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MORPHOLOGICAL STUDY OF GASTRIC LESIONS DEVELOPING IN THE RAT UNDER SEVERAL DAMAGING CONDITIONS: MODIFICATIONS INDUCED BY PRETREATMENT WITH ZINC ACEXAMATE

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Abstract

Lesions developing in the gastric mucosa of the rat after exposure to different gastric damaging agents (100 mg/kg aspirin, and 70% or 100% ethanol) were assessed by scanning electron microscopy. The severity of the lesions was quantified according to morphological criteria. Modifications in the severity of these lesions induced by pretreatment with zinc acexamate were also analyzed. The scanning electron microscope revealed that with the exception of absolute ethanol, which caused distinctive morphological features, lesions found under the different experimental agents shared a common pattern of progression. Ultrastructural lesions on surface epithelial cells preceded further alterations of parietal cells. After the integrity of the epithelial cells was lost, detachment of the parietal cells occurred, probably, through peptic digestion of the connections between cells and their extracellular matrices. Pretreatment of animals with zinc acexamate increased the presence of mucus on the gastric surface and significantly prevented the progression of lesions towards the severest stages. Ultrastructural damage of surface epithelial cells was not influenced by this treatment, but detachment of damaged cells was clearly diminished. These data confirm the protective effect of zinc acexamate against gastric aggressions. Moreover, our studies confirm the notion that mucus secretion and maintenance of continuity on the gastric lumen by surface epithelial cells is of critical importance in preventing the gastric damage induced in these experimental models.

Key Words: Scanning electron microscopy, light microscopy, gastrotoxic agents, rat gastric mucosa, gastric glands, surface epithelial cells, parietal cells, aspirin, ethanol.

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Introduction

Development of gastric mucosal damage induced by a variety of ulcerogenic agents has been studied in different experimental models [20]. The macroscopical quantification and morphological assessment of these gastric injuries have contributed to the understanding of the mechanisms involved in experimentally induced lesions which, in turn, has facilitated the development of pharmacologic therapies for the human pathological condition.

To date, macroscopical criteria used to quantify experimental lesions include measurement of the ulcer length by morphometry [8] and the use of arbitrary scales [1, 11]. The light microscope allows the determination of the depth of the injury and morphological modifications in the gastric mucosa. Unfortunately, observations in the light microscope are often restricted to a limited portion of the stomach and fine ultrastructural changes taking place on the gastric surface might be overlooked. The scanning electron microscope (SEM), initially introduced for studies on gastric topology [21, 22], permits investigation of gastric lesions at ultrastructural level [4, 15, 17, 19, 35]. The possibility of accessing different areas of the same sample, the great depth of field, and the three-dimensional viewing of the surfaces provide detailed structural information that is of great help in reconstructing the natural progression of the lesions. Morphometric evaluation by SEM is certainly limited [25], though some authors have developed morphological criteria that can be used to stage the severity of gastric lesions [34].

Zinc acexamate (ZAC) has been demonstrated to prevent the development of experimental gastric lesions [9, 10, 12]. The protective effect of ZAC seems to result from its combined action on aggressive and defensive mechanisms at the gastric mucosal level. ZAC improves gastric mucosal defense preventing the disruption of the mucosal barrier [11, 12], stabilizing biological membranes [24], and improving gastric microcirculation [6]. This zinc compound has shown additional inhibitory effects on pepsin and acid secretion [7]. A recent investigation on ultrastructural gastric damage induced by aspirin revealed that ZAC was capable of preventing

formation of deep erosions, but did not completely prevent ultrastructural damage of surface epithelial cells [5].

In the present study, SEM was used to investigate morphological changes taking place in the gastric mucosa of the rat subjected to different damaging agents which are known to result in the development of severe gastric ulcerations. Severity of the experimental lesions was quantified according to morphological criteria. Modifications in the severity of these lesions induced by pretreatment with ZAC were also analyzed.

Methods

General

Female Wistar rats of similar weight (200 ± 10 grams) and age (8-9 weeks old) were used [13]. Animals were distributed in 4 groups consisting of 20 animals each. One of these groups was used as a control and the remaining three groups were subjected to experimental gastric damage according to the methods described below. Animals were deprived of food but allowed free access to water during the 24 hours that preceded the induction of gastric damage. Half of the animals of each experimental group received a pretreatment with 100 mg/kg ZAC (p.o.) 60 minutes before exposure to the correspondent gastric aggressive agent.

Experimental models of gastric lesion

Acetylsalicylic acid (ASA): Gastric damage was induced by oral administration of 100 mg/kg ASA. Animals were sacrificed 1 hour later [5].

Ethanol: To induce gastric lesions, 1 ml of 100% or 1 ml of 70% ethanol was administered p.o. Animals were sacrificed 1 hour later [26].

Sacrifice and sampling

Animals were killed by cervical dislocation. The stomachs were reached through medial laparotomy and an incision was made in the duodenum. A polytene cannula was placed into the stomach via the oesophagus and the cavity washed with 10-15 ml of 0.15 M phosphate buffered saline (PBS) pH 7.2. Immediately afterwards, the stomach was perfused with fixative solution (2.5% glutaraldehyde in PBS). Once the solution flowed out through the duodenal incision, the pylorus and the oesophagus were ligated. Stomachs were removed from animals and immersed in fixative. After 20 minutes, stomachs were opened along the greater curvature and macroscopically inspected. Representative stripes (6 mm x 5 mm) were obtained from the fundic region of each stomach, placed in fresh fixative solution, and fixation was continued for 24 hours at 4 °C.

Sample processing for scanning electron microscopy

Fixed stomachs were washed in PBS pH 7.2 and postfixed for 45 minutes in 1.0% buffered osmium tetroxide. Samples were washed again in the buffer and dehydrated in graded series of ethanol and transferred to pure isoamyl acetate. Samples were dried by means of the critical point method, and coated with gold up to a thickness of 400 Å in a sputter coating unit (Polaron E5000). Specimens were coded so that observers were unaware of the treatments. Observations were performed in a Hitachi S2300 SEM operated between 15 and 20 kV.

