LECTURE

Luminal acid elicits a protective duodenal mucosal response

Jonathan D. Kaunitz[1] **and Yasutada Akiba**[1,2]

[1]*Greater Los Angeles Veterans Affairs Healthcare System; CURE: Digestive Diseases Research Center; and Department of Medicine, School of Medicine, University of California Los Angeles, Los Angeles, CA, USA*

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Abstract. Measuring duodenal epithelial intracellular pH (pH_i), blood flow and mucus gel thickness (MGT), we studied duodenal defense mechanisms *in vivo* so as to more fully understand the protective mucosal response to luminal acid. Exposure of the mucosa to physiologic acid solutions promptly lowered pH_i, followed by recovery after acid was removed, indicating that acid at physiologic concentrations readily diffuses into, but does not damage duodenal epithelial cells. Cellular acid then exits the cell via an amiloride-inhibitable process, presumably sodium-proton exchange (NHE1). MGT and blood flow increase promptly during acid perfusion; both decrease after acid challenge and are inhibited by vanilloid receptor antagonists or by sensory afferent denervation. Bicarbonate secretion does not increase during acid perfusion but increases following acid challenge. Inhibition of cellular alkali uptake by anion transport inhibitors lowers pH_i, and increases mucosal injury, whereas inhibition of apical alkali secretion alkalinizes pH_i and diminishes injury. These observations support the following hypothesis: luminal acid diffuses into the epithelial cells, lowering pH_i. Acidic pH_i increases the activity of a basolateral NHE, acidifying the submucosal space and increasing cellular alkali loading. The acidic submucosal space activates capsaicin receptors on afferent nerves, increasing MGT and blood flow. With continued acid exposure, a new steady state with thickened mucus gel, increased blood flow, and a higher cellular buffering power protects the mucosa from acid injury. After acid challenge, mucus secretion, blood flow and pH_i return to normal, while bicarbonate secretion increases. Through these integrated mechanisms, the epithelial cells are protected from damage due to repeated pulses of concentrated gastric acid. (Keio J Med 51 (1): 29–35, March 2002)

Key words: bicarbonate secretion, cellular buffering power, intracellular pH, mucosal blood flow, mucus secretion

Introduction

The location of the duodenal epithelium just distal to the gastric antrum and proximal to the pancreaticobiliary ducts uniquely exposes it to a highly variable pH environment due to peristaltically conveyed pulses of concentrated gastric acid combining with secreted bicarbonate. Since the duodenum does not have the inherent acid protective structural properties of its 'neighbors to the North', namely the stomach and esophagus, whose intercellular junctions severely curtail transepithelial ionic permeation,[1,2] the duodenum, being leaky, has evolved alternate means for defense against acid (Fig. 1).

In this review, we will systematically describe the current knowledge concerning duodenal defense mechanisms, ending with a scheme that integrates these mechanisms into a coherent protective response to luminal acid. Moreover, we will discuss how these mechanisms might be altered in the disease cystic fibrosis, in which the duodenum appears to be highly resistant to luminal acid.[3–5]

Duodenal Luminal pH

Antral peristalsis and pyloric opening exposes the proximal duodenum to cyclical variations of luminal pH. These cycles are more pronounced post-prandially, and

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Reprint requests to: Dr. Jonathan D. Kaunitz, Greater Los Angeles Veterans Affairs Healthcare System; CURE: Digestive Diseases Research Center; and Department of Medicine, School of Medicine, University of California Los Angeles, Bldg. 114, Room 217, West Los Angeles VA Medical Center, 11301 Wilshire Blvd., Los Angeles, CA 90073 USA, e-mail: jake@ucla.edu
[2] Present affiliation: Department of Internal Medicine, School of Medicine, Keio University

