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M. Al-Tikriti
The University of Tennessee

F. Al-Bagdadi
Louisiana State University

R. W. Henry
The University of Tennessee

J. Hoskins
Louisiana State University

C. Titkemeyer
Louisiana State University

See next page for additional authors

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THE NORMAL STRUCTURE OF REGIONAL FELINE GASTRIC MUCOSAE: SCANNING ELECTRON MICROSCOPIC STUDY

M. Al-Tikriti[a*], F. Al-Bagdadi[b], R. W. Henry[a], J. Hoskins[c], C. Titkemeyer[b], G. Strain[d]

[a]Department of Animal Science, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901-1071. [b]Department of Veterinary Anatomy and Fine Structure, [c]Department of Veterinary Clinical Sciences, [d]Department of Veterinary Physiology, Pharmacology and Toxicology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA, 70803.

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Abstract

Regions of cat's stomach can be identified by looking at the surface epithelial cells by scanning electron microscopy (SEM). The luminal surface of cells of the cardiac region were elongated, of the fundus rounded, of the corpus polygonal shaped, and of the pyloric region diamond shaped. The quantity and distribution of microvilli covering the epithelial cells varies, being abundant and evenly distributed in the cardiac region and gradually decreasing in number toward the gastro-duodenal junction, where they were confined to cell perimeters. The colliculi varied in shape and distribution from few in the fundus and corpus to numerous in the pyloric region. Large numbers of gastric pits were present in the corpus. They diminish toward both the cardia and gastro-duodenal junction. The cardiac and pyloric glands were coiled. The gastric glands (glandula gastrica propria) were straight tubules in the fundus and coiled in the corpus. All luminal surfaces of glandular epithelial cells were covered with microvilli, but the regional distribution of microvilli on the cell was variable. Parietal, mucous neck, and chief (zymogen) cells were identified by their cytoplasmic structure. Parietal cells had long apical microvilli, mucous neck cells contained large numbers of globular mucous granules, and chief cells were vacuolated. A few G cells (Endocrinocytus gastrointestinalis) were seen in the cardiac region, large numbers in the pyloric region, and not found in fundus or corpus.

Key Words: Cardiac, Gastric, and Pyloric glands; Digestive system; Feline; Gastric mucosa; Scanning electron microscopy.

*Address For Correspondence: M. Al-Tikriti, Department of Animal Science, College of Veterinary Medicine, The University of Tennessee, Knoxville, TN 37901-1071. Phone No.: (615) 546-9230 Ext. 328.

Introduction

The gastric mucosa forms the interface between the internal and external environments. It is involved in many physiological processes, and its morphology varies with function (Ito, 1974; Miller and Revel, 1975; Wood and Dubois, 1983). Surface topography of the stomach is used for diagnostic information when evaluating biopsies for pathophysiologic studies (Burhol, 1968; Takagi et al., 1974). Due to high resolution, great depth of field, rapidity of tissue preparation, and three dimensional viewing of tissue surface, scanning electron microscopy affords a unique tool for surface morphological studies especially in the gastrointestinal tract (Pfeiffer, 1971). Many studies of the gastric mucosa (both normal and diseased) have been reported in man and animals (Ogata and Murata, 1969; Pfeiffer, 1970a,b; Capoferro, 1974; Takagi et al., 1974). Similar studies of the stomach of cats are less numerous (Al-Tikriti et al., 1984, 1987), even though the cat has been used as an experimental animal (Biancani et al., 1982; Clerc and Mei, 1983; Clerc, 1983; Al-Tikriti, 1984). The objective of this study is to describe the normal surface mrphology of various regions of the gastric mucosa as well as the surface morphology of the various glandular epithelia in the cat's stomach.

Materials and Methods

Animals

Twelve healthy adult cats of mixed breeds and sexes were used in this study. The cats were conditioned in the Louisiana State University vivarium for two weeks. The cats were fed regularly, but were fasted 24 h before euthanasia.

Necropsy and Collection of Samples

The cats were euthanatized by intravenous injection of T-61 Euthanasia Solution (American Hoechst Corporation). The abdominal and thoracic cavities were immediately opened and the stomach removed, washed, and fixed utilizing the procedure described by Al-Tikriti et al., (1986). After washing the stomach gently with saline and gloved hand, the stomach was moderately stretched with mucosal surface up, and the edges were

pinned to a paraffin tray. Subsequently, the stomach was thoroughly washed with saline solution and submerged in a solution of 5% glutaraldehyde in phosphate buffer (pH 7.4, 0.15M). The stomach was left overnight in fixative at 4°C. The next day, the stomach was washed with phosphate buffer (pH 7.4, 0.2M). Samples were cut from the gastro-esophageal junction, cardiac region, fundus, corpus, pyloric region, and gastro-duodenal junction.

Scanning Electron Microscopic Procedure

After initial fixation, the samples were washed three times with phosphate buffer (pH 7.4, 0.2M). Subsequently, the samples were postfixed for 90 minutes in 1% osmium tetroxide in phosphate buffer (pH 7.4, 0.15M), washed three times (10 minutes each) with phosphate buffer, and dehydrated in a graded series of acetone solutions (70%, 80%, 95%, with three changes in 100% acetone). Eventually, the samples were dried in a critical point drying apparatus with liquid CO_2. The samples were mounted on aluminum stubs, sputter coated with a 15nm layer of gold-palladium, and examined with a Cambridge Stereoscan 150 Mark 2 SEM, operating at 20kV.

Results

Gastro-esophageal junction

At the gastro-esophageal junction, the epithelium abruptly changes from stratified squamous (Epithelium stratificatum squamosum) to simple columnar epithelium (Epithelium simplex columnare) (Fig. 1). At low magnification, the gastric pit (Foveola gastrica) openings, through which the cardiac glands (Glandula cardiaca) open, were observed (Fig. 2). The openings were of different shapes and sizes, and some of them showed flakes of precipitated mucus oozing from their ostia. The surface of the cardiac region (Pars cardiaca) was marked by low ridges that separate the ostia of the gastric pits. In addition to debris of exfoliated cells, mononuclear leukocytes passing between the surface epithelial cells were observed (Fig. 2).

