Pathogenesis of Acute Gastric Stress Ulcers

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Acute ulcerations of the stomach and of the duodenum have been recognized since the nineteenth century when they were described in association with severe body burns and lesions of the brain. It has become apparent that the ulcers may follow a variety of stress conditions in man, including multiple injuries and sepsis, but the pathway by which the various disease states lead to acute ulceration remains unsolved. There have been numerous attempts to reproduce these lesions experimentally, including stimulation or ablation of different parts of the brain, the administration of various pharmacologic agents, and the employment of restraint stress. Stimulation of the anterior hypothalamus and destruction of the posterior portion have been shown to result in increased gastric acidity and the production of ulcers. Also, restraint-induced ulcers are prevented in part or totally by prior vagotomy or by the administration of anticholinergic drugs, whereas adrenalectomy or cholinergic and sympatholytic agents afford no protection. These observations all tend to indict vagal overactivity as the principal effector in the induction of stress ulcers.

In a separate study employing restraint stress, we observed that bacterial products of intestinal origin may play an important role in the central stimulation of the hypothalamus and vagal nuclei and that the prior oral administration of a nonabsorbable antibiotic affords significant protection against the development of the ulcers. However, the parts played by the various motor and secretory effects of the vagal action, and the relative importance of vascular changes and the gastric epithelial mucin in the development of the mucosal lesions have not been clarified. A correlative histologic, ultrastructural, and mucin histochemical study of the gastric mucosa in immobilized rats was undertaken, therefore, in an effort to delineate the various factors involved in the pathogenesis of the stress lesion.

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Material and Methods

Groups of Charles River CD-strain rats averaging 125 gm. in weight were fasted for 12 hr. and then completely immobilized in wire mesh, using the modified method of Brodie and Hanson, for 4, 8, 12, 16, and 20 hr. Polymyxin B was administered orally to half the animals; 25 mg./100 ml. was added to the drinking water and 6 mg. was given by gavage in 2 ml. of tap water for 3 days prior to the restraint. Qualitative cultures of the feces were performed using thioglycollate broth and Endo-agar plates. The antibiotic treatment resulted typically in a reduction of the E. coli, whereas the other enteric organisms were not regularly affected. Only those animals which were demonstrated to be coliform-free were included in the antibiotic-treated group.

The rats were sacrificed by decapitation, and the stomachs and intestines were examined in situ in order to estimate the state of contraction or dilatation. Bacteriologic cultures were obtained from the cecal contents. The stomachs were excised, opened along the greater curvature, and examined under a magnifying lens for the presence of mucosal lesions. Complete necropsies were performed in selected cases. For the light-microscopic studies, circumferential sections from the midportions of both the corpus and antrum were obtained in all cases, including those without gross ulceration. In some instances, parallel sections of the entire glandular portion were examined. The tissues were fixed in 10% neutral formalin and embedded in paraffin; the sections were stained with hematoxylin and eosin and the periodic acid-Schiff procedure (PAS), the latter for the demonstration of epithelial mucin.

Ultrastructural studies were performed on the stomachs of 6 untreated and 6 polymyxin-treated rats without ulceration after 4 hr. of stress, and 4 untreated animals with ulcers after 8 hr. of stress. Small blocks of tissues, from the midportion of the glandular corpus, 1 mm. in thickness, were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 2–4 hr., washed in buffer solution, and refixed in 1% osmium tetroxide in 0.1 M phosphate buffer at pH 7.2 for 1–2 hr. Following dehydration in graded ethyl alcohols and embedding in Epon 812, 1-μ and ultrathin sections were cut. The 1-μ sections were stained directly with 1% methylene blue in borate buffer at pH 7.4 and, after removal of the Epon by saturated sodium hydroxide solution, with the PAS procedure. The ultrathin sections were doubly stained with saturated lead hydroxide and 1% uranyl acetate solutions and examined with a Phillips EM 200 electron microscope.

Results

Mucosal ulcers were found in both the treated and the untreated animals, but polymyxin provided partial protection, especially during the first 12 hr. of the stress period (Table 1) as reported previously.

Gross Features

In all the unstressed and in most of the 4-hr. stressed rats, the polymyxin-treated animals revealed marked muscular relaxation of the stomach and intestines (Fig. 1). Ulcers were never present in animals with dilated stomachs. In contrast, contraction of the gastrointestinal tract was observed in many of the antibiotic-treated animals after longer periods of restraint and in all of the untreated rats. Ulcers were always associated with gastric contraction, often to a marked degree (Fig. 1). In addition,
the polymyxin-treated animals showed a moderate congestive enlargement of the liver and spleen as compared to the untreated group. Except for irregular fresh pulmonary hemorrhages which were seen in all stressed animals, no abnormalities were observed in the other organs.

