Histochemical and Morphologic Changes in Gastric Mucosa of Dogs on Ulcerogenic Regimen

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STRESS generally is regarded as an important factor in the pathogenesis of peptic ulcer. The formation of stress ulcer appears to be associated with hyperacidity since stressful stimuli such as insulin hypoglycemia increase acid secretion in man and monkey. The latter hypersecretion is mediated by the vagus nerve and the adrenal gland.[1,2] Gastric secretion responds to both types of stimulation, and a potentiating effect on the secretion has been demonstrated with a combination of a vagomimetic agent and adrenocorticoid.[3] The possibility was tested that together these stimuli might be ulcerogenic. It was found that dogs subjected to combined vagal and adrenal stimulation for 3 months developed acute gastric erosions and gastritis, while control dogs treated with vagal or adrenal stimulation showed no such change.[4]

The mechanism whereby chronic vagal and adrenal stimulation effect such changes has not been elucidated. The mucous secretions derived from and coating the gastric epithelium presumably comprise a barrier to physical or chemical trauma including autodigestion. The ulcerogenic effects of vagoadrenal stimulation might be related to an influence on the mucous barrier.

Histochemical investigations have revealed the complex heterogeneity of the carbohydrate-rich gastric secretions in the dog.[5-9] Each type of epithelial cell forms a histochemically distinctive mucosubstance. In the surface epithelium, the superficial cells secrete predominantly an acid mucin rich in carboxyls, whereas the deep foveolar cells produce a mucin, the acidity of which derives from sulfate esters. Mucous neck cells and parietal cells, on the other hand, form neutral mucosaccharides containing residues with periodate-reactive vicinal glycols, presumably hexoses or deoxyhexoses. The zymogen cells and pyloric glands form periodate-unreactive sulfated mucosubstances which appear to be

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unique among epithelial secretions in their susceptibility to hyaluronidase.

The observations reported here concern histochemical changes in carbohydrate-rich constituents and histologic alterations associated with the experimental gastric erosions which developed in dogs on combined vagal and adrenal stimulation. A preliminary account of this study was reported recently.[10]

Materials and Methods

Four healthy adult female mongrel dogs, weighing 15–20 kg, were studied. Throughout the experiment, all the dogs were fed on a standard diet of commercial dog food (Kal-Kan) and 2 g of sodium chloride daily. Each dog was fed once daily, in the afternoon, with a measured quantity to provide approximately 70 calories/kg/day. The dogs were weighed at weekly intervals.

Each dog was prepared with an esophagostomy. After a 6-week period of recovery from the operation, combined vagal and adrenal stimulation was initiated. Vagal stimulation was achieved by sham feeding in the morning for 15 min daily. The esophageal mucosa was everted through the stoma of the esophagostomy during sham feeding. Adrenal stimulation was accomplished by intramuscular injection of 40 units of ACTH gel daily. At the end of 3 months, these dogs were sacrificed.

On the day of sacrifice, the dog, fasted for 18 hr to minimize variation in stored epithelial secretions, was anesthetized with intravenous Nembutal. The stomach and duodenum were fixed briefly in vivo with a solution of 2% calcium acetate in 10% neutral formalin solution and then were opened along the greater curvature and examined for gross lesions. The mucosa and muscularis mucosa were then separated from the muscular wall. Strips of mucosa, 5 mm wide, were taken along the entire length of the stomach from six equidistant curvatures and were rolled concentrically on a stick for continued fixation and subsequent embedding as described previously.[5]

The histochemical methods employed as before [5,7] for characterizing the carbohydrate-rich secretions of dog stomach included the alcian blue pH 2.5-periodic acid-Schiff (PAS) method,[11] an alcian blue pH 1.0-PAS sequence, a sequence of aldehyde fuchsin or high iron diamine followed by alcian blue, and azure A at pH 4.0. In general, the mucosubstance content of epithelial cells and phagocytes was evaluated with all of these methods; that of mast cells with azure A, aldehyde fuchsin, and high-iron diamine; and that of zymogen cells with the latter two stains plus pH 1.0 alcian blue. Sections were also stained with hematoxylin and eosin (H&E) for morphologic examination and with the von Kossa procedure for visualization of inorganic calcium deposits. Specimens from a normal control dog processed with those of the present experimental animals resembled exactly those from the 8 normal dogs previously examined, and failed to reveal the pathologic changes described here for the dogs treated with adrenovagal stimulation.

Results

Surface Epithelium

Morphologic Examination

In normal dogs, tall columnar epithelium covered the most-superficial mucosa uniformly, and lower epithelium lined the foveolae. A

vacuolated stratum with an empty appearance separated the subnuclear Golgi zone from underlying basement membrane in the superficial but not in the foveolar part of the surface epithelium (Fig 1 and 3).

In Dogs 1, 2, and 4 of the experimental group, microscopic examination confirmed the presence of mucosal erosions as noted grossly (Tables 1 and 2). The erosions lacked both superficial and foveolar surface epithelium; the tissue loss did not involve mucous neck cells or subjacent gastric glands (Fig 19–23). Dilated and congested vessels, stroma with infrequent collections of leukocytes (Fig 23), and intervening mucous neck glands comprised the floor of the erosion. In addition to erosions, focal areas with altered epithelium were noted (Fig 2).

Histochemical Examination

The most-superficial surface epithelium covering the stromal papillae usually stained for carboxymucin, and foveolar epithelium stained for sulfomucin in the dogs on the experimental regimen, as was the case in control dogs. The erosions lacking surface epithelium were evident in sections stained for mucosubstance, as in adjacent sections stained routinely.

In addition, scattered gastric foci in the dogs on the combined regimen revealed a striking histochemical change which involved the superficial but not the foveolar surface epithelium. This change possibly corresponds with the foci of altered epithelium noted in routinely stained sections and is referred to as the "lesion with decreased mucosaccharide." In these foci the abundant-to-scant cytoplasm in cells of the superficial convolutions stained lightly or not at all for mucin with the several basic dyes and with the PAS method, whereas the foveolar epithelium stained normally (Fig 12 and 13). Adjacent superficial epithelial cells often varied markedly from one to another in the staining intensity of stored secretions (Fig 12). Thick accumulations of surface mucus indicative of hypersecretion covered the surface of the superficial and often of the foveolar epithelium in these foci. Since similar changes occurred in the epithelium bordering erosions, this lesion may represent a pre-erosion alteration.

In the experimental animals, the amount and the stratification of the neutral mucosubstance varied more than normally in the Golgi region of the superficial and to a lesser extent of the foveolar surface epithelium (cf Fig 4 and 5 with Fig 3). The PAS-reactive Golgi material was particularly diminished in the lesions with decreased mucosaccharide.

