counting were converted to volume fractions (see APPENDIX) by use of Eq. A5, α for each crypt, and γ = π/4; the resulting correction factors ranged from 1.02 to 1.15.

Individual goblet granule masses were measured to monitor directly volume changes in this apically located cell compartment. Volume of goblets (Vgob) was calculated from
width (w) and height (h) of the granule mass by use of Eq. 2, with the assumption of a spheroidal shape

\[ V_{\text{gob}} = \frac{\pi}{6} w h \]  

(2)

Eccentricity [prolate (\(e_{\text{prolate}}\)) and oblate (\(e_{\text{oblate}}\))] in goblet shape was calculated using Eq. 3 as a measure of distortions to this cellular compartment

\[ e_{\text{prolate}} = \frac{1}{1 - \left(\frac{w}{h}\right)^2}^{1/2} \]  

(3a)

\[ e_{\text{oblate}} = -\frac{1}{1 - \left(\frac{h}{w}\right)^2}^{1/2} \]  

(3b)

For oblate goblets the ratio of width to height was inverted, and the resulting eccentricity was assigned a negative value. These definitions allow numerical comparisons of dissimilar goblets, with eccentricity ranging from \(-1.0\) to \(+1.0\) as shape changes from short and wide (oblate) to tall and narrow (prolate). Calculated goblet volumes (Eq. 2) were corrected for underestimation due to off-center optical sectioning of goblets within the crypt midline images. Separate frequency histograms of raw width and height measurements were deconvoluted to obtain an estimate of actual mean width and height (58). Comparing these actual mean widths and heights with means of raw measured values gave a volume correction factor of 1.19 to adjust goblet volume calculations. Eccentricity values were not adjusted, because the distributions of width and height produced similar underestimation, such that the ratio (Eq. 3) was unaffected. Time courses of goblet volume changes were obtained by measuring the same goblet through a series of images taken during various stimulatory conditions. In this analysis, only goblets that were clearly sectioned near the center during the entire sequence were used. The average control volume from these measurements was similar to the corrected volume obtained with the larger sample of all visible goblets.

Speed of refractile objects moving in the lumen was measured from video recordings. Transit time between cursors (10- to 30-µm separation, depending on rate of movement) was used to calculate speed. Size of objects was obtained from width (orientation with lumen diameter) and length. Object volume was calculated by assuming a spheroidal shape, as for goblet granule masses (Eq. 2).

Values are means ± SE. Statistical comparisons were made using a two-tailed Student’s t-test for paired comparisons, with significant difference accepted at \(P < 0.05\). For unpaired comparisons between groups, significant difference was accepted at \(P < 0.05\) from ANOVA with the method of Newman and Keuls.

RESULTS

Isolated colonic crypts imaged with DIC microscopy (Fig. 1) exhibited the two predominant cell types associated with this epithelium: columnar and goblet cells (3, 14, 21, 40, 48). Focusing at the midline of the crypt showed the full height of the epithelium, with apically located granules above basally located nuclei. Surrounding the crypt epithelium was a pericryptal sheath of myoepithelial cells (36, 44), which was apparent as a fibrous layer with flattened nuclei. Goblet cells, in particular, were distinct because of a densely packed cluster of highly refractile granules stored in the apical pole, which had a characteristic ovate profile. Generally, goblet cells were separated by columnar cells having a more hourglass-shaped profile that arched over the neighboring goblet cells. Thus the luminal surface often was dominated by columnar apexes, even though goblet granules filled more than one-half of the apical epithelial volume.

Crypts also were obtained from colonic epithelium of patients with IBD (ulcerative colitis and Crohn’s disease; Fig. 1). During dissection ulcerated regions of colon with active colitis had a fragile surface epithelium and a sparse number of recognizable crypts (4 specimens with active colitis were examined by dissection microscope; data not shown). Those crypts present (Fig. 1B) were generally large in diameter (>100 µm). Goblet cells appeared less full of mucous granules than did normal crypts. Colonic epithelium from patients with nonactive ulcerative colitis (Fig. 1C) or Crohn’s disease (Fig. 1D) was indistinguishable on dissection from normal epithelium in crypt density or crypt size. Crypts from all IBD patients had more focal spots of short epithelial height than did normal crypts. Colonic epithelium of tamarins had densely packed crypts, even in specimens with severe colitis. As for crypts from patients with IBD, the tamarin epithelium had focal spots of short cell height (Fig. 1E) and relatively depleted granule stores in goblet cells. Results from tamarin crypts are specifically indicated; all other results are for human crypts.

Crypt dimensions were measured and assigned to three groups (Table 1): normal (see METHODS), IBD, and tamarin. A distinction also was made for crypts from the normal group that were opened for luminal perfusion. Differences in size among these groups were not apparent statistically. The focal spots of short epithelial height seen in crypts from patients with IBD and in tamarin crypts were not extensive enough to cause a decrease in average epithelial height; however, the lumen diameter of perfused crypts and crypts from patients with ulcerative colitis had a tendency to be larger.

Secretory stimulation. Addition of secretagogues that stimulate mucus and fluid secretion produced several changes in crypt geometry, as well as epithelial cell morphology. The cholinergic agent carbachol (CCh) stimulated mucus release from goblet cells, seen as shrinking of apical granule clusters and as light flashes, presumably the result of refractile contents exiting individual granules (4, 20, 46). In some cases, evanescent plumes could be seen forming above the sites of flashes, which then drifted away from the cell surface. Flashes ceased within a few seconds of CCh removal. Concurrent with goblet granule release, crypt lumens dilated (Fig. 2). Histamine also stimulated mucus release from goblet cells, as indicated by numerous apical flashes. Stimulation with prostaglandin E₂ (PGE₂) or adenosine produced fluid secretion and recession of columnar cell apices without any apparent release from goblet cells (Fig. 3). Occasional light flashes were seen at columnar cell apices, but these events were not consistently observed. Fluid flow toward the crypt orifice was visualized by the rapid movement of refractile particles along the lumen. The cholinergic response of tamarin crypts was dramatic, with nearly complete disgorging of mucous granules.
and large dilation of the lumen (Fig. 4). Large plumes of mucus stayed tethered to the cell of origin (Fig. 4 C).

Subsequent stimulation with adenosine (Fig. 4 D) further shortened the epithelium and produced rapid fluid flow along the lumen that eventually ripped mucous plumes from goblet cells.

Crypt shape changed during secretory stimulation through an increase in lumen diameter and a small decrease in crypt diameter (Fig. 5). After sequential stimulation with these two classes of secretagogues (goblet type and fluid type), the crypts maintained an enlarged lumen diameter (Fig. 5 D). The changes in crypt and lumen diameters with each secretagogue (Fig. 5, B and C) are consistent with the decreased epithelial volume expected from release of apically stored mucus. Change toward a smaller crypt diameter and larger lumen diameter also could have resulted from a simple crypt elongation caused by cells becoming wider and shorter without losing volume. Including a measurement of length to monitor a consistent group

<table>
<thead>
<tr>
<th>Condition</th>
<th>Crypt Diameter, µm</th>
<th>Lumen Diameter, µm</th>
<th>Epithelial Height, µm</th>
<th>Crypt Length, µm</th>
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<tr>
<td>Normal</td>
<td>73.5±3.4 (8)</td>
<td>16.2±2.8 (8)</td>
<td>28.7±1.5 (8)</td>
<td>433±25 (6)</td>
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<tr>
<td>Normal (perfused)</td>
<td>80.6±4.4 (6)</td>
<td>26.2±4.3 (6)</td>
<td>27.2±2.7 (6)</td>
<td></td>
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<tr>
<td>IBD</td>
<td>87.1±11.2 (3)</td>
<td>25.4±6.3 (3)</td>
<td>30.8±3.9 (3)</td>
<td>399±75 (2)</td>
</tr>
<tr>
<td>Tamarin</td>
<td>67.6±3.2 (4)</td>
<td>17.4±3.2 (4)</td>
<td>25.1±2.6 (4)</td>
<td>383±36 (4)</td>
</tr>
</tbody>
</table>

Values are means ± SE of number of observations in parentheses. IBD, inflammatory bowel disease.
of crypt epithelial cells would provide an unambiguous calculation of epithelial volume. Secretagogue-induced changes in epithelial volume. Epithelial volume of crypts was monitored during secretagogue stimulation by picking a consistent segment of crypt length over which to measure crypt diameter and lumen diameter. Segment length was defined from the boundaries between identifiable cells positioned near the edges of the recorded image fields. A representative experiment from the central portion of a normal crypt is shown in Fig. 6. Volumes (Fig. 6B) were calculated from the measured crypt dimensions (Fig. 6A; see methods). Epithelial volume decreased with CCh addition and did not return in the subsequent control period. With addition of PGE2, a decrease in epithelial volume that was not recovered on return to control conditions also occurred.