Figure 1 (on the facing page). Scanning electron micrographs of rat gastric surfaces: (**A**) Normal appearance of the undamaged gastric mucosal surface in a control animal showing the smoothly undulated epithelial surface layer delimiting the openings of gastric glands and presence of the mucus layer (arrow). Treatment with aspirin caused the disappearance of the mucus layer and induced a wide range of lesions often in the same animal. (**B**) Focal erosions of the mucosal surface progressing towards the mucous neck cells. (**C**) Intercellular junctions are preserved (arrow). (**D**) Destruction of the interfoveolar surface epithelial cells with exposure of the architectural organization of the gastric glands (arrow). (**E**) Shedding of mucous and parietal cells from the basal lamina. Membranes of these cells appear well preserved. (**F**) Complex image of severely damaged mucosa with marked exfoliation, presence of blood cell elements and fibrous material. Bar = 20 μm.

Semi-quantitative morphometric analysis

The fundic mucosa of each stomach was systematically examined at low and high magnification in the SEM. A progressive score of gastric damage from 0 (no lesion) to 5 (severest damage) was established. This scoring system is similar to that used by Winters *et al.* [34] with minor modifications:

0. Gastric surface coated with mucus and normal morphology of the epithelial cells in those areas where they are visible.

1. Ultrastructural damage of isolated surface epithelial cells.

2. Ultrastructural damage affects groups of surface epithelial cells usually of the same gastric gland, but the integrity of the epithelial surface is still preserved.

3. Detachment of surface epithelial cells with exposure of the reticular framework (basal lamina); mucous neck cells are visible on the reticulum.

4. Dehiscence of cellular components of gastric glands from the basal lamina.

5. Bare reticulum with single or isolated groups of cells visible in deep portions of the remaining reticulum.

The maximum score (severest damage) observed for each stomach was recorded and used for statistical studies. Mann-Whitney rank sum test was used for statistical comparisons.


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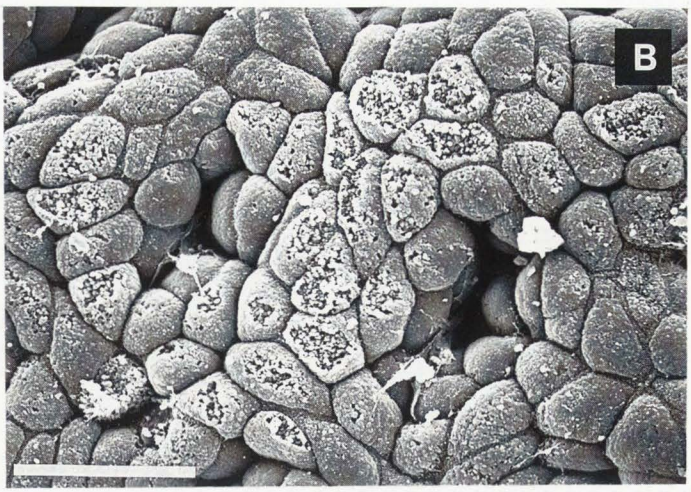
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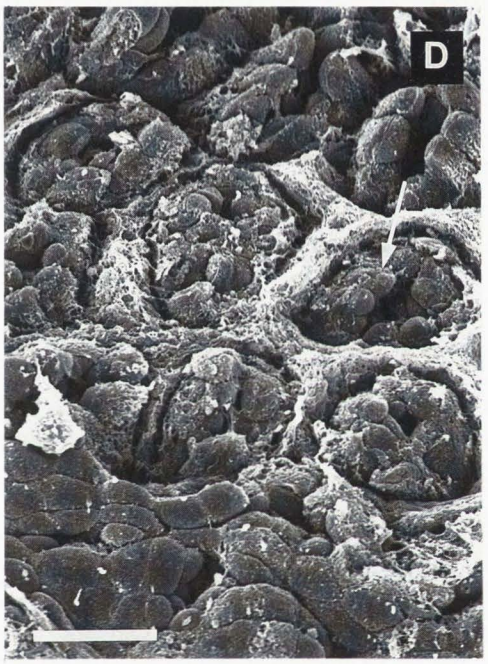
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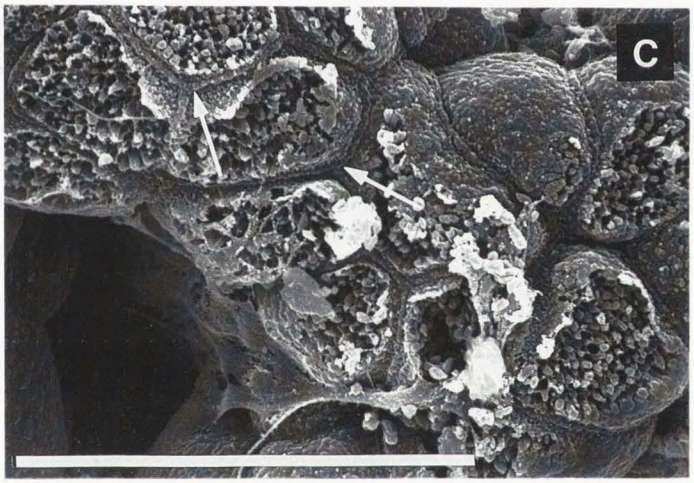
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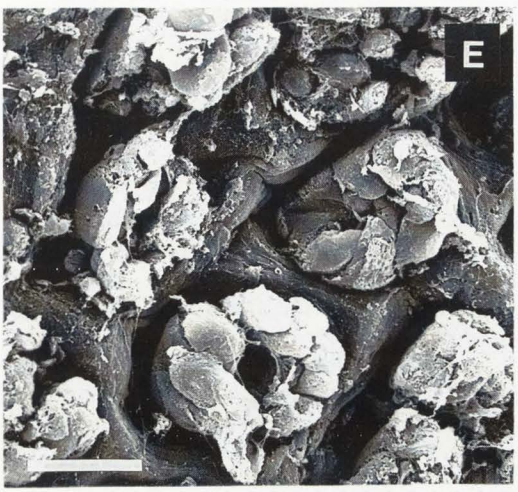
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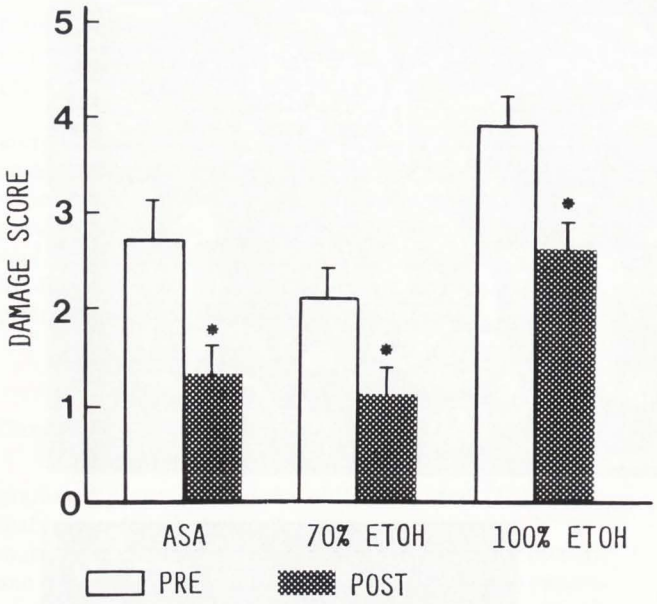