Downward displacement of ballooned mucous cells constituted another striking change from normal in the surface epithelium of experi-

Table 1. Changes in Dog Gastric Mucosa After 3 Months on Ulcerogenic Regimen

	Normals	Dogs on sham feeding and ACTH			
		1	2	3	4
Chief cell mucosubstance	+++	± to ++	++ to +++	0 to ±*	0 to +†
Mucosal mast cells‡	+++ to ++++	0 to ++	0 to +++	0 to ±	0 to ++
Goblet-like mucous cells in surface epithelium	0 to ±	+ to ++	+ to ++	++*§	++§‖
Mucous cells in lamina propria	0 to ±	+ to ++	+ to ++	0	0 to ±
Giant cells in lamina propria	0 to ±	0	0	++ to +++	++ to +++†
Intermediate zone cysts	0	0	0	+++*	± to ++‖
Ballooned parietal cells in distal corpus	0	+ to +++	0 to ++	0	0 to +
Curvature with erosion(s)	0	AG	PG	0	L
Curvature(s) with numerous displaced mucous cells	0	PG	G, PG	G, PG	AL

The values represent relative prevalence or extent of each feature throughout four gastric curvatures including the lesser (L), anterior lesser (AL), greater (G), posterior greater (PG), and anterior greater (AG) for each dog except that the lesser curvature was not included for Dog 3.

* Except AL curvature showed + to ++ zymogen cell mucosaccharide, few or no depressed mucous cells, and no intermediate zone cysts.

† Except in L and PG curvatures, zymogen mucosubstance was + to ++, and in L curvature giant cells of the lamina propria were +.

‡ Values refer to prevalence of mast cells in gastric lamina propria. All animals with decreased mast cells in the gastric mucosa showed more abundant and basophilic mast cells in the esophageal mucosa and gastric submucosa, although these cells were somewhat decreased in the latter sites compared with normal.

§ Downward dislocated mucous cells were enlarged and had a goblet-like appearance.

‖ Only AL curvature revealed the depressed, goblet-like mucous cells and more than ± intermediate zone cysts.

mental Dogs 3 and 4 (Fig 6 and 8; Tables 1–3). Specimens from these dogs exhibited numerous dislocated cells in which excessive mucous secretion distended the apical half or more of the cell in a goblet-type configuration. These enlarged goblet-like cells protruded deep to the apical layer of mucous secretion in the superficial surface epithelium and lay at the level of the nuclei or subnuclear Golgi region of neighboring epithelial cells. The hyperplasia of goblet-like cells and of epithelial cells occurred predominantly in the proximal stomach and in curvatures most involved with other changes (Table 2). The stratified appearance imparted by the depressed, mucin-filled goblets clearly differed from

Table 2. Changes According to Gastric Curvature in 2 Dogs on Combined Sham Feeding-ACTH Treatment

Curvature	Chief cell mucosubstance	Mucosal mast cells	Goblet-like mucous cells in surface epithelium*	Giant cells in lamina propria	Intermediate zone cysts	Erosions
Dog 3						
AL	+ to ++	0 to ±	0 to ±	++	0	0
G	0	0	++	++	+++	0
PG	0	0	++	+++	+++	0
Dog 4						
L	+ to ++	0 to +	0	0 to +	±	+
AL	0	0	+++	+++	++	0
G	± to ++	0	0	++	±	0
PG	+ to +++	0	0	++	0	0

The values represent relative prevalence or extent of each feature throughout several gastric curvatures including the lesser (L), anterior lesser (AL), greater (G) and posterior greater (PG).
* These cells occurred mainly in the cardiac region and proximal half of the corpus.

Table 3. Carbohydrate Histochemistry of Normal and Pathologic Structures in the Dog Stomach

Staining procedure	Control dogs			Dogs subjected to adrenovagal stimulation			
	Superficial surface epithelium	Foveolar surface epithelium	Mast cells	Goblet-like mucous cells in epithelium	Mucous cells in lamina propria	Giant cells in lamina propria	Cysts in lamina propria
Aldehyde fuchsin-alcian blue sequence	B–PB	P	P	B–PB–P	BP–P	BP–P	BP–P
High-iron diamine-alcian blue sequence	B–NB	N	N	BN–N	NB–N	O–B	O–B–BN
Alcian blue pH 2.5-PAS sequence	BP	BP	O–B	BP	BP	O–B	R–BP
Diastase-PAS	R	R	O	R	R	O–Pk	R
Azure A pH 4.5	B	V	P	V	—	B	B
von Kossa	O	O	O	O	—	F	F

The letters indicate strong staining observed consistently: B, blue; F, brown; N, black; P, purple; Pk, pink; R, red; V, violet; O, no staining.

pseudostratification attributable to the plane of section being tangential to either the most-superficial epithelium or to a foveolar opening.

The depressed goblet-like cells occurred not only as single cells, but occasionally as groups of two or more cells (Fig 8). In addition, cellular hyperplasia of the surface epithelium occurred in those gastric regions showing proliferation of depressed goblet-like cells. This epithelial hyperplasia resulted in formation of one or more acinar buds projecting laterally from the vertically oriented lumen (Fig 7).

The cytoplasmic secretory material in the depressed goblet-like cells stained weakly or strongly for sulfomucin or a mixture of sulfo- and carboxymucins in different cells. The goblet hyperplasia was less apparent in sections stained with H&E (Fig 6) than in those stained histochemically.

The first and second dogs on the combined stimulation regimen showed a moderate number of relatively small mucous cells which were displaced downward, lying deep in the surface epithelium at or below the level of the neighboring nuclei (Fig 4 and 5; Table 1). The depressed mucous cells in these dogs appeared contracted below normal size, rather than distended as in the other 2 dogs on the treatment; however, they disclosed mucosaccharide with histochemical stains. Such downward displaced mucous cells occurred very infrequently in sections of stomachs from the control dogs.

Zymogen Cell

In the zymogen cells of all 4 experimental dogs, vacuolation of the apical cytoplasm and loss of basophilia of the basilar chromidial substance, was evident with routine H&E staining (cf Fig 16 and 17). As indicated especially by aldehyde fuchsin staining but also by other methods, the vacuolated zymogen cells were depleted of sulfated mucosaccharide (Fig 14 and 15; Tables 1 and 2). The degree of mucosaccharide depletion was greatest proximally and varied in different gastric curvatures.

Parietal Cell

In the experimental dogs, some of the parietal cells appeared ballooned and failed to stain with routine or histochemical methods. These ballooned cells occurred in the deep portion (Fig 18) of the more distal gastric glands; the superficial and relatively proximal parietal cells usually retained their normal morphology and PAS reactivity.