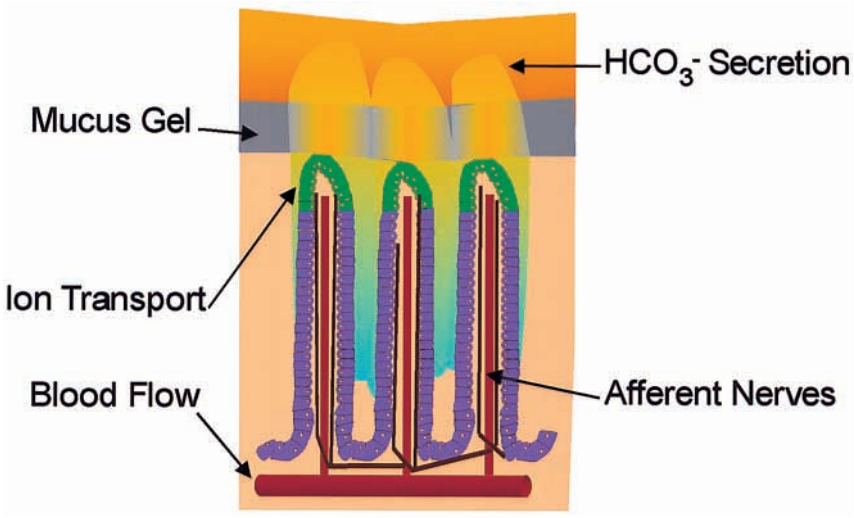

Fig. 1 Duodenal defense mechanisms. All of these mechanisms are believed to defend the mucosa against luminal acid, although bicarbonate secretion is the most accepted.

vary luminal pH between two and seven on a scale of minutes.[6–8] These rapid shifts of pH presumably arise from alternate exposure to gastric acid and secreted bicarbonate of epithelial and pancreatic origin. In contrast, gastric luminal pH is sustained on a minute-to-minute scale, although it does of course vary over a 24 hr period. Rapid shifts of duodenal pH are likely to create intense stress on the epithelial cells to maintain constant intracellular pH (pH_i) in order to maintain function and prevent irreversible necrosis due to intracellular acidification. Thus, a potent defensive system must be in place to prevent cellular acidification during mucosal acid challenge.

Defense Mechanisms

The most studied duodenal defense mechanism is epithelial bicarbonate secretion. Other potential defense mechanisms include the mucus gel and mucosal blood flow. Reparative processes such as restitution from injury are beyond the scope of this review and will not be further addressed.

Bicarbonate secretion

Bicarbonate secretion is a logical duodenal defense mechanism for the following reasons: 1) Duodenal bicarbonate secretion/cm^2 epithelium is much greater than gastric bicarbonate secretion,[2,9–11] 2) pH electrode studies suggest that epithelial bicarbonate secretion creates a layer of neutral pH next to the mucosa,[12–14] 3) *Helicobacter pylori* infection complicated by duodenal ulcers is associated with diminished bicarbonate secretion, and eradication of helicobacter infection restores duodenal bicarbonate secretory capacity.[15,16] The mechanism by which bicarbonate is secreted from the epithelial cell is controversial. Formerly, it was thought that CO_2 diffusing into the cell was converted to bicarbonate and protons by cellular carbonic hydrase. Bicarbonate was then secreted across the apical membrane by anion exchange. More recent studies, including molecular immunolocalization and studies of pH_i, do not fully support this mechanism.[17–19] What appears to also be present is that bicarbonate is transported from the blood across the basolateral membrane by a variant of the sodium-bicarbonate transporter, or NBC, in response to decreased pH_i resulting from exposure to luminal acid. Since inhibiting or eliminating the apical membrane chloride channel cystic fibrosis transmembrane regulator (CFTR) greatly attenuates bicarbonate secretion,[20,21] the CFTR has been implicated in the mechanism of bicarbonate secretion, although it is unknown whether it serves directly as a bicarbonate channel, or indirectly to preserve transmembrane electrical or ion gradients.

Role of alkali loading

We have re-examined the role of bicarbonate secretion in overall duodenal defense from acid, and, in doing so, have formulated a novel hypothesis with regard to the role of bicarbonate transport. To test these possibilities, we developed a technique for the measurement of pH_i, blood flow, and mucus gel thickness in the duodenum of anesthetized rats.[22] With this system, we could perfuse solutions of varying pH through a chamber placed over the exposed duodenal mucosa, thereby simulating changes in luminal pH. With this system, we

exposed the mucosa to a brief pulse of acid, which promptly decreased pH_i. This fall of pH_i, even with mildly acidic perfusates, suggested that acid could readily penetrate the overlying mucus gel and the mucosa, therefore calling into question the role of pre-epithelial bicarbonate neutralization in duodenal mucosal defense. With removal of the acid challenge, pH_i was elevated to supernormal values, which indicated that cellular buffering power has increased, not decreased, during acid challenge. Furthermore, a second acid challenge acidified pH_i less than the first, further confirming that acid exposure was associated with cellular alkali loading and increased cellular buffering power. This somewhat surprising finding was confirmed by comparison with prior studies conducted in a variety of systems, in which acid pulses were followed by pH_i overshoot, indicative of cellular alkali loading in cells containing a plasma membrane alkali-loading mechanism such as sodium-bicarbonate cotransport.[23]