Relative changes in epithelial volume were measured in the central portions of crypts during stimulation by CCh, histamine, PGE2, and adenosine (Fig. 7). Normal crypts had a sustained ~4% decrease in epithelial volume induced by CCh (Table 2). The only crypts that did not exhibit a decrease in epithelial volume (Fig. 7A) were three perfused crypts with dilated lumens that appeared relatively depleted of goblet granules before stimulation. Tamarin crypts had the largest responses to CCh. As a group, the responses to histamine showed no distinct change in epithelial volume (Fig. 7B), even though flashes indicative of granule release events were seen during stimulation. Addition of PGE2 produced a sustained ~5% decrease in epithelial volume (Fig. 7C, Table 2), with only one perfused normal crypt not showing a decline. Similarly, adenosine addition

Fig. 2. Cholinergic stimulation. Goblet granule clusters (arrowheads) responded to a cholinergic agonist. Individual granules are discernible within some of these clusters. Crypt orifice is to right. Scale bars, 10 µm. A: midline DIC image from central portion of a normal crypt in control condition. B: crypt in A 5 min after beginning of cholinergic stimulation (100 µM carbachol (CCh)). Lumen diameter increased concurrent with loss of apically stored material from goblet cells. Release of mucin granules produced craters in some goblet cells.

Fig. 3. Prostaglandin E2 (PGE2) stimulation. Response of columnar cells (arrowheads) to a fluid secretagogue. Crypt orifice is to left. Scale bars, 10 µm. A: midline DIC image from central portion of a normal crypt in control condition (after return from stimulation with 2 µM CCh). A large globule of goblet mucus (~10 µm diameter) can be seen in lumen (under 2nd downward arrowhead from left), remaining from earlier release event. B: crypt in A 5 min after beginning of stimulation with PGE2 (2 µM). Lumen diameter increased through a recession of columnar apexes without any apparent change in goblet cells.
resulted in a decrease in epithelial volume (Fig. 7D); a crypt from the patient with nonactive ulcerative colitis was relatively unresponsive but lost volume during subsequent PGE2 stimulation.

The epithelial volume decreases that were sustained after return to control (Fig. 7) are consistent with a volume loss due to release of cellular material (presumably mucus) from apical stores (Figs. 2–4). Transient portions of epithelial volume responses may reflect changes in cytoplasmic volume during secretion that recover after removal of the stimulus. The portion of epithelial volume contributed by apical granules in goblet and columnar cells (Vaz) was estimated for each crypt from the radial extent of the apical goblets (see METHODS); Vaz ranged from 0.29 to 0.48 and averaged 0.35 ± 0.02. Loss of epithelial volume (Fig. 7) was normalized to the individual measures of Vaz to provide an indication of how much of the stored material was released (Table 2, Fig. 8). The total amount of Vaz released after stimulation by goblet- and fluid-type secretagogues was largest in those crypts from patients with ulcerative colitis and from tamarins (Fig. 9) but still did not exceed the measured control apical stores.

The contribution of goblet granules to Vaz was measured by point counting in control crypt images (see METHODS). For normal crypts the goblet fraction was 0.65 ± 0.03 (n = 8). Crypts from patients with IBD had goblet fractions statistically indistinguishable from crypts from normal patients (0.64 ± 0.04, n = 3). The CCh-induced loss of Vaz (Table 2) can be transformed by using the goblet fraction to estimate the percentage of goblet granule volume released in normal crypts: ~30%. For crypts from the two patients with ulcerative colitis, goblet volume release was 55–75%. Tamarin crypts released ~80% of goblet volume. Similarly for PGE2-Stimulated volume loss (Table 2), normal crypts released ~50% of nongoblet (columnar cell) Vaz. Release from this columnar cell apical zone was 80–100% for crypts from patients with ulcerative colitis and from tamarins.

Crypt diameter and length relationships may be controlled in part by the contractile state of the pericryptal sheath (36, 44). Normal crypts maintained length within 1% of control values during stimulation with CCh (n = 6) and then shortened by ~2% on return to control conditions. Perfused crypts (n = 5) shortened by ~2% during CCh stimulation and maintained that shortening on return to control conditions. Crypts from patients and tamarins with ulcerative colitis (2 humans and 2 tamarins) lengthened by ~4% during CCh stimulation and then shortened to roughly control length on return to the control condition. During PGE2 stimulation, normal crypts (perfused and nonperfused) maintained length within 1% of control values (n = 7) and then shortened by ~2% on return to control conditions. Crypts from patients and tamarins with IBD (n = 3: active colitis, Crohn’s disease, and tamarin) shortened by ~3% during PGE2 stimulation and maintained that shortening on return to control. Together with the decreases in crypt diameter (Figs. 5 and 6), these changes in crypt length suggest that the peri-
cryptal sheath responds to secretagogues and contributes to maintenance of crypt dimensions.

Luminal fluid flow. Fluid flow along the lumen was apparent from movement of refractile particles. Sustained movement of these objects in the lumen was seen only during secretagogue stimulation. Presumably, the objects were made up of released mucus and other cellular debris. In some crypts, particles were small (~3 µm diameter) and seen infrequently, whereas in other crypts the lumen was crowded with globular objects. Stimulation by CCh of a crypt with globular objects already present in the initial control condition (Fig. 10A) produced a measurable particle flow toward the crypt orifice. Removal of the stimulus stopped flow with a lag of ~1 min, although the flashes indicative of granule release stopped within a few seconds. PGE₂ produced particle speeds of ~10 µm/s throughout the period of stimulation, whereas CCh stimulation resulted in particle speeds comparable to PGE₂ for only 2–3 min, after which movement slowed and became undetectable.

Generally, particles moved as discrete entities, with faster speeds nearer the center of the lumen, as expected for laminar flow. Volume flow through these crypt sections can be estimated from the speed of the fastest particles and the cross-sectional area of the lumen. The fastest speeds in Fig. 10 correspond to a...
crypt fluid flow of ~10 pl/min during CCh stimulation that increased to ~350 pl/min during PGE₂ stimulation (see DISCUSSION); fluid flow decreased to ~210 pl/min after CCh addition with PGE₂. Retrograde flow consistent with fluid absorption was not observed. For tamarin crypts, maximal fluid flow with PGE₂ or adenosine stimulation tended to slowly push extruded goblet mucus globules along the lumen walls rather than as free objects in the center of the lumen. The crypt from the patient with active colitis (Fig. 1B) formed a continuous filament (~15 μm diameter) of presumptive goblet mucus in the center of the lumen that was dragged toward the crypt orifice during PGE₂ stimulation. Particle movement in the fluid surrounding the filament was comparable to that in Fig. 10, but the speed of the filament was only ~0.5 μm/s. Volume flow of mucus carried in the filament was ~5 pl/min, which stopped at the end of PGE₂ stimulation.