Superficial Lamina Propria

The histochemical stains disclosed mucus-containing cells in the

superficial lamina propria in experimental Dogs 1 and 2 (Fig 4 and 5; Tables 1 and 3). These stromal mucous cells extended beyond the basement membrane a short distance into the superficial lamina propria, usually at or near the tips of the stromal papillae. The histochemical reactivity of the acid mucosubstance in the stromal mucous cells resembled that of the sulfo- and carboxymucins in the surface epithelium and differed distinctly from that of the heparin in stromal mast cells or neutral mucosubstance in the infrequent stromal macrophages (Table 3). The lamina propria of control dogs very rarely contained cells with histochemically demonstrable acid mucosubstance.

Dogs 3 and 4 revealed multinucleated giant cells which were located close beneath the surface epithelium and usually near the tip of the papillae in the proximal half of the stomach. In H&E-stained sections the giant cells contained several nuclei and abundant pink cytoplasm with coarse granules (Fig 6). Histochemical methods disclosed the presence in these cells of sulfated mucosubstance, which differed from that of the secretion in surface epithelium but to a limited extent resembled that of mast cells (Fig 8 and 9; Tables 1 and 3). Focal areas in the cytoplasm of the giant cells usually reacted moderately to strongly with the von Kossa method for localizing calcium. The curvatures of the stomach most involved differed in the 2 dogs and corresponded in general with those most affected with the other changes described excluding erosions (Table 2).

The distal corpus and intermediate zone of the stomach in Dogs 3 and 4 were the sites of another abnormality consisting of variable-sized, irregular cysts (Fig 10; Table 1). The content of the cysts exhibited sulfated mucosaccharide (Fig 10) and calcium, as did the cytoplasmic substance of the giant cells, but the cysts' contents differed from that of the giant cells in some histochemical reactions (Table 3). Deposits histochemically similar to those in the cysts were seen in spaces in infrequent neighboring giant cells (Fig 11), suggesting that the cysts may be late degenerative stages of such giant cells.

The cysts usually occupied the superficial half of the mucosa. Lying in the stroma between glands, the cysts caused no obstruction of glands. The type of giant cell seen in the proximal stomach terminated where cysts began, so that these two lesions did not overlap.

Mast Cell

The experimental dogs lacked the heavy infiltrate of mucosaccharide-rich mast cells normally present throughout the gastric lamina propria (cf Fig 14 and 15; Table 1). Mast cell depletion, although clearly apparent with any of the mucosaccharide staining methods, was least

evident in aldehyde fuchsin-stained sections. The latter was the most sensitive method for demonstrating dog mast cell granules and allowed visualization of a moderate number of weakly reactive mast cells, indicating degranulation as well as depletion of mast cells in the combined-regimen dogs. Reversing the normal pattern, mast cells in the submucosa of the stomach and the mucosa of the distal esophagus outnumbered those in the lamina propria of the stomach in the experimental dogs.

Discussion

Altered capacity of the surface epithelium to secrete a "mucous barrier" has been proposed as a possible defect predisposing to gastric ulceration.[12] Indeed, histochemical studies have demonstrated decreased mucous secretion in and bordering stress ulcers produced by restraint.[13] The diminished histochemical reactivity for mucin in the lesions with decreased mucosaccharide observed here seems consistent with the biochemical evidence for decreased sialic acid in gastric secretion after cortisone,[14] particularly in view of the fact that the superficial epithelium showing diminished basophilia is the location of carboxymucin.

Acute vagal stimulation by means of feeding or drug administration is known to increase the formation of gastric mucosubstance [15-17] and, in a recent histochemical and autoradiographic study, was shown to induce secretion and subsequent increased mucous synthesis by the crypt (foveolar) cells.[18] Possibly in the present study the observed depletion of mucosubstance in the superficial surface epithelium reflects predominance of the cortisone action. On the other hand, this diminished mucin reactivity may result from an increased secretory rate and failure of the (cortisone-inhibited) biosynthetic mechanisms to keep pace with secretory demands imposed by chronic vagal stimulation. Actually, diminished mucin has been observed in histochemical studies of vagal-mediated ulcers induced in rats by restraint stress.[13] The abnormal, thick layer of mucus overlying the lesions with decreased staining of superficial cells suggests hypersecretion by these cells. Alternatively, the thick mucous layer covering these foci may indicate a qualitatively atypical secretion. Clearly, the response to the chronic, combined stimulation involving principally the superficial cells differs from the response to acute vagal stimulation, which affects primarily the crypt cells.[18] Autoradiographic studies with selective precursors of mucosubstance such as $S^{35}O^{=}_{4}$ or tritiated glucose, by determining rates of mucosaccharide turnover in the epithelium, might evaluate whether the foci with decreased mucin in the superficial epithelium result from hypersecretion or retarded biosynthesis of acid mucosubstance.

Although focal in nature, the lesions showing diminished mucous secretion in the superficial epithelium and associated morphologic alterations appear more related to development of erosions than do the other epithelial and stromal abnormalities noted. These foci of superficial epithelium with diminished mucin, although encountered separately, often bordered erosions and exhibited a gradient of increasing change from the more distant periphery, toward the edge, and finally to the center of the erosion. Thus, lateral to the erosion histochemically altered superficial epithelium was encountered, whereas at the periphery of the erosion superficial epithelium was missing, and in the center of the lesion foveolar epithelium was absent as well. This spectrum of changes suggests that the erosion develops in conjunction with initial alteration of the mucinous secretion of the most-superficial surface epithelium.

The superficial extent of the erosions, and their lack of a leukocytic infiltrate and fibrosis, leave some question about these being chronic lesions. Stomachs of 4 dogs, examined grossly and by routine light microscopy after 1 month on the combined regimen, lacked erosions; of 2 dogs examined more fully at 2 months, neither exhibited erosions, but 1 disclosed the lesion which consists of decreased reactivity of epithelial mucin and is thought to precede erosions. Additional experience at shorter experimental intervals will be needed to determine whether the erosions are acute lesions which develop early in treatment; whether they appear only after prolonged treatment; and whether they may occur, heal, and recur regularly during the course of the experiment, perhaps giving rise thereby to other changes.

The pathogenesis of the displaced, goblet-like mucous cells remains an intriguing question. The distribution of the hyperplastic goblet-like cells does not correspond particularly with that of the erosions (Table 2), and the relationship between these two changes is uncertain. The goblet-like cells, crowded in and displaced beneath the adjacent epithelium (Fig 8), may result from epithelial hyperplasia. The crowding from increased cell replication for unknown reasons might force these cells toward the basement membrane rather than the lumen. Secretion would then accumulate in such dislocated cells deprived of access to the lumen, effecting their ballooned distention. The lateral glandular budding occasionally observed in the areas showing the goblet-like cells (Fig 7) further indicates the occurrence of epithelial hyperplasia in the specimens. Such hyperplasia possibly results from regeneration of eroded epithelium and conceivably might require cycles of epithelial erosion and replacement. Further morphologic or autoradiographic studies of mitotic activity in the gastric epithelium of dogs

subjected to adrenovagal stimulation perhaps would clarify whether the rate and site of epithelial cell replication is altered in connection with, or independent of, erosion and regeneration cycles.