Further studies indicated that this alkali loading was inhibited by the stilbene anion transport inhibitor DIDS (4,4′diisothiocyanostilbene-2,2′-disulfonic acid). When exposed to two short acid pulses, pH_i decreased less during the second challenge; again strongly suggestive that cellular buffering power was increased during acid exposure. Again, DIDS inhibited this adaptive effect.[24] Our studies were thus consistent with alkali loading being induced by luminal acid exposure by a DIDS-inhibitable mechanism. This finding was expected insofar as primary isolated duodenal epithelial cells recovered from acid exposure by a mechanism consistent with the activity of an NBC,[25] and that bicarbonate-secreting pancreatic duct cells have a basolateral membrane NBC.[26] Recently, our laboratory, in collaboration with Dr. Ira Kurtz, have confirmed the presence of the pancreatic-type isoform of NBC1 (pNBC1) in the basolateral membrane of rodent proximal duodenal epithelial cells using immunohistochemistry.[27,28]

Alkali loading during acid challenge, which increases cellular buffering power and attenuates the fall of pH_i, is an attractive means of defending the epithelium from acid challenge. To address how bicarbonate secretion is related to this observation, we performed parallel experiments in which bicarbonate secretion was measured in a perfused duodenal loop exposed to the same pH perfusion sequence as the measurements of pH_i. Bicarbonate secretion was measured by the conventional acid back-titration technique, but also by measurement of total dissolved CO_2 content of the effluent collected from the perfusion with a CO_2 electrode. We found that titratable alkalinity increased substantially during acid perfusion. Surprisingly, total CO_2 content decreased somewhat at the same time.[24] To account for the large discrepancy between the back-titration experiments and the measurement of effluent CO_2 content, we performed control experiments in which we measured total CO_2 content in the perfusates and effluent of perfused duodena. We found that there was a finite loss of CO_2 during duodenal perfusion, in agreement with prior studies[29] but inadequate to explain the discrepancy.

To help interpret the data, we postulated three means by which acid can disappear from the lumen: back-diffusion of acid, back-diffusion of CO_2, and neutralization by secreted bicarbonate. If all of the acid disappearance measured by back-titration occurred by bicarbonate neutralization, the effluent CO_2 content should increase to the same extent as did titratable acidity, which was not the case. If the perfusate bicarbonate was converted into CO_2, which then back-diffused into the epithelium, we would still predict a large increase of effluent CO_2 content. Hence, the best means of explaining the discrepancy between effluent CO_2 content and acid disappearance is by postulating that most of the acid loss during perfusion with pH 2 solution is due to acid back-diffusion. In that case, bicarbonate secretion must have been unchanged or perhaps decreased during acid challenge. The implications of these data, combined with our measurements of pH_i, support our hypothesis that increased cellular buffering, and not bicarbonate secretion, was the primary duodenal defense mechanism from acid. Acid was not neutralized at the duodenal surface, since cellular pH_i clearly decreased during acid challenge, and since acid back-diffusion was the major means of acid loss when perfused over the mucosa. Furthermore, since bicarbonate secretion was unchanged during acid perfusion, and only increased after acid removal, secreted bicarbonate is unlikely to be protective, since its increased secretion is present only when it is not needed *i.e.* when luminal acid is no longer present. Supporting the concept that bicarbonate secretion is not enhanced during luminal acid stress are the observations that lowered pH_i deceases cellular bicarbonate concentration, inhibiting bicarbonate exit, and that CFTR permeability to the related anion chloride is diminished at acidic pH_i due to lack of CFTR phosphorylation.[30]