Figure 2. Bar diagrams representing average maximum scores obtained under the different experimental conditions. Results are expressed as mean ± standard error. Empty bars represent values obtained in animals subjected only to gastric aggressions. Dashed bars represent the values obtained in similar groups of animals that received a pre-treatment with zinc acexamate (100 mg/kg). Asterisk denote statistical significant differences as revealed by the Mann-Withney test: * $p < 0.05$.

Results

Morphology of gastric lesions after different experimental conditions

In the SEM, the gastric mucosa of control animals appeared smoothly undulated. Frequently the presence of a continuous layer of mucus prevented the detailed observation of the luminal mucosa. Cells of the gastric mucosal surface were easily distinguishable in areas not covered by mucus. Figure 1A shows the normal appearance of the undamaged gastric mucosal surface in a control animal revealing the convex surface epithelial cell convolutions surrounding the openings of gastric glands and the presence of mucus. Results of the semi-quantitative analysis of the gastric damage observed after the different experimental models are summarized in Figure 2. The severity of the lesions varied with the different damaging agents. Absolute ethanol caused the severest lesions followed by aspirin and 70% ethanol.

Destruction of the mucus layer and damage of surface epithelial cells was commonly observed in animals receiving ASA. The cellular damage was evidenced by cell membrane destruction that was often seen even in animals with no apparent macroscopical injury (Figures 1B and 1C). Occasionally, this cellular damage was the only sign of gastric lesion in aspirin-treated animals. However, more severe lesions such as sloughing

Figure 3 (facing page, at left). At low magnification (**A**), the presence of large areas denuded of gastric mucosa (lower left) alternating with undamaged areas (upper right) was a common finding after administration of absolute ethanol. A more detailed observation of these apparently undamaged areas (**B**) reveals that sealing cells have lost their normal appearance progressing to doughnut-like shapes that eventually slough off allowing the visualization of the basal lamina. Treatment of animals with 70% ethanol resulted in severe lesions of the gastric mucosa (**C**), but epithelial cell membrane disruption similar to that observed after aspirin was commonly observed. Bar = 30 μm (in **A**); 20 μm (in **B, C**).

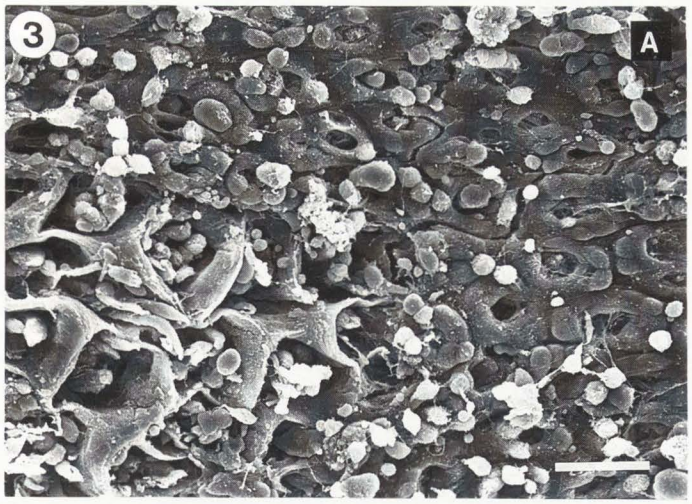
Figure 4 (facing page, at right). Appearance of the gastric mucosa in animals pretreated with zinc acexamate (ZAC). Oral administration of ZAC to control animals (**A**) resulted in an increased presence of mucus covering the mucosal surface. Surface epithelial cells appear well preserved in those areas where the mucus layer was discontinued (insert).

Pretreatment of animals with ZAC prevented the detrimental actions of the different gastric damaging conditions: (**B**) An increased presence of mucus with a fibrillar disposition was observed in those animals that were exposed to aspirin (100 mg/kg) after ZAC. At low magnification the mucosal surface beneath this mucus layer appeared well preserved. Extensive disruption of the superficial mucosal layer observed in animals only exposed to aspirin was prevented. Studies at higher magnification showed cellular damage on isolated cells. Surface epithelial cells seem more affected than those located deeper in the neck of mucous glands (insert).