At higher magnification the elongated epithelial cells were delineated. Their surfaces were covered with minute microvilli of irregular shapes and length (Fig. 3). A side view of the gastro-esophageal junction showed that the cardiac glands were coiled and sectioned profiles of different shapes were observed (Fig. 4). Higher magnification revealed the glands were lined by polygonal glandular epithelial cells (Exocrinocytus cardiacus). The luminal surfaces of these cells had microvilli which were more numerous at their perimeter (Fig. 5). Among the glandular epithelial cells were a few cells characterized by long microvilli projecting from their luminal surface (Fig. 6).

Fundus ventriculi

The surface of the fundus had ridges separating the ostia of the gastric pits. A few mononuclear leukocytes were observed between the epithelial cells (Fig. 7). Many gastric pits had flakes of precipitated gastric juice occluding their ostia. The gastric pits of the fundus were more numerous than in the cardiac region. The pit ostia were surrounded by circumferentially oriented epithelial cells.

At higher magnification, the epithelial cells were rounded with a dome-like surface. The surface was covered by short microvilli (Fig. 8). The microvilli were more numerous near the cell perimeter. A side view of the fundic region revealed the gastric glands (Glandula gastrica propria) arranged in tall straight columns from the Lamina muscularis mucosae to the surface epithelium (Fig. 9). Individual glandular cells were recognized as polygonal in shape with knob-like projections within the column of cells. Cells concentrated in the isthmus of the fundic glands appeared to be parietal cells (Exocrinocytus parietalis).

Corpus ventriculi

The surface epithelium of this region also had high ridges of short length, separating the numerous ostia of the gastric pits (Fig. 10). The ostia of the pits were of different sizes and shapes. A few mononuclear leukocytes were seen squeezed between the epithelial cells.

The epithelial cells were polygons with short microvilli concentrated at the perimeter of the cell. Some cells showed colliculi of different sizes (Fig. 11). A side view of the corpus region showed cross-sectional profiles of the long, coiled gastric glands (Fig. 12). The mucous neck cells (Mucocytus cervicalis) in the gastric glands were characterized by irregular globules which corresponded to mucous granules (Fig. 13). Their luminal surfaces had longitudinal ridges surrounded by microvilli. The chief cells (Exocrinocytus principalis) were concentrated predominantly in the basal portion (Pars principalis) of the gastric glands. Their cytoplasmic matrix contained empty vacuoles of various sizes (Fig. 14). Their luminal surfaces were covered by short microvilli. The parietal cells had relatively long microvilli projecting into the lumen of the gastric glands (Fig. 15).

Fig. 1. Gastro-esophageal junction. E - esophagus C - cardiac region. Bar = 200 µm.

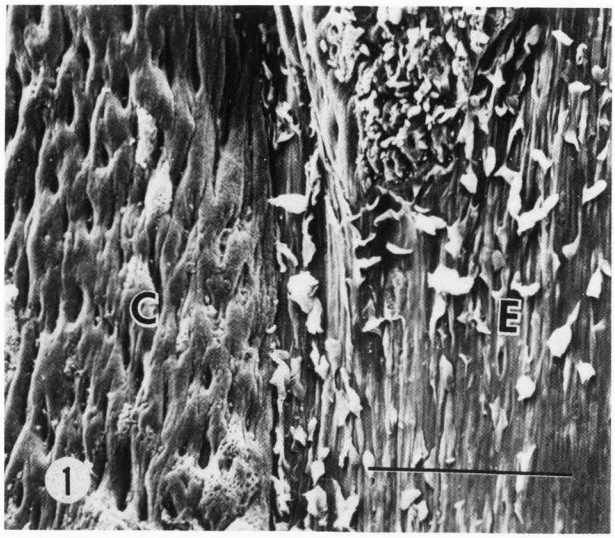
Fig. 2. Low magnification of cardiac gland region with ostia of gastric pits (P) and mononuclear cells (Mc). Bar = 50 µm.

Fig. 3. High magnification of cardiac surface epithelial cells which are covered by minute microvilli. Bar = 8 µm.

Fig. 4. Side view of wall of the gastro-esophageal junction. Cardiac glands are simple coiled. E = esophagus, C - cardiac region. Bar = 200 µm.

Fig. 5. Low magnification of cardiac glands. Luminal surface of glandular cells are covered with microvilli (Mv). (*) - indicates area of figure 6. Bar = 10 µm.

