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SCANNING ELECTRON MICROSCOPIC STUDY OF THE SURFACE OF FELINE GASTRIC EPITHELIUM: A SIMPLE METHOD OF REMOVING THE COATING MATERIAL

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Abstract

Scanning electron microscopic examination of the gastric surface epithelial cells is often hindered by the presence of a coating material. Several methods for removal of coating material on feline gastric mucosa were utilized. The cleansed tissues were evaluated using the scanning electron microscope to assess damage caused by the use of various cleansing methods to surface epithelial cells. The stretched stomach washed several times, including rubbing the mucosal surface with gloved fingers, yielded the best results with no apparent damage to the surface epithelial cells. Flushing unstretched stomachs with saline only did not adequately remove coating material. Flushing unstretched stomachs with saline while stroking the surface with a cotton tipped applicator stick removed debris but damaged the surface epithelium.

Key Words: Feline, Gastric Mucosa, Scanning Electron Microscopy, Mucus, Mucus removed, Stomach, Digestive System.

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Introduction

Recent application of SEM to study the surface morphology of the alimentary mucosa has progressed rapidly. The presence of opaque materials coating the mucosal surface of the stomach poses a problem when studying its surface morphology. These materials include mucus mixed with debris of exfoliated cells as well as food particles. These materials obscure the surface morphology of the epithelial cells. Several mechanical procedures such as washing, ultrasonication (Takagi et al., 1974), brushing (Zalewsky and Moody, 1979), and the use of enzymes followed by mechanical agitation (Wood and Dubois, 1981) have been used. Unfortunately, none of these procedures have yielded both excellent visualization of surface morphology and minimal damage to the surface epithelial cells. The present study was conducted to remove this material without altering the normal gastric morphology.

Materials and Methods

Animals and Sample Collections

Twelve non-fasted mixed-breed cats of either sex were used. The cats were euthanatized by intravenous injection of T-61 euthanasia solution. The peritoneal (abdominal) and pleural (thoracic) cavities were immediately opened and the stomach was exposed. For the removal of the stomach, the esophagus was severed one centimeter proximal to the cardia (gastroesophageal junction), and the duodenum was severed one centimeter distal to the pylorus. The stomach was removed leaving a small amount of major (greater) omentum attached. The serosal surface was cleared of fat and the omentum, and the stomach was opened along the major (greater) curvature as marked by the line of attachment of the major omentum. Collected stomachs were randomly assigned to be processed by one of the following methods.

Unstretched Stomach

Using this method, eight stomachs were randomly divided into two groups:

A - Four stomachs were washed several times by flushing with saline solution. Subsequently, sections were taken from the corpus and antrum regions and were washed with 0.2M phosphate buffer, pH 7.4. The samples were fixed overnight in 5% glutaraldehyde in 0.15M phosphate buffer, pH 7.4. The next day, the samples were washed several times with 0.2M phosphate buffer, pH 7.4. After washing, they were

post-fixed in 1% osmium tetroxide for 1½ h. Then the samples were processed for SEM evaluation.

B - Four stomachs were washed several times by flushing with saline solution. At this time, the mucosal surface was stroked with a cotton tipped applicator stick several times in an attempt to remove the coating materials. Subsequently, samples were taken from the corpus and antrum regions and processed as outlined in A.

Stretched Stomach

C - Four stomachs were used in this method. Each opened stomach was washed several times by flushing with saline. During the washing process, the mucosal surface was gently rubbed by the hand covered by a surgical glove. The stomach was moderately stretched, mucosal surface up, by having its edges pinned to a paraffin tray (Fig. 1). It was thoroughly washed in a saline solution and then submerged in a solution of 5% glutaraldehyde in 0.15M phosphate buffer, pH 7.4. It was left in fixative overnight at 4°C. The next day, the stomach was washed with 0.2M phosphate buffer, pH 7.4. Samples were collected from the corpus and antrum regions and processed for SEM examination.

