Epithelial Response of the Rat Gastric Mucosa to Chronic Superficial Injury

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Chronic injury to the healthy gastric mucosa with noxious agents such as aspirin or alcohol induces a progressive strengthening of the stomach wall against these insults. The present study examined the histologic response of the rat gastric mucosa to chronic destruction of the superficial mucosa for one month with hypertonic saline. The number, position and morphology of proliferating, parietal, G and D cells were followed during mucosal injury and one month of recovery. The results showed that chronic injury reduced parietal cell numbers by about 30 percent, particularly in the middle of the mucosal thickness where a clear zone was formed by hypertrophy of mucous neck-like cells. G cells were also reduced by about 50 percent, but there were no changes in D cells. Chronic injury induced a marked increase in the number of antral (+112 percent) and fundic (+250 percent) proliferating cells. Conclusion: The rat gastric mucosa responds to chronic superficial injury by down-regulation of acid secretory cells and gastrin secreting cells and an up-regulation of proliferating cells. The appearance of a prominent layer of mucous neck-like cells may indicate a new secretory function for these cells.

INTRODUCTION

Circumstantial evidence from patients ingesting noxious agents such as non-steroidal anti-inflammatory drugs and various mixtures of ethanol indicates that chronic insult to the gastric mucosa may be an important factor in the development of some gastropathies [1]. Nevertheless, most experimental studies of gastric damage and repair utilize a single, large dose of a strong necrotizing agent such as absolute ethanol to produce gross lesions in the stomach. These experimental protocols clearly have advantages for screening potential gastro-protective drugs and to monitor the sequelae of healing events, but they probably have little clinical relevance [2].

Relatively few experimental studies have monitored the progression of gastric mucosal responses after successive doses of an orally administered damaging agent [3-12]. Surprisingly, repeated exposure to a strong necrotizing agent such as 80 percent ethanol induces a progressive mucosal resistance despite the agent's initial production of hemorrhagic lesions. The mucosa also becomes progressively protected against the effects of strong necrotizing agents when it is chronically insulted with a mild damaging agent which destroys only the superficial epithelium. Very little is known about the mechanisms by which augmented mucosal protection is acquired under these conditions. A previous study showed that chronic superficial insult resulted in rapid re-establishment of the

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[e]*Abbreviations:* PBS, phosphate buffered saline; BrdU, 5'-bromo-2'-deoxyuridine.

mucosal surface epithelium and changes in the composition of antral mucus, but other histological responses to the repeated injury are unknown [12]. With this in mind, the present study utilized a clinically relevant model of chronically insulted gastric mucosa to examine potential responses in the number, position and morphology of selected epithelial cells. Histochemical and immunohistochemical techniques were used to localize parietal cells, proliferating cells, and cells containing gastrin (G cells), and somatostatin (D cells) in the fundic and antral mucosa of rats subjected to chronic superficial mucosal injury for one month followed by one month of recovery. The results showed that chronic injury induced significant changes in the number and position of these cells, which may reflect the development of mucosal protection.

MATERIALS AND METHODS

Male Sprague-Dawley rats (150-250 g, Charles River Breeding Laboratories, Wilmington, DE) were fed standard laboratory chow and kept on a 12-hr light/dark cycle. Except for a 30-min dosing period, all rats were allowed water *ad libitum*.

The experimental group of rats was placed on a four week regimen of chronic intragastric dosing with hypertonic saline followed by four weeks without dosing. Previous studies had shown that 2 M NaCl destroyed the superficial gastric epithelium without inducing hemorrhagic lesions [12]. Each rat received a single bolus (two to three ml) of 2 M NaCl orogastrically every 48 hours between 1:00 and 2:30 p.m. During this part of the animals' diurnal cycle they were somnolent and their stomachs nearly void of food, which allowed direct access of saline to the gastric mucosal surface. After dosing, the rats were returned to their cages and deprived of food and water for 30 min followed by free access to both. During the four-week recovery period when rats were not dosed, they were given free access to both food and water and were handled in the same manner as the untreated control group. The two control groups of rats received either physiological saline (0.9 percent) in the same manner as the experimental rats or were left untreated.

Rats were sacrificed at two and four weeks after the initiation of the dosing regimen and four weeks after its termination. At the termination of each segment of the experiment, rats were anesthetized with ether, the stomachs were exposed by laparotomy, and the gastroesophageal and gastroduodenal junctions were ligated taking care not to occlude blood vessels and nerves. The nonglandular stomach was pierced with a 26-gauge needle, and three to four ml of either Carnoy's or Bouin's fixative was injected to gently inflate the gastric wall. Optimal immunohistochemistry for light microscopic identification of proliferating cells and parietal cells was achieved using Carnoy's fixative. Bouin's fixative was optimal for immunohistochemical localization of G and D cells. The same fixative as used for intragastric inflation also was poured over the external stomach surface and the preparation left *in situ*. After 20 min, the animals were killed and the ligated stomach was excised and submersed in fixative for an additional one to three hr at room temperature. The stomach was hemisected along the greater and lesser curvatures and full thickness pieces of the gastric wall were excised with a razor blade. Two strips of fundus (1.5-2.0 x 0.5 cm), each from the greater to lesser curvature adjacent to the limiting ridge, and one piece (0.5 x 0.5 cm) of antrum were excised. These tissues were processed for light microscopy as previously described [12] or for routine histochemistry and immunochemistry as described below.