The origin of the giant cells in the superficial lamina propria of dogs on the ulcerogenic regimen is not known. That these multinucleated cells represent fused mast cells may be suspected from their location where mast cells are normally abundant, from the coincidental absence of the mast cells in the same area, and from their content of sulfated mucosaccharide. The giant-cell mucosubstance resembles heparin of mast cells in some respects (Table 3), but differs in its lack of affinity for either azure A or high-iron diamine. Stearic hindrance to combination with the dye introduced by some associated component might account for such diminished basophilia, however.

The downward displacement of epithelial mucous cells toward the base of the epithelium and across the basement membrane into the lamina propria suggests another process by which the giant cells could have developed. Macrophages undertaking phagocytic disposal of aberrant epithelial cells in the lamina propria might fuse to form the mucosaccharide-rich giant cells of the lamina propria. The latter consideration need not exclude participation of mast cells in the genesis of the giant cells, since mast cells appear to have some association with phagocytic activity.[19] The von Kossa reactivity noted in these giant cells, demonstrating their content of calcium, perhaps is related to their marked basophilia. Calcification in these cells provides another instance of calcium deposition at the site of acid mucosaccharide comparable to that in cartilage of aged rats and in renal or biliary concretions.

The observed decrease in the mast cell infiltrate could be significant in regard to a possible mechanism for ulcerogenesis. A previous report described degranulation of mucosal mast cells in the rat stomach following ACTH or glucocorticoid treatment, noting in further agreement with the present results the relative resistance of the submucosal mast cells to these hormones.[20] Possibly, histamine released from the mast cells predisposes to ulcerogenesis through an effect on gastric vascular dilatation or acid secretion. Support for this view derives from the demonstration of associated microcirculatory changes and decreased mast cells in the stomachs of rats developing gastric ulcers during restraint.[21] However, doubt has been expressed that mast cells contribute importantly to the overall histamine content of the stomach, and other cells, such as Schwann cells, have been considered as a source of histamine.[22]

A point of interest in connection with the depletion of mucosubstance in the zymogen cells of the test animals concerns the known antipeptic

action of sulfated polysaccharide.[23,24] The sulfated mucosaccharide visualized histochemically in dog gastric zymogen cells could function in regulating activity of pepsin in the chief cells and their secretion, and its depletion by an ulcerogenic regimen might result in decreased peptic control and predilection to ulcer development. Whether sulfated mucosaccharide is more diminished than pepsin in the animals investigated here is not known, but in animals responding to acute vagal stimulation both sulfated mucosubstance and pepsinogen appeared to be depleted.[18]

Coincident with the development of gastric erosions in the dogs subjected to vagoadrenal stimulation, a definite disturbance in the composition of the carbohydrate moiety of gastric mucus has been found.[25] The overall change in the biochemical composition of the mucus can be expressed as a significant decrease in the carbohydrate (total) to protein ratio in the gastric secretion. Mucoproteins owe their characteristic resistance to proteolysis to their carbohydrate components.[26] A decrease in these components relative to the protein should render a mucoprotein molecule more subject to hydrolysis by gastric proteolytic enzymes.

Summary

Dogs subjected to combined sham feeding and adrenocorticotropic hormone (ACTH) were examined for changes in the histology and carbohydrate histochemistry of the stomach. This combined stimulation exerted an ulcerogenic influence on the canine gastric mucosa, inducing erosions of the superficial surface epithelium. Gastric erosions involved loss of superficial, and to a lesser extent of foveolar, surface epithelium. The floor of the erosion consisted of mucous neck glands, congested vessels, collagen, and infrequent leukocytes.

Foci observed at the border of erosions and elsewhere in the gastric mucosa showed diminished staining for mucinous secretion in the most-superficial, but not the foveolar, part of the surface epithelium. An abnormal, thick layer of mucus overlay these foci.

Other epithelial changes included downward displacement of surface epithelial mucous cells; focal hyperplasia of enlarged and depressed, goblet-like cells in the surface epithelium; depletion of sulfated mucosaccharide in chief cells; and ballooning of some of the deeper and more distal parietal cells.

Stromal changes included the presence of mucous cells in the superficial portion of the stromal papillae; presence of mucosaccharide-rich, calcium-containing giant cells and cysts in the lamina propria; and degranulation or depletion of the normally abundant stromal mast cells.

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[*Illustrations follow*]

Legends for Figures

All photomicrographs illustrate mucosa from the proximal or midcorpus of the dog stomach, except Fig 18, which shows intermediate zone. Unless stated otherwise, the sections were stained with hematoxylin and eosin.

Fig 1. Gastric surface epithelium and stromal papilla from control dog. Note regularly aligned spaces at base of superficial epithelial cells and compression or absence of such spaces at base of foveolar cells. Nuclei in the latter appear more condensed and less elongated. The three nuclei at the level of these basal spaces *(arrow)* are presumably in leukocytes. A space between the most superficial nuclei appears empty except for a pyknotic nucleus. Since cells with similar spaces containing material showing histochemically reactive mucin are seen infrequently in sections stained for carbohydrate, these spaces may represent degenerating mucous epithelial cells about to be extruded. Such spaces between epithelial cells and leukocytes infiltrating the basal vacuolated layer are rare in control specimens. × 400.

Fig 2. Focal change in superficial surface epithelium of experimental dog. Note decrease in apical cytoplasm and disorderly arrangement of both epithelial cell nuclei and spaces at base of epithelial cells. Several nuclei suggestive of leukocytes infiltrate layer of basal vacuoles. A space, empty except for a pyknotic nucleus *(arrow)*, occupies the basal layer. Similar-appearing cells containing mucin often are seen in this location in sections stained for carbohydrate, indicating that these are depressed and apparently degenerating epithelial mucous cells. × 400.

Fig 3. Surface epithelium and stromal papilla from control dog. Acidic mucous secretion stored apically in surface epithelial cells stains intensely. Above and especially beneath unstained nuclei lie evenly stratified Golgi zones which stain for neutral mucosubstance *(short arrows)*. Mucinous material in Golgi zones varies, being nearly absent in most-superficial epithelium. Orderly layer of empty-appearing spaces lies between Golgi zones and basement membrane. A cell in the lamina propria, presumed to be a macrophage, contains reactive material rich in neutral mucosaccharide *(long arrow)*. Such cells are infrequent in control specimens, and stromal cells demonstrative of acid mucosubstance are rarely if ever observed in control dogs (cf Fig 4 and 5). Alcian blue pH 2.5-PAS. × 400.