Stimulated release of cellular contents. Loss of epithelial volume during stimulation by goblet- and fluid-type secretagogues appeared to be restricted to specific cell types. Goblet cells were the site of cholinerigic volume loss. Release of mucus from apical granules began at the luminal margin, occasionally progressing to make deep craters within the apex of goblet cells, presumably by fusion of individual pits (Fig. 11). Columnar cell apexes continued to arch over goblet cells during CCh stimulation, suggesting a lack of volume release from this cell type. An en face view of the epithelium (Fig. 12) shows the craters centered within the goblets and the spatial arrangement of goblet and columnar cells within the epithelium. Craters generally did not persist, with goblet granule clusters instead rearranging to form smaller spheroids.

The response to CCh of a crypt from the patient with active ulcerative colitis was consistent with hypersensitivity of goblet cells (Fig. 13). As with the crypt from a tamarin with moderate colitis (Fig. 4), released mucus remained tethered to the cells of origin, giving a cobblestone appearance to the apical surface. The crypts from tamarins with severe colitis had a similar appearance on CCh stimulation.

Stimulation of fluid secretion with PGE₂ or adenosine did not produce any noticeable changes in granules of goblet cells. Although apical vesicles or vacuoles were not generally discernible in columnar cells (Figs. 11 and 14), fluid secretion was accompanied by selective recession of columnar cell apical borders (Fig. 14), consistent with loss of apically stored contents.

Volume of stored mucus was calculated for individual goblet cells (Table 3) by measuring the width and height of the apical granule cluster, with the assumption of a spheroidal shape (see METHODS). Goblet clusters in crypts from patients with IBD were smaller than those in crypts from normal patients. Eccentricity of the goblet granule cluster (ratio of width to height, Eq. 3) provides an indication of granule arrangement within the goblet cluster; goblet granule clusters in perfused crypts and tamarin crypts were more nearly spherical than those of normal crypts, suggesting less restrictive packing. The relationship between control goblet volume and eccentricity is shown in Fig. 15. Distortion of large goblets into more prolate shapes suggests a constraint on packing larger granule volumes into the tubular crypt structure, with goblet crowding around the lumen.

Volume stored in individual goblets was measured before and after maximal stimulation by CCh. The goblet volume released by human crypts was ~25% of the control value (Fig. 16A). Release at 2 and 10 μM CCh was >90% of the value at 100 μM, indicating that the half-maximal stimulating concentration for CCh was ~0.2 μM. In response to histamine, ~30% of control goblet volume was released in human and tamarin crypts (Fig. 16B). Addition of atropine, a muscarinic antagonist, before and during histamine stimulation did not alter the response (data not shown).
Incomplete discharge of goblet stores during maximal activation suggests stimulatory mechanisms (cholinergic and histaminergic) that limit granule release. Goblets from tamarin crypts, however, released nearly all the stored contents with CCh stimulation (Fig. 16A) but not with histamine stimulation (Fig. 16B), supporting a selective lack of restrictive cholinergic regulation in these tamarins.

The two independent measures of CCh-induced goblet volume release (Figs. 7A and Fig. 16A) were compared quantitatively by using the goblet fraction of epithelial volume; Fig. 17 shows the goblet volume changes obtained from these individual (goblet volume) and global (epithelial volume) measures. Normal human and tamarin crypts had volume changes close to the line of identity, supporting a conclusion that these two measures of volume release represent the same cellular events. Crypts from the two patients with ulcerative colitis deviated significantly from the line of identity, suggesting that measurements of individual goblet volumes underestimate total volume released in these crypts. The larger response measured with epithelial volume changes could result from a columnar cell contribution in ulcerative colitis, but a more likely explanation is that smaller goblet clusters seen in ulcerative colitis crypts (Table 3, Fig. 13) were underrepresented in measurements of goblet volume, since these small clusters were excluded because of difficulty in reliable tracking through the sequence of stimulation.

**Table 2. Relative volume response to secretagogues**

<table>
<thead>
<tr>
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<th>Epithelial</th>
<th>Apical Zone</th>
<th>Cell-Specific Apical Zone</th>
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<tbody>
<tr>
<td>CCh</td>
<td>-4.2 ± 1.4‡ (11)</td>
<td>-19.0 ± 3.7‡ (8)</td>
<td>-29.2 ± 6.5‡ (8)</td>
</tr>
<tr>
<td>PGE₂</td>
<td>-4.7 ± 1.8‡ (7)</td>
<td>-17.7 ± 4.6‡ (6)</td>
<td>-50.6 ± 13.1‡ (6)</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed as percentage; number of crypts is in parentheses. Change in relative volume was calculated as difference between control period after stimulation and control period preceding stimulation. Carbachol (CCh) was added at 2–100 µM (maximum stimulation). Prostaglandin E₂ (PGE₂) was added at 2–5 µM (maximal stimulation). *Goblet. †Columnar. ‡Significantly different from zero, P ≤ 0.05.
Responsiveness of individual goblet cells. The distribution of CCh-induced fractional goblet volume release for individual cells is shown in Fig. 18A. Two distinct peaks occurred: one near zero volume release and one at higher release. This bimodal distribution is consistent with a nonresponding group of goblet cells dispersed among responding goblet cells. Comparison of the fraction of goblet cells responding within a crypt to the average initial goblet volume (Fig. 18B) indicated a greater proportion of nonresponding cells in crypts with larger goblet clusters. A similar relation for histamine stimulation (Fig. 18C) suggested that the histamine response was not related to goblet size. In those crypts exposed to both CCh and histamine (3 crypts, 27 goblets), 43% of goblet cells responded (defined as in Fig. 18) to both agents, but the CCh-nonresponsive cells responded to histamine and the histamine-nonresponsive cells responded to CCh. Apparently all these goblet cells were capable of stimulated release of granule contents.

The nonresponding goblet cells were not present in any apparent pattern within the central portion of the crypts. In tamarin crypts, after CCh stimulation had dramatically depleted central goblet cells of granules (Fig. 4), those goblet cells in the most distal 25% of the crypt (closest to the surface epithelium) retained large stores of granules (data not shown).

The group of responding cells was used to provide a time course of the responses to the goblet secretagogues CCh and histamine. A secretory response from a representative crypt is shown in Fig. 19A. Adenosine did not
ALTER GOBLET VOLUME, BUT SUBSEQUENT ADDITIONS OF HISTAMINE AND CCH PRODUCED RAPID LOSSES OF GOBLET VOLUME. THE SHAPE OF GOBLETs ALSO CHANGED ON STIMULATION. GOBLETs BECAME MORE SPHERICAL DURING ADENOSINE ADDITION BUT RETURNED TO A PROLATE SHAPE IN CONTROL CONDITIONS. AN IRREVERSIBLE CHANGE TOWARD SPHERICAL OCCURRED WITH SUBSEQUENT CCH ADDITION; HISTAMINE DID NOT DRAMATICALLY ALTER GOBLET SHAPE. AVERAGE TIME COURSES FOR CCH AND HISTAMINE GOBLET VOLUME RESPONSES HAD SIMILAR KINETICS AND AMPLITUDE (Fig. 19B). THE CCH RESPONSE OF GOBLET CELLS MEASURED FROM EPITHELIAL VOLUME CHANGES WAS INDISCRIMINABLE FROM THE GOBLET VOLUME MEASUREMENT, FURTHER SUPPORTING THAT THESE INDEPENDENT MEASURES REFLECT THE SAME CELLULAR EVENT. GOBLETs FROM TAMARIN CRYPTS HAD A SIMILAR HALF-TIME FOR GOBLET VOLUME RELEASE, BUT NEARLY ALL THE CONTENTS WERE RELEASED. A TIME COURSE FOR VOLUME RELEASE FROM THE APEICAL ZONE OF COLUMNAR (NOGOBLET) CELLS INDICATES A SECRETORY RESPONSE OF COMPARABLE SPEED FOR FLUID SECRETAGOGUES (Fig. 19C).