In animals subjected to absolute ethanol (**C**), the most severe erosions were prevented. A thin layer of adhering mucus capping surface epithelial and neck mucous cells was evident (arrow). Bar = 20 μm.

off the more superficial cells from the secretory channels were observed in the same stomachs (Figure 1D). At this stage, cells still present in the secretory channels remained apparently attached to the basal lamina and did not show major alterations of their membranes. At a more advanced stage of the same phenomenon, shedding of cells from the secretory channels leaving the basal lamina largely denuded was more frequent (Figures 1E and 1F).

Extensive exfoliation of surface epithelial cells and exposure of the basal lamina were frequently observed in the stomachs from rats receiving absolute ethanol. Disruption and cavitation of surface epithelial cells seen after aspirin were extremely unusual after absolute ethanol. Deep erosions developing after administration of this concentrated necrotizing agent affected wide portions of the gastric mucosa and extravasated blood cells were often present. At low magnification (Figure 3A), gastric erosions were found adjacent to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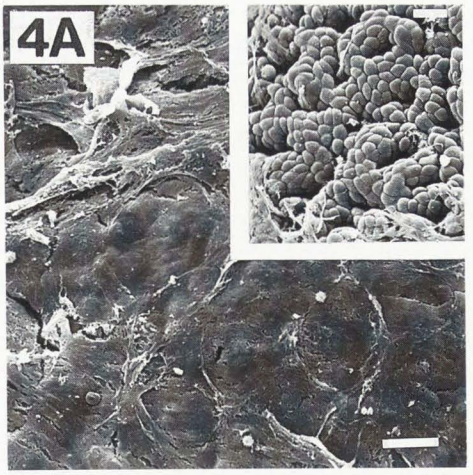
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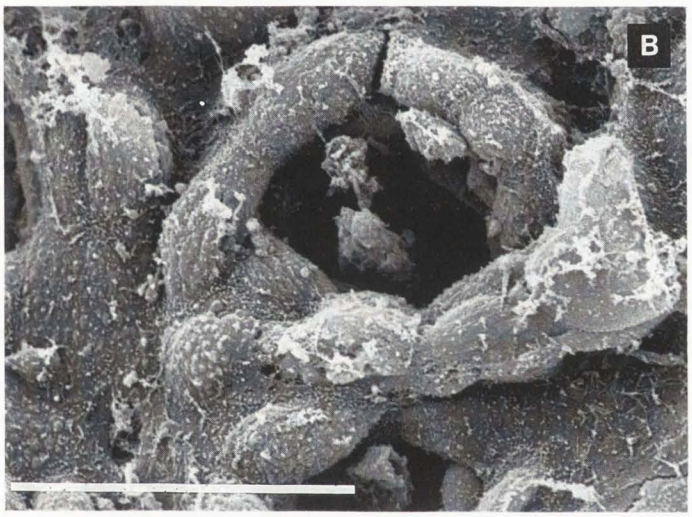
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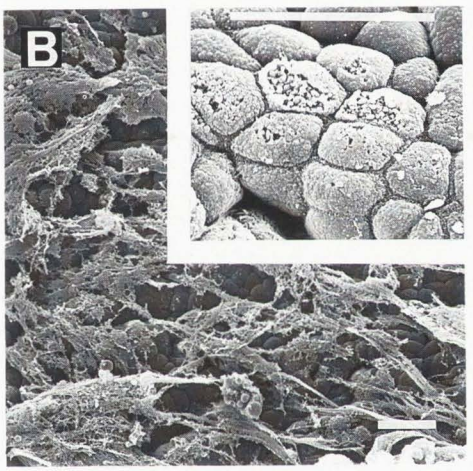
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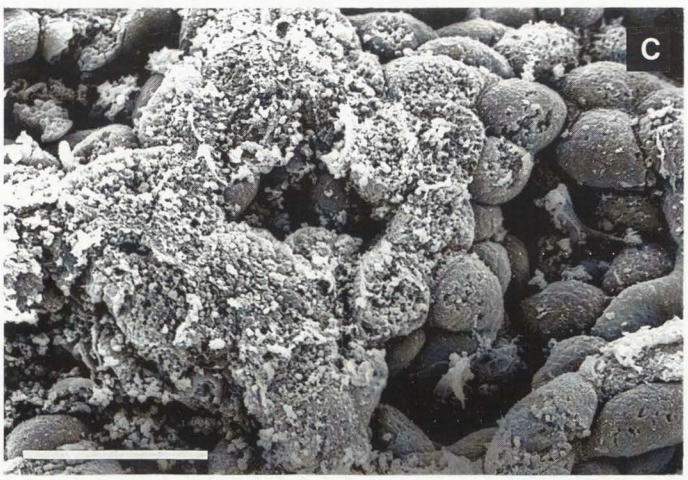
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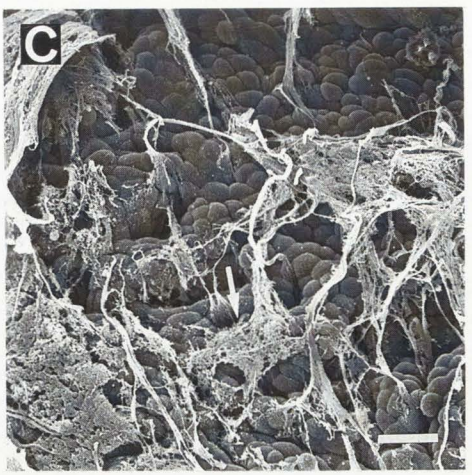
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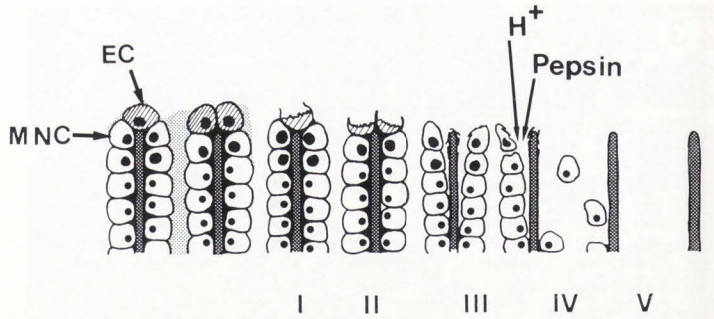