We further tested this hypothesis by measuring epithelial injury under conditions in which alkali loading is either inhibited or enhanced, in order to confirm that bicarbonate loading, and not secretion is the primary defensive mechanism. To accomplish this, DIDS, and the anion channel inhibitor NPPB (5-nitro-2-(3-phenylpropylamino) benzoic acid), added to the perfusate, respectively either decreased or increased pH_i. Changes of pH_i also correlated with mucosal injury susceptibility. The most striking finding was that NPPB inhibited bicarbonate secretion but increased pH_i and decreased injury susceptibility. Thus, NPPB 'uncoupled' bicarbonate secretion from mucosal protection, a novel

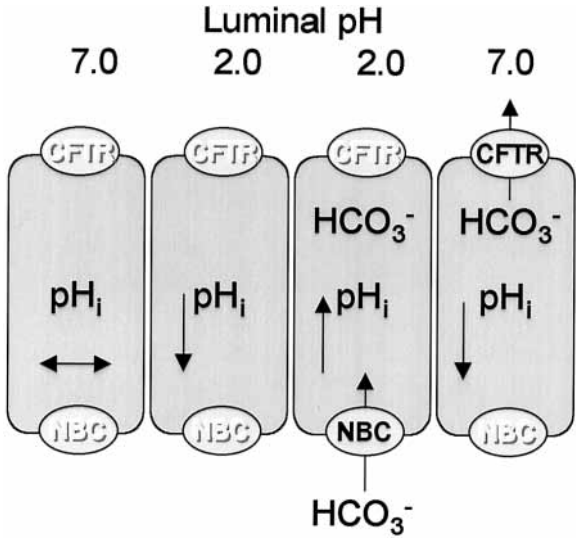

Fig. 2 Sequential response of duodenal epithelial cells to luminal acid. In the left panel, steady-state $pH_i = \sim 7.1$ when no acid is present. In the succeeding panels to the right, luminal acid rapidly acidifies the epithelial cells. Low pH_i decreases CFTR conductance and intracellular [HCO_3^-], suppressing HCO_3^- secretion. Low pH_i also increases the activity of the basolateral sodium-bicarbonate cotransporter (pNBC1), which in turn increases cellular bicarbonate concentration. When luminal pH returns to neutrality, acid diffuses out of the cell. The excess intracellular alkali raises pH_i over baseline (overshoot), which activates CFTR and DRA, which then increases bicarbonate secretion. In the disease cystic fibrosis, a dysfunctional CFTR and DRA limit apical HCO_3^- exit, which raises pH_i.

finding that casts further doubt on the primacy of bicarbonate secretion on mucosal protection.[27] In this proposed mechanism, shown in Fig. 2, bicarbonate secretion occurs to remove excess alkali from the cell, when excess intracellular bicarbonate is no longer needed after acid challenge.

Blood flow

Mucosal blood flow is an accepted component of upper gastrointestinal barrier function. In the stomach, for example, interventions that attenuate the hyperemic response to acid perfusion increase mucosal injury.[31–33] The data derived from studies of the duodenum are less conclusive, however. One potential confounder is that stimuli of bicarbonate secretion and blood flow are co-regulated, making it difficult to determine the relative importance of blood flow in terms of overall barrier function.

With our technique, we were able to measure blood flow, pH_i, and mucus gel thickness simultaneously. This technique enabled us to formulate novel conclusions about the relative contribution of blood flow to overall barrier function. Mucosal blood flow, as measured by laser-Doppler flowmetry, increased in response to acid perfusion.[22,34] This response differs from the gastric mucosa, which must be either injured or pre-treated with gastrin or other compound in order to induce this acid response.[35,36] Our studies revealed some novel observations about the nature of duodenal blood flow and its regulation. For example, inhibition of sodium-proton exchange (NHE) with the potent amiloride analog dimethylamiloride inhibited the hyperemic acid response. Interestingly, acidification of the cytoplasm by alternate means such as with ammonium pre-pulse or valinomycin increased blood flow, also inhibitable by dimethylamiloride.[22] These studies suggested that acid must pass through the epithelial cell and exit via by NHE prior to eliciting a hyperemic response. In further studies, we examined the sensing mechanisms underlying the hyperemic response. Capsazepine, an antagonist to the recently cloned vanilloid receptor, abolished the hyperemic response to acid, confirming the involvement of vanilloid receptors in the acid response. Further studies also confirmed that the hyperemic response was mediated by a well-known pathway that includes afferent sensory nerves, nitric oxide release the neuropeptide calcitonin gene-related peptide (CGRP), but was not inhibited by indomethacin, a non-selective inhibitor of cyclooxygenase. These studies provided data supporting our proposed mechanism of duodenal acid-induced hyperemia, including acid diffusion into the epithelial cell, basolateral extrusion via NHE, activation of vanilloid receptors on afferent nerves, CGRP release, with activation of endothelial nitric oxide synthesis, with production of vasodilatory nitric oxide. A scheme of proposed regulatory mechanisms for blood flow is shown in Fig. 3.