EXPANSION OF EXTRUDED GOBLET MUCUS WAS PARTICULARLY APPARENT IN THE CRYPT FROM THE MODERATELY AFFECTED TAMARIN (Fig. 4). FOR SEVEN GOBLETs IN THIS CRYPT THAT APPEARED TO RELEASE ALL THE MUCIN GRANULES, A MUCUS EXPANSION RATIO WAS CALCULATED. VOLUME OF THE EXTRUDED MUCUS WAS MEASURED FROM WIDTH AND HEIGHT (Eq. 2) AND COMPARED WITH GOBLET VOLUME BEFORE CCH STIMULATION. THE AVERAGE EXPANSION RATIO WAS 2.1 ± 0.1 AND WAS HALF-MAXIMAL AT ~5 MIN AFTER ONSET. PARTICLES MOVING IN THE LUMEN (PRESUMPTIVE GOBLET MUCUS, Fig. 10) WERE OFTEN LARGER THAN EXPECTED FOR THE EFFLUENT OF A SINGLE GOBLET CELL (>200 fl: 335 fl AVERAGE GOBLET VOLUME: 0.3 MEAN FRACTIONAL RELEASE · 2-FOLD EXPANSION RATIO), SUGGESTING THAT THESE SECRETORY PLUMES COULD COALESC.

CHANGES IN GOBLET SHAPE DURING SECRETAGOGUE-STIMULATED RELEASE OF APICALLY STORED CONTENTS ARE CONSISTENT WITH A CONSTRAINT ON PACKING WITHIN THE CRYPT STRUCTURE. LOSS OF GOBLET VOLUME DURING CCH STIMULATION WAS ASSOCIATED WITH A ROUNGING OF THE GOBLET PROFILE (Figs. 19A AND 20A), CONSISTENT WITH RELIEF FROM DEFORMING FORCES AS VOLUME IN THE APEICAL ZONE DECREASED. STIMULATION WITH PGE2 OR ADENOSINE LED TO SIMILAR SHAPE CHANGES WITHOUT LOSS OF VOLUME FROM GOBLETs (Figs. 19A AND 20B), CONSISTENT WITH LOWER DEFORMING FORCES DUE TO VOLUME RELEASE IN NEIGHBORING COLUMNAR CELLS. RETURN OF GOBLET ECCENTRICITY AFTER REMOVAL OF FLUID SECRETAGOGUES MAY OCCUR THROUGH COMPACTION AS CRYPT LENGTH SHORTENS, PRESUMABLY THROUGH THE ACTION OF THE PERICRYPTAL SHEATH. THE PROLATE SHAPE OF CONTROL GOBLET CLUSTERS BECAME MORE SPHERICAL WITH SECRETAGOGUES THROUGH CHANGES IN WIDTH AND HEIGHT (Table 4, Fig. 20C). RESPONDING GOBLETs BECAME SHORTER AND NARROWER WITH CCH, WHEREAS NONRESPONDING GOBLETs BECAME SHORTER BUT WIDER. DURING PGE2 OR ADENOSINE STIMULATION, ALL GOBLETs BECAME SHORTER AND WIDER. THESE GOBLET SHAPE CHANGES INDICATE THAT CELLS NEIGHBORING GOBLET CELLS, PRESUMABLY COLUMNAR CELLS, LOSE VOLUME ON STIMULATION WITH PGE2 OR ADENOSINE.

DISCUSSION

A MAJOR TASK OF THE COLONIC EPITHELIUM AS THE MOST DISTAL SITE ALONG THE ALIMENTARY TRACT IS TO REABSORB FLUID. IN CONTRAST TO THIS CONSERVATION FUNCTION, THE COLONIC EPITHELIUM ALSO SECRETES FLUID. THIS FLUID HAS A DISTINCT ELECTROLYTE AND MACROMOLECULAR COMPOSITION. THE LARGEST MACROMOLECULE SECRETED IS MUCUS (9), WHICH SERVES TO BUFFER CELLS AGAINST ABRASION BY LUMINAL CONTENTS AND TO CREATE A MICROCLIMATE (UNSTIRRED LAYER) NEAR THE EPITHELIAL SURFACE. MUCUS RELEASED IN CRYPTS OF LIEBERKÜHN IS CLEARED INTO THE COLONIC LUMEN BY FLUID SECRETION. AS SHOWN IN RESULTS, CRYPTS FROM HUMAN COLON RELEASE APICALLY STORED MATERIAL (PRESUMABLY MUCUS) FROM GOBLET AND COLUMNAR CELLS. SEPARATE CONTROL OF THESE MUCUS SECRECTIONS OCCURS THROUGH DISTINCT TYPES OF SECRETAGOGUES.

EPITHELIAL CELL TYPES IN COLONIC CRYPTS. THE COLONIC EPITHELIUM IS COMPRISED OF SEVERAL CELL TYPES THAT FORM THE TWO MAJOR EPITHELIAL STRUCTURES: THE SURFACE EPITHELIUM AND THE CRYPTS OF LIEBERKÜHN. SIMILAR TO OTHER
mammals (3, 14), human colonic epithelium contains two predominant cell types: columnar and goblet cells (21, 40, 48). Columnar cells have been distinguished further on the basis of ultrastructural features associated with the degree of differentiation. Enteroendocrine cells, which release signaling molecules, generally make up <5% of the cells. Goblet cells are distinguished by the large number of mucous granules in the apical pole. Although in crypts these cells lack the characteristic narrow basal pole seen in the surface epithelium, crypt goblet cells have a rigid cytoskeletal arrangement that maintains the round profile of the granule mass (43, 51). In living crypts, with use of DIC microscopy, goblet cells can be recognized readily by the ovate shape of this granule cluster because of the highly refractile contents of the granules (Figs. 1–4 and 11–14). Columnar cells fill the spaces between goblet cells; distinctions among these nongoblet cells were not apparent. Deformation by the more rigid goblet cell neighbors produced a narrow waist between basal and apical poles, giving columnar cells an hourglass appearance. The apical poles of columnar cells fan out over goblet cells, such that only the small apexes of goblet cells contact the lumen. Whereas in rabbits and mice the apical poles of columnar cells are filled with large vacuoles (3, 14), columnar cells in human colon have

Fig. 11. Cellular response to cholinergic stimulation. Addition of CCh stimulated progressive release of mucin granules, producing apical craters in goblet cells (arrowheads), without evidence of release from columnar cells (bars with *). Scale bars, 5 µm. Three pairs of images are shown for control and stimulated conditions (A1 and B1, A2 and B2, and A3 and B3). A: control goblet cells had broad clusters of granules. Columnar cells arched over goblet cells, such that only goblet apices were in contact with lumen. B: during CCh stimulation (100 µM for ~10 min), pits formed in apices of goblets, with some becoming deep invaginations into apical granule clusters. C: goblet during CCh stimulation (~10 min) shows an apical crater connected to lumen (apical margin is along lower edge of goblet, with lumen below). Individual ~0.9-µm-diameter granules (arrowhead) also are visible. Scale bar, 2 µm.
small apical vesicles (21, 40, 48). Thus goblet and columnar cells have apically stored products positioned for release into the lumen. The general epithelial appearance is of goblet ovals pointing toward the lumen and fan-shaped deltas of columnar cells with a wide luminal extent.

Mucus secretion by goblet and columnar cells. Secretory control of columnar and goblet cells in colonic crypts is distinct. Release of mucus from goblet cells is stimulated by cholinergic agonists and by histamine (35); goblet cells do not release mucus in response to vasoactive intestinal peptide (VIP) or cAMP and theophylline (35), agents that stimulate fluid secretion (13). Responsiveness was assessed in fixed specimens by the presence of apical cavitation in goblet cells, a procedure that would miss modest stimulation of granule release. A morphometric assessment of cholinergic stimulation indicated that goblet cells that did not cavitate, particularly in the surface epithelium and crypt base, did show a decrease in mucous granules (37, 39). From these studies of fixed colonic tissue, the strongest goblet secretagogues are cholinergic agonists and histamine, but other agents are not conclusively ruled out as minor secretagogues. In living human colonic crypts, with use of morphometric assessment, it was found that CCh and histamine stimulated goblet granule release (Figs. 11 and 16), but the fluid secretagogues PGE2 and adenosine did not cause goblet granule release (Figs. 14, 19, and 20, Table 4). Although these results are not comprehensive for all potential agonists, goblet granule release was separable from sustained fluid secretion.