All tissue samples were embedded in paraffin, sections cut five micrometers thick, placed on glass slides, deparaffinized and rehydrated using standard techniques.

Some sections were stained with hematoxylin and eosin or Masson's Trichrome for histochemistry. Other sections were prepared for immunohistochemistry by pretreatment for five min in three percent H_2O_2 to eliminate endogenous peroxidase activity, then rinsed in phosphate buffered saline (PBS)^e. Exposure of tissue sections to the four different antisera

listed below was performed at room temperature (except where specified) in a sealed, moisturized chamber.

Proliferating cells were labeled with antisera to 5'-bromo-2'-deoxyuridine (BrdU), which had been injected into rats for one hr according to previously published techniques [13].

Parietal cells were localized using a monoclonal antibody directed against H+/K+-ATPase [14]. This antibody was made by standard hybridoma techniques using as antigen pig gastric microsomal vesicles highly enriched in H+/K+-ATPase. Immunoblot analysis shows antibody specificity for the α subunit of H+/K+-ATPase, with an apparent molecular mass by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of 94 kDa (not shown). The antibody stains only parietal cells in pig, rabbit, rat, human, dog, guinea pig, and gerbil gastric mucosa. In these cells, tubulovesicular and secretory canalicular membranes are the site of antibody reactivity. Sections were preincubated in one percent bovine serum albumin (BSA) for 30 min at room temperature then incubated in H,K-ATPase monoclonal antibody at a 1:100 dilution in PBS overnight at 4°C. The slides were rinsed in PBS then incubated in peroxidase-labeled goat anti-mouse IgG at a 1:100 dilution in PBS for 20 minutes.

Gastrin (G) cells were localized using specific antisera according to previously published techniques [15].

D cells were localized using a previously described sheep anti-somatostatin antiserum (8093) generated against somatostatin-14 [16]. The antiserum cross-reacts with both somatostatin-14 and somatostatin-28 and does not cross-react with gastrin, glucagon bombesin, pancreatic polypeptide, β-endorphin or neurotensin. Sections were preincubated in a 1:100 dilution of blocking serum (normal rabbit serum) in PBS for 30 min then incubated overnight at 4°C in a 1:1000 antiserum dilution in PBS. The sections were rinsed in PBS then incubated in a 1:50 dilution in PBS of rabbit anti-sheep IgG (secondary antibody) for 20 min, rinsed in PBS, then incubated in a 1:100 dilution of sheep peroxidase-antiperoxidase in PBS for 20 min.

The final step for each of the four antiserum procedures described above was incubation of the tissue sections in a 0.04 percent solution of 3.3 diaminobenzidine tetrahydrochloride in Tris buffer, pH 7.3, to which 33 ml of 3.0 percent H_2O_2 was added just prior to use. The slides were reacted approximately 10 min at room temperature in this mixture, rinsed several times in PBS and counterstained with cresyl violet or light green to more readily visualize tissue architecture. Negative controls were run in conjunction with all specific antiserum staining.

Quantitative light microscopical analyses of each cell type and mucosal thickness measurements were done at 400-fold magnification in tissue sections in which the gastric pits and glands were oriented perpendicular to the plane of section. An ocular micrometer was placed parallel to the *muscularis mucosae* and the number of immunoreactive cells counted in a standard length of 280 microns. At least 20 standard lengths (5.6 mm) were counted for each rat. Mean values for each rat were determined and these numbers used to calculate mean values for each experimental group. To determine the position and size of the proliferative zone, the following distances were measured at 280 μm intervals as described above: a) from the most luminal (uppermost) positively staining (BrdU) cell to the mucosal surface; b) from the uppermost positive cell to the lowermost positive cell in the upper 1/3 to 1/2 of the mucosa; c) from the lowermost positive cell to the base of the gastric gland. In the antrum the proliferating zone lies at the base of the blind-ended gastric pit-isthmus. Because of this anatomical arrangement, the accuracy of the measurement from lowest BrdU-positive cell to "base of gland" as described above could not be confirmed. Mucosal thickness was measured from the base of the gastric glands to the top of the interfoveolar epithelium at each standard length (280 microns).

RESULTS

Consistent with previously published data, the present study showed that chronic insult to the gastric mucosa with 2 M NaCl did not produce hemorrhagic lesions [12] and that damage was confined to the superficial mucosa. Light microscopy confirmed that a consistent exfoliation of the interfoveolar and upper gastric pit epithelia occurred immediately after each exposure to hypertonic saline with subsequent epithelial restitution by foveolar mucous cells [12].