Fig. 6. High magnification of the labeled area (*) in figure 5. G cell has long microvilli (Mv) projecting into the lumen of the cardiac gland. Bar = 4 µm.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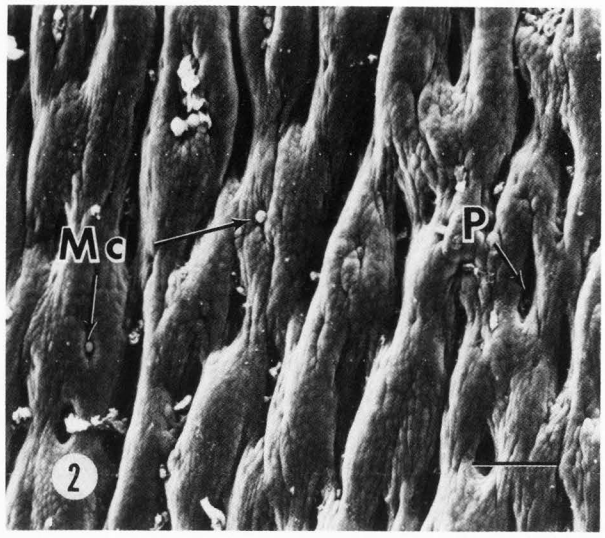
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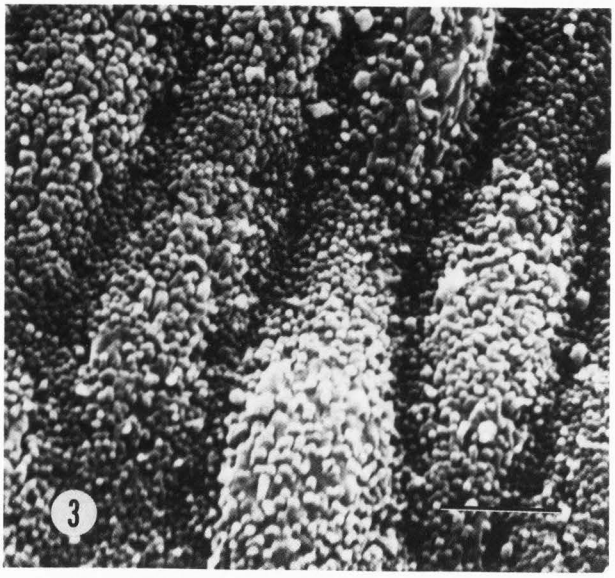
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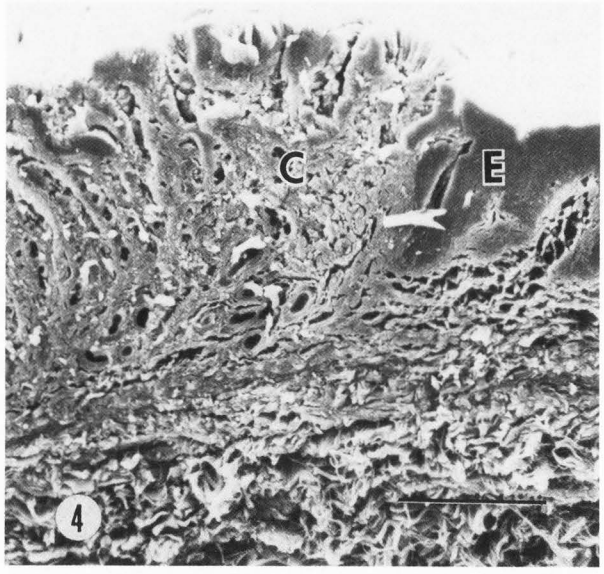
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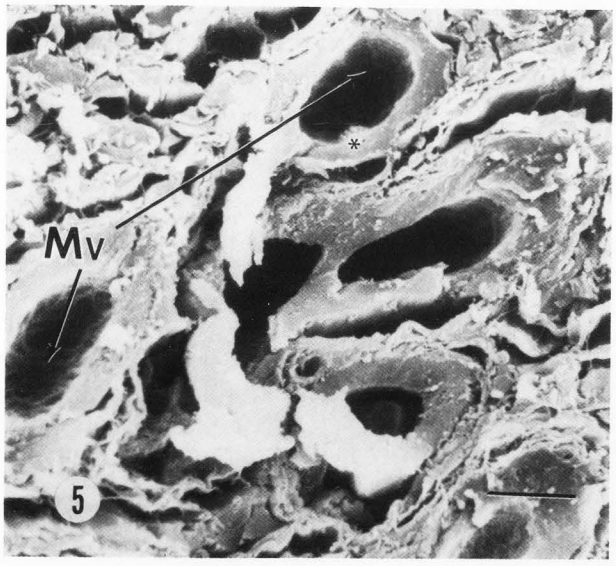
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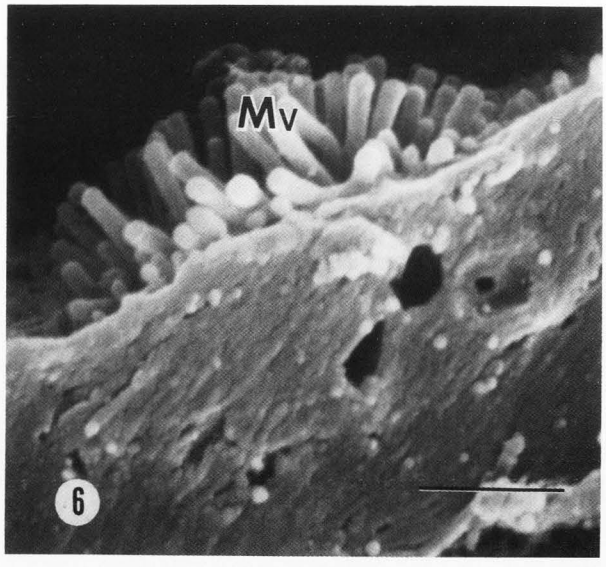
Mv
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Pars pylorica

Although there was no true line of demarcation, two morphologically different portions were observed. 1. The pyloric antrum (Antrum pyloricum) was characterized by its surface thrown up in a labyrinthine fashion which obscured the ostia of the gastric pits (Fig. 16). 2. The pyloric canal's (Canalis pyloricus) surface was marked by long anastomosing ridges which separated the ostia of the gastric pits (Fig. 17). These ridges gradually become taller as they are changing to the finger-like projections found in the duodenum (Fig. 18). At higher magnification, the individual epithelial cells were clearly delineated and diamond shaped (Fig. 19). Their free surface was relatively convex. The majority of the cell surfaces were covered with numerous colliculi. However, a few short microvilli were present at the cell's perimeter and intercellular space. A side view of the pyloric region showed that the pyloric glands (Glandula pylorica) were coiled and scattered in solitary groups in the lamina propria mucosae (Fig. 20).

The pyloric glands were lined by cuboidal to low columnar glandular epithelial cells (Figs. 21 and 22). Their luminal surfaces were covered with evenly distributed, abundant, short microvilli, especially near the neck of the gland (Fig. 21). In the basal portion of the gland, their surfaces were covered by microvilli and the perimeter of the cells appeared as ridges. These ridges were extensively covered by short microvilli (Fig. 22). Cells with long microvilli were seen in large numbers, especially at the base of the pyloric glands (Fig. 23).

Discussion

The present investigation provides a description of the mucosae of the cat's stomach and associated glands. New techniques have been developed to allow examination of subepithelial structures and cellular components with the scanning electron microscope. These include fracturing the tissue or by preparing it with blunt dissection (Hattori and Fujita, 1974; Boyde, 1975; Miller and Revel, 1975; Mackercher et al., 1978). In this study, blunt dissection was used successfully to view the gastric glands and other structures in the Lamina propria mucosae. The gastric pits are tubular and descend from the epithelial surface to the isthmus of the gland at which point the parietal cells open. The gastric glands appear as straight columns in the fundus but coiled in the corpus.

Three types of cells were identified in the gastric glands. The parietal cells, when viewed from the side, had knob-like projections and long microvilli projecting into the lumen of the gland. This description was similar to that reported in the golden hamster (Hattori, 1974). The zymogen (chief) cells were recognized by the presence of empty vacuoles in their cytoplasmic matrix. These empty vacuoles were originally filled with zymogen granules (Granulum zymogeni) that were dissolved during tissue preparation. The mucous neck cells were characterized by the presence of globule-like granules in their cytoplasm similar to those described by Mackercher et al. (1978) in the human.

