Virchows Archiv A Pathol Anat (1989) 414:217–222

Virchows Archiv A
Pathological Anatomy and Histopathology

The gastric mucosal barrier: tight junction structure in gastritis and ulcer biopsies

Zoltan Posalaky, Irene Posalaky, Dennis McGinley, and Rita A. Meyer
Department of Pathology, St. Paul-Ramsey Medical Center, 640 Jackson Street, St. Paul, Minnesota 55101;
Veteran's Administration Hospital, Minneapolis, Minnesota;
University of Minnesota Medical School, Minneapolis, Minnesota, USA

Summary. Tight junctions of the human gastric mucosa were examined using quantative freeze-fracture methods. Biopsies examined were from patients with gastric diseases including gastritis, ulcers, and pernicious anemia. No significant differences were seen in strand number or tight junction complex depth among the biopsies analyzed, however, anomalous tight junction structures were observed. Discontinuities in the tight junction complex and hyperplastic tight junctions (extensions of the apical tight junction strands radiating over the lateral plasma membrane) were seen. These alterations were not associated exclusively with either the diagnosis of gastritis or ulcers. However, a higher frequency of tight junction breaks was seen in stomach biopsies diagnosed as gastritis while those diagnosed as ulcers displayed a higher occurrence of hyperplastic tight junctions.

Key words: Gastric mucosa – Gastritis – Peptic ulcer – Tight junctions – Freeze fracture

Introduction

The gastric mucosal barrier is thought to be comprised of several components including the plasma membrane, tight junction complexes, and extracellular mucous (in combination with other secretions, e.g. bicarbonate) (Davenport 1967; Powell 1981). The tight junctions or zonulae occludentes are membrane specializations near the cell apex that essentially form a "zipper" around the cell and act as a passive barrier to diffusion of molecules from the gastric lumen into the paracellular space. Tight junctions appear to be a major component in barrier systems of other epithelia (Staehlin 1974) and therefore are probably an essential component of the gastric mucosal barrier (Meyer et al. 1984). It is generally believed that patients with chronic gastritis and gastric ulcer suffer from a disturbance in the gastric mucosal barrier which allows exposure of the mucosal cells to the adverse action of the acid-peptic contents of the stomach. Disturbances in barrier function of other gastrointestinal systems has been shown to be associated with tight junction abnormalities (Walker and Porvaznik 1978; Porvaznik 1979; Madara and Trier 1980; Marin et al. 1983). Therefore we have investigated the possibility of alterations in the structure of gastric mucosal tight junctions in patients with gastritis and ulcers using the freeze-fracture method.

Offprint requests to: Z. Posalaky

Materials and methods

Gastric mucosal biopsies were obtained from twenty-two adults some of whom had been previously diagnosed by endoscopy or x-ray (one case) as having peptic ulcer or gastritis, while others were biopsied because they had complained of epigastric pain or had signs of gastrointestinal bleeding or both. The age of the patients (11 male and 11 female) varied from 24–85. Routine esophagogastroduodenoscopy was done with an Olympus GIF-P-2, K, or Q endoscope advanced under direct visualization. Biopsies were taken at ulcer margins or adjacent to endoscopically abnormal stomach areas. Part of the biopsy was used for routine diagnostic purposes and part for this study. Light microscopically the biopsies were diagnosed as: 1) "no significant pathologic changes" (3 patients); the mucosa is normal in appearance with intact surface and foveolar mucus producing epithelial cells that have basally oriented nuclei (throughout the stomach) and usually straight gastric glands (fundus, corpus, antrum) lined with chief and parietal cells (fundus and corpus), 2) chronic gastritis, active (mild to severe) (9 patients); this was based on the intensity of the inflammatory process and it's effects on the gastric mucosal structure including epithelial erosions and degenerative or regenerative changes of the glandular or surface epithelium, 3) chronic gastritis (mild to severe) with ulcers (9 patients); this was based on the existence of a tissue defect that extends beyond the muscularis mucosa, the ulcer base was covered by a homogeneous necrotic layer of cells of varying width. Biopsies were taken from the ulcer margins and showed inflammation, focal foveolar hyperplasia and in some cases coexistence of a necrotic layer with underlying vascular granulation tissue.