Mucus secretion

The role of mucus in duodenal mucosal defense is the subject of only a few studies. The most accepted hypothesis is that mucus stabilizes the pre-epithelial pH gradient, with neutral pH measured near the mucosa, preventing acid from entering the epithelial cells.[12,37–39] Mucus secretion is also co-regulated by the same neurohormonal and phamacologic stimuli that increase other defense mechanisms such as bicarbonate secretion and blood flow, making it a likely candidate for being a secondary defense mechanism.

With our technique, we could optically and non-invasively measure mucus gel thickness in our anesthetized preparation.[40] We found, for example, that mucus gel thickness rapidly increases in response to perfused acid, but equally rapidly decreases in thickness when the acid challenge is removed. Measurement of effluent mucus glycoprotein content was consistent with increased sloughing of mucus into the perfusate when mucus was rapidly secreted, indicating that there is a

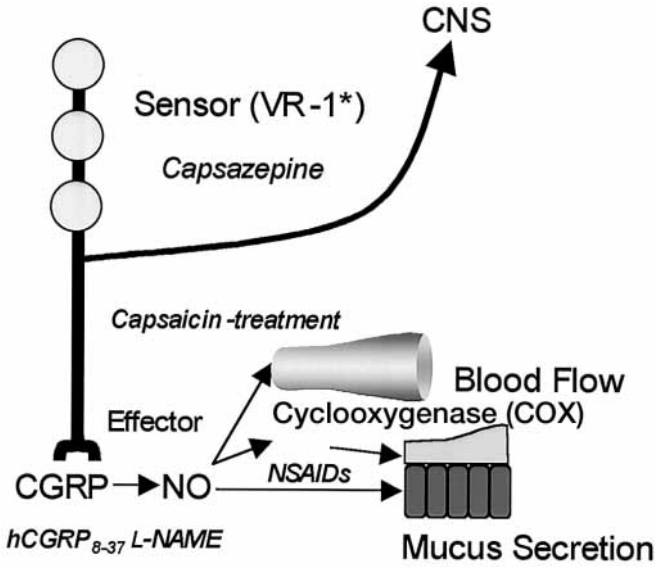

Fig. 3 The capsaicin pathway. Mucosal responses to luminal acid are mediated by this pathway, which includes an acid sensor, which is a vanilloid receptor, afferent nerves, and an effectors mechanism dependent on the secretion of calcitonin gene-related peptide (CGRP) and nitric oxide synthase (NOS). Prostaglandin synthesis is also involved in the regulation of mucus gel thickness. Italics denote inhibitors of each component.

dynamic relation between mucus secretion and erosion, as has been previously hypothesized.[41] When the secretion slowed, the rapid sloughing remained, thinning the gel until a new steady state occurred. A scheme depicting our concept of how mucus gel thickness is regulated is shown in Fig. 4. Further studies showed that the capsaicin pathway, involving acid-sensing vanilloid receptors, afferent nerves, CGRP, and nitric oxide, regulates mucus gel secretion and that non-selective COX inhibition with indomethacin abolishes the mucus secretory response to all secretagogues, suggesting a fundamental role of prostaglandins in duodenal mucus secretion.[42]

Clinical Correlate

We have formulated the ''CF paradox''[27] in which we pose the question: why are duodenal ulcers not increased in patients with CF? Patients with CF, for example, have high normal acid secretion,[43] and hence must take gastric antisecretory medications in order to diminish esophageal acid reflux and to prevent acid-mediated inactivation of pancreatic enzymes.[44,45] Furthermore, pancreatic and duodenal bicarbonate secretion are presumably impaired by the disease,[21] and the duodenal pH is lower than normal.[46] Combined with the frequent prevalence of chronic lung disease, these patients should be a high risk for peptic ulceration. Clinical experience, and the literature, however, do not support an increased incidence of peptic ulceration is this population, but rather, it appears that the prevalence of peptic ulceration may actually be diminished.[5] Our hypothesis that elevated cellular buffering power is present when the CFTR is dysfunctional is supported by independent observations: 1) resting pH_i is elevated in cells derived from individuals with a dysfunctional CFTR[47] and 2) the apical anion exchanger DRA, the only other known apical exit pathway for bicarbonate, is downregulated when the CFTR is dysfunctional,[48,49]