Chronic exposure to hypertonic sodium chloride did not induce significant changes in fundic or antral mucosal thickness (Table 1).

Histochemical staining showed normal morphology in both the fundus and antrum of control rats dosed with physiological saline and those which were not dosed (Figure 1).

The histological architecture of fundic mucosae was altered in rats subjected to chronic superficial injury. The most notable feature visible even at extremely low magnification was the presence of a weakly staining portion of the middle and upper portions of gastric glands. Collectively, these glandular regions formed a band which occupied the middle 1/4 to 1/3 of the fundic mucosa (Figure 1). This area, termed here "clear zone," was composed predominately of cells with some morphological characteristics of mucous neck cells (unpublished observation). The clear zone appeared within two weeks of the initiation of the hypertonic saline insults, which was the earliest time point at which tissue sections were observed in this study. The clear zone diminished significantly four

Table 1. Changes in the mucosal thickness and proliferative zone of rat fundic and antral mucosa after chronic superficial mucosal damage and recovery.

			Proliferative Zone (PZ)		
	n	Mucosal thickness	Proliferating cells	PZ thickness	PZ to surface	PZ to base
I. FUNDUS						
Control	8	653.6±35.7	47.3±2.5	89.5±5.1	182.1±9.9	403.7±21.7
Chronic dosing						
2 weeks	4	592.3±35.5	128.6±11.5*	136.4±13.3*	161.5±13.5	302.6±22.4
4 weeks	4	718.8±42.3	147.0±19.1*	144.1±4.7*	155.7±5.6	410.5±41.5
Recovery						
2 weeks	4	599.6±11.9	39.7±5.2	101.5±4.7	161.1±2.4	335.7±11.5
4 weeks	8	556.1±63.3	41.3±4.4	76.3±5.1	147.2±22.3	331.6±43.6
II. ANTRUM						
Control	8	233.9±12.0	63.2±4.5	58.2±4.5	146.7±8.1	-
Chronic dosing						
4 weeks	4	255.1±23.7	134.0±12.1*	69.3±3.7*	168.0±28.4	-
Recovery						
4 weeks	4	203.1±18.9	74.1±16.8	50.5±10.7	152.6±9.8	-

Values are means ± standard error of the mean. Proliferating cells are number of immunoreactive cells per mm length of tissue. All other values are in micrometers. "PZ to surface" and "PZ to base" refers to mucosal surface and gland or pit base, respectively. The PZ in the antrum lies near the isthmus base. PZ measurements were not made from PZ to base. Asterisks indicate statistically significant differences ($p < .05$) compared to respective control values. n = number of rats.

weeks after the chronic injury was terminated, but there was still evidence of its existence in some tissue sections. Antral tissue did not show the development of a clear zone or other morphological alterations subsequent to chronic mucosal insult, but changes in mucus chemistry have been described previously [17].

Proliferative cells were immunohistochemically identified by punctuate, darkly staining deposits in the nuclei of cells synthesizing DNA [13]. Fundic tissue from control rats showed a clustering of immunopositive cells defining the proliferative zone in the gastric pit-isthmus region. Rarely was a positive cell observed outside the proliferative zone
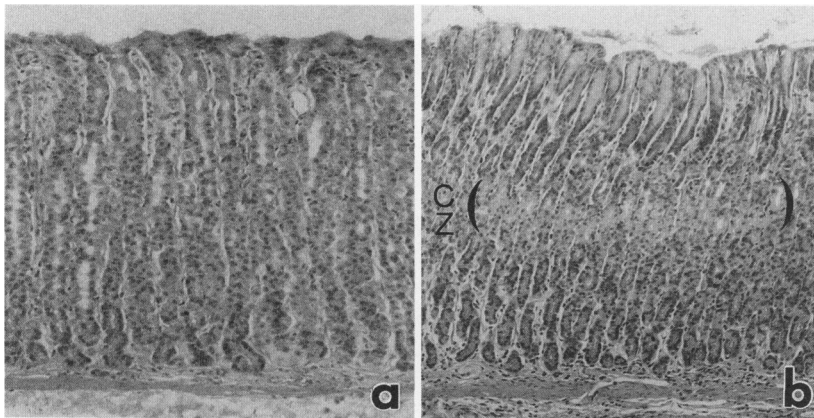