Figure 5. Schematic representation of the sequence of morphological changes inferred from this study. Stages: **I-II** = damage of surface epithelial cells; **III** = dehiscence of mucous cells located in the neck of gastric glands; **IV** = detachment of deeper cell elements; **V** = exposure of the naked basal lamina. Loss of continuity of surface endothelial cells facilitates acid and enzymatic attack on the abluminal sides of remaining cellular elements in the gastric gland.

areas where surface epithelial cells remained apparently preserved without signs of cellular damage. A more detailed observation of these cells revealed morphological changes such as flattening of the surface convexity, absence of membrane villi and progression of the cells sealing the secretory channel to a doughnut-like appearance (Figure 3B).

Gastric erosions also appeared after administration of 70% ethanol though they were less frequent and affected only limited portions of the gastric mucosa. Damage of surface epithelial cells, similar to that observed after aspirin, was again observed when ethanol was used at this lower concentration (Figure 3C).

Effect of pretreatment with Zinc Acexamate

Pretreatment of rats with ZAC resulted in a significant decrease in the severity of the lesions usually developing after exposure to the different gastric damaging agents. Statistically significant decreases ($p < 0.05$) in maximal scores obtained in the semi-quantitative morphological evaluation were observed in animals that had received ZAC (Figure 2).

An increase in the presence of mucus without changes in the normal appearance of the gastric mucosa was observed in animals of the control group that received only ZAC (Figure 4A).

Detailed morphological studies in ZAC-pretreated animals revealed that this compound prevented the development of the most severe lesions induced by the different experimental conditions. Superficial erosions of the gastric mucosa with dehiscence of mucous neck cells and deep erosions with exposure of bare basal lamina (scores 3-4) commonly observed after the different damaging agents were only occasionally seen in animals pretreated with ZAC. Conversely, ultrastructural damage of random or grouped surface epithelial cells (scores 1-2) was more frequently observed in those animals subjected to gastric damaging agents that had been pretreated with ZAC (Figures 4B and 4C).

Disruption of the mucus layer usually observed with aspirin treatment alone was less evident in ZAC-treated animals (Figure 4B). An increase in mucus output was also noticed in the group of animals subjected to 70% and 100% ethanol-induced injury (Figure 4C).

Discussion

The present study describes ultrastructural changes observed in stomachs of young adult rats subjected to different gastric damaging agents (100 mg/kg aspirin, and 70% or 100% ethanol). Maintenance of the continuity of surface epithelial cell layer seems to be of critical importance in the prevention of gastric damage developing in these experimental models. Studies performed in similar groups of animals receiving oral pretreatment with 100 mg/kg ZAC indicate that an increase in mucus secretion together with an improvement in the resistance of the epithelial mucosal coverage play a crucial role in the prevention of severe ulcerations developing under the experimental conditions used in our investigations.

Our SEM studies indicated that, with the exception of absolute ethanol which caused distinctive morphological lesions, aspirin- and 70% ethanol-induced lesions in the rat shared a similar pattern of progression. As reflected in Figure 5, a common sequence of events was recognized in our studies. First, injury of surface epithelial cells was evidenced in the form of cell membrane destruction that was only noticeable at high magnification. This type of cellular damage is often overlooked in macroscopical evaluations [5]. The ultrastructural damage affected initially random cells and extended to mucous neck cells. In a more advanced stage, the integrity of contiguous surface epithelial cells sealing gastric glands was lost resulting in exposure of the underlying cellular components of gastric glands to the gastric juice. When groups of these surface epithelial cells were found sloughing off the gastric foveolae in some areas, more severe lesions were likely to appear in other areas of the same stomach. These findings confirm previous observations suggesting that the surface epithelial cells conform a mosaic that seals the gastric glands and protects other cell components against the chemical content of the gastric lumen [27, 30, 31]. When several pieces of this mosaic are damaged, the architectural integrity is lost and the underlying cellular components of the gastric glands are exposed to the gastric content. Consequently, the dissociation of these cellular components from the basal membrane would be greatly facilitated.

A number of interpretations to the presence of cellular components attached to the basal lamina have been given in the literature. Terms such as migration, repair, and regeneration have been applied to cells attaching to or detaching from the basal lamina [16-19, 34]. Based on the analysis of images obtained in the present study, we suggest that dehiscence of the cell elements from the gastric gland occurred by peptic digestion. In fact, enzymatic digestion is currently used in the laboratory to separate different cell components from their original tissues or to detach cultured cells from their support [28, 32]. This interpretation does not preclude that some of the enzymatically detached cells might form colonies in other areas of the gastric mucosa, thus contributing to the process of repair described by other authors [16, 18, 29]. As previously stated, this enzymatic attack does not necessarily result in a complete cellular destruction [18]. In fact, a good ultrastructural preservation of the morphology of those cells still attached to the basal lamina was observed in our studies, suggesting that these cells are still viable. Despite the good ultrastructural preservation, morphological classification of detached cellular elements exclusively through images provided by the SEM would be uncertain.

Studies performed in animals pretreated with ZAC indicated that this compound protected against severe gastric damage developing under the different experimental gastric aggressions used in our experiments. Our observations suggest that ZAC increased the presence of mucus in control animals and partially preserved the integrity of the mucus sheets, apparently disrupted by the different gastric damaging agents. These results substantiate previous observations in the light microscope suggesting quantitative and qualitative changes in mucus production in rats treated with ZAC [5, 6, 10-12].