Multiple samples from three partial gastrectomy specimens (removed for benign gastric ulcers) were taken at the margin

of the ulcer and 3–6 cm away from the lesion in grossly normal appearing areas. Three to five samples were acquired depending on the amount of stomach tissue removed. The age of the patients (2 male and 1 female) varied from 60–75.

Informed consent was obtained from all subjects in this study and the study was approved by the St. Paul-Ramsey Medical Center Human Subjects Review Committee (IRB review #240).

Biopsy specimens were fixed immediately after removal and surgical resection specimens were submerged in saline and removed from the operating room. They were fixed within 5–10 min after removal. All specimens were fixed in 2% glutaraldehyde −3% paraformaldehyde in 0.1 M cacodylic acid buffer with 0.1 M sucrose at pH 7.2. For freeze-fracture the tissue was submerged in 30% glycerol-fixative solution for at least 1 h, then frozen in Freon 22 cooled by liquid nitrogen and stored in liquid nitrogen until fractured. The samples were fractured and replicated at −110° C in a Balzers 301 freeze-etch machine equipped with a Pt gun and quartz thin film crystal monitor. Replicas were cleaned overnight in dimethylformamide followed by an overnight treatment in full strength Clorox bleach with 0.2 N NaOH and were rinsed in two washes of double distilled water. They were examined in a JEOL 100B electron microscope at 80 KV.

All morphologic measurements were done by the same person (R.M.) on coded tissue samples and without knowledge of tissue source. For freeze-fracture quantitation at least two freeze-fracture runs were done producing a minimum of 8 replicas per stomach. The replicas were examined and large fractured areas of membrane with a tight junction complex present (defined as an interface, IF) were photographed and printed at a final magnification of 40000. For each tight junction complex, the average depth and average number of horizontal strands were recorded using methods similar to those employed by others (Claude and Goodenough 1973). The average depth of the tight junction complex was determined by measuring the distance between the upper-most and lower-most strands (excluding aberrant loose strands) both at the center and one-fourth of the distance from each end of the exposed tight junction complex. The number of strands on the tight junction complex was counted by use of an overlay that consisted of a line parallel to the microvillar surface and lines perpendicular to this line every 0.5 μm. The intersection of these perpendicular lines with the tight junction strand was counted and averaged using a point-counting program in an image analysis system. The ladder-like extensions of tight junctions occurring at boundaries where three cells form junctions were excluded from these measurements. The total length of the occluding junctional complex parallel to the apical cell surface in a given micrograph was also measured with an Apple II computer equipped with a Hipad digitizing tablet. The data were analyzed by the student's t-test. A probability value of <0.05 was considered statistically significant.

Results

The tight junctions complexes of the three gastric biopsy specimens, classified by light microscopy as having no significant pathologic change were comparable in strand number ($x \pm SE(x) = 3.9 \pm 0.1$) and complex depth ($x \pm SE(x) = 0.45 \pm 0.02$ μm). The tight junction complexes of these biopsies were composed of interwoven strands with occasional free ended strands extending basally from the apical complex and had very few breaks (Table 1).