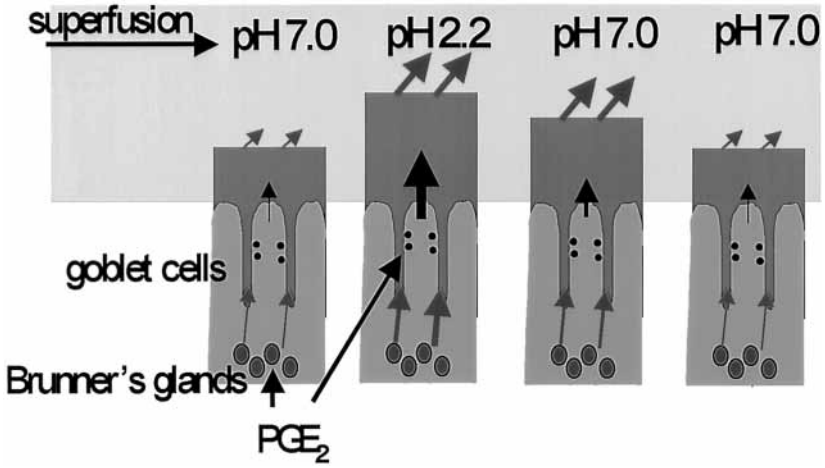

Fig. 4 Dynamic regulation of mucus gel thickness in response to luminal acid. In the left panel, alkaline mucus secretion and the rate of sloughing into the lumen are balanced. Luminal acid creates a sudden exocytotic burst of mucus secretion from goblet cells and Brunner's glands, which thickens the gel. The newly secreted mucus sloughs into the lumen at a higher rate, resulting in a new steady-state gel thickness. Removal of luminal acid decreases mucus secretion, decreasing gel thickness, and also initiates synthesis of new mucus granules.

which would support the concept that bicarbonate is 'trapped' in the cell in CF, raising pH_i and increasing cellular buffering power.

We propose that the impairment of duodenal bicarbonate secretion in the disease may explain why this population may be protected from peptic ulceration. Recall that the CFTR and apical DRA are needed for duodenal bicarbonate secretion. If the cells can alkali load normally via a basolateral NBC1 and by in-diffusion CO_2 converted to HCO_3^- by carbonic anhydrase, but cannot secrete bicarbonate across the apical membrane due to defective CFTR and DRA functioning, cellular buffering may be abnormally high due to the dysfunction of the two major apical bicarbonate exit pathways. This increased cellular buffering power may then protect the epithelial cells from undue acidification due to low luminal pH.

Summary and Conclusions

Study of the duodenal response to acid has yielded fresh insights into how mucosal surfaces protect themselves from a hostile environment. Cellular alkali loading in response to acid is plausible and fits well with the existing body of data correlating ulcer disease with bicarbonate secretion, in that bicarbonate secretion is the end result of alkali loading, although it may not, in and of itself, be protective against acid. The alkali-loading hypothesis also fits well with the clinical observation of the sparing of CF patients from ulcer disease. Although the signal for alkali loading may be decreased pH_i, enhanced blood flow and mucus secretion in response to acid challenge rely on well-described pathways that are present in many tissues,[50–52] and serve as a useful paradigm for epithelial responses to environmental stimuli.

In the future, we plan to study the proposed two-step mechanism (separate basolateral uptake and apical exit processes) by which duodenal bicarbonate secretion may occur. We also would like to rigorously test the alkali-loading hypothesis in CF knockout and other mouse models, and also to further study the duodenal acid response. Other planned studies include the role of the high CO_2 duodenal luminal environment in mucosal acidification. Through these studies, we hope to understand how environmentally exposed epithelia deal with potentially damaging conditions, with the hope that further understanding and insight might be gained about diseases that affect the gastrointestinal tract and other epithelia.

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