Fig 4. Surface epithelium and stromal papilla of experimental dog. Downward displaced epithelial cell with mucus accumulation lies beneath nuclei *(short arrow)*. Mucous cells with secretion stained like that in epithelium extend into lamina propria *(long arrows)*. Subnuclear spaces in superficial epithelium appear absent or disordered. Alcian blue pH 1.0-PAS. × 400.

Fig 5. Experimental dog. Several downward displaced cells with acidic mucosubstance *(short arrows)* are seen in basal epithelium. One of these toward the left lies in a dilated space. Mucus-laden cells are present also in upper lamina propria at far right *(long arrow)*. Alcian blue pH 2.5-PAS. × 250.

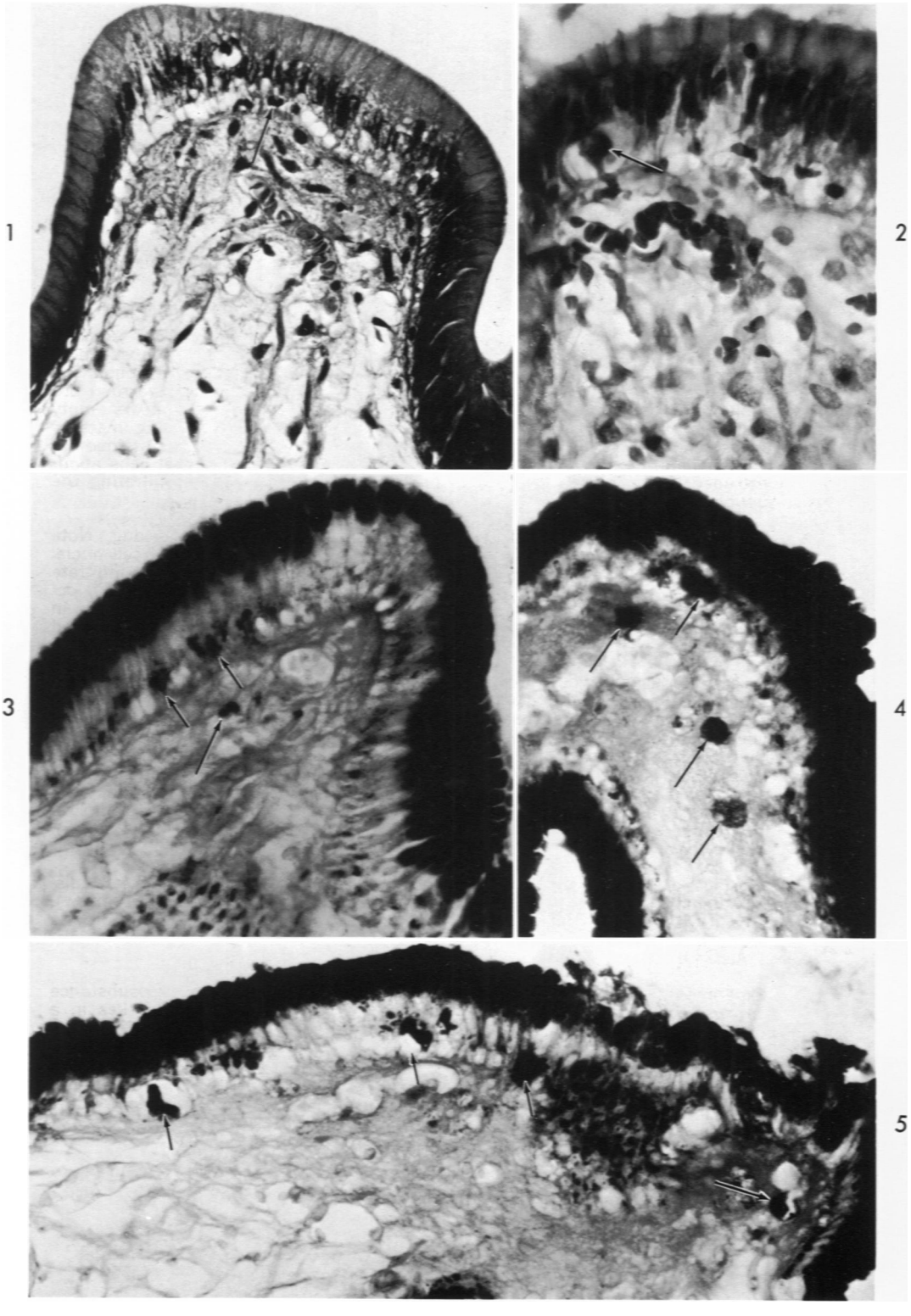

Fig 6. Experimental dog. Enlarged cells distended with mucus in a goblet-like configuration (*arrows*) lie at level of nuclei deep to apical layer of mucous secretion in superficial epithelium. Compressed pyknotic nuclei lie at bottom of ballooned mucous cells, and spindled pyknotic nuclei of neighboring cells bend around goblet-like cells. A neighboring cell with empty-appearing cytoplasm and pyknotic nucleus underlies nuclear layer. A giant cell partially surrounded by dilated spaces in lamina propria borders epithelium closely. × 400.

Fig 7. Experimental dog. Several gastric foveolae showing hyperplasia of distended mucous cells with budding of gland-like formations (*arrows*) from central lumen. × 400.

Fig 8. Experimental dog. Numerous ballooned mucous cells with goblet-like contour are displaced downward in epithelium. Mucous secretion in goblet-like cells and adjacent surface epithelium in this dog stained lighter than that in controls. Staining of goblet cells reflected predominantly a content of sulfomucin like that of normal foveolar epithelium. Intense staining of giant cells in lamina propria (*arrows*) indicates their content of sulfated mucosaccharide. Aldehyde fuchsin-alcian blue. × 400.