In the biopsies diagnosed light microscopically as having gastritis or ulcerative changes, the tight

Table 1. Freeze-fracture quantitation of the tight junction complexes (gastric biopsies)

Stomach Diagnosis-Region	No. stomachs examined	No. of interfaces	Total length of occluding junctions measured (μm)	% total No. IFs[a] with hyperplastic tight junctions	No. of discontinuities in complex (% total IFs)	Average distance between discontinuities in the complex (μm)
"No significant pathologic change						
antrum	3	157	849	0	4 (1)	212
Gastritis						
cardia	1	16	125	0	0	
antrum	4	115	583	0	37 (11) ($p=0.01$)	16
corpus	4	167	911	0.6 (n.s.)[c]	55 (12) ($p=0.01$)	16
All regions combined	9	298	1576	0.3	92 (11)	17
Pernicious Anemia						
antrum	1	22	125	0	8 (36) ($p=0.001$)	16
Ulcer[b]						
antrum	3	127	764	35.5 ($p=0.001$)	14 (5) ($p=0.04$)	54
corpus	6	592	3216	44.5 ($p=0.001$)	83 (6.5) ($p=0.02$)	39
All regions combined	9	719	3980	43	97 (6)	41

[a] Interface (IF) is defined as 58 μm² of fractured membrane surface that has indication of another cell present
[b] This group includes gastric biopsy specimens and gastrectomy specimens from the margin of the ulcer
[c] Student's t test; vs "no significant pathological change" group; n.s. = no significant difference

junction complexes exhibited an array of configurational abnormalities. The anomalies in the tight junction complexes included: a) proliferation of tight junction strands (hyperplastic tight junctions) which were frequently observed on the lateral plasma membrane running from the apical tight junction complex to the base of the cell (Fig. 1); the apical tight junction complexes on the majority of these plasma membrane interfaces (IF) appeared relatively intact; b) variability in the number of strands comprising the tight junction complex, ranging from 1 to 10 strands (Fig. 2); c) large discontinuities which were observed in tight junction complexes that otherwise appeared fairly extensive (Fig. 3); multiple somewhat smaller discontinuities were seen in complexes showing other irregularities such as a decreased number of tight junction strands (Fig. 4). None of the configurations cited were associated exclusively with either gastritis or ulcerative disease.

No significant differences were seen in tight junction complex depth ($x \pm SE(x) = 0.70 \pm 0.04$) and complex strand number ($x \pm SE(x) = 3.7 \pm 0.2$) when the individual biopsies were compared to each other. There was also no differences seen when they were grouped and compared as gastritis v.s. ulcerative disease. However, a higher frequency of tight junction complex discontinuities was observed in biopsies diagnosed as having gastritis. There was on the average 1 discontinuity per 17 μm of tight junction complex measured in the gastritis group compared to 1 discontinuity per 41 μm of complex measured in the ulcer group (Table 1). In the biopsies diagnosed as having ulcerative changes, a higher percentage of IFs had hyperplastic tight junctions (Table 1). Overall there appears to be more configurational abnormalities in the ulcer group (49%, Table 1) than in the gastritis group (11.3%, Table 1).

A sample from a patient with pernicious anemia and gastritis was also examined and displayed a high percentage of IF's with discontinuities in the tight junction complex (Fig. 5).

Multiple samples from three gastrectomy specimens were also examined. Samples were taken from the ulcer margin and at one centimeter intervals from margin. All of the samples revealed abnormalities in the tight junction complexes that were similar to those seen in gastric ulcer biopsies (Table 2).

Discussion

We have used the freeze-fracture technique to study the tight junction complexes of human gastric biopsies and partial gastrectomy specimens. These were from patients with chronic gastritis with or without ulceration and exhibited varying degrees of pathology. Samples from stomachs that showed no significant light microscopic pathologic changes contained few tight junctional alterations. In contrast, all samples from stomachs with light microscopic abnormalities displayed aberrant tight junctional arrangements.

The gastric mucosal tight junctional defects that were repeatedly documented in this study include: 1) tight junction strand discontinuities, 2) variability in the number of strands comprising the tight junction complex, and 3) tight junction strand proliferation (i.e. hyperplastic tight junctions). Biopsies diagnosed as gastritis revealed a higher frequency of tight junction complex discontinuities, while those also showing ulcerative changes were seen to have a higher percentage of IF's containing hyperplastic tight junctions.