One of the problems of studying the surface morphology of the stomach in various animals is the presence of opaque materials coating the gastric mucosa. This coating material obscures structure detail of the gastric epithelial cells. Different methods of removing this coat have been tried unsuccessfully (Takagi et al., 1974; Zalewsky and Moody, 1979; Wood and Dubois, 1981). In this study we successfully used the method recently described by Al-Tikriti et al. (1986) to remove this coating material. Even so, a few ostia of gastric pits were occluded by precipitated flakes of mucus and cell debris. The number, shape, and size of gastric pit ostia varies regionally. The ostia were small and few in number in the cardiac region. They reached their greatest number in the corpus and gradually decreased in number toward the pylorus. Variation in number of ostia is attributed to the increased number of gastric glands in the corpus region. In addition, the large openings of the gastric pits in the corpus suggest that these glands produce larger amounts of secretory product than the cardiac and pyloric glands. Hence, larger glandular openings are required to discharge their secretory products.

At higher magnification, the surface epithelial cells of the cat appear as gently sloping domes. The gastric epithelial cells vary in size and shape. They are elongated in the cardiac region, rounded in the fundus, polygonal in the corpus, and diamond shaped in the pyloric region. The surface epithelial cells are covered with microvilli similar to that reported in man and ferret (Pfeiffer, 1970a,b). The microvilli cover the epithelial cells of all regions of the stomach. However, they differ in their concentration and distribution due to different physiological activities (Wood and Dubois, 1983). Microvilli covered the entire surface of the

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Fig. 7. Low magnification of fundus showing ostia of gastric pits surrounded by circumferentially oriented epithelial cells. Mononuclear cell (Mc). Bar = 100 µm.

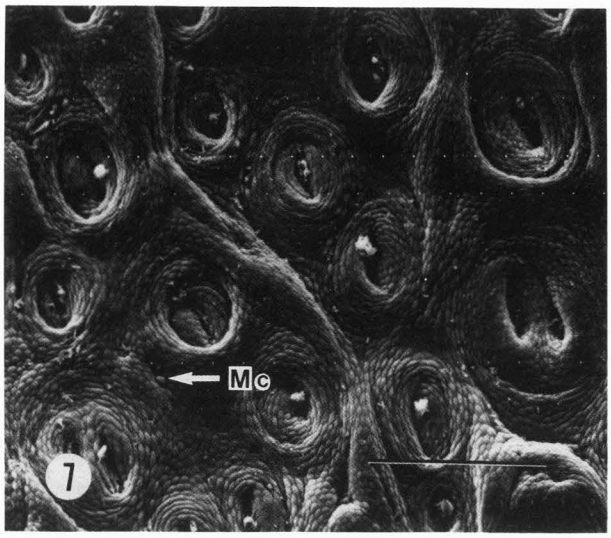
Fig. 8. High magnification of fundus. Epithelial cells have convex surfaces covered with microvilli. Bar = 8 µm.

Fig. 9. Side view of fundus. Gastric glands are in columns. Mm - muscularis mucosae, Pc - parietal cells. Bar = 200 µm.

Fig. 10. Low magnification of corpus showing widely opened gastric pits. Bar = 100 µm.

Fig. 11. High magnification of surface epithelial cells of corpus showing few microvilli. Co - colliculi. Bar = 8 µm.