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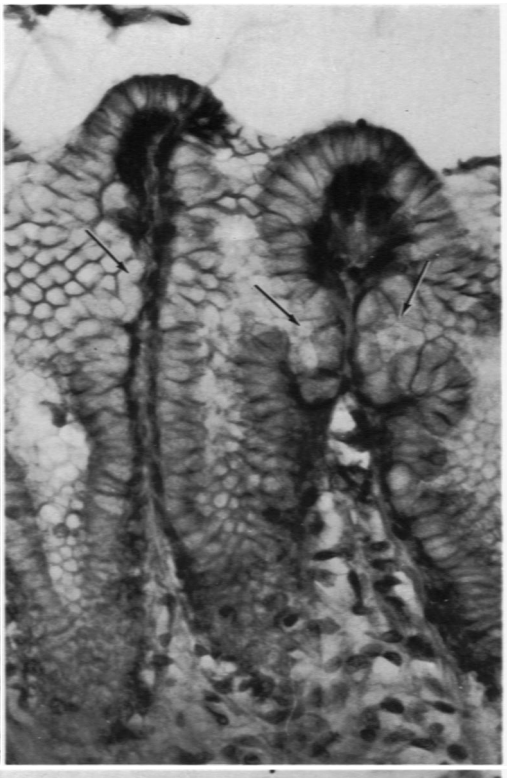

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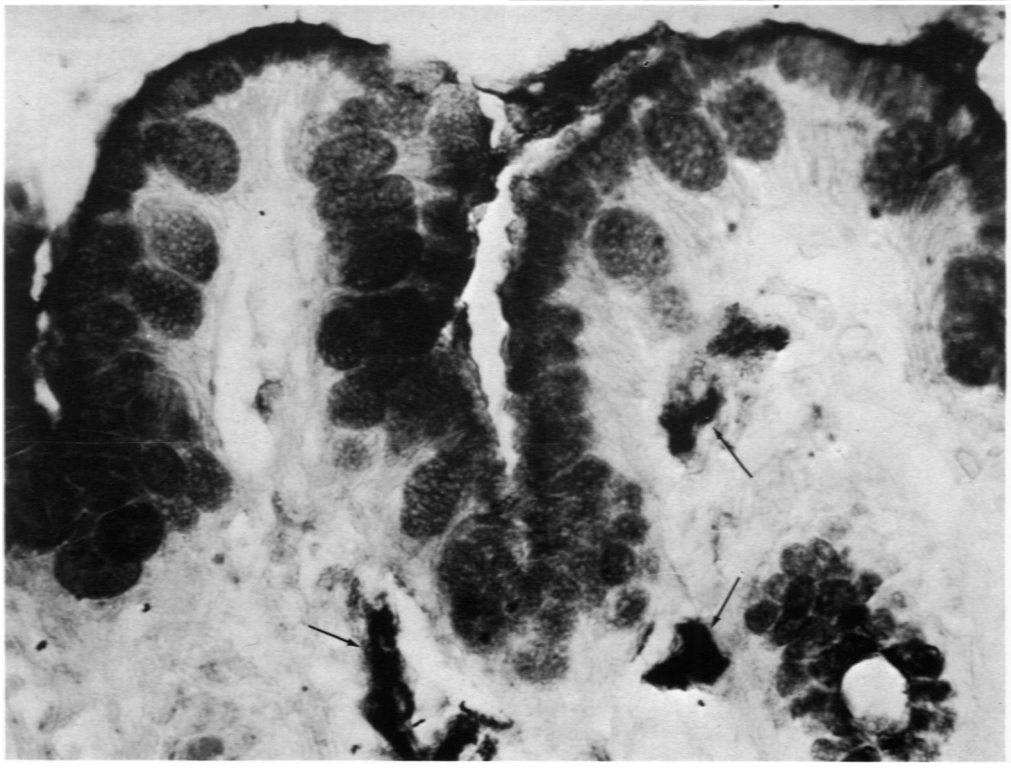

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Fig 9. Experimental dog. Superficial lamina propria encloses numerous giant cells (*arrows*) which contain abundant sulfated mucosaccharide. Aldehyde fuchsin-alcian blue. × 250.

Fig 10. Experimental dog. Cysts (*arrows*) near midlevel of mucosa in intermediate zone contain material rich in sulfated mucosaccharide. Aldehyde fuchsin-alcian blue. × 100.

Fig 11. Experimental dog. A small cyst with a multinucleated giant cell forming the cyst wall. Hematoxyphilic material like that in neighboring larger cysts nearly fills the cyst space. Such structures suggest that cystic degeneration of giant cells may occur in genesis of larger cysts. × 1000.

Fig 12. Experimental dog. Staining of superficial epithelium at top of this tangentially sectioned mucosal area varies in neighboring cells from negligible (*arrows*) to intense (cf normal superficial epithelium, Fig 3). Lumens contain abundant, lightly stained secretion. Since weak staining of mucous secretion in superficial epithelial cells occurred usually in proximity or at border of erosions, this change possibly precedes loss of epithelium in erosions. Alcian blue pH 2.5-PAS. × 250.

Fig 13. Experimental dog; lesion with decreased mucosaccharide. Weakly stained apical secretion in most superficial epithelium (*arrows*) contrasts with heavily stained secretion in foveolar epithelium. Subnuclear Golgi zones appear absent or weakly stained in superficial epithelium. A thick layer of darkly stained surface mucus like that covering the epithelium here does not normally adhere to the surface epithelium. Alcian blue pH 2.5-PAS. × 400.

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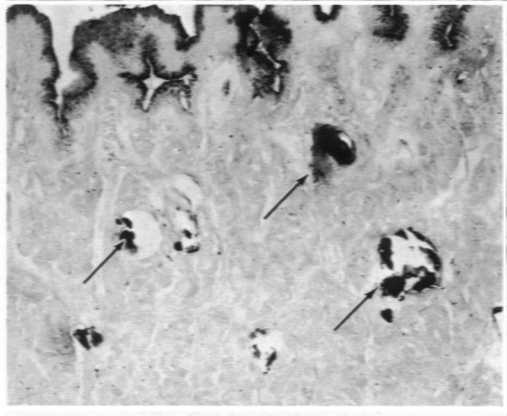

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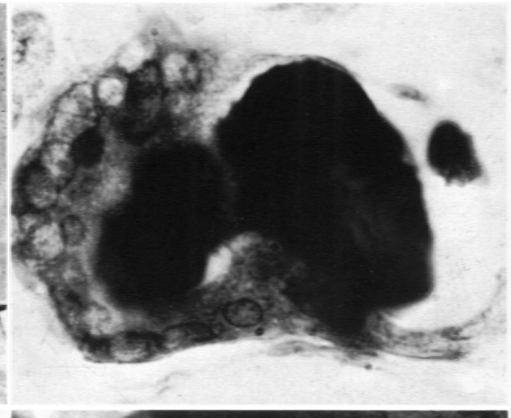