Tight junction complexes that have strand discontinuities often show a reduction in junctional depth and strand number and an increase in the number of free ending basal strands, but these changes were not as striking as those displayed by cells with hyperplastic tight junctions. Proliferation of tight junction strands (i.e. hyperplastic tight junctions) has been described in a number of pathological conditions including, Crohn's disease (Marin 1983), celiac sprue (Madara 1980), endotoxin assault (Walker and Porvaznik 1978) and irradiation (Walker and Porvaznik 1978; Porvaznik 1979) and exposure to proteolytic enzymes (Orci et al. 1973, Shimono and Clementi 1977). The latter experiments may be particularly relevant to this study since they may suggest that pepsin, which has bypassed the gastric mucosal barrier could be stimulating tight junction proliferation directly. The extensive development of tight junctions has also been associated with mitotically active cells (Tice et al. 1979), non-specific chemical stimuli (Robenek 1980), and have even been observed in excised prostate tissue that had been incubated for as little as three min (Kachar and Pinto da Silva 1981). Tight junction proliferation has been attributed to cell injury and disassembly of tight junction complexes (Porvaznik 1979) or viewed as a possible stage in the repair and reformation of new complexes (Walker and Porvaznik 1978). The fact that hyperplastic tight junctions are seen with greater frequency in specimens from stomachs with ulcerative changes may be a reflection of the more stressful environment in which these cells must exist.

The cause and significance of the demonstrated tight junctional breaks in this barrier is not quite