Secretion of mucus is thought to play a critical role in gastric defense against luminal aggressions. Mucus would act not only as a chemical [3] but also as a physical barrier [2] against further damaging conditions. Gastric mucus is intrinsically resistant to the gastric juice. Mucous cells are expected to resist unfavorable chemical conditions developing in the gastric lumen. However, routine light microscope studies performed in the same samples used for SEM revealed that even mucous cells located in the neck of gastric glands disappeared in severely damaged areas (data not shown). The most plausible explanation seems to be that adherent mucus would act as a protective cap for the mucosal epithelium [33], but it is ineffective against enzymatic aggressions acting on their abluminal side. The latter event is likely to occur when surface epithelial cells have been already damaged or lost (detail in Figure 5).

The importance of the continuity of the surface epithelium is reinforced by the observations in ZAC treated animals. This compound did not prevent ultrastructural damage of surface epithelial cells, but significantly reduced the progression of this damage towards aggravated ulcers. Indeed, ZAC markedly reduced the detachment of damaged epithelial cells which resulted in a better preservation of this protective layer. Zinc compounds are known to increase stability of cell membranes against labilizing conditions [23, 24] and to stabilize cytoskeletal proteins located at cell adhesion sites [14]. These cell stabilizing mechanisms are likely to be related to the resistance of epithelial cells to aggressive conditions.

Of the different damaging conditions tested in our studies only absolute ethanol showed a distinct pattern of evolution of gastric lesions. One hour after the administration of this damaging agent, apparently well preserved surface epithelial cells coexisted in the same microscopic fields with areas where severe erosions were evident. Similar morphological patterns have been perceived as an early sign of repair [17-19, 34]. To us, this interpretation is not completely satisfactory because repair should be faster under conditions causing less injury (70% ethanol). However, our studies performed with this lower concentration of ethanol showed morphological features that resembled more those described for aspirin. These observations suggest that absolute ethanol causes specific lesions through mechanisms that do not parallel those involved in other experimental models of gastric aggression used in our studies.

In summary, different methods of gastric aggression showed a common pattern of ultrastructural modifications. Pretreatment of animals with ZAC prevented severe gastric damage through increase in mucus secretion and maintenance of the continuity of the mucosal epithelium. Loss of the superficial layer of surface epithelial cells is a critical event for the development of gastric lesions in these experimental models.

References

1. Adami F, Marazzi-Uberti C, Turba C. (1964) Pharmacological research on gefarnate, a new synthetic isoprenoid with an antiulcer action. Arch. Int. Pharmacodyn. **147**: 113-145.

2. Allen A, Bell A, McQueen S. (1984) Mucus and mucosal protection. In: Mechanisms of Mucosal Protection in the Upper Gastrointestinal Tract. Allen A, Flemström G, Garner A, Silen W, Turnberg LA (eds). Raven Press, New York, pp 195-202.

3. Allen A, Hutton DA, Leonard AJ, Pearson JP, Sellers LA. (1989) The role of mucus in the protection of the gastroduodenal mucosa. Scand. J. Gastroenterol. **21**: 71-77.

4. Black BA, Morris CP, Wallace JL. (1985) Effects of acid on the basal lamina of the rat stomach and duodenum. Virch. Arch. (Cell Pathol.) **50**: 109-118.

5. Bravo L, Escolar G, Navarro C, Fontarnau R, Bulbena O. (1990) Effect of zinc acexamate on gastric lesions induced by aspirin: a morphological study. Eur. J. Pharmacol. **190**: 59-65.

6. Bulbena O, Esplugues JV, Escolar G, Navarro C, Esplugues J. (1989) Effects of zinc acexamate on

blood flow and prostanoid levels in the gastric mucosa of the rat. Prostagland. Leuk. Essent. Fatty Acids **36**: 119-123.

7. Bulbena O, Esplugues JV, Escolar G, Gil L, Navarro C, Esplugues J. (1990) Zinc acexamate inhibits gastric acid and pepsinogen secretion in the rat. J. Pharm. Pharmacol. **42**: 252-256.

8. Cho CH, Ogle CW. (1978) A correlative study of the antiulcer effect of zinc sulphate in stressed rats. Eur. J. Pharmacol. **48**: 97-105.

9. Escolar G, Camarasa J, Navarro C, Vernetta C, Bulbena O. (1987) Antiulcerogenic activity of zinc acexamate in different experimental models. Meth. Find. Exp. Clin. Pharmacol. **9**: 423-427.

10. Escolar G, Navarro C, Sendrós S, Bulbena O. (1987) Effect of cold-restraint stress and zinc acexamate on gastric mucus production in intact glands. Arch. Int. Pharmacodyn. Ther. **290**: 128-137.

11. Escolar G, Bulbena O. (1989) Zinc compounds, a new treatment in peptic ulcer. Drugs. Exp. Clin. Res. **15**: 83-89.

12. Esplugues JV, Bulbena O, Escolar G, Martí-Bonmatí E, Esplugues J. (1985) Effects of zinc acexamate on gastric mucosal resistance factors. Eur. J. Pharmacol. **109**: 145-151.

13. Farinati F, Cardin F, Dimario F, Battaglia G, Lamizzaro R, Penon G, Naccarato R. (1988) Gastric ulcer and stomach aging: pathophysiological and clinical implications. Gerontology **34**: 297-303.

14. Geiger B, Avnur Z, Rinnerthaler G, Hinssen H, Small VJ. (1984) Microfilament-organizing centres in areas of cell contact: cytoskeletal interactions during cell attachment and locomotion. J. Cell. Biol. **99**: 835-915.

15. Hollander D, Tarnawski A, Krause WF, Gergely H. (1985). Protective effect of sucralfate against alcohol-induced gastric mucosal injury in the rat. Gastroenterology **88**: 366-374.