Fig. 12. Side view of corpus region. Gastric glands are coiled. Mm - muscularis mucosae. Bar = 200 µm.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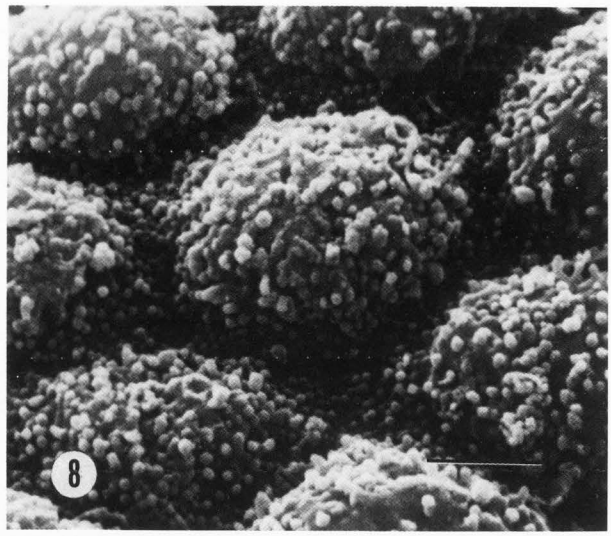
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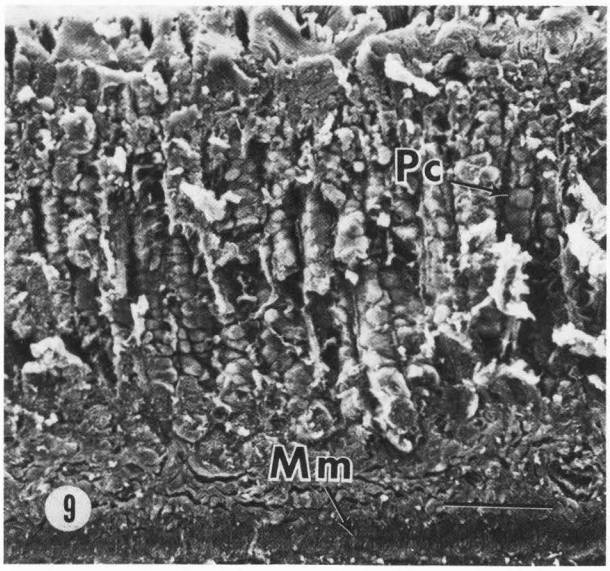
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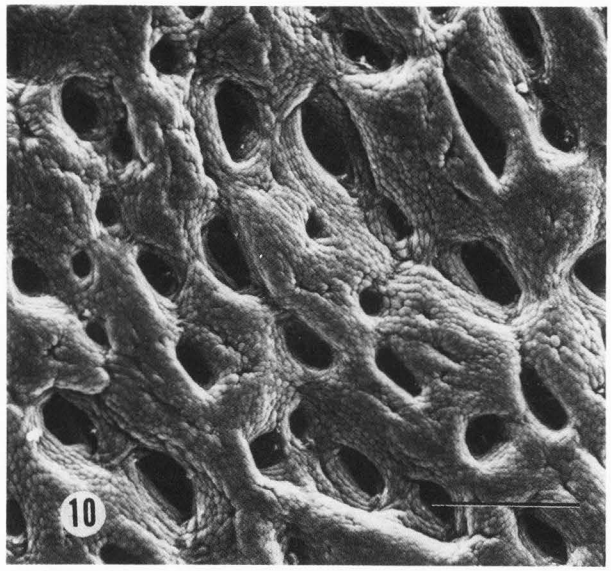
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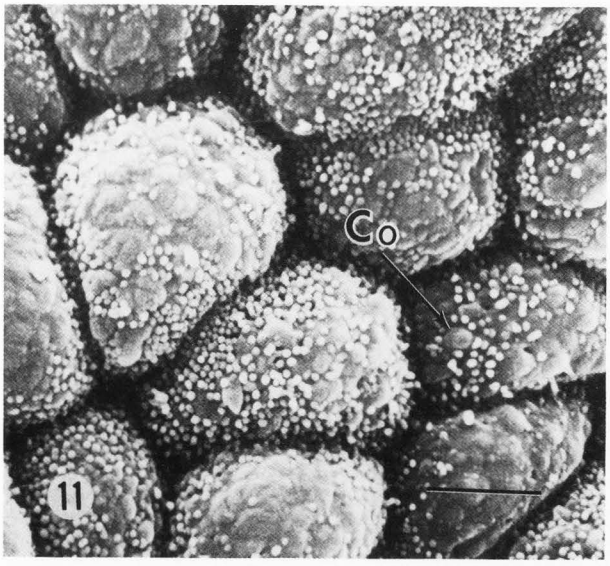
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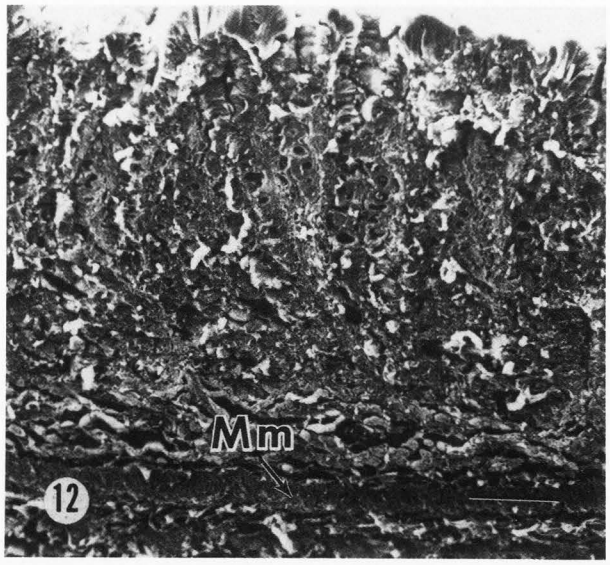
Mm
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cells of the cardiac and fundic regions, but they decrease in number and concentrate near the perimeter of the epithelial cells in the corpus and pyloric region. Similar distribution of microvilli has been described in man (Mackercher, et al., 1978) and in the ferret (Pfeiffer 1970a,b). In addition,another surface modification is the presence of colliculi of varying sizes. These colliculi are found in increased quantity in the corpus and pyloric region. Colliculi are thought to be formed by mucous secreting granules which gather beneath the cell membrane (Takagi et al., 1974).

It is of interest that various workers have demonstrated increased numbers of degenerated gastric epithelial cells (Pfeiffer, 1970a). In this study, cellular debris and small holes in the surface epithelial cells were observed in the cardiac and pyloric regions. This suggests that the stomach undergoes continual degeneration and regeneration at a slow rate similar to that described by Pfeiffer (1970a,b) in man and ferrets and Townsend (1961) in the rat.

Often mononuclear leukocytes were observed on the surface epithelium. They were seen in large numbers in the cardiac and pyloric region. These were the interepithelial lymphocytes described by Douglas and Weetman (1975). It has been suggested that they have an immunological function, transporting antibodies into the lumen to be released when they degenerate (Burrows and Havens, 1948; Fichtelius, 1967).

A few endocrine cells (Endocrinocytus gastrointestinalis) were observed in the cat cardiac and pyloric regions. They are characterized by long microvilli projecting from their luminal surface into the gland lumen. They likely play a role in the mechanism of cellular stimulation by either chemical or mechanical stimuli (Solcia et al., 1967, 1975). These endocrine cells are considered to be G cells. Their identification is based on the observation that the G cell contacts both the basal lamina and the lumen of the gland (Solcia et al., 1975, Al-Tikriti et al., 1987). G cells have been identified structurally in the pyloric region of the cat and man (Vassallo et al., 1969, 1971, Forssmann and Orci, 1969) and in the cardiac mucosa of the rat (Forssmann and Orci, 1969). However, their presence and function in the cardiac region of the cat remains enigmatic.

Acknowledgments

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Fig. 13. Side view of corpus region. Luminal surface of gastric gland is covered with microvilli. Mn - mucous neck cells. Bar = 8 μm.

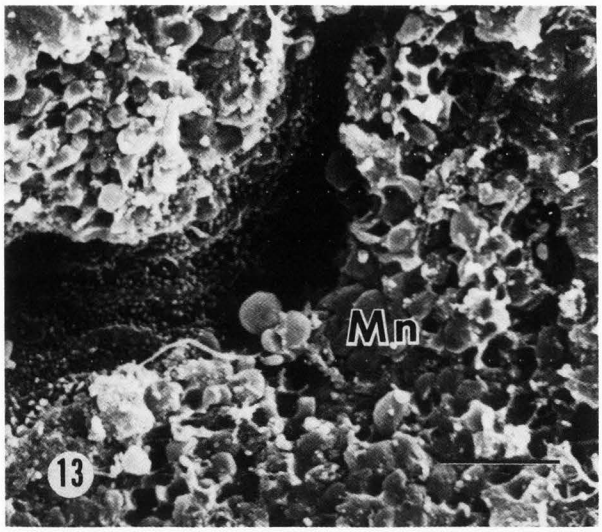
Fig. 14. Side view of corpus region. Chief cells surround lumen (L) of gastric gland. Cv - cytoplasmic vacuoles. Bar = 20 μm.