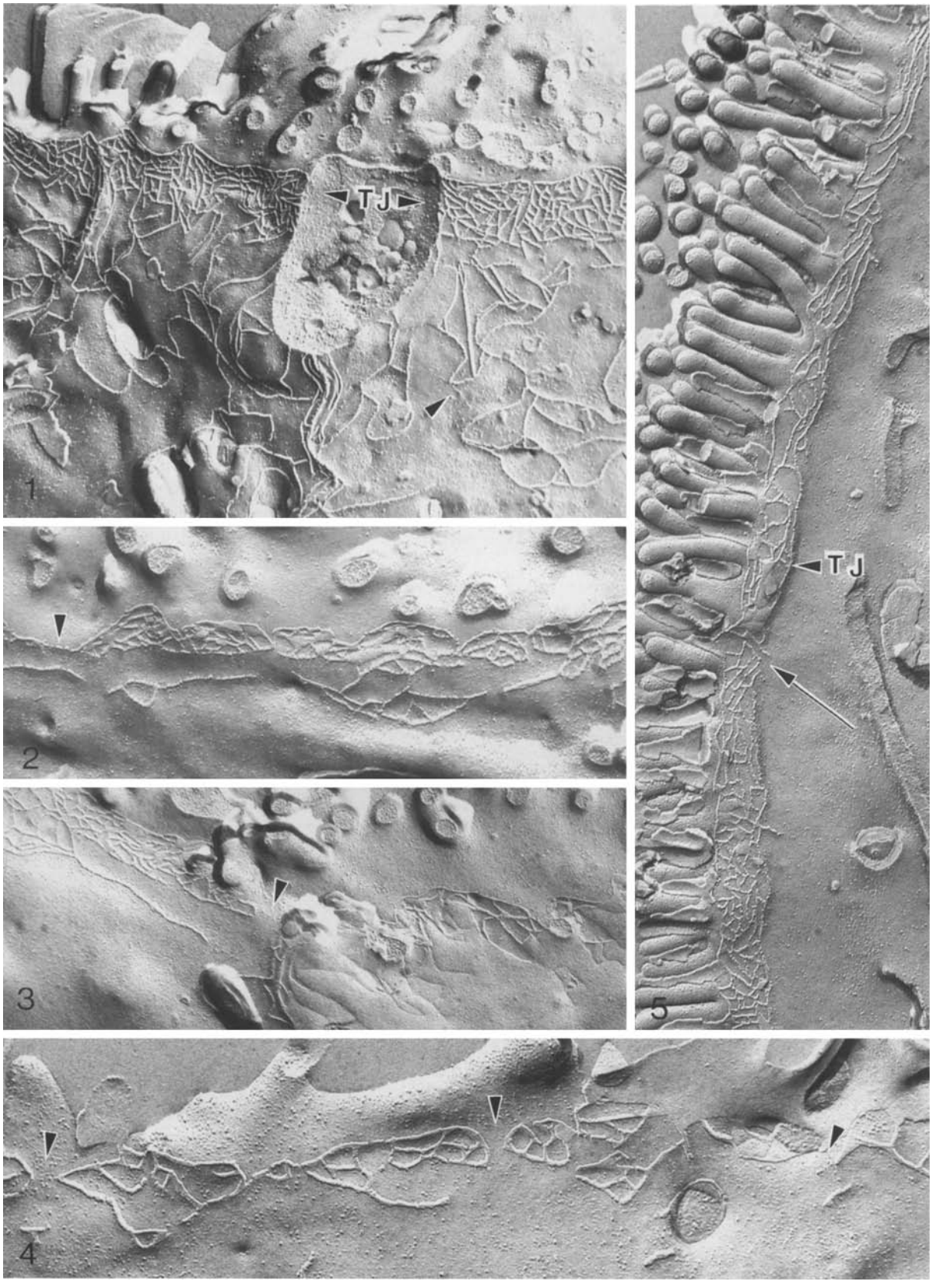
TJ
1
2
3
TJ
5
4

Table 2. IFs[a] classification of stomachs with multiple samples (gastrectomy specimens)

Stomach-Region	% of IFs with hyperplastic tight junctions	% of IFs with discontinuities in the tight junction complex
	(positive interfaces/ total interfaces)	(positive interfaces/ total interfaces)
1 a (ulcer margin – corpus)	54 (23/43)	0 (0/43)
b (corpus)	87 (48/55)	4 (2/55)
c (corpus)	60 (35/58)	4 (2/58)
2 a (ulcer margin – corpus)	25 (7/28)	0 (0/28)
b (corpus)	27 (16/59)	4 (2/59)
c (antrum)	38 (22/58)	0 (0/58)
3 a (ulcer margin – antrum)	2 (1/46)	13 (6/46)
b (corpus)	14 (7/52)	10 (5/52)
c (corpus)	96 (53/55)	0 (0/55)
d (corpus)	13 (5/38)	0 (0/38)
e (corpus)	90 (70/78)	5 (4/78)

[a] Interface (IF) is defined as 58 μm^2 of fractured membrane surface has an indication of another cell present

clear. Morphologically the gastric mucosal barrier consists of the apical plasma membrane and tight junctional complexes of the surface epithelial cells, and an overlying layer of mucous. That tight junction discontinuities would allow back diffusion of hydrogen ions is strongly suggested by the results of experiments in which aspirin was used to induce tight junction alterations identical to the ones described here (Meyer et al. 1986). In this study changes in tight junction permeability was indicated by the penetration of the light microscopically intact canine gastric epithelium by heavy metal tracers. These findings may support the assumption that in the human certain inflammatory, "irritative" factors can also cause tight junction breaks that could cause increased mucosal permeability and lead to more serious cellular damage and ulcer.