Crypt columnar cells have vesicles in the apical pole that are positioned to release contents into the lumen. Columnar cell vacuoles of rabbit distal colonic crypts stain histochemically distinct from goblet cell granules, and this vacuolar material was present in the crypt lumen after PGE2 stimulation (14). During PGE2 stimulation, imaged with video microscopy in living crypts, the apical pole of columnar cells became empty of refractile material, consistent with exocytotic release (14). Release of material was also apparent in guinea pig distal colonic crypts by use of electron microprobe analysis (16). Columnar cell apical vacuoles had a characteristic composition of high Ca2+ and sulfur with
low Na\textsuperscript{+} and Cl\textsuperscript{−} in control conditions that changed to high Na\textsuperscript{+} and Cl\textsuperscript{−} with low Ca\textsuperscript{2+} and sulfur during PGE\textsubscript{2} stimulation, consistent with access of the vacuole interior to the extracellular space. Together these observations clearly indicate a release of columnar cell material into the crypt lumen during stimulation by a fluid secretagogue. Intracellular ion concentrations of crypt columnar cells also changed as expected for a Cl\textsuperscript{−} secretory cell, with PGE\textsubscript{2} stimulation producing an increase in Na\textsuperscript{+} (16) and a drop in Cl\textsuperscript{−} (15). Thus columnar cells of colonic crypts respond to fluid secretagogues by actively secreting Cl\textsuperscript{−} and by releasing a macromolecule into the lumen, indicating a cellular link between fluid and mucus secretion. Living human colonic crypts responded to PGE\textsubscript{2} and adenosine by secreting fluid (Figs. 4 and 10) and releasing apically stored material from columnar cells (Figs. 3, 8, and 14). Cholinergic stimulation did not alter columnar cells

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<thead>
<tr>
<th>Volume, fl</th>
<th>Eccentricity</th>
<th>N (n)</th>
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<tbody>
<tr>
<td>Normal</td>
<td>335 ± 27</td>
<td>+0.74 ± 0.03</td>
</tr>
<tr>
<td>Normal (perfused)</td>
<td>310 ± 37</td>
<td>+0.45 ± 0.11*</td>
</tr>
<tr>
<td>IBD</td>
<td>230 ± 27*</td>
<td>+0.70 ± 0.02</td>
</tr>
<tr>
<td>Tamarin (severe)</td>
<td>137 ± 12*</td>
<td>+0.22 ± 0.15*</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, number of crypts; n, number of individual goblets. *Significantly different from normal, P ≤ 0.05.
Consistent with a segregation of secretory responses to specific cell types, colonic epithelial cell lines have been used extensively to study fluid and mucus secretion. The capacity for fluid secretion generally is detected by measuring electrogenic Cl\(^-\) secretion, which produces the osmotically driven flow of fluid. Mucus secretion has been measured by various methods that detect the presence of released glycoproteins.

**Fluid secretion.** Stimulation of human colonic crypts with goblet-type (CCh) or fluid-type (PGE\(_2\)) secretagogues stimulated fluid flow toward the crypt orifice (Fig. 10), consistent with active fluid secretion. This transepithelial fluid flow is driven by the osmotic gradient developed by active Cl\(^-\) secretion; human colonic epithelium can secrete fluid, as indicated by stimulated Cl\(^-\) secretion at distal and transverse sites (47). Isolation of crypts from the interstitium may alter the secretory osmotic gradient by removing any component of interstitial hypotonicity that may normally develop. Fluid flow stimulated by PGE\(_2\) was larger and more sustained than that stimulated by CCh (Fig. 10). In addition, significant flow toward the crypt orifice in the tamarin crypt was not apparent with CCh but increased dramatically with adenosine (Fig. 4). This difference in fluid secretory rates is consistent with measurements of Cl\(^-\) secretion, which indicate that cholinergic stimulation produces only a transient increase in Cl\(^-\) secretion compared with a sustained increase by fluid secretagogues such as PGE\(_2\) (5, 13). Reduction in PGE\(_2\)-stimulated fluid secretion with CCh (Fig. 10) may reflect the inhibiting action of CCh on Cl\(^-\) secretion measured in T84 cells (57). Thus the two types of mucus secretion (goblet cell derived and columnar cell derived) are consistent with a segregation of secretory responses to specific cell types.

**Colonic epithelial cell lines.** Epithelial cell lines derived from colonic tumors have been used extensively to study fluid and mucus secretion. Capacity for fluid secretion generally is detected by measuring electrogenic Cl\(^-\) secretion, which produces the osmotically driven flow of fluid. Mucus secretion has been measured by various methods that detect the presence of released glycoproteins.

The T84 cell line secretes Cl\(^-\) in response to VIP, prostaglandin E\(_1\) (PGE\(_1\)) and CCh (5). Release of mucus by T84 cells was stimulated with CCh, PGE\(_1\), and VIP (28, 32); electron micrographs showed that only ~5% of the cells contained large apical granules, so that the mucus secretion may have emanated from a small minority of the cells. Several subclones of the HT-29 cell line have been developed (HT29.Cl16E, HT29.B6, and HT29.18N2) that have a morphology similar to goblet cells (apically located granules). Forskolin and CCh stimulate a short-circuit current consistent with Cl\(^-\) secretion in HT29.Cl16E cells (33). With HT29.B6 cells, VIP and PGE\(_1\) stimulate Cl\(^-\) secretion (25). The HT29.Cl16E cell line releases mucus in response to CCh, neurotensin, VIP, and ATP (1, 33). Mucus release from HT29.18N2 cells is stimulated by a PGE\(_2\) derivative (38). The ability of these cultured colonic epithelial cell lines to release mucus in response to goblet secretagogues (CCh) and fluid secretagogues (PGE\(_2\) and VIP) suggests that these cell lines combine the traits of goblet and columnar cells, either within a single cell or in a culture of cells with mixed differentiation.

**Fluid secretion.** Stimulation of human colonic crypts with goblet-type (CCh) or fluid-type (PGE\(_2\)) secretagogues stimulated fluid flow toward the crypt orifice (Fig. 10), consistent with active fluid secretion. This transepithelial fluid flow is driven by the osmotic gradient developed by active Cl\(^-\) secretion; human colonic epithelium can secrete fluid, as indicated by stimulated Cl\(^-\) secretion at distal and transverse sites (47). Isolation of crypts from the interstitium may alter the secretory osmotic gradient by removing any component of interstitial hypotonicity that may normally develop. Fluid flow stimulated by PGE\(_2\) was larger and more sustained than that stimulated by CCh (Fig. 10). In addition, significant flow toward the crypt orifice in the tamarin crypt was not apparent with CCh but increased dramatically with adenosine (Fig. 4). This difference in fluid secretory rates is consistent with measurements of Cl\(^-\) secretion, which indicate that cholinergic stimulation produces only a transient increase in Cl\(^-\) secretion compared with a sustained increase by fluid secretagogues such as PGE\(_2\) (5, 13). Reduction in PGE\(_2\)-stimulated fluid secretion with CCh (Fig. 10) may reflect the inhibiting action of CCh on Cl\(^-\) secretion measured in T84 cells (57). Thus the two types of mucus secretion (goblet cell derived and columnar cell derived) are consistent with a segregation of secretory responses to specific cell types.
nar cell derived) are accompanied by different amounts of fluid secretion.