Fig. 15. Side view of corpus. Parietal cells (Pr) have long microvilli. L - lumen of gastric gland. Bar = 8 μm.

Fig. 16. Low magnification of pyloric antrum. Gastric pits are obscured by irregular ridges of surface epithelium. Bar = 100 μm.

Fig. 17. Low magnification of pyloric canal. Ostia of gastric pits are separated by long anastomosing ridges. Bar = 400 μm.

Fig. 18. Gastro-duodenal junction. P - pylorus, D - Duodenum. Bar = 400 μm.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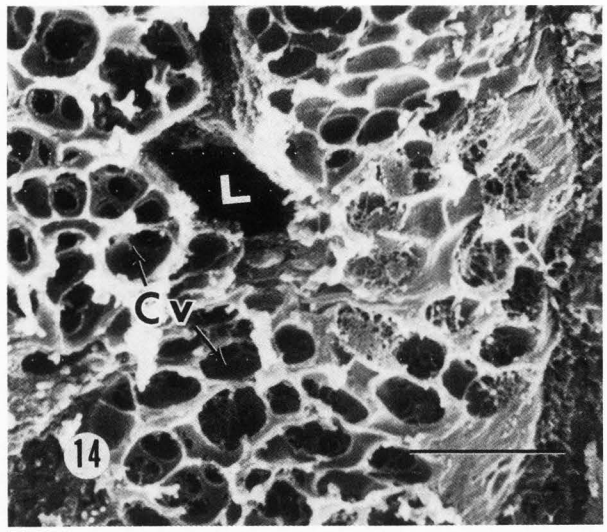
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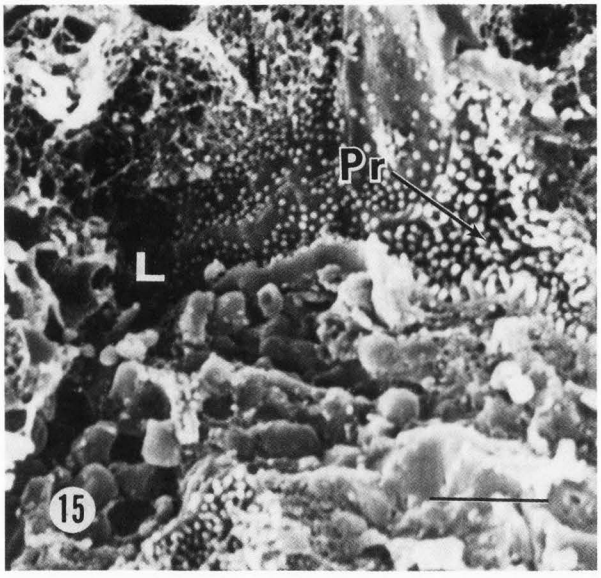
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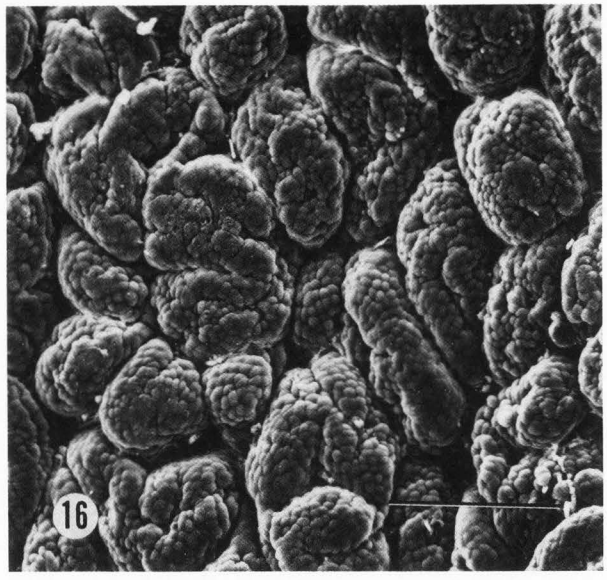
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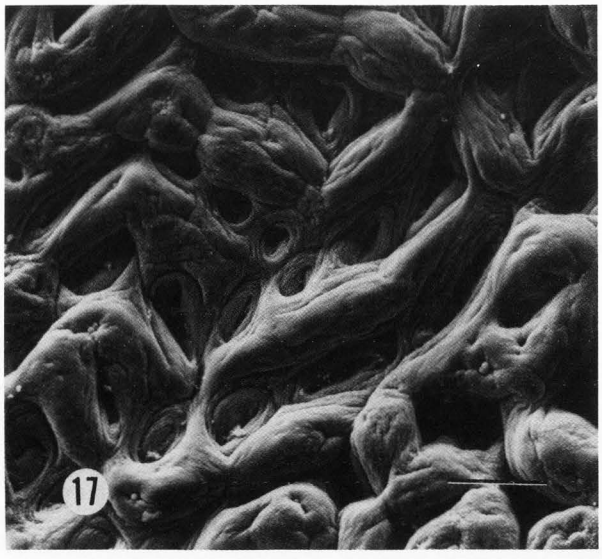
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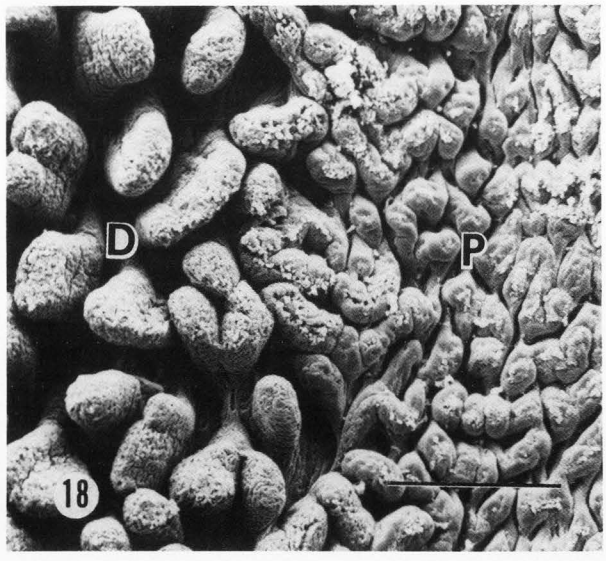
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Discussion with Reviewers

T. Makita: Zymogen granules in chief cells have been preserved in preparation for transmission electron microscopy. Why were they dissolved in this SEM study?