Figure 1. Tissue sections of fundic mucosa stained with Masson's trichrome. a) The control mucosa appears normal. b) The stomach exposed to chronic superficial injury has developed a zone of lightly staining cells (clear zone, CZ) in the middle of the mucosal thickness. Magnification = 160x.
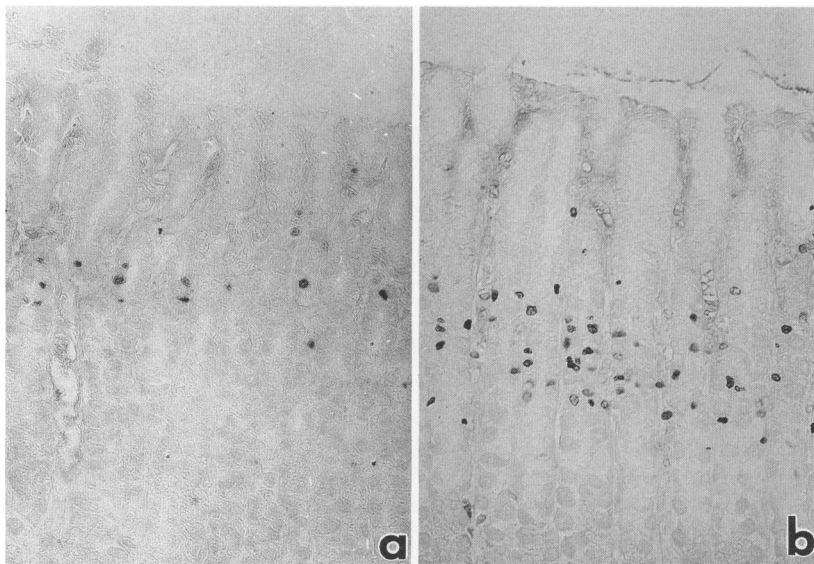

Figure 2. Tissue sections immunohistochemically stained to identify proliferating cells. a) Control fundic mucosa shows the epithelial proliferative zone near the junction of the gastric gland isthmus. b) Fundic mucosa from experimental rats shows significant expansion of the epithelial proliferative zone and increase in the number of proliferative cells. Magnification = 230x.

(Figure 2). In control rats, the proliferative zone occupied slightly less than 15 percent of the fundic mucosal thickness (Table 1).

The distribution and density of proliferating cells in the fundus and antrum were rapidly and significantly increased after chronic injury to the superficial gastric mucosa (Table 1 and Figures 1 and 2). The fundic proliferative zone widened by about 50 percent and the number of proliferating cells increased about 250 percent within two weeks of mucosal damage as seen in Figure 2 and Table 1. These effects remained elevated for the additional two weeks of dosing and then returned to control levels when the mucosa was allowed to recover (Table 1). The marked increase in proliferative zone thickness was accounted for by the presence of immunoreactive cells both more deeply into the gastric gland and further towards the mucosal surface. Nevertheless, neither distance, mucosal surface to proliferative zone, nor gland base to proliferative zone was significantly different from control rats (Table 1). In spite of a widening of the proliferative zone in experimental rats, it lay luminal to the clear zone (Figure 2).

Proliferating cells in the antrum occupied the lowermost region of the gastric pit-isthmus, as shown previously, because there is not a comparable gastric gland in this region of the stomach [17, 18]. The proliferative zone occupied about 25 percent of the total antral thickness (Table 1).

Antral tissue also responded to superficial mucosal injury by a significant increase (112 percent) in the number of proliferating cells (Table 1). In addition, the proliferative zone widened, being 18 percent greater than in control rats. In experimental rats, the proliferative zone occupied 27.1 percent of the mucosal thickness which was not significantly different from the control tissue. Because the proliferative zone lies near the base of the gastric pit, any widening of the proliferative zone moves the boundary of the zone towards the gastric lumen. However, our data showed that there was not a significant change in this distance despite a significant change in proliferative zone thickness. This would indicate that the proliferative zone also expanded towards the base of the gastric pits, a dimension which was not measured here.

Cells immunoreactive for gastrin (G cells) were located in the lower 1/2 and predominantly in the base of the antral pits where they were interspersed among the proliferating cells as reported previously [19]. One month of mucosal injury reduced the density of these cells by approximately 50 percent, which was reversed to control levels during the recovery period (Figure 3). There did not appear to be a difference in either the distribution or morphology of these cells during the experimental regimen.

Cells immunoreactive for somatostatin (D cells) were distributed differently in the antrum than in the fundus although the relative number of cells did not change during the chronic injury regimen (Table 2). In the antrum, D cells were interspersed among the G cells as well as sometimes being relatively near the gastric lumen and thus above the proliferative zone as described previously [19]. In the fundus, D cells were scattered along the length of the gastric gland but never in the gastric pit and rarely in the isthmus.

Parietal cells were identified by darkly staining deposits along the apical plasma membrane as well as in the cytoplasm (Figure 4). In untreated rats, these cells were distributed from the lower gastric pit region to the gland base, becoming progressively larger and more deeply stained along this axis. Chronic injury for both two weeks and four weeks reduced the number of these identifiable acid-secreting cells by approximately 30 percent (Figure 5). This reduction was accounted for largely by a nearly complete depletion of positively staining cells in the middle 1/4 to 1/3 of the mucosa (Figure 4) coincident with the development of the clear zone. There was also a reduction in the number of parietal cells in the isthmus and gastric pit and the gland which lay above and below the clear zone, respectively. In addition, the parietal cells in chronically injured mucosa showed markedly lighter immunostaining than parietal cells below the clear zone. Parietal

Table 2. Density of somatostatin immunoreactive cells.