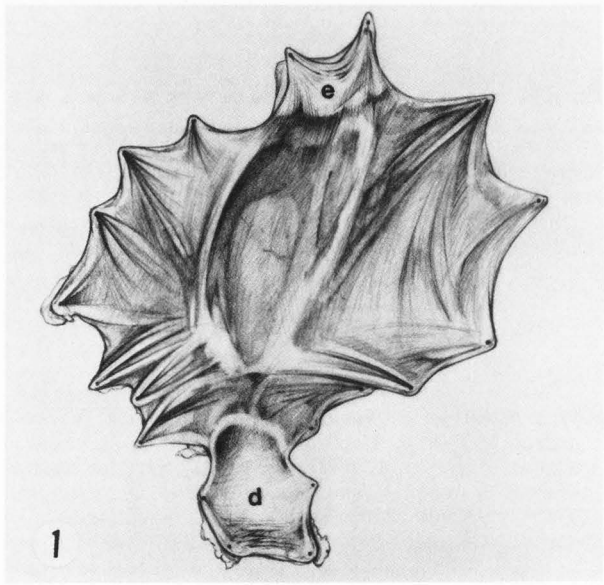


Fig. 1: Schematic drawing, showing the stomach of the cat pinned open with mucosal surface up, opened along the major curvature. e - esophagus, d - duodenum.

Scanning Electron Microscopic Procedure

Samples from the corpus and antrum regions were post-fixed in 1% osmium tetroxide in phosphate buffer, pH 7.4 for 1½ h. Tissues were dehydrated using an ethanol series of 50%, 70%, 95%, and three changes of 100%. The samples were critical point dried, mounted on aluminum stubs, and sputter coated with 150 Angstroms gold-palladium. They were examined with a Cambridge Scanning Electron Microscope operating at 20 kV.

Results

Unstretched Stomach

Observation of samples (A), flushed with saline only, reveal an abundance of coating material still intact (Fig. 2). Intercellular clefts are detectable in some areas. In addition, mucous flakes and particles are still trapped in the grooves between the mucosal folds. A few holes are seen on the surface epithelium. Those holes are most likely from exfoliated dead epithelial cells (Fig. 2).

Samples (B), flushed with saline and massaged with a cotton tipped applicator stick show little evidence of coating material on or between the mucosal folds (Fig. 3). The absence of debris or secretions between individual epithelial cells allows clear visualization of well defined cell boundaries. However, several cells demonstrate the damaging effects of the applicator stick to their surfaces, partial loss of their luminal cell membranes (Fig. 3).

Stretched Stomach

Observation of samples (C) reveal no coating material left on their surface (Figs. 4, 5). The intercellular clefts between the epithelial cells are clearly visible. The ostia of the gastric pits are of different sizes and shapes (Fig. 4), and are not occluded by mucus or debris of dead cells. In addition, the surface epithelial cells are not damaged. At higher magnification, the surface epithelium is seen in more detail (Fig. 6). The microvilli are not damaged and uniformly cover the entire surface epithelial cells (Fig. 6). Minimal mucus and cellular debris are seen. The intercellular clefts between the epithelial cells are clearly visible and the cells are covered by microvilli.