Authors: The zymogen granule is a membrane bound secretory vesicle. The membrane was well preserved. However, the content is lost during processing most likely due to the fact that these specimens are not prepared by freeze fracture. When the membrane is cut during sampling the fluid content is lost. We are looking only on the surface, therefore only cut granules are shown. The granules located deep in the tissue most likely are well preserved. In TEM the embedding media will impregnate and hold the content of the granule in situ.

T. Makita: Are you also of opinion that mononuclear cells are interepithelial lymphocytes transporting antibodies into the lumen? You mentioned that they were numerous in the cardiac and pyloric regions. Did you mean that immunoactivity of these two regions were higher than other regions?

Authors: It has been shown that mononuclear cells are interepithelial lymphocytes. However, their role in transporting antibodies into the lumen is questionable. It is possible that the immunoactivity of these regions is higher than other regions based on the evidence of the numerous lymphocytes in these regions.

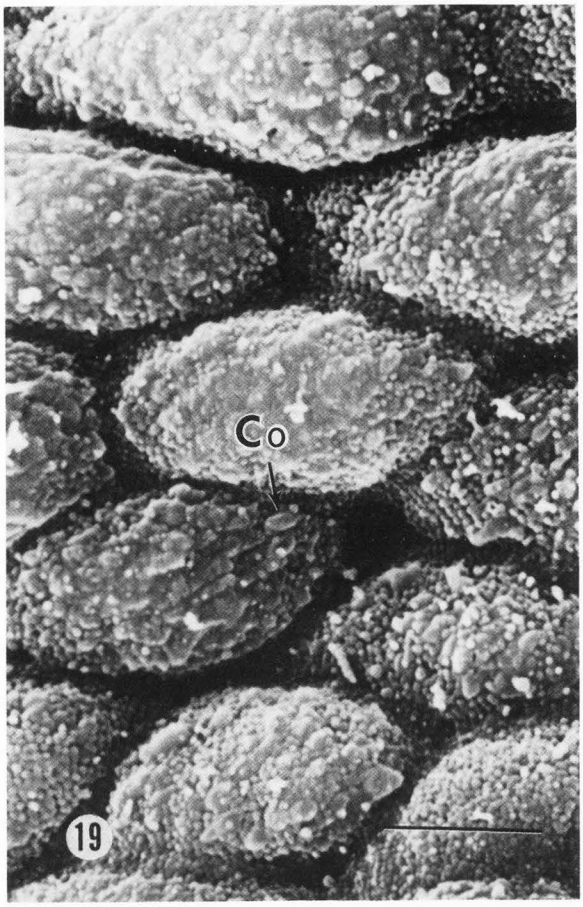
Fig. 19. High magnification of surface epithelial cells of pylorus. Individual cells are delineated and majority of their surface is covered with colliculi (Co). Bar = 20 μm.

Fig. 20. Side view of the pyloric region. The pyloric glands are coiled and scattered in solitary groups (arrows). Bar = 100 μm.

Fig. 21. Pyloric gland region (neck of the gland). Luminal surface of pyloric gland cells is extensively and evenly covered with microvilli. Bar = 20 μm.

Fig. 22. Pyloric gland region (base of the gland). Luminal surface of pyloric gland cells is extensively covered with microvilli (Mv). Bar = 40 μm.

Fig. 23. Pyloric gland. Three G cells with long microvilli lie in the base of the gland. Bar = 8 μm.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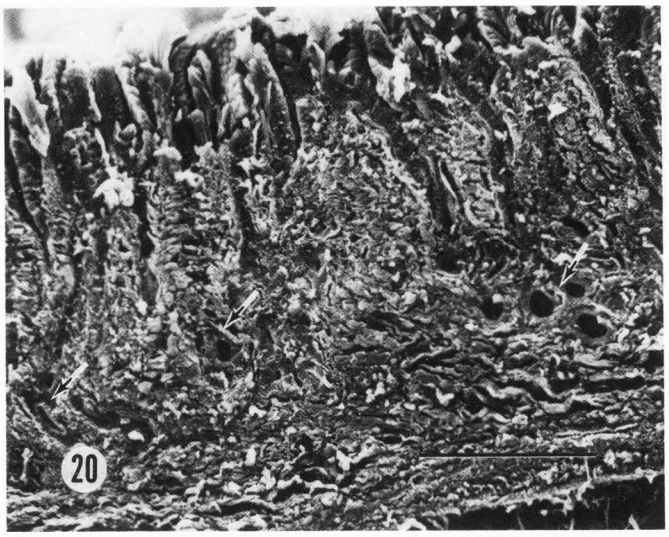
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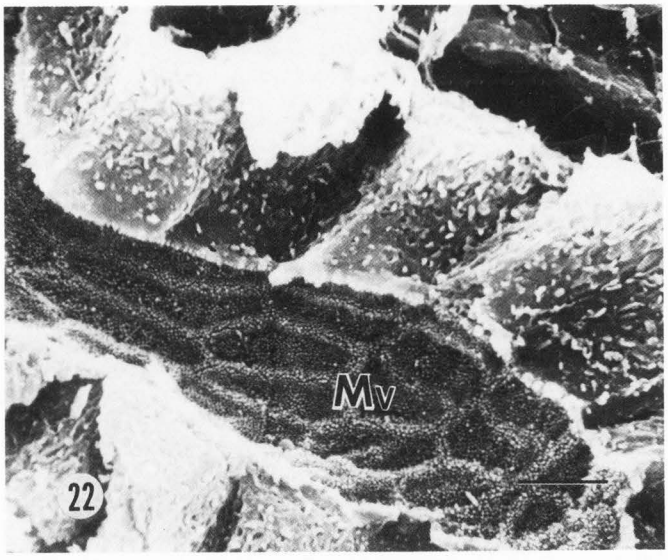
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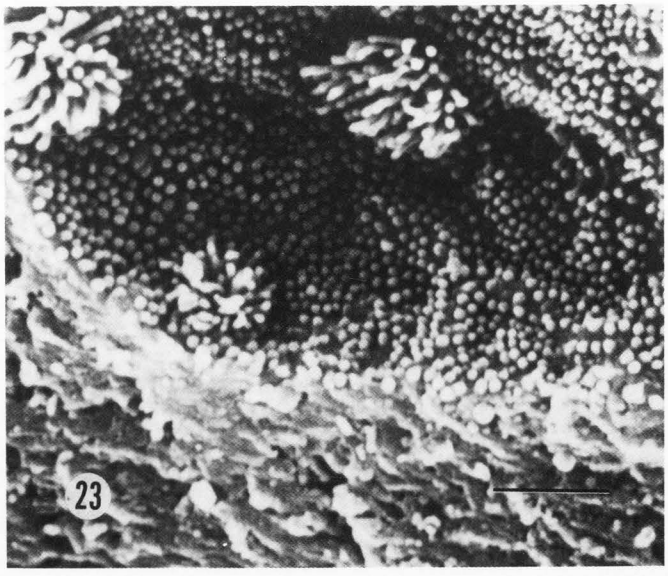
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T. Makita: Do you agree with the opinion that long microvilli on the G cell's free surface plays a role in the mechanism of cellular stimulation? If so, are longer microvilli more sensitive to stimulation than shorter ones?
Authors: Yes we agree, refer to Solcia et al. (1967, 1975) (text references) and [Solcia E., Vassallo G., Capella C., (1970). In: Origin, chemistry physiology and pathophysiology of the gastrointestinal hormones. Grentzfeidt W, (ed.) Schattauer, Stuttgart p. 3.] One function of microvilli is to increase the surface area providing more contact with materials. Therefore, longer microvilli would be more sensitive to stimulation than shorter ones and can be stimulated by chemicals farther away from the cell plasmalemma. This could allow earlier transmission of information about glandular activity.