A further insight into the role of the tight junctions in the gastric mucosal barrier has been gained from studies of effect of cytoprotective agents on this system. We have studied the effects 16, 16 dimethyl prostaglandin E_2 ($dmPGE_2$) on the canine gastric mucosa, exposed concurrently to unbuffered aspirin (Meyer et al. 1987). As was mentioned previously, tight junction alterations similar to those described in this paper are seen following aspirin administration (Meyer et al. 1986); aspirin also lowers mucous output and induces a drop in transmucosal potential differences (Miller 1983). Among the cellular responses elicited by $dmPGE_2$ is an increase in mucous and non-parietal cell alkaline (e.g. bicarbonate) secretion; $dmPGE_2$ also prevents the typical decline in transmucosal resistance associated with aspirin exposure (Miller 1983). In our experiments $dmPGE_2$ reduced significantly the light microscopic surface epithelial damage caused by aspirin; results that were comparable to other similar studies. Interestingly, when the freeze-fracture technique was used to study the structure of the tight junctions in stomachs treated with $dmPGE_2$ + aspirin, junctional defects were as numerous as those seen in stomachs treated with aspirin alone. The possibility that increased mucous and bicarbonate secretion may be protecting the epithelium and maintaining transmucosal resistance by retaining and neutralizing H+ ions provides an attractive theory that has been proposed by others (Allen and Garner 1980; Allen 1981; Thomson 1981; Morris and Harding 1984). Furthermore, the efficacy of agents that increase mu-

Fig. 1. This micrograph shows hyperplastic tight junctions (*arrowheads*) on a surface mucous cell extending basally from an intact apical tight junction complex (TJ) and over the entire lateral plasma membrane. The specimen was taken from a patient with gastric ulcers. Mag. ×23400

Fig. 2. The gland area tight junction complex shown here demonstrates the variability in strand number seen within the complex. The strand number ranges from 1 (*arrowhead*) to 7 strands (*asterisks*). The gastric biopsy was from a patient with gastric ulcers. Mag. ×35500

Fig. 3. This demonstrates a large discontinuity in the tight junction complex (*arrowhead*) of a surface epithelial cell. The sample was from a patient with gastritis. Mag. ×12900

Fig. 4. Multiple discontinuities (*arrowheads*) in the apical tight junction complex of a gland area cell is illustrated here. Additionally, a decrease in strand number and a decrease in the number of microvilli (MV) can be seen. The specimen was from a patient with gastric ulcers. Mag. ×44100

Fig. 5. This freeze-fracture electron micrograph illustrates a surface epithelial cell tight junction complex (TJ) with a subtle discontinuity (*arrow*). The gastric biopsy was from a patient with pernicious anemia (stomach antrum). The gastric lumen (L) and epithelial microvilli (MV) are labelled. Mag. ×29500

cous production would seem to imply that mucous secretion in ulcer patients was insufficient either qualitatively or quantitatively or both, and may suggest that, in addition to tight junction defects, these patients also possess a deficiency in this component (mucous) of the gastric mucosal barrier as well.

The fact that samples of stomach distant (3–6 cm) from the ulcer site showed tight junctional abnormalities would seem to indicate that the presence of an ulcer can effect a considerable portion of the stomach. It should be noted that patients with peptic ulcer often suffer from gastritis as well, and therefore there is the possibility that tight junction changes in non ulcer areas may be due to an underlying gastritis. However, the repeated observation of hyperplastic tight junctions in locations removed from the ulcer is not consistent with their infrequent occurrence in specimens from patients with gastritis alone.

One of the samples in this study was from a patient with pernicious anemia (p.a.) and antral gastritis. Pernicious anemia is usually associated with chronic atrophic gastritis and is thought to be due to an immune reaction against gastric parietal cells. Although an antral presentation is not common in p.a. and the pathogenesis of this case differs from the other cases included in this study, it is nevertheless interesting that the frequency of tight junctional discontinuities seen in this sample was very similar to that seen in other gastritis samples.

In summary, patients with gastritis and gastric ulcers possess a defective gastric mucosal barrier and thus are subject to mucosal injury as a result of the back diffusion of hydrogen ions. Damage to the gastric tight junction complexes, which are a component of the barrier, certainly contributes to this loss of competence. However, alteration of other components of the barrier would also seem to be involved as well.

Acknowledgments. The authors wish to thank Dr. J. Pries for supplying specimens, and Karen Cloutier for preparing the manuscript. This work was supported by grant #8326 FROM THE ST. PAUL RAMSEY FOUNDATION.