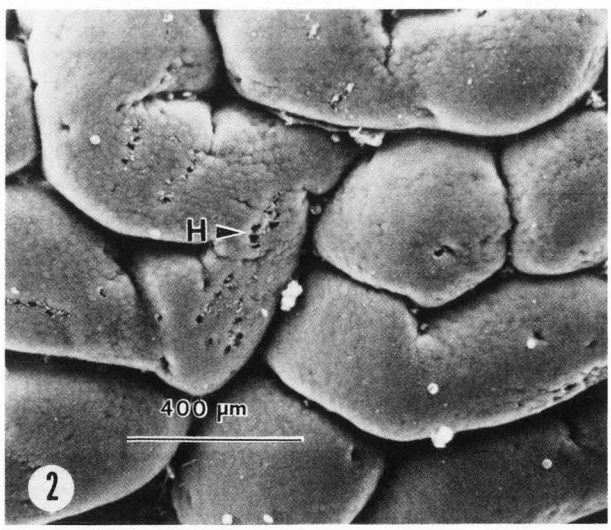


Fig. 2: Surface epithelial cells from the antrum, prepared by vigorous flushing with saline only (method - A). The cell surfaces are covered by coating material. A few holes (H) left by exfoliated cells are observed.

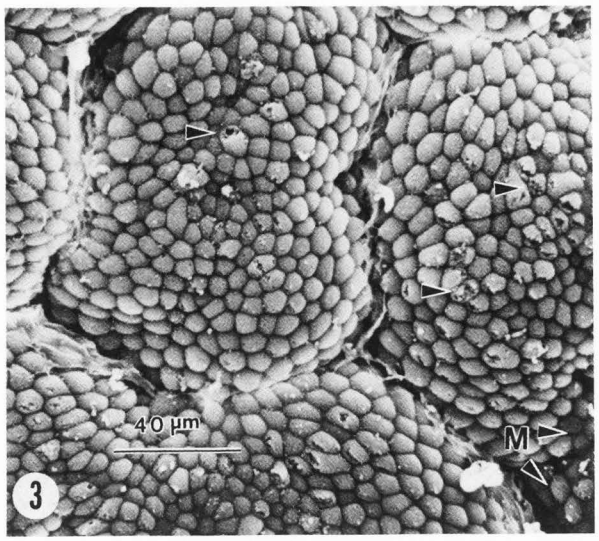
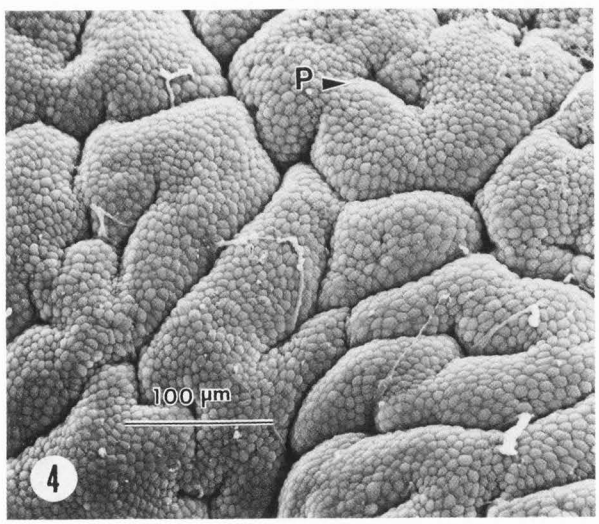
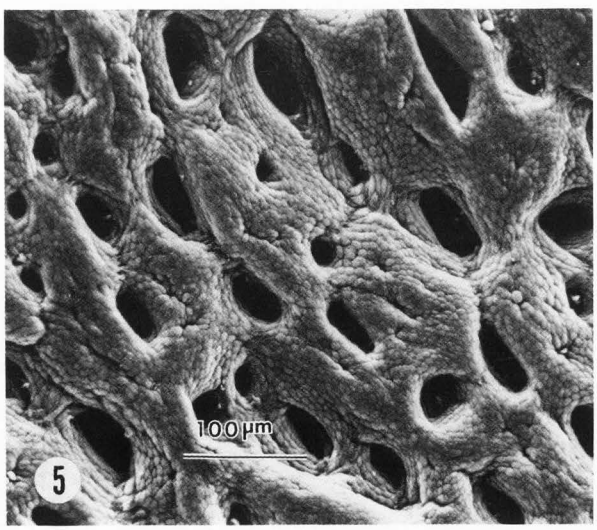


Fig. 3: Surface epithelial cells from the antrum, prepared by flushing and massaging with a cotton tipped applicator stick (method -B). A small amount of coating material is present (M). Arrows indicate damaged cells.