16. Hollander D, Tarnawski A, Gergely H. (1985) Protection against alcohol-induced gastric mucosal injury by aluminum-containing compounds-Sucralfate antacids and aluminum sulfate. Scand. J. Gastroenterol. **21**: 151-153.

17. Ito S. (1987) Functional gastric morphology. In: Physiology of the Gastrointestinal Tract. Johnson LR (ed.). Raven Press, New York, 817-851.

18. Lacy ER, Ito S. (1989) Mechanisms of rapid epithelial restitution of the superficial rat gastric mucosa after ethanol injury. In: Ulcer Disease: New Aspects of Pathogenesis and Pharmacology. Szabo S, Pfeiffer CJ (eds.). CRC Press, Boca Raton, FL, pp 65-83.

19. Lacy E, Ito S. (1984) Rapid epithelial restitution of the gastric mucosa after ethanol injury. Lab. Invest. **51**: 573-583.

20. Lee YH, Bianchi RG. (1971) Use of experimental peptic ulcer models for drug screening. In: Peptic Ulcer, Pfeiffer CJ (ed.). Munksgaard, Copenhagen, pp 329-349.

21. Ogata T, Murata F. (1969) Scanning electron microscopic study on the rat gastric mucosa. Tohoku J. Exp. Med. **99**: 65-71.

22. Pfeiffer CJ. (1970) Surface topology of the stomach in man and the laboratory ferret. J. Ultrastruct. Res. **33**: 252-262.

23. Pfeiffer CJ, Cho CH, Cheema A, Saltman D. (1980) Reserpine-induced gastric ulcers: protection by lysosomal stabilization due to zinc. Eur. J. Pharmacol. **61**: 347-353.

24. Pfeiffer CJ, Bulbena O, Esplugues JV, Escolar G, Navarro C, Esplugues J. (1987) Anti-ulcer and membrane stabilizing actions of zinc acexamate. Arch. Int. Pharmacodyn. Ther. **290**: 128-137.

25. Pfeiffer CJ, Bulbena O. (1989) Gastrointestinal surface changes: Interpretation problems and indexing possibilities (A Review). Scanning Microscopy **3**: 929-935.

26. Robert A, Nezamis JE, Lancaster C, Hanchar AJ. (1979) Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl and thermal injury. Gastroenterology **77**: 433-443.

27. Robert A. (1979) Cytoprotection by prostaglandins. Gastroenterology **77**: 761-767.

28. Romano M, Razandi M, Ivey KJ. (1990) Effect of sucralfate and its components on taurocholate-induced damage to rat gastric mucosal cells in tissue culture. Dig. Dis. Sci. **35**:467-476.

29. Tarnawski A, Hollander D, Stachura J, Krause WJ, Gergely H. (1985) Prostaglandin protection of the gastric mucosa against alcohol injury - a dynamic time-related process. Gastroenterology **88**: 334-352.

30. Tasman-Jones C. (1986) Pathogenesis of peptic ulcer disease and gastritis: importance of aggressive and cytoprotective factors. Scand. J. Gastroenterol. **21**: 1-5.

31. Tasman-Jones C, Maher C, Thomsen L, Lee SP, Vanderwel M. (1987) Mucosal defences and gastroduodenal disease. Digestion **37**: 1-7.

32. Terano A, Ivey KJ, Stachura J, Sekhon S, Hosojima H, McKenzie WN Jr, Krause WJ, Wyche JH. (1982) Cell culture of rat gastric fundic mucosa. Gastroenterology **83**: 1280-1291.

33. Wallace JL, Whittle JR. (1986) The role of extracellular mucus as a protective cap over gastric mucosal damage. Scand. J. Gastroenterol. **21**: 79-84.

34. Winters C, Hinsull SM, Gregory S. (1991) A scanning electron microscopic morphological and semi-quantitative evaluation of rat stomach treated with colloidal bismuth subcitrate and alcohol. Scanning Microscopy **5**: 541-548.

35. Wood LR, Dubois A. (1983) Scanning electron microscopy of the stomach during modifications of acid secretion. Am. J. Physiol. **244**: 475-479.

Discussion with Reviewers

Reviewer I: The (semi-)quantitative ratings are only based on the most severe lesion found. However, the size and number of the damaged areas should be consid-

ered, especially in evaluating effects of different damaging conditions. How can general effects be evaluated on the basis of, maybe even a single small, though severe, lesion, without considering the condition of the whole gastric epithelium.

Authors: In the studies referred in our manuscript, all the stomachs were routinely subjected to a macroscopical inspection and subsequent grading of lesions according to criteria already published. The problem with the macroscopical grading of gastric mucosal lesions is that criteria that are useful to evaluate lesions appearing in one experimental model are inappropriate for other experimental conditions. Even measurement of lesions in mm^2, a very accurate method for the geometrically shaped lesions induced by ethanol, would underestimate scattered lesions caused by anti-inflammatories.

We agree with the reviewer that the method we describe might appear simple or limited for a precise morphometric evaluation. In our opinion, this method takes into consideration the experience gained from macroscopical evaluations and incorporates the advantage of the more detailed information provided by the SEM. Basically, in our method the severity of the lesions in each stomach is judged on the basis of their maximal depth. This method shares common points with a previous and more elaborated morphometric method described by Winters *et al.* [34]. In our hands, the semi-quantitative method we describe provides numerical data that parallels with those obtained in macroscopical methods for grading ulcers. This is the reason that prompted us to delete the table showing macroscopical evaluations of gastric lesions that otherwise would have been repetitive.

We want to emphasize that in our experience, which is generally in accordance with the literature published on experimental gastric ulcer, the possibility of a single small, though severe, ulcer is unlikely in the rat. The only experimental model that produces such type of ulcers in the rat is the submucosal injection of acetic acid [36]. In this experimental model, the size of the round shaped ulcer can be easily quantified macroscopically by measuring the diameter in mm.

