Role of Mucus in Proton Secretion

Until recently, the path by which secreted acid traversed the mucus gel has never been well understood. In the past, the impedance of the flux of secreted acid by mucus was either simply ignored, or was considered to occur by diffusion.

The role of viscous fingering

In the early 1980's, the technique of *in vivo* microscopy was used to visualize the effects of acid secretion at the mucosal surface. One of the most striking findings was accomplished with the use of the dye Congo red, a pH indicating dye that adheres to the luminal surface of the mucus gel. Upon stimulation of acid secretion, a color change was noted in the dye in small areas overlying the openings of the gastric pits, indicating that local acidification had occurred. This finding was interpreted as being consistent with tunneling of secreted acid through gastric mucus through discrete channels.[20] Further support for the tunneling hypothesis was provided several years later, when *in vitro* experiments confirmed that the general concept of viscous fingering applied to the specific case of acid traversing mucus. Acid injected through a small gauge needle under pressure will tunnel through a gel, which is quite different than its behavior when injected into a liquid, which is free mixture with the liquid. When mucus pH was reduced to <4, tunneling was not observed, presumably due to the increase in mucus viscosity.[21] On the basis of these two complementary observations, the concept of secreted acid tunneling through the mucus gel by the process of viscous fingering was generally accepted. Further data supporting this hypothesis has been provided largely by Holm's group in Sweden. With the use of sensitive micro-transducers, fluctuating pressures were measured in the lumen of the gastric glands, consistent with the existence of a gland luminal hydrostatic pressure. Further studies have examined the regulation of gland luminal pressure by acid secretagogues and antisecretory agents[22,23] and also have demonstrated the existence of muscle fibers surrounding the glands, the contraction of which would presumably generate intraluminal pressure. Pressure, however, can only be generated against a resistance, which is postulated to be generated by constriction of the luminal opening of the gastric pit simultaneously with squeezing the gland. Such a constriction, and direct observation of secreted acid traversing the mucus gel by viscous fingering, however, have not been reported.

Measurement of gel pH with in vivo confocal microscopy

The most recent investigation of mucus pH has been done with the help of a new technique, developed by Drs. Montrose and Chu in collaboration with Dr. S. Tanaka of my laboratory[24] in which confocal microscopy was used to non-invasively measure mucus gel pH *in vivo*. A ratiometric technique was used to quantitatively report fluorescence response from pH-dependent and pH -independent fluorescent dyes as a measure of pH. Images of pH had spatial resolution of better than 1 μm, and were collected in 1 second. Figure 1 depicts confocal reflectance images of the mucosa parallel (**A**) and perpendicular (**B**) to the mucosal surface. Gel pH was then measured with either pH 3 or pH 5 superfusion (to simulate the fasted and fed states, respectively), and in the presence of secretagogues or proton pump inhibitors. During pH 3 superfusion, juxtamucosal pH was near pH 4 and increased to over 5 after PGE_2, consistent with prior observations of an alkaline juxtamucosal layer (Fig. 1C). Nevertheless, with pH 5 superfusion, juxtamucosal pH was 4.2 (Fig. 1D), and could be acidified further to 3.7 during maximal acid stimulation with pentagastrin. The mucus gel, due to its content of cellular debris, was easily visualized (Fig. 1B) and often was exceeded in thickness by the depth of the acid layer. This acid layer was uniform above the mucosal surface, and the depth of the layer was related to the rate of superfusion. Prior work suggested that viscous fingers of secreted acid should have been observed to be 15 μm in diameter,[20] but no inhomogeneity was observed, even during maximal acid stimulation, and even when observed directly over the mouths of the pits. By contrast there was some inhomogeneity observed during pH 3 superfusion, when juxtamucosal pH was high but when there was no evidence of active acid secretion (only base secretion was evident). In this condition, underlying base (presumably bicarbonate) secretion appeared to emanate mostly from the surface cells lining the gastric pits. These data were interpreted as being inconsistent with the viscous tunneling hypothesis for acid secretion. Instead, secreted acid diffused laterally and then towards the lumen. The reason why these experiments differed from prior studies is not yet clear, but was not due to a lack of robust acid secretion or an inability to detect an alkaline surface pH.

Asymmetry of Proton Diffusion

Protons are constantly traversing the gastric mucus in two directions. Secreted acid emanating from the gastric pits crosses the mucus before reaching the lumen, whereas luminal acid must cross the gastric mucus before reaching the epithelial cells and deeper struc-