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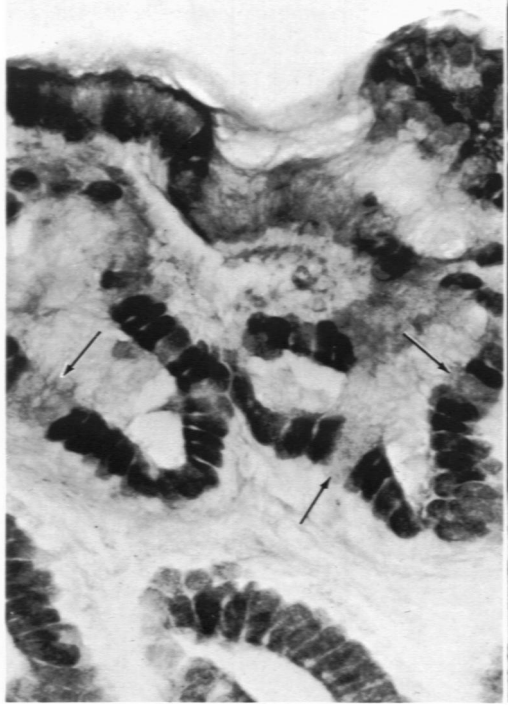

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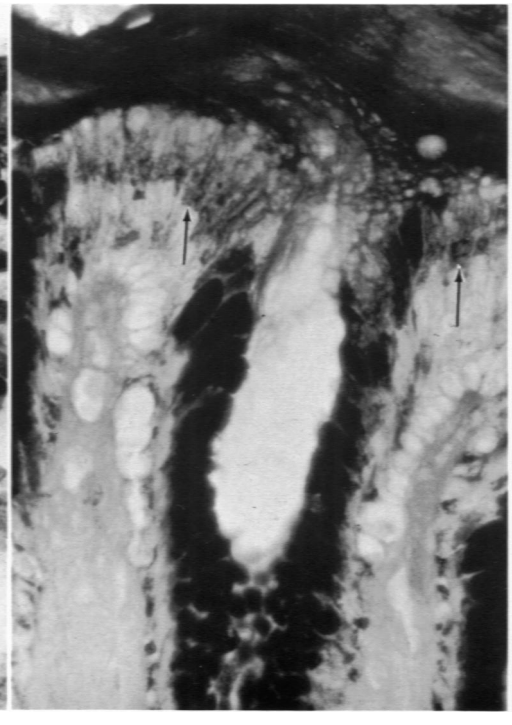

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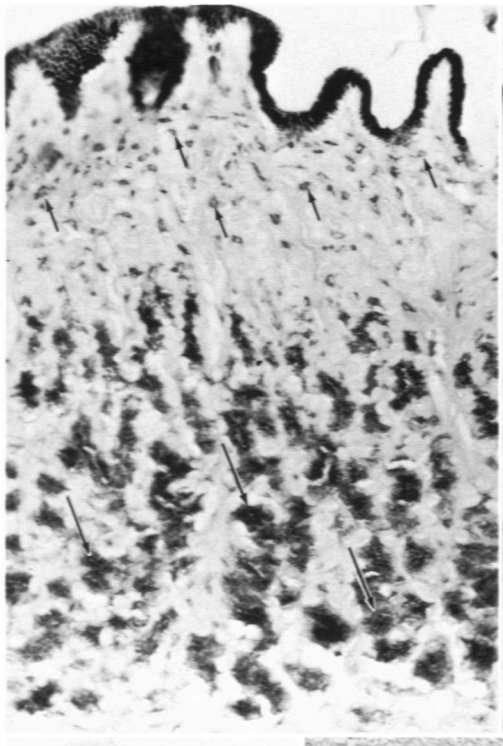
Fig 14. Normal dog. Numerous well-stained mast cells in lamina propria (*short arrows*) and abundant strongly stained secretion in zymogen cells (*long arrows*) are characteristics of gastric corpus in control dogs. Sulfated mucosubstance is present in these sites. Aldehyde fuchsin-alcian blue. × 100.

Fig 15. Experimental dog. Note absence of staining normally conspicuous in mast cells and chief cells (cf Fig 14), and persistence of normal staining in surface epithelium.

Fig 16. Normal dog. Chief cells predominant in bottom two-thirds of gastric glands are outlined by ribonucleic acid-rich, hematoxyphilic cytoplasm at base of cells. × 60.

Fig 17. Experimental dog. Chief cells lack basophilia normally conspicuous at base of cell (cf Fig 16). × 40.

Fig 18. Experimental dog. The more-superficial, normal-appearing parietal cells toward top of micrograph show the moderate staining of cytoplasm that is usually seen in these cells (*short arrows*). Most of the parietal cells toward the bottom are distended with unstained cytoplasm (*long arrows*). Alcian blue pH 1.0-PAS. × 400.



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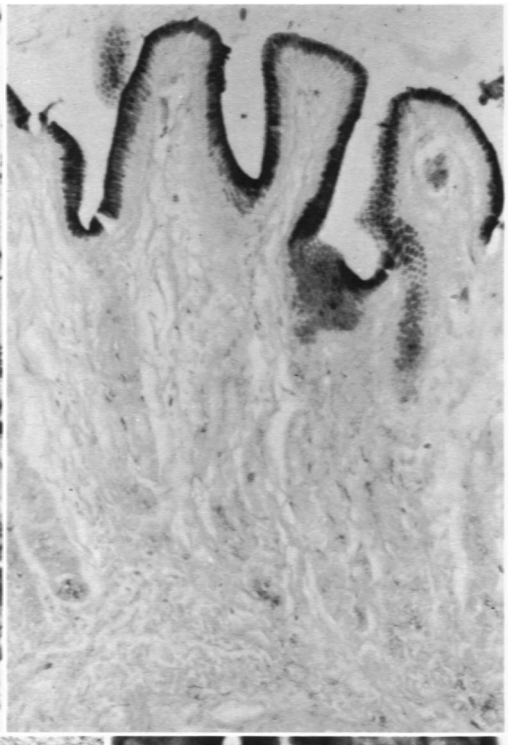

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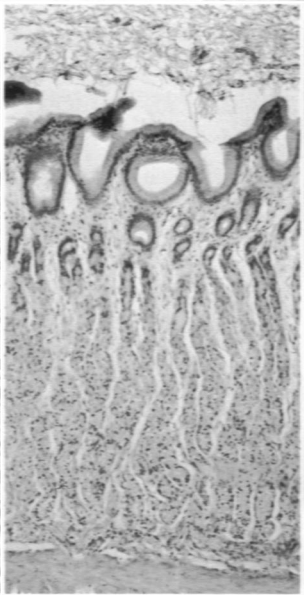