The appearance of the ulcers was similar in both the untreated and the treated groups; they were typically linear, hemorrhagic, and superficial, and they measured up to 1 mm. in thickness and 6 mm. in length (Fig. 2). They were located parallel to and near the crest of the rugae in the midportions of either the anterior or the posterior walls of the glandular corpus. In general, the size of the ulcer was proportional to the duration of the stress period, and multiple lesions were commonly seen after 12 hr. or longer of restraint. In addition, independent of the existence of ulcers, a few petechiae were often noted in the gastric mucosa; these were present in both the corpus and the antrum and were observed more commonly in the untreated animals. No lesions were found in the gastric rumen or the duodenum.

**Histologic Features**

Examination of the mucosal ulcers revealed on cross section a roughly wedge-shaped zone of necrosis (Fig. 3) characterized further by a superficial area of ulceration and hemorrhage and a peripheral region of coagulative necrosis (Fig. 4). This had the appearance, in general, of an infarction of the mucosa with superficial hemorrhagic ulceration. The necrosis involved approximately the superficial half of the mucosa after 4 hr. of stress and, with increasing restraint, extended to three-quarters of the thickness. At all times, the basal portion of the mucosa, including the muscularis mucosae, appeared to be spared.

Dilatation of small veins, venules, and capillaries was constantly observed in the gastric mucosa of the untreated animals, and this was apparent as well in the early stages before the development of the ulceration. These vascular changes, together with tiny hemorrhages, were also present at the edges of the lesions, and a neutrophilic reaction was first noted.
after 8 hr. The inflammatory response became more prominent thereafter, and occasional small venous thrombi were observed in the adjacent mucosa after 16 hr. The remaining gastric mucosa revealed occasional tiny hemorrhages without necrosis and focal venous dilatation. No constant histologic abnormalities were noted in the other organs.

**Histochemical Features**

No epithelial mucin was demonstrable in the ulcerated areas, as evidenced by the PAS reaction. In addition, in the adjacent mucosa there was often noted a reduction or total loss of mucin granules within the surface and gastric pit cells. In the stomachs without ulcers, however, no depletion in mucin content could be identified in either the treated or the untreated groups.

**Ultrastructural Features**

**Group 1 (polymyxin-treated, 4 hr. stress, no ulcers).** The muscular coats, including the muscularis mucosae, were composed of closely aligned smooth-muscle cells, without evidence of interstitial edema (Fig. 5). The muscle cells in most animals were in a state of relative relaxation, exhibited by infrequent infoldings of cellular and nuclear borders, widely spaced dense zones, and a generally longitudinal orientation of the myofilaments. The surface vesicles were mainly of normal appearance and size with a median diameter of 80 μ. No abnormalities were noted in the submucosa or mucosal cells. Small multivesicular bodies, 540–860 μ in diameter, were observed rarely in the parietal cells (Fig. 6).

**Group 2 (untreated, 4-hr. stress, no ulcers).** The external muscularis and submucosa were unremarkable. The muscle cells in the muscularis mucosae were separated and appeared disoriented (Fig. 7); in most areas the surface vesicles were enlarged with a median diameter of 110 μ (Fig. 8). Occasional cells showed prominent cellular and nuclear infoldings and dense zones, indicating a state of relative contraction. No alterations were noted in the mucous and chief cells. A few parietal cells, however, contained enlarged multivesicular bodies, up to 2 μ in diameter, and an apparent increase in the number and length of the surface microvilli (Fig. 9 and 10). The mucosal venules and capillaries showed prominent dilatation and thinning of the walls, but no structural abnormalities were noted in the cells of the walls.

**Group 3 (untreated, 8-hr. stress, ulcers present).** Contraction of the fibers in the muscularis mucosae was seen and, in addition, separated muscle cells with enlarged surface vesicles were present in both the muscularis mucosae and the external muscularis. The median diameter of
the vesicles was 140 mμ. The peripheral zone of coagulative necrosis around the ulcerated area was apparent. Interstitial edema, large lipid bodies, and autophagocytic vacuoles were demonstrated in the surface mucous cells adjacent to the ulcers, representing early signs of degeneration of these cells (Fig. 11). In the nonulcerated areas of mucosa, parietal cells were often observed with many enlarged multivesicular bodies, marked intracellular edema, and occasional big cytoplasmic vacuoles.