Particle speeds \( (v) \) can be used to calculate the Reynolds number \( (Re) \) for luminal fluid flow, which provides a comparison of inertial and viscous forces acting in the crypt lumen; high Re indicate dominance of inertia, and low Re indicate a more viscous regimen \((56)\). Lumen diameters can be used for the characteristic length \( (l) \), and estimates of fluid viscosity \( (\eta) \) and density \( (\rho) \) can be taken as the values for water \((Re = \rho lv/\eta)\). For a human crypt secreting fluid as in Fig. 10, Re is 0.0004. Such a low Re clearly indicates the dominance of viscous forces during crypt fluid secretion. More realistic estimates of fluid viscosity would be higher, because of secreted mucus, which would reduce Re further. The consequences for crypt fluid flow are that turbulence does not occur and a laminar flow condition develops immediately as flow starts.

An estimate of the fluid secretory rate for an entire crypt can be calculated from the observed particle speeds and lumen dimensions. Volume flow past a specific point is related to the cross-sectional area of the lumen \((\pi d^2/4)\) and the velocity profile across the lumen \((56)\). For laminar flow, total volume flow \((J_v)\) is equal to one-half the product of cross-sectional area and speed at lumen center \((J_v = \pi v_{max} d^2/8\), where \(v_{max}\) is maximum velocity); the highest speeds in Fig. 10 should correspond to the center of the velocity profile. Because the particle speeds were obtained about halfway along the crypt, volume flow at the crypt orifice would be twice as great (with the assumption of uniform secretion over the entire crypt). The resulting total volume

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\text{Fig. 17. Comparison of volume release measurements. Average relative volume change of individual goblet granule clusters (V_{gob}) in crypts produced by CCh stimulation (mean } \pm \text{ SE) is shown in relation to relative volume change of apical goblet zone (goblet fraction of epithelial volume (V_{ep})): } \bullet, \text{ Normal; } \odot, \text{ normal-perfused; } \triangle, \text{ ulcerative colitis (N, nonactive); } \mathbf{v}, \text{ tamarin. Dashed line, line of identity. Ulcerative colitis crypts are significantly off line of identity. All other values are not significantly different from line of identity.}
\]

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\text{Fig. 18. Responsiveness of apical goblets. A: relative frequencies of CCh-induced fractional goblet volume release for all goblet cells in normal crypts (n = 74). Fitted curve is sum of 2 Gaussian distributions; peak at small fractional release contains 23% of goblets with a mean of 0.04 } \pm \text{ 0.01 and peak at higher fractional release contains 77% of goblets with a mean of 0.29 } \pm \text{ 0.02. B: fraction of CCh-responsive goblet cells for each crypt relative to average control goblet volume (mean } \pm \text{ SE). } \bullet, \text{ Normal; } \odot, \text{ normal-perfused; } \triangle, \text{ ulcerative colitis (N, nonactive); } \bullet, \text{ Crohn's disease; } \mathbf{v}, \text{ tamarin. Responding goblet cells were defined as those with a volume release fraction } \leq 0.20. \text{ C: fraction of histamine-responsive goblet cells for each crypt relative to average control goblet volume (mean } \pm \text{ SE). Symbols as in B. Responding goblet cells were defined as those with a fractional goblet volume release } \geq 0.20.}
\]
flow for the crypt in Fig. 10 is ~350 pl/min during PGE2 stimulation. Normalizing for crypt length (Table 1) converts this fluid secretory rate to ~900 pl·min⁻¹·mm⁻¹. PGE2-stimulated fluid secretion was ~40 pl·min⁻¹·mm⁻¹ in isolated rabbit colonic crypts (24). In isolated perfused rat colonic crypts (49), VIP reversed a fluid absorption of 350 pl·min⁻¹·mm⁻¹ to a fluid secretion of 350 pl·min⁻¹·mm⁻¹; cholinergic stimulation reversed absorption to a fluid secretion of 330 pl·min⁻¹·mm⁻¹. Interestingly, a goblet secretagogue and a fluid secretagogue stimulated similar rates of fluid flow with these rat colonic crypts, in contrast to the results for human colonic crypts. Fluid absorption may be difficult to detect in the present study, since the method relies on movement of refractile particles that might be relatively scarce during absorption. In addition, rat colonic crypts were thought to be absorptive because of the absence of the pericryptal sheath (49); all dissected human and tamarin crypts retained the pericryptal sheath (Fig. 1). Glands of the tracheal epithelium, which secrete fluid and mucus, can be stimulated to secrete fluid at much higher rates, ~10 nl/min (42, 53).

Secretory capacity for the human colon can be estimated from the output of an individual crypt. Because crypt density in the human colonic epithelium is ~25,000/cm² (48), the colonic mucosal surface area of ~4,000 cm² indicates a total number of crypts in the colon of ~10⁸. The total daily output of continuously stimulated maximal crypt fluid secretion would be ~50 liters. Such a rate clearly could not be sustained, but the ability to overwhelm the absorptive capacity of the colon is apparent.

The hydrostatic pressure gradient necessary to push fluid toward the crypt orifice can be obtained from the Hagen-Poiseuille equation \( \Delta P = \frac{128 \eta l J}{(\pi r^4)} \), where \( \Delta P \) is pressure gradient) by using lumen dimensions, total crypt volume flow, and fluid viscosity (56). Viscosity is undoubtedly higher for the secreted fluid than for water because of the presence of secreted mucus. Colonic mucus contains soluble and insoluble forms (2), with the insoluble, or gel form, probably representing goblet granule mucin. The less dense columnar cell material (14, 16) is more likely a soluble form, which would be most pertinent for determining fluid viscosity. Mucus viscosity depends on source, concentration, and shear encountered (8, 22, 34) but could range from 10-to 1,000-fold that of water. From this range of fluid viscosity, the expected pressure difference would be 0.1–10 mmHg, consistent with a low pressure-flow system.

Perfusion of the crypt lumen via pipettes mimics the state of fluid secretion and allows introduction of compounds to the apical surface of crypt epithelial cells. The measured perfusion rate was ~4 nl/min in the study of fluid transport with perfused rat colonic crypts (49), which is comparable to the estimated rate of 1–5
nl/min in this study of human colonic crypts. These luminal perfusion rates are 3- to 15-fold faster than the secretory flow at crypt orifice produced with PGE₂ (Fig. 10), which may contribute to the altered appearance (Figs. 5 and 15) and response (Fig. 7) of perfused crypts compared with intact crypts. Higher perfusion rates are produced by higher applied hydrostatic pressure, which has been reported to be deleterious to rabbit colonic crypts (26). Although luminal perfusion has the advantage of providing access to the apical membrane, nonphysiological fluid flow may lead to abnormal cellular responses.

Mucus secretory mechanism. Mucus release by epithelial cells, and goblet cells in particular, has been measured by several methods. These techniques can be separated into two broad categories: those that detect mucus released (1, 28, 32, 38) and those that measure depletion of mucus from epithelial cells (4, 35, 37, 39, 46, 50, 52). Detection of mucus secreted from crypts is difficult, because collecting the mucus would require not only release from the cell but clearance from the crypt lumen. Also, differences in cellular responsiveness are difficult to determine from collection methods. For these reasons, a morphological approach was chosen for the present study. Mucus volume released was measured (see METHODS) by two means: decreases in epithelial volume and decreases in goblet volume. Epithelial volume changes include goblet and columnar cell events, so that qualitative observations are required to indicate which cell types produced a particular response (Figs. 11 and 14). Goblet cell mucus granule volume release could be measured directly, Table 4. Changes in size and shape of mucous goblet