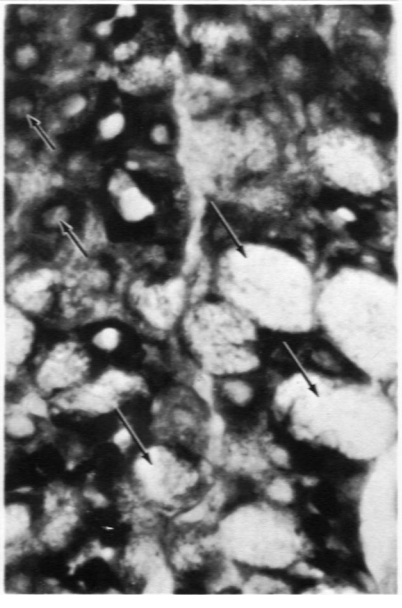

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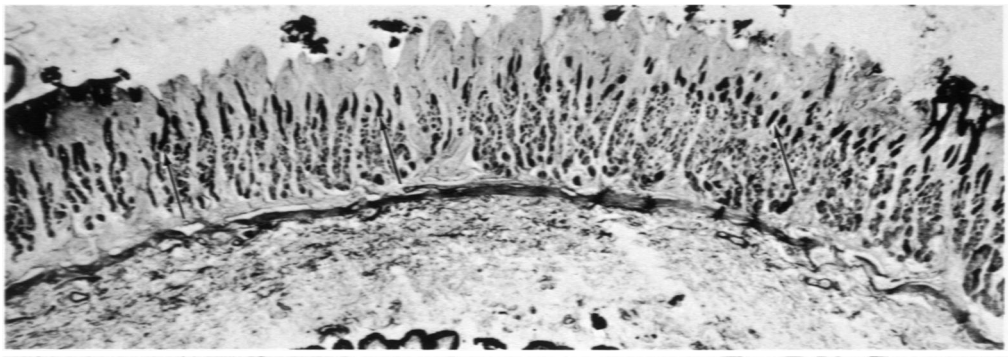
Fig 19. Experimental dog. Gastric erosion. Superficial and foveolar surface epithelium is absent, but epithelium lining mucous neck (*arrows*) and gastric glands remains intact. Alcian blue pH 2.5-PAS. × 25.

Fig 20. Experimental dog. Section adjacent to that in Fig 19 at border of erosion showing absence of surface epithelium in right half of micrograph. Floor of erosion consists of connective tissue which contains congested vessels and extravasated erythrocytes but no leukocytic infiltrate. Epithelium lining mucous neck and gastric glands appears intact. × 250.

Fig 21. Experimental dog. Section adjacent to that in Fig 20 at border of erosion. Apical mucous secretion in the most superficial epithelium bordering erosion (*arrow*) is depleted or unstained. Strong staining persists, however, in secretion in epithelium of subjacent foveolae and mucous neck glands. Alcian blue pH 2.5-PAS. × 250.

Fig 22. Experimental dog. Gastric erosion showing loss of superficial surface epithelium and retention of normal staining foveolar epithelium and mucous neck glands. Alcian blue pH 2.5-PAS. × 100.

Fig 23. Experimental dog. Edge of gastric erosion. Stroma in floor of this erosion was the only one, of several observed, to contain a leukocytic infiltrate. × 100.



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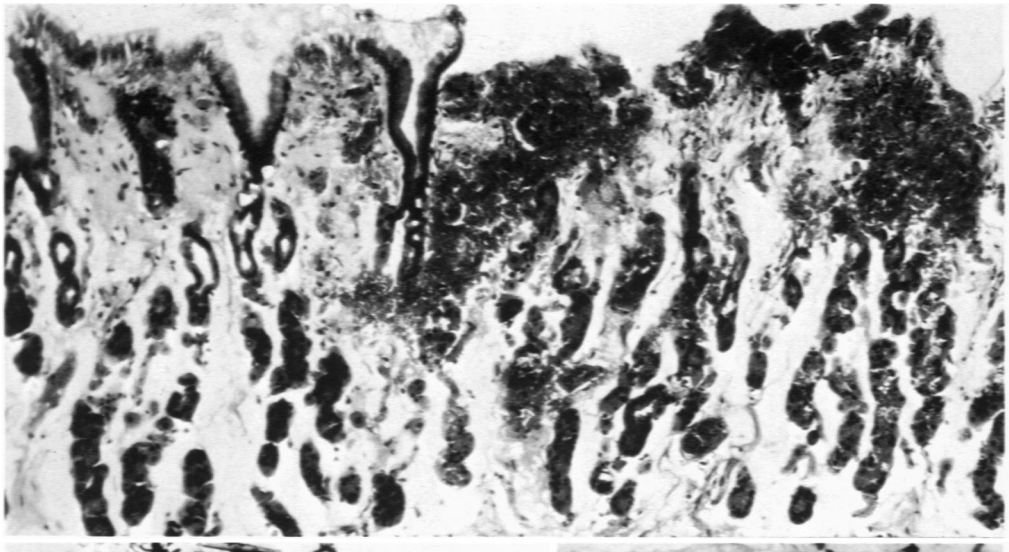

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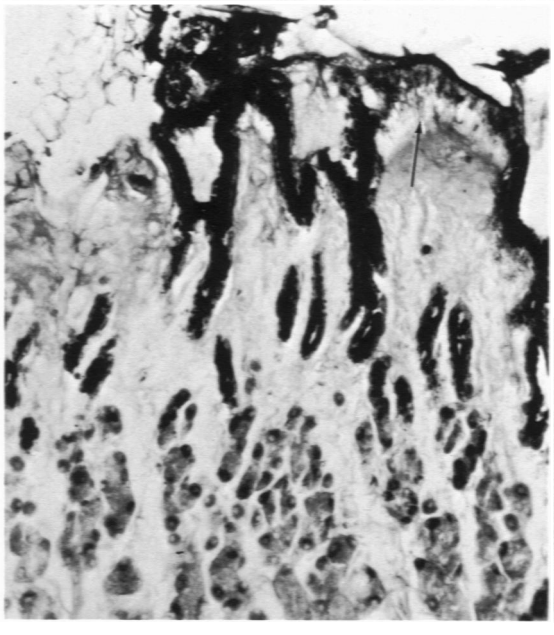

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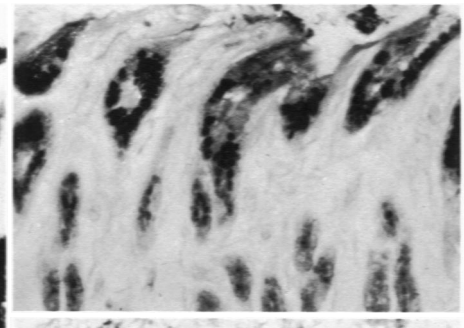

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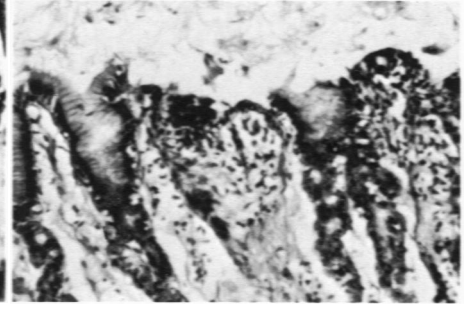

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