References

Allen A, Garner A (1980) Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. Gut 21:249–262

Allen A (1981) The structure and function of gastrointestinal mucus. In: Harmon JW (ed) Basic mechanisms of gastrointestinal mucosal cell injury and protection. Williams and Wilkins, Baltimore, pp 351–367

Claude P, Goodenough DA (1973) Fracture faces of zonulae occludentes from "tight and leaky" epithelia. J Cell Biol 58:390–400

Davenport HW (1967) Salicylate damage to the gastric mucosal barrier. N Engl J Med 276:1307–1312

Kachar B, Pinto da Silva P (1981) Rapid massive assembly of tight junction strands. Science 213:541–544

Madara JL, Trier JS (1980) Structural abnormalities of jejunal epithelial cell membranes in celiac sprue. Lab Invest 43:254–261

Marin ML, Greenstein AJ, Geller SA, Gordon RE, Aufses AH (1983) A freeze-fracture study of Crohn's Disease of the terminal ileum: Changes in epithelial tight junction organization. Am J Gastroenterol 78:537–547

Meyer RA, McGinley D, Posalaky Z (1984) The gastric mucosal barrier: Structure of intercellular junctions in the dog. J Ultrastruct Res 86:192–201

Meyer RA, McGinley D, Posalaky Z (1986) Effects of aspirin on tight junction structure of the canine gastric mucosa. Gastroenterology 91:351–359

Meyer RA, McGinley D, Posalaky Z (1987) The effects of 16, 16-dimethyl prostaglandin E_2 + aspirin on the gastric mucosal barrier. Virchows Arch [A] 412:119–125

Morris GP, Harding PL (1984) Mechanisms of mucosal recovery from acute gastric damage: Roles of extracellular mucus and cell migration. In: Allen A, Flenström G, Garner A, Silen W, Turnberg L (eds) Mechanism of mucosal protection in the upper gastrointestinal tract. Raven Press, New York, pp 209–214

Miller TA (1983) Protective effects of prostaglandins against gastric mucosal damage: Current knowledge and proposed mechanism. Am J Physiol 245:G601–G623

Orci L, Amberdt M, Henquin JC, Lambert AE (1973) Pronase effect on pancreatic Beta cell secretion and morphology. Science 180:647–649

Porvaznik M (1979) Tight junction disruption and recovery after sublethal gamma-irradiation. Radiat Res 78:233–250

Powell DW (1981) Barrier function of epithelia. Am J Physiol 241:G275–G288

Robenek H, Doldissen M, Themann H (1980) Morphological changes of tight junctions of the rat liver after chronic administration of N-nitrosomorpholine (NMM) as revealed by freeze-fracturing. J Ultrastruct Res 70:82–91

Shimono M, Clementi F (1977) Intercellular junctions of oral epithelium 2. Ultrastructural changes in rat buccal epithelium induced by trypsin digestion. J Ultrastruct Res 59:101–112

Staehlin LA (1974) Structure and function of intercellular junctions. Int Rev Cytol 39:191–283

Tice LW, Carter RL, Cahill MB (1979) Changes in tight junctions of rat intestinal crypt cells associated with changes in their mitotic activity. Tissue Cell 11:293–316

Thomson ABR (1981) Unstirred water layers: A basic mechanism of gastrointestinal mucosal cytoprotection. In: Harmon JW (ed) Basic mechanisms of gastrointestinal mucosal cell injury and protection. Williams and Wilkins, Baltimore, p 327

Walker R, Porvaznik M (1978) Disruption of the permeability barrier (zonula occludens) between intestinal epithelial cells by lethal doses of endotoxin. Infect Immun 21:655–658

Walker R, Porvaznik M (1978) Association of leukopenia and intestinal permeability with radiation-induced sensitivity to endotoxin. Life Sci 23:2315–2322

Received July 30, 1988 / Accepted November 7, 1988