Fig. 4: Surface epithelial cells from the corpus, prepared by flushing and rubbing with gloved hand (method - C). There is little evidence of coating material, and the gastric pits (P) are not occluded.

Fig. 5: Surface epithelial cells from the antrum, prepared by (method - C). Little evidence of coating material or cell damage is observed.





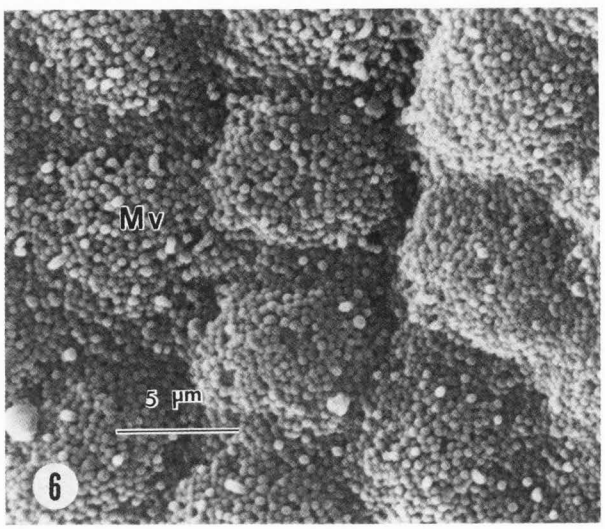


Fig. 6: High magnification of surface epithelial cells from the corpus (method - C). Fine surface detail is seen clearly with no evidence of coating material or damage to microvilli. The surface is covered by evenly distributed microvilli (Mv).

Discussion

Observation of gastric surface epithelial cells using scanning electron microscopy is often hindered by the presence of an opaque material coating the mucosa. Former methods of solving this problem often resulted in damage to the surface epithelium or incomplete removal of the coating material thus reducing the visibility of its undamaged structure. Several mechanical methods for removal of the coating material have been used with moderate success. The best results to date were by Wood and Dubois (1981) who used glycosidic enzymes followed by mechanical action (agitation in Sorensen's phosphate buffered saline).

Washing the stomach several times by flowing liquid will remove food particles and cellular debris, but not the mucous coating material (Takagi et al, 1974). Zalewsky and Moody (1979) after 24 h of fixation lightly brushed the gastric surface with filter paper. The moderate success of their research

prompted method B used by the authors of this paper. Method B (stroking of the surface with a cotton tipped applicator stick and then flushing with saline before fixation) removed most of the coating material of the gastric surface of the unstretched stomach. Unfortunately, it produced damage to the surface epithelial cells.

Method C (gloved finger rubbing the stretched stomach) removed the mucous coating material from the surface epithelial cells. It also did not damage the surface epithelial cells. In addition, it did not damage the delicate microvilli which cover the surface epithelium. This method is effective in removing the coating material of the gastric mucosa in both the corpus and antrum regions. Moderate stretching of the stomach before fixation causes spreading of the mucosal folds and thus eases the process of removing the coating material.

This method (C) of preparation is simple, rapid, and economical. It allows the removal of the surface coating material without damage to the epithelium. This should facilitate scanning electron microscopic studies of gastric mucosa of both normal and pathological specimens.

References

Takagi T, Takabayashi S, Tokuyasu K, Tsuji K (1974). Scanning electron microscopy on the human gastric mucosa; fetal, normal and various pathological conditions. Acta. Pathol. Jap. 24:233-247.

Wood LR, Dubois A (1981). SEM studies of gastric cell surface epithelium: An evaluation of various methods of preparation. Virchows Arch [cell pathol] 35:207-212.

Zalewsky CA, Moody FG (1979). Mechanisms of mucus release in exposed canine gastric mucosa. Gastroenterology 77:719-729.