A.W. Stinson: Were the junctional areas between the cardiac-fundic, fundic-corpus, and corpus-pyloric regions as distinct as that between the gastro-esophageal junction?
Authors: No. The gastro-esophageal junction is distinct because the mucosal lining is of two different epithelia (stratified and simple columnar epithelium). Since the rest of the stomach is lined by simple columnar epithelium, distinct demarcation between regions of the stomach is not possible. However, different regions can be identified by the number of gastric pits and the shape and height of the ridges between gastric pits.

S. Siew: Could you explain what is meant by "rubbing" the mucosa with saline?
Authors: This method was developed and described by Al-Tikriti et al. (1986) (text reference). It is an effective method for cleaning the mucosal surface of mucus and cellular debris. The stomach is flushed several times with saline, and the mucosal surface is gently rubbed by the fingers covered with a surgical glove. The stomach is moderately stretched with the mucosal surface up. The edges are pinned to a paraffin tray. After thorough washing with saline solution, the stretched stomach is flooded and submerged in the fixative solution.

S. Siew: In your experience of comparative pathology, are there significant differences between the gastric function of the feline species and other non-ruminant mammals, such as man?
Authors: There are histological similarities and differences between the feline and human stomach. In both species, the stomach is unilocular and lined by glandular mucosa. However, the mucosa differs in thickness: 1 mm in man and approximately 500 µm in cat (Al-Tikriti and Henry "unpublished data"). The number of gastric glands which open into pits are: 3-7 glands in man [Helander HF, (1981). The cells of gastric mucosa. Int. Rev. Cytol. 70:217-282] and 2-3 glands in cat [Al-Tikriti (1984.) (text reference)]. The distribution of parietal cells in the gastric gland is: in man they are concentrated in the neck and isthmus, whereas in cat it is not uncommon to find parietal cells in the basal portion of the gastric gland. All these factors contribute to gastric function, therefore, we expect more gastric juice secretion in man than in the cat, but both are of similar chemophysiological content.

J. E. Breazile: Reference is made to the number (density) of gastric pits in the different regions of the stomach. Was there any attempt to determine the density through actual counts of gastric pits per unit area of mucosal surface?
Authors: This paper contains only morphological description of feline stomach. Future results will contain quantitative measurement.

J. E. Breazile: Considering the turn over rate of gastric epithelial cells, and rate at which they can alter their morphology in response to gastric contents, what alterations, if any, would you expect to occur due to the 24 h fast preceding collection of the materials?
Authors: Based on previous studies [Helander HF (1986). Ultrastructure of gastric fundus glands of refed mice. J. Ultrastruct. Res. 10:160-175] no major changes were observed in the mucosa. The greatest ultrastructural changes are observed in zymogen cells. They show reduced diameter of zymogen granules, the Golgi apparatus occupying a smaller area, and the rER having reduced cisternal space. In the parietal cells there is a reduction in the number of microvilli and in the diameter of the intracellular canaliculi. Other cells should remain unchanged.

J. E. Breazile: You indicate that the diameter of the gastric pits represent the secretory capacity of the associated gastric glands. Is there any experimental evidence that this association can be made?
Authors: Yes, there is some experimental evidence that shows morphological changes of gastric pits. It was found that the gastric pits become more shallow following a meal [Willems G, Vansteenkiste Y, Smets P, (1971). Effects of food ingestion on the cell proliferation kinetics in the canine fundic mucosa. Gastroenterology 61:323-327].

J. E. Breazile: You conclude that less cellular debris, as well as fewer small holes in the epithelial cells observed in this study, indicate a slower turn-over rate of mucosal epithelial cells in the cat than have been observed in man, the ferret and the rat. Is it likely that the method of mucosal preparation for this study contributed to this finding?
Authors: The turn-over time has been calculated to be between 2 and 8 days for the surface mucous cells and mucous neck cells [Castrup HJ, Fuchs K, Schiller U (1973). Zur Zellerneuerung der menschlichen magenschleimhaut: Autoradiographiche in vitro-untessuchungen as magenschleim-hantbiopsieh. Res. Exp. Med. 161:311-320]. The method we used will get rid of nearly all mucus and cellular debris but not the holes which should be differentiated from that of artifact origin. However, another possibility is sample collection. It may be that our samples happened to be from an area that does not show epithelial turnover.