	Control	Chronic dosing	Recovery
Fundus	30.3±1.0[1]	24.9±3.3	27.2±6.0
Antrum	9.9±2.5	12.5±1.8	11.2±2.4[1]

Values are mean ± standard error of the mean number of cells per mm tissue. n = 4 rats for all groups except (1) in which n = 3. Chronic dosing and recovery were for four weeks each.
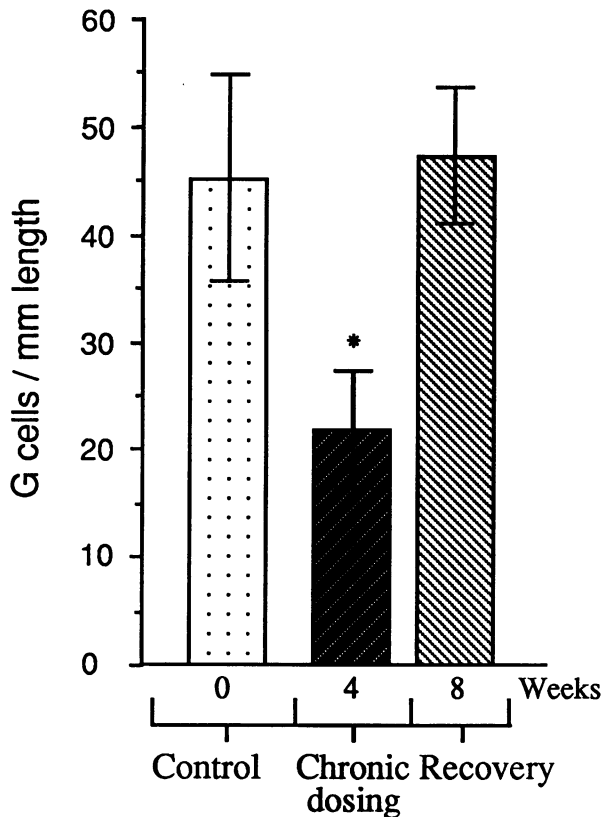

Figure 3. Histogram showing the density of antral G cells in control rats, those exposed to 1 month of chronic superficial injury and one month recovery. Asterisk = $p < .05$.

cell density and immunoreactivity returned to control values with the cessation of injury for four weeks and the concomitant disappearance of the clear zone.

DISCUSSION

The gastric mucosa of the healthy rat like that of its human counterpart responds to chronic injury by becoming progressively more resistant to acute insult [3-12]. In the present study we sought to determine which, if any, epithelial components of the rat mucosa were altered morphologically as the stomach adapted to these repeated insults. The results showed an injury-induced shift in epithelial cell type density and position, which may reflect protective responses in the mucosa.

Our previous studies showed that the chronically injured gastric mucosal surface returned to epithelial integrity faster than did the unchallenged mucosa after injury [12]. We speculated that this reaction might be attributable to relatively younger mucous cells more
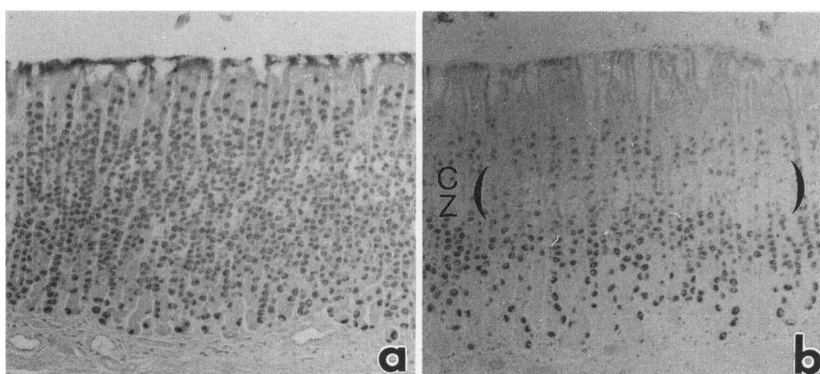
Figure 4. Tissue sections showing distribution of immunohistochemically identifiable parietal cells in a) control fundic mucosa and b) experimental rats in which they are markedly reduced in the middle of the mucosa forming a clear zone (CZ). Magnification = 160x.
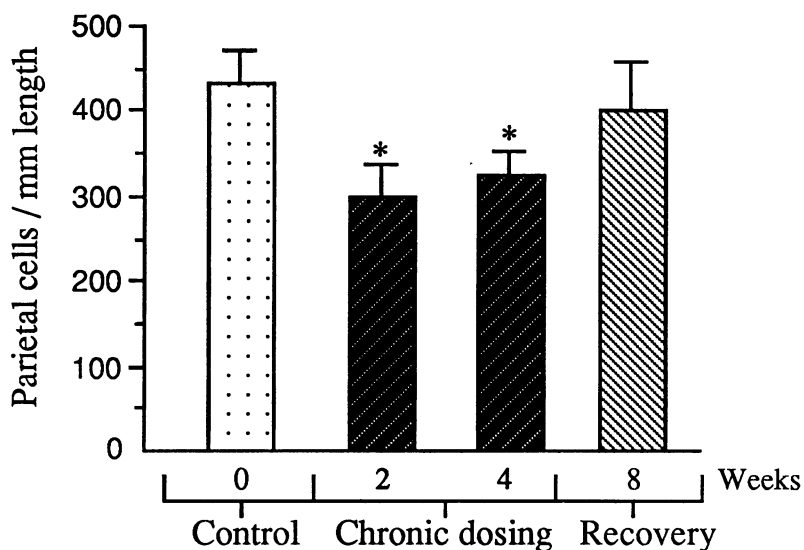