Discussion

The present study of the gastric-stress lesions emphasizes the number and variety of the cellular changes and the complexity of the factors involved in their causation. Evidence of vagal overactivity, including prominent muscular contraction and increased gastric acid concentration, is clearly present in the early stages before the development of the ulcerations. Also seen in this pre-ulcer stage are dilatation of the mucosal veins and ultrastructural alterations in the muscularis mucosae consisting of disorientation of the muscle cells with enlarged surface vesicles and interstitial edema. Since these features are not a sign of muscular contraction alone,27 they would appear either to represent early injury to this structure or to be a reflection of increased vascular permeability. In either case, the appearance of these changes at a time when the mucosa is intact suggests that localized ischemia may play an important preconditioning role. This is supported by the subsequent demonstration of predominantly coagulative necrosis in the mucosal lesions. The concept that ischemia plays a vital role in the induction of gastric ulcers was originally championed by Virchow; more recently, Bonfils et al.28 have emphasized that a vascular factor is important in restraint-induced ulcers. Furthermore, Guth and Hall28 have shown that congestion of the surface mucosa precedes the development of ulcers in this area in restrained rats, and Myren28 noted reduced India-ink filling of gastric mucosal vessels in mice with experimental ulcers.

The early presence of gastric contraction and the specific linear shape of the lesions further suggest that the ischemia may result in part from the muscular contraction and extrinsic compression of the intramural vessels. A similar hypothesis has been proposed in the pathogenesis of ulcerative colitis in man30 to explain the occurrence of longitudinal ulcerations along regions with teniae coli. Vascular thrombi are seen only after many hours and are clearly secondary to the adjacent necrosis. The local action of various vasoactive substances has been postulated by others,28 and it remains possible that these may contribute to the ischemic state. Furthermore, although it is customary to consider that vagal activity
causes vasodilatation and increased gastric blood flow,\textsuperscript{31} it is conceivable that an excess in parasympathetic stimuli may lead instead to local vasoparalysis and consequently result in vascular insufficiency. This mechanism has been postulated in the experimental induction of ulcerative colitis in dogs.\textsuperscript{32}

The role of gastric acid in the production of stress ulcers has been controversial. In studies employing pyloric ligation, Menguy\textsuperscript{16} demonstrated a decrease and Bonfils \textit{et al.}\textsuperscript{13} an increase in total acid following restraint. Brodie, Marshall, and Moreno\textsuperscript{38} employed gastric fistulas in their study and showed a decrease in volume but an increase in acid concentration. In our studies,\textsuperscript{19} following restraint stress the acid concentration in the animals with normal intestinal bacterial flora was almost twice that of the antibiotic-treated group. Furthermore, enlarged multivesicular bodies and surface microvilli are found only in the parietal cells of the untreated group in the early stages, and such bodies have been seen in several species following stimulation of acid production by various methods, including histamine administration and vagal excitation.\textsuperscript{34-36} It appears, therefore, that gastric acid production is increased in the immobilized animals, and that the acid is free to act on a mucosa already damaged by ischemia and to contribute to the ulcer formation. It should be emphasized, however, that this secretory effect of vagal stimulation is not the sole factor in the development of the mucosal lesion.

Finally, one must consider the role of the epithelial mucin of the surface mucosa which represents the natural barrier to acid digestion and ulceration.\textsuperscript{37} The administration of cortisone has been shown to result in decreased mucin, as well as in mucosal ulcerations both in man\textsuperscript{7} and in unrestrained rats.\textsuperscript{38} However, this factor does not appear to be operative in restraint-induced ulcers, since prior adrenalectomy is deleterious\textsuperscript{11,14,15} and cortisone treatment offers partial protection.\textsuperscript{11} Although it had been thought that gastric mucus secretion was increased by vagal stimulation,\textsuperscript{39,40} more recent studies have indicated that this secretion is continuous in the rat and that any increase in mucus is a local response to a rise in acidity.\textsuperscript{41} In any case, the present histochemical and ultrastructural observations show that mucin depletion does occur in restraint stress, but that the area of involvement is strictly confined to the site of ischemia. When this occurs, the luminal acid is free to act on the unprotected mucosa, resulting in ulceration.

Although ultrastructural changes are noted in the muscularis mucosae after 4 hr. of stress and in the external muscularis after 8 hr., overt necrosis and ulceration are limited to the mucosa. This was found to be the case in both the present and earlier studies\textsuperscript{12,14,16} employing a restraint period
of 24 hr. or less. However, Rohm, Seybold, and Pirtkein have demonstrated that the ulcerations extend to the submucosa after 48 hr. and to the muscularis propria after 72 hr. of immobilization.