<table>
<thead>
<tr>
<th>Condition</th>
<th>Δ Volume, fl</th>
<th>Δ Eccentricity</th>
<th>Δ Width, μm</th>
<th>Δ Height, μm</th>
<th>N (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCh</td>
<td></td>
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<tr>
<td>High responders</td>
<td>-158 ± 8*</td>
<td>-0.29 ± 0.11*</td>
<td>-0.62 ± 0.23*</td>
<td>-3.00 ± 0.56*</td>
<td>6 (47)</td>
</tr>
<tr>
<td>Nonresponders</td>
<td>-6 ± 7</td>
<td>-0.11 ± 0.03*</td>
<td>+0.39 ± 0.15*</td>
<td>-0.91 ± 0.25*</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Fluid secretagogues</td>
<td></td>
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<tr>
<td>Goblets</td>
<td>+1 ± 9</td>
<td>-0.17 ± 0.06*</td>
<td>+0.43 ± 0.05*</td>
<td>-0.85 ± 0.23*</td>
<td>5 (66)</td>
</tr>
</tbody>
</table>

Values are means ± SE; N, number of crypts; n, number of individual goblets. Δ values were calculated from stimulated condition compared with prior control condition. High responders released ≥20% of goblet volume (see Fig. 18); nonresponders released <10% of goblet volume. *Significantly different from zero, P ≤ 0.05.
since the apical granules formed a compact distinct grouping. Unfortunately, a similar measure for columnar cells was not possible, because the extent of apical vesicles could not be distinguished consistently through the time course of secretory stimulation. Comparison of these two measures for cholinergic responsiveness of goblet cells (Fig. 17) indicates that epithelial volume can reliably report on the mucus volume released by goblet cells, suggesting that the fluid secretagogue response measured as epithelial volume change (Figs. 7, C and D, and 19C) also reflects mucus release.

Goblet cell mucus secretion. Similarity in the time courses for the CCh response of epithelial and goblet volume (Fig. 19B) suggests that cytoplasmic volume changes during this stimulation are minor and limited to a small transient decrease during the first minute (seen as excess volume loss in the epithelial volume trace compared with the goblet volume trace). Histamine stimulation of goblet granule release (Fig. 16) was not apparent in epithelial volume measurements (Fig. 7B), suggesting that some or all of the crypt cells had cytoplasmic swelling that obscured the volume released from mucous granules. Shape changes of goblet granule masses during histamine stimulation also are consistent with swelling, because goblet eccentricity stayed prolate during volume loss contrary to the CCh response (Fig. 19A). Cytoplasmic swelling would have compressed goblets laterally so that a more spherical shape could not occur. Thus cholinergic and histaminergic stimulation of goblet cells can be distinguished, in part, on the apparent cytoplasmic swelling induced by histamine.

Goblet cells release mucus by an exocytotic mechanism in which mucous granules fuse with the apical membrane (51). Maximal cholinergic stimulation leads to deep cavitation of the goblet granule cluster by compound exocytosis (50). This fusion of granules to other granules that have already fused with the apical membrane produces craters in the apex of goblet cells and results in loss of granule membrane as successive fusions isolate some membrane fragments. Volume of the granule pool in control conditions (Table 3) was similar to that in rabbit distal colonic goblet cells (43); from the size of an individual granule, \(-0.4\text{ fl}\) (Fig. 11), the number of granules in a normal goblet was \(\sim 850\). Individual exocytotic events were visible in living crypts as changes in light intensity of granules (flashes) that presumably result from a decrease in mucous refractivity as expansive release from granules occurs. These exocytotic flashes have been seen in other goblet cells (4, 20, 52); the half-time for the process is \(\sim 0.2\text{ s}\) (46). Supramaximal stimulation of human colonic goblet cells only occasionally produced the deep cavitations seen in rabbit distal col (50). Instead, small craters were apparent at the apex of goblets (Figs. 2 and 11). As the volume of goblet granule clusters decreased (Figs. 16 and 19), the remaining granules rearranged to make goblets shorter and narrower (Fig. 20). This more subtle response is similar to goblet cells in colonic surface epithelium (39) and small intestinal villi (37). A more dramatic goblet response was observed in a crypt from the patient with active ulcerative colitis (Fig. 13) and in crypts from tamarin colon (Figs. 4, 16, and 19). Although large quantities of mucus were visibly released, cells appeared to rearrange behind the departing mucus by becoming shorter without a noticeable cavity. For the tamarin with moderate colitis (Fig. 4), many of the granules appeared to be jettisoned intact as a group, presumably when more peripheral granules fused in a compound fashion to cut off connection with the cell. Within \(\sim 10\text{ min}\) of extrusion, the appearance of the mucous plume became homogeneous, suggesting a lysis of these granules. These results with human crypts support an involvement of compound exocytosis in goblet release but suggest that remaining granules are moved within the cell to alter cell shape.

Expansion of mucus results from electrostatic repulsion of multiple anionic residues on these molecules (54). Packing of mucus into granules is facilitated by charge screening with divalent cations such as \(\text{Ca}^{2+}\) (55). Goblet granules in the colonic epithelium contain high concentrations of \(\text{Ca}^{2+}\) and \(\text{Mg}^{2+}\) (16) that presumably serve this purpose of condensing the filamentous mucous molecules. The massive release from tamarin goblet cells (Fig. 4) indicated almost twofold expansion for extruded mucus. Because this enlargement is much smaller than the 600-fold expansion seen for slug goblet granules (55), charge screening must be occurring in the crypt lumen to limit mucus extension. Such a mechanism would have the advantage of not blocking the crypt lumen, allowing easier passage of goblet mucus out of the crypt.

Observing living colonic crypts allowed individual goblet cells to be followed through the course of stimulated granule release. Some goblet cells were unresponsive to cholinergic stimulation, 23% (Fig. 18A), and tended to have larger goblet volumes (Fig. 18B), consistent with retention of granules. Apparently, unresponsiveness lasted long enough to fully pack the goblet zone. Responsive goblets, on average, released 29 ± 2% of mucus stores (none > 70%, Fig. 18A). This incomplete release suggests that an inhibitory control restricts granule emptying and can block release in about one-fourth of the goblet cells.

Columnar cell mucus secretion. The fluid secretagogues PGE\(_2\) and adenosine produced decreases in epithelial volume that were sustained after return to the control condition (Fig. 7, C and D), which suggests that the lost volume resulted from mucus release from columnar cells (14), as visualized by the recession of the apical cell margin (Fig. 14). Further support for this cellular specificity is the lack of goblet volume decrease (Fig. 19A, Table 4). A contribution of cytoplasmic volume change to the fluid secretagogue response is more difficult to assess than with goblet secretagogues because of the lack of a direct measure of apical vesicle volume. Crypt diameters have been used as an indicator of epithelial volume during VIP stimulation of rat colonic crypts isolated without the pericryptal sheath (7). Although changes in crypt diameter were altered by furosemide, a blocker of \(\text{Na}^+\)-dependent \(\text{Cl}^-\) entry, interpretation of these changes as indicative of epithelial volume is problematic without measurements of lumen diameter and crypt length. Intracellular \(\text{Cl}^-\)
concentration decreased transiently during PGE$_2$ stimulation in isolated rabbit colonic crypts, consistent with cell shrinkage that returned to near the initial value in ~2 min (15). The membrane electrical potential difference of rabbit colonic crypt cells also depolarized transiently during PGE$_2$ or adenosine stimulation, returning to near the initial value in 1–2 min (27). These results support an activation process for fluid secretagogues in which cytoplasmic volume decreases and returns to control levels; this response results from Cl$^-$ and K$^+$ channels opening followed by increasing Cl$^-$ influx, which supplies continued Cl$^-$ secretion. Therefore, the contribution of cytoplasmic changes to epithelial volume would appear to be constrained to early time points, such that sustained volume decreases in human colonic crypts (Figs. 7, C and D, and 19C) reflect release of apically stored mucus from columnar cells.