Figure 5. Histogram showing the density of parietal cells in control rats, those exposed to chronic injury, and those allowed to recover from injury for one month. Asterisks = $p < .05$.

rapidly reaching the upper foveolus from the lower gastric pit and isthmus to replace the exfoliated surface cells. If this were indeed the case, then increases in cell proliferation after chronic damage would be necessary to replace the constantly damaged mucosal surface. Using techniques to localize cells which were synthesizing DNA, we showed in the present study that a significantly greater number of new cells in both antrum and fundus were being added to the epithelial population in response to superficial injury. Because the fate of these proliferating cells was not followed, we can only assume that a significant fraction of them were terminally differentiated into mucous cells, which migrated from the pit-isthmus region of their origin onto the mucosal surface. This assumption seems warranted since the

increase in proliferating cells appeared to balance the loss of exfoliated cells from the superficial mucosa as reflected in the absence of significant changes in mucosal and gastric pit thickness during the experimental regimen.

Although there is evidence from previously published work that various cell types within the epithelium can replicate from fully differentiated parent cells, the general consensus currently is that a single population of pluripotent stem cells in each gland gives rise to all other epithelial cell types within the gastric mucosa through various cell lineages [20-27]. Karam and Leblond [23-27] have shown in the mouse that a single population of stem cells gives rise to three cell lineages, which subsequently become fully differentiated pit (mucous cell), parietal, and zymogenic (chief) cells, respectively (Figure 6). Although each cell-type's life span is probably different in the rat than that described for mice, the general lineage plan is no doubt quite similar in these two rodents. Therefore, we have used the lineage patterns described by Karam and colleagues to interpret the data in the present study [23-28].

Figure 6 shows the mouse stem cell progeny consisting of pre-pit cell precursor cells (67 percent), preparietal cells (nine percent), and pre-neck cell precursors (24 percent) along the three lineages, respectively [28]. The former two cell types give rise almost entirely (98-99 percent) to pre-pit and pre-neck cells but a small fraction of each (one to two percent) gives rise to pre-parietal cells which ultimately mature into parietal cells. Both pre-pit and pre-neck but not pre-parietal cells divide, thus enriching their own population [28]. The mucous neck cell, although apparently incapable of further division, progressively develops into a terminally differentiated zymogenic (chief) cell. The determinants of daughter cell fate are unknown but, as shown below, this must be a tightly regulated event to balance changes in epithelial cell dynamics.

Anatomically, the pre-pit cells migrate luminally from the proliferative zone as they mature into pit cells. Subsequently they reach the interfoveolar epithelium and are exfoliated through necrosis or apoptosis. This transit takes approximately 3.1 days under normal conditions in the mouse [28]. When the interfoveolar epithelium is destroyed, it is replaced rapidly by migrating pit cells from the isthmus.
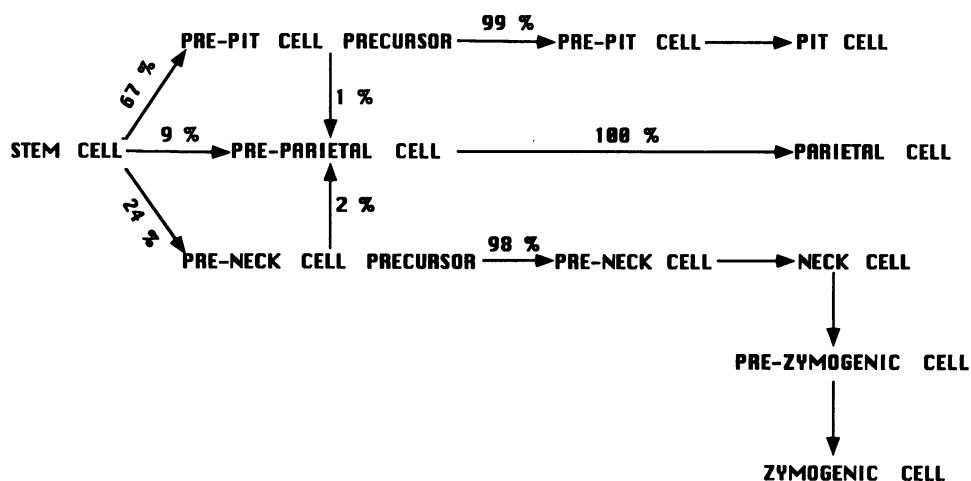

Figure 6. **Schematic representation of cell lineage pattern in mouse fundic mucosa (modified from Reference 28).**

The pre-parietal cells migrate from the isthmus in two directions, along the gland axis towards its base as well as luminally into the lower pit. The life span for the pre-parietal cell and parietal cell is three and 54 days, respectively, in the mouse and 164 days in the rat [28, 29]. The turnover time for a neck cell is 12.5 days and zymogenic cell is 194 days in mice.