In the unlikely event that a single deep ulcer (severe prognoses in humans) was found in a rat, our morphometric method would have assigned that stomach a high score which seems correct to us. Perhaps, a situation in which our method would be less reliable would be the case of very superficial lesions affecting wide areas of the gastric mucosa. In such a case, the severity of the total lesion might be underestimated. Fortunately, in the latter case the detailed topographic information provided by the SEM helps in re-qualifying such lesion and a comment would be made following the initial score obtained. Such comments, when necessary, are included in the description of the results.

Reviewer I: Presence or absence and the (again quantitative) extent of mucus seems crucial. Do the authors think that their preparation protocols (PBS-washing, glutaraldehyde-fixation, dehydration,...) allows the preservation of the mucus cover in a reproducible manner, comparable to the *in-vivo* appearance?

Authors: The method of sample preparation used in our investigations is commonly used for ultrastructural studies. The reviewer is correct, it has been widely discussed that such methods are not optimal for the preservation of the mucus layer that coats the gastric mucosa. It is likely that the mucus-cover, as observed after conventional preparation procedures, may in fact differ from its *in-vivo* appearance. However, most of the problems described for these conventional methods is that they dehydrate, shrink and distort the appearance of the mucus gel [37, 38]. It is always recommended to use unfixed sections of the gastric mucosa when accurate morphometric evaluation of mucus presence is required.

If our information is correct, extraction of mucus would limit the significance of our results if they showed decreased mucus presence, but would have little impact in our case where we found increased presence of mucus after zinc acexamate. Another possibility is that the quality rather than the quantity might have been altered under some experimental conditions. Our previous experience with zinc acexamate indicates that this compound affects both quantitatively and qualitatively the presence of mucus [5, 6, 10-12].

Reviewer II: No time course experiment was performed in the present study. Are there any particular reason for terminating the experiments 1 hour after the damaging treatment?

Authors: Our studies have been performed according to protocols currently accepted for acute studies in rat experimental models. It seems obvious that conditions can be modified to achieve certain experimental objectives. Dosage of damaging agents or time exposure to damaging conditions can be increased even to the point that pharmacological treatments (effective in human practice) would be ineffective in the animal models. In the experiments referred in our manuscript, experimental conditions were maintained for one hour. The literature and our own experience demonstrates that lesions induced during this period of time (under the damaging conditions employed) can be totally or partially reversed by pharmacological agents.

Reviewer II: Do you mean that if one specimen is graded II then the whole stomach (fundic part) will be classified as a grade II damage even though the other specimens have a normal ultrastructural appearance?

Authors: The answer to the reviewer's comment is yes. When an specimen is graded II, we assume that the whole stomach is graded II. Although this assumption may seem hazardous, please consider some additional information: **a**) after the initial 20 minute-fixation, stomachs are always macroscopically inspected; **b**) it is unusual, in the experimental models used, that gastric lesions affect isolated areas that are not included in the sample; **c**) in our experience, which is generally in ac-

cordance with the published literature on experimental gastric ulcer, the possibility of a single small, though severe, ulcer is unlikely in the rat. The only experimental model that produces such type of ulcers in the rat is the submucosal injection of acetic acid [36].

Taking into consideration the frequency of distribution of lesions in the experimental models used and the access to a wide area of each stomach provided by the SEM, the possibility of under- or over-estimating gastric lesions is more limited than it may initially seem. We think that the possibility of error, though it exist, is similar to that implied in histological procedures in sections. It is always possible that a group of sections examined in the light microscope may not contain the injured area!

Reviewer II: In the **Discussion** it is speculated a lot about the importance of mucus in protection against these various damaging conditions. The authors may be right, but there are many other possible explanations for the protective effect of ZAC. It is generally accepted that HCl plays a key role in the development of ulcers disease. Is it not possible that ZAC may reduce HCl secretion and thereby provide some protection? Have you compared the effects of ZAC with conventional H_2-blockers or omeprazol?

Authors: You are correct, zinc acexamate possesses inhibitory effects on pepsin and acid secretion. This effect on peptic and acid secretions may partially explain the protective effect observed in experimental models where acid secretion plays a role. However, this anti-secretory mechanism cannot explain the effect of zinc acexamate on experimental models such as ethanol in which acid secretion plays a limited role [9, 11]. Since this manuscript is based on ultrastructural techniques, results are discussed on the basis of the morphological findings.

Effects of zinc acexamate have been compared with those of cimetidine and ranitidine in experimental [11, 39] and clinical studies [40]. Results of experimental studies suggest that zinc acexamate may possess additional advantage over cimetidine and ranitidine in models of chronic gastric ulcer. Meta-analysis of grouped clinical trials suggest that zinc acexamate effectiveness in the treatment of peptic ulcer is similar to that of ranitidine (but with less side effects in the group of patients treated with zinc acexamate).

Additional References

36. Okabe S, Pfeiffer CJ. (1971) The acetic ulcer model: A procedure for chronic duodenal or gastric ulcer. In: Peptic Ulcer, Pfeiffer CJ (ed.). Munksgaard, Copenhagen, pp 13-20.

37. Allen A, Hutton DA, Leonard AJ, Pearson JP and Sellers LA. (1986) The role of Mucus in the protection of the gastroduodenal tract. Scand. J. Gastroenterol. **21**: 71-77.

38. Bollard JE, Vanderwee MA, Smith GW, Tasman-Jones C, Gavin JB. (1986) *In-situ* preservation of mucus in the colon of the rat. Dig. Dis. Sci. **31**: 1338-1344.

39. Navarro C, Escolar G, Bravo ML, Jiménez E, Bulbena O. (1990) Effect of Zinc Acexamate and Ranitidine on Chronic Gastric Lesions in the Rat. Digestion **45**: 121-129.

40. Jiménez E, Bosch F, Galmés JL, Baños JE. (1992) Meta-analysis of efficacy of zinc acexamate in peptic ulcer. Digestion **51**: 18-26.