We have previously shown that bacterial products of intestinal origin play an important role in the central hypothalamic and vagal stimulation in restraint stress and that this excitation may be initially prevented by a reduction in the coliform bacteria of the gut. The animals with the reduced intestinal flora, as a result of the oral polymyxin treatment, fail to show the heightened motor and secretory effects of vagal overactivity in the early stages of restraint; rather, prominent gastrointestinal relaxation and dilation are apparent and persist throughout the period of protection. Furthermore, the muscular and parietal cell changes noted ultrastructurally are not observed in the antibiotic-treated animals at this time.

**Summary**

A correlative histologic, ultrastructural, and mucin histochemical study of the gastric mucosa has been performed in rats with restraint-induced ulcers to delineate the various pathogenetic factors involved. The fully developed ulcers are linear mucosal infarctions, characterized by a peripheral zone of coagulative necrosis and a superficial region of ulceration and hemorrhage. Signs of vagal overactivity, including prominent muscular contraction and increased gastric acid production, are apparent in the stressed animals before the development of the mucosal lesions. Also seen in this early stage are dilatation of the mucosal veins and ultrastructural signs of damage to the muscularis mucosa. These latter features, together with the subsequent demonstration of predominantly coagulative necrosis, suggest that localized ischemia may play an important preconditioning role. It is further suggested that the ischemia may result in part from the muscular contraction and extrinsic compression of the intramural vessels and that this leads to degeneration of the surface mucous cells. Although the gastric acid production is increased, it is probably not the sole cause of the mucosal lesions; rather, the ulceration results from the action of the luminal acid on a mucosa that has been previously damaged by ischemia.

Animals with reduced intestinal bacterial flora, as a result of oral polymyxin treatment, failed to show the heightened motor and secretory effects of vagal overactivity and the ultrastructural changes in the muscle in the early stages of restraint; this was accompanied by a significant reduction in ulcer development. This supports the concept that in restraint stress circulating bacterial products of intestinal origin may contribute to the excessive central hypothalamic and vagal stimulation which gives rise to gastric ulceration.
References


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Legends for Figures

Fig. 1. Comparison of untreated (left) and polymyxin-treated (right) rats after 4 hr. of restraint. Note gastrointestinal contraction in untreated animal in contrast to marked relaxation and dilatation in antibiotic-treated rat.

Fig. 2. Rat stomach showing bilateral, linear, and hemorrhagic ulcers in glandular corpus.
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Fig. 3. Section of mucosal lesion, showing superficial hemorrhage and ulceration. Basal portion of mucosa appears spared. Hematoxylin and eosin. × 64. Fig. 4. Section of mucosal lesion showing superficial hemorrhage and peripheral zone of coagulative necrosis. Intact mucosal cells are present in lateral portions. Hematoxylin and eosin. × 102. Fig. 5. Muscularis mucosae of stomach from 4-hr. stressed, antibiotic-treated animal without ulcer. Muscle cells are closely aligned, and surface vesicles are of normal size. Infoldings of nuclei and cytoplasm are infrequent, dense zones are widely separated, and myofilaments are well oriented, indicating a state of muscular relaxation. Lead hydroxide and uranyl acetate stains. × 14,100. Fig. 6. Parietal cell of 4 hr. stressed, antibiotic-treated animal without ulcer, which appears in resting state. There are few microvilli (MV), tiny multivesicular bodies (arrows), and no intracellular edema. Lead hydroxide and uranyl acetate stains. × 8230.
Fig. 7. Muscularis mucosae of stomach from 4 hr. stressed, untreated animal without ulcer. Muscle cells appear disoriented and separated by interstitial edema, and surface vesicles are prominent. Compare with Fig. 5. Lead hydroxide and uranyl acetate stains. \( \times 10,100 \).

Fig. 8. Muscularis mucosa of stomach from 4 hr. stressed, untreated animal without ulcer. Surface vesicles of muscle cells are enlarged. Lead hydroxide and uranyl acetate stains. \( \times 60,200 \).
Fig. 9. Parietal cell of 4 hr. stressed, untreated animal without ulcer. There is a marked increase in number and length of the microvilli (MV), and 2 enlarged multivesicular bodies (arrows) are present, indicating stimulation. Compare with Fig. 6. Lead hydroxide and uranyl acetate stains. \( \times 8230 \).

Fig. 10. Stimulated parietal cell of 4 hr. stressed, untreated animal without ulcer. Markedly enlarged multivesicular bodies (arrows) and prominent intracellular edema are present. Compare with Fig. 6. Lead hydroxide and uranyl acetate stains. \( \times 10,400 \).

Fig. 11. Surface mucous cells of stomach, adjacent to ulcer in an 8 hr. stressed, untreated animal. There is marked interstitial edema, and cells contain large lipid bodies, numerous autophagocytic vacuoles and a decreased number of mucin granules, indicating degeneration. Lead hydroxide and uranyl acetate stains. \( \times 7940 \).