Results of the present study indicate that chronic superficial injury induces more cells to synthesize DNA deep within the proliferative zone of the fundic and antral mucosa. These increased numbers could be due to either more cells undergoing proliferation and/or shortening of the cell cycle time. This study did not differentiate between these two parameters. In addition, we are not certain which of the three cell types (stem, pre-neck, pre-pit), capable of cell division in the proliferative zone, were increased in response to superficial injury.

The significant reduction in parietal cell mass after mucosal injury that we observed in the present study suggests an imbalance between production and loss of these cells. Two possible explanations may shed light on the process. First, the pre-parietal cell lineage from stem cells may be "down-regulated" so that fewer cells matured into parietal cells. Second, an increased number of fully differentiated parietal cells were lost from the epithelial population by necrosis or by apoptosis without a concomitant increase in pre-parietal cell production. Our ultrastructural observations of the gastric mucosa have shown that parietal cells are extremely sensitive to luminal perturbations and rapidly exfoliate in the face of challenge (unpublished observations). The fact that parietal cells nearest the gastric lumen were not severely depleted like those deeper in the mucosa (in the clear zone), as explained below, makes us question the likelihood that luminal sodium chloride selectively destroyed these deeper cells. Nevertheless, the reduced number and H^+/K^+-ATPase immunoreactivity of parietal cells nearer the gastric lumen may suggest a direct effect of the NaCl insult.

Although we did not do regional cell counts across the thickness of the mucosa, it is apparent that the reduced parietal cell mass was due to a loss of these cells primarily in the clear zone and secondarily above and below it. The cell type taking the place of the parietal cell in the clear zone has many histological characteristics of the mucous neck cell with markedly increased mucous granules. Because the lineage of these cells was not traced, it is possible that they are a new cell type arising in the face of gastric damage. This possibility is not unreasonable since new cell types arise in the colon in chronic ulcerative states [30]. The current study does not reveal why this loss of parietal cells appears to be focal and, therefore, cannot account for the reduced number of parietal cells. It seems unlikely that the generation of new parietal cells from stem cells has been terminated since some proliferating cells adjacent to the clear zone would be likely to migrate towards the gastric gland and, therefore, differentiate into parietal cells. Because there remained an identifiable population of parietal cells below the clear zone, although their density was decreased compared to controls, and because fully differentiated parietal cells do not divide, there arises the question of how parietal cells cross the "clear zone" in what appears to be reduced numbers and then appear below the clear zone in normal density. Three possibilities may account for this phenomenon. First, changes in cellular size and geometry may have falsely accounted for reduced numbers of parietal cells. A combination of hypertrophy of adjacent mucous neck cells (unpublished observations) and parietal cell hypotrophy would show fewer immuno-positive parietal cells in each tissue section even though the numbers of the two cell types remained the same. Observations of the clear zone indicate that this may indeed account for some of the reduced parietal cell numbers because the clear zone is accounted for by increased numbers and mucus content of mucous neck-like cells. Second, the antibody used to identify these cells may not reflect their true numbers. Parietal cells were identified by

immunolocalization of H^+/K^+-ATPase, an integral membrane protein of tubulovesicular and secretory canalicular membranes of acid-secreting parietal cells. It may be that the synthesis or activation of this protein is down-regulated as these cells pass through the clear zone. We cannot exclude the possibility, although seemingly remote, that parietal cells "de-differentiate" as they cross the clear zone and regain their full complement of H^+/K^+-ATPase as they emerge into the chief cell-laden gastric gland. Third, all the parietal cells in the isthmus region (above the clear zone) may be destined to migrate upwards towards the gastric pit. In the normal mucosa, some of the proliferating cells in this region migrate up and others migrate down towards the gastric gland [28]. This scenario would require alterations in either the number of pre-parietal cells or changes in numbers of mature parietal cells lost from the mucosa. Supportive studies for this notion have shown that selective destruction of parietal cells either by blocking the H_2 receptor or the H,K-ATPase induces their early death, as well as stimulating the production of a greater number of pre-parietal cells, although this area of research is controversial [31-33]. Nevertheless, clearly there is a feedback mechanism from the dying parietal cells to the stem cell for pre-parietal cell regulation. We are unaware of the signal pathway for this regulation, but the reduced number of parietal cells caused by superficial injury most likely reflects a decreased acid output that would teleologically appear advantageous at a time when the superficial mucosa is constantly being assaulted.