Columnar cells of colonic crypts release a material stored in apical vesicles (vacuoles), but the mechanisms of release are much less precisely known than for goblet cells. Stimulation by fluid secretagogues in rabbit distal colon leads to release via opening of a narrow connection between vacuole and crypt lumen (14). Viewed in these crypts with DIC, initially the apical pole of the columnar cell becomes empty (less refractile) and then collapses, such that goblet cells appear closer together. Because in human crypts the apical vesicles were small, it was not possible to view the emptying, but the collapse of the apical zone was observed as a recession of the apical margin of columnar cells, with goblet cells becoming closer together (Fig. 14). The time course of the PGE$_2$ response of human crypts (Fig. 19C) indicates that volume release was essentially complete at 10 min of stimulation, whereas fluid secretion continued. Apparently, fluid secretion does not depend on constant mucus release, even though both are initiated by the same agents.

IBD and secretory processes. The IBD of ulcerative colitis and Crohn’s disease lead to epithelial damage and altered function (31, 41). Cell death may be the cause of the focal points of short epithelial height seen in inflammatory crypts (Fig. 1). In addition, the larger diameter of crypts from patients with ulcerative colitis may reflect an early stage in cancer progression (31, 41, 59). Whether epithelial changes are simply a consequence of immune cell activity during inflammation or constitute part of the cause for the pathology is uncertain.

One diagnostic feature is depletion of goblet cell mucus in ulcerative colitis compared with normal mucosa or Crohn’s disease, whereas Crohn’s disease mucosa contains only slightly less mucus than normal mucosa (29). In colon from guinea pigs with experimental colitis, the depletion of mucus was due to less mucus in goblet cells rather than a decrease in goblet cell number (19). In addition to mucus depletion, the composition of colonic mucus is altered in ulcerative colitis (41), and some of the change may relate to the different mucus types in goblet and columnar cells (11). In the colonic crypt goblet cells from patients with ulcerative colitis and those from tamarins with severe colitis, stores of mucus were smaller (Table 3, Fig. 15), consistent with the interpretation that goblet cells are present in ulcerative colitis but are simply more difficult to identify because of reduced mucus content.

Mucus depletion may have resulted from chronic stimulation by inflammatory mediators released in vivo, but the response to CCh in vitro was larger than for normal crypts. Tamarin crypts released nearly the complete apical supply of mucous granules (Figs. 4 and 16A). Both crypts from patients with ulcerative colitis (active and nonactive disease) had indications of hypersensitive goblet cells. Mucous plumes were apparent (Fig. 13), and CCh-induced volume release was larger than in normal patients (Fig. 8A). The disparity between CCh-induced epithelial volume changes and individual goblet changes may result from preferential (or hypersensitive) release from goblets of small size: small goblets would be difficult to monitor through the time sequence of stimulation and would be underrepresented in direct measures of goblet volume. Whereas all tamarin goblets were hypersensitive (Figs. 16 and 18), the largest (and easiest to monitor) goblets in human crypts were not hypersensitive, and some were unresponsive. In contrast, tamarin and human ulcerative colitis goblet cells did not appear to be hypersensitive to histamine, although an increased sensitivity in cells with limited granule supplies cannot be excluded. Thus goblet cells in ulcerative colitis are hypersensitive to cholineric stimulation, but those few that were the least responsive would have retained more granules and have the largest granule stores. Fluid secretion also is increased in ulcerative colitis (41), which may be a direct consequence of elevated in the inflammatory mediator PGE$_2$ (17). Because isolated ulcerative colitis crypts did not secrete fluid until stimulated, the action of these fluid secretagogues was removed by the isolation of crypts from the mucosa. The epithelial volume released in response to PGE$_2$ or adenosine (Fig. 8, B and C) also suggests a hypersensitivity to these secretagogues in human and tamarin ulcerative colitis. Hypersecretion for release of goblet cell mucus and columnar cell mucus would exacerbate hyperstimulation occurring through immune responses. These results do not indicate whether epithelial cells contribute to initiation of the inflammatory response, but the crypt cells have an augmented mucus secretory response.

APPENDIX

A translation of relative area in crypt midline optical sections to relative volume requires assumptions about crypt geometry. For random sections, area measures will reflect volume (58). The rigid geometry of crypts can be used to compensate for the nonrandom nature of midline optical sectioning. Crypts can be modeled with roughly equal numbers of goblet and columnar cells dispersed evenly in the epithelium (10). A unit volume for the apical zone of the crypt can be defined as a truncated pie piece (a bite removed from the tip to create the lumen) with a single goblet in each; a series of these prismoid unit volumes reconstruct the apical zone around the circumference of the lumen. The goblet granule cluster can be approximated as a frustum of a right circular cone. Volume within the pie piece, but not in the frustum, would be the apical zone volume contributed by columnar cells. Volume of the frustum ($V_{fr}$, Eq. A1) can be
calculated from the height (h) and two widths (diameters), the smaller at the lumen (\(w_{\text{l}}\)) and the larger (w) at the base of the goblet:

\[
V_c = \frac{\pi}{12} h (w^2 + w w_{\text{l}} + w_{\text{l}}^2)
\]  

(A1)

Volume of the unit apical zone (\(V_{\text{au}}\), Eq. A2) can be obtained from the frustum measurements, since the frustum is inscribed within the unit volume.

\[
V_{\text{au}} = \frac{1}{2} h (w^2 + w w_{\text{l}})
\]  

(A2)

The fraction of volume (\(f_v\)) contributed by goblets (Eq. A3) is obtained by dividing \(V_c\) by \(V_{\text{au}}\)

\[
f_v = \frac{\frac{\pi}{6} (1 + \alpha + \alpha^2)}{\alpha} = \frac{w_{\text{l}}}{w}
\]  

(A3)

As \(\alpha\) ranges from 0.0 to 1.0, \(f_v\) would increase from \(\pi/6\) to \(\pi/4\). The fraction of area (\(f_p\)) contributed in midline section by goblets (Eq. A4) is obtained from the ratio of the frustum profile to the apical unit area.

\[
f_p = \frac{\frac{\pi}{3} (1 + \alpha + \alpha^2)}{3\alpha} = \frac{1}{\gamma}
\]  

(A4)

Because the lumen circumference is made up in increments of unit volumes, the ratio \(w_{\text{l}}/w\) can be obtained from the measured diameters, \(\alpha = d/D\). As \(\alpha\) approaches 1.0, the tubular crypt epithelium would transform into a flat sheet, and the frustums would become cylinders. In this limit, \(f_v\) is equivalent to \(f_p\) and \(\gamma = \pi/4\). Sectioning the crypt optically at the midline and measuring area along the crypt segment is equivalent to sectioning unit volumes at various angles, by rotating a unit around the axis defined by the center of the crypt. Thus quasi-random sectioning of the unit volume is equivalent to sectioning unit volumes at various angles, by rotating a unit around the axis defined by the center of the crypt. Therefore, the angle subtended at the crypt axis by the wedge of the unit volume (pie piece) is equal to \(2\theta\), where \(i = \arcsin(2w_{\text{au}}/D)\). The ratio \(i/n\) gives the fractional deviation of the unit volume from the midline. For goblet frustums, Eq. A7 gives the average area from sectioning (\(A_{\text{au}}\))

\[
A_{\text{au}} = \frac{wh}{1 + \alpha} \sum_{i=0}^{n} \sec(i\pi/n) \left[ 1 - \tan^2 \left( \frac{i\pi}{n} \right) \right] / (n + 1)
\]  

(A6)

Combining Eqs. A6 and A7 results in Eq. A4 and provides an explicit expression for \(\gamma\) (Eq. A8) in the frustum model of goblets

\[
\gamma = \frac{\sum_{i=0}^{n} \sec(i\pi/n) \left[ 1 - \tan^2 \left( \frac{i\pi}{n} \right) \right]}{\sum_{i=0}^{n} \sec(i\pi/n)}
\]  

(A8)

In the limit of many sectioning profiles (large \(n\)), \(\gamma\) is about \(\pi/4\). The number of profiles sampled in a typical crypt image was 20–30. For the range of observed crypt diameters (D) and cell widths, \(\gamma\) is negligibly larger than \(\pi/4\) (by a factor of 1.001–1.003).

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