In the mouse, mucous neck cells are precursors to chief (zymogenic) cells. If this pattern is present in the rat, it presents a conundrum with respect to the findings in the present investigation (Figure 6). The significantly increased numbers of mucous neck-like cells in the "clear zone" of the mucosa in experimental rats would suggest an increased number of chief cells if their rate of differentiation remains constant. Although we did not quantitate the chief cells, their numbers were not strikingly changed even though there was some hypertrophy in experimental rats. However, the time frame of our experiments may have been too short to account for major differences in the chief cell population since these cells have a reported life span of 194 days in the mouse and possibly longer in the rat [28]. Mucous neck cells have a life span of 12.5 days in mice and then continue their differentiation (42 days) into pre-zymogenic cells before maturing into zymogenic cells. Since we did not observe an accelerated loss of chief cells, we can only speculate that the increased number of neck cells must reflect an up-regulation of either pre-neck cell precursors or pre-neck cells.

The effects of chronic injury in the gastric antrum were similar to that of fundus, in that a marked increase in proliferative cells occurred. It should be noted that in control rats not only was the absolute number of proliferative cells per unit length greater in the antrum but the relative number per tissue mass (mucosal thickness) was almost four times greater in the antrum than fundus. We did not observe a clear zone in the antrum because there is not a population of mucous neck cells in that region or in gastric glands. This observation lends strength to the argument that mucous neck cells are not precursors to mucous cells lining the pit and interfoveolar regions.

Because gastrin is a potent stimulator of mucosal epithelial cell proliferation, we examined the number of antral G cells as a potential indicator of cell proliferation and regulation [34]. We were surprised initially to find that chronic injury induced a marked decrease in the number of G cells in light of increases in cell proliferation in both antrum and fundus. We did not measure serum gastrin, and thus, the number of G cells may not reflect gastrin secretory levels, although this is unlikely from previous studies of hyper- and hypogastriemia [35]. Although the G cells reside within the proliferative zone where they could act in a paracrine fashion to affect the production of new epithelial cells, the morphologic data here suggests that gastrin does not induce the stem cells to increase epithelial cell proliferation in the antrum.

Numerous studies have shown that gastrin released from antral epithelial cells has potent trophic actions on the fundic mucosa. For example, elevated serum gastrin levels are correlated with increased parietal cell mass without an increase in zymogenic cells [36, 37]. Willems and Lehy [37] found a significant increase in production of new parietal cells following gastrin treatment in the mouse. These authors postulated that this cell population increased because of fundic stem cell DNA synthesis and shortened maturation time. Likewise, Blom and Erikonin [38] showed gastrin was directed at stem cells but also was specific for parietal cells since the number of chief cells in the gastric mucosa was not altered. Conversely, anterectomy produces fundic atrophy and particularly significant decreases in parietal cell mass [39]. These data taken together with those of Karam and Leblond [23-27] showing three separate lineages from stem cells, suggest that gastrin targets the pre-parietal cell lineage and not those of the pre-pit or pre-mucous neck. This observation supports our current data showing a positive correlation between the reduced number of both G cells and parietal cells independent of the elevated number of proliferating cells. Further evidence to support this notion comes from the lack of trophic effect of gastrin on the antral mucosa which has no pre-parietal cell lineage [40, 41]. In fact, there appears to be a negative feedback or inhibitory action of gastrin in the antrum since in patients with Zollinger-Ellison syndrome (parietal cell hyperplasia, hypergastrinemia, G cell hyperplasia) there is significant antral hypoplasia with concomitant reduction in epithelial mitotic indices [42, 43].

Although it has been suggested that G cells may proliferate from stem cells, there are no data to suggest that the different epithelial cell types in the antrum may arise from separate cell lineages as is the case for the fundus. Data from the present investigation suggests that there are different cell lineages in the rat antral mucosa. As the superficial antral epithelium was constantly being replaced by increases in mucous cells from the proliferative zone, the number of G cells significantly decreased, suggesting regulation of at least two separate cell lineages.

Gastrin has not only a trophic effect on parietal cell population but stimulates acid secretion in these cells. Since one of the antagonists to gastrin's effect on parietal cells is somatostatin, we examined the number, size and position of the D cells in both the antral and fundic mucosa. Somatostatin blocks the trophic action of gastrin [44, 45] and acts as a paracrine inhibitor of gastric secretion through receptors on the G cell in the antrum [46]. With the understanding that as serum gastrin levels decrease, serum somatostatin rises, we found an increase, although not statistically significant, in the number of somatostatin-containing cells concomitant with G cell and parietal cell reduction in experimental rats. Without having measured serum somatostatin levels, the physiological significance of the changes cannot be discerned. Somatostatin-containing D cells are also present in the fundic mucosa, where there are no G cells to act on. We found no change in D cell population in the fundus, suggesting that chronic injury did not alter the function of these cells.

Taken together, the results of this study indicate that the rat gastric mucosa responds to chronic superficial injury by down-regulation of the acid secretory (parietal) cell and G cell populations with simultaneous increases in proliferative cells. There are no doubt other cellular and biochemical adjustments of the mucosa to chronic injury and these are currently under investigation.

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