# Normal histology and the effect of acute mechanical stress on the esophagus epithelium in the guinea pig

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With the aim of investigating histologically the effect of acute mechanical stress on keratinized mucosa of the guinea pig, examination of the esophagus was carried out on 50 adult animals. Twenty-eight served as normal controls, while in 22 animals the esophagus was dilated *in vivo* 10 min by means of polyethylene tubes of varying diameter before killing the animals. In histological sections from six non-dilated esophagi the length of the epithelio-mesenchymal border (basement membrane) was measured at a distance of 15 and 20 mm from the larynx. No difference was found when measured at the two levels in one and the same animal, whereas there was a significant variation between different animals. Macroscopically the esophageal mucosa showed a complex system of circularly arranged keratin crests, divided into segments. Histologically the configuration of the epithelium-connective tissue union showed a regularly scalloped pattern. The spinous cells were arranged in arcades stretching from one epithelial ridge to the next. The stratum granulosum was distinct with deeply stained keratohyalin granules. The stratum corneum was divided into a narrow heavily stained deeper zone and a loosely structured, lightly stained upper zone. *In vivo* dilation of esophagi resulted in a uniform flattening of the epithelium without break in continuity of the tissue. The scalloped epithelium-connective tissue boundary was evened out. The length of the basement membrane of the dilated esophagus was found to be considerably greater than in control animals. The investigation demonstrated that lining epithelium is pliable to a great extent and adapted for marked elongation.

*Key-words:* Epithelium; esophagus; guinea pigs

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Under physiological conditions the mucous membrane of the upper part of the alimentary tract is subjected to a variety of compressing and stretching forces especially during mastication and swallowing.

As part of a series of investigations (*Fejerskov*, 1970; *Philipsen*, 1971; *Fejerskov*, 1971a, 1971b, 1971; *Fejerskov & Philipsen*, 1971) into the histology and cytology of rodent keratinized mucous membrane epithelium under normal and experimental altered conditions, it was found of interest to study the changes in the structure of the epithelium under acute mechanical stress.

The effect of mechanical stress on keratinized squamous epithelium in masticatory mucosa such as palatal mucosa (mucoperiosteum) has been studied in dogs

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by *Scapino* (1967) and *Kydd et al.* (1969). When subjected to load, the epithelial ridges were found to decrease in depth and exhibited a completely flattened appearance and the connective tissue papillae were obliterated. The extent of the alteration varied with the force and the duration of applied force. The *in vivo* response of lining mucosa to loading does not seem to have been investigated, probably because the experimental conditions are difficult to standardize in a highly movable mucosal area such as the cheek or lip.

Preliminary studies have shown that the structure of the epithealial lining of the cheek, lip and esophagus in guinea pigs is basically similar (*Philipsen & Fejerskov*, 1972). Furthermore, the type of attachment of the mucosa to underlying structures is the same in the three areas. The esophagus rather than the cheek or lip was chosen for the present investigation because it is possible through dilatation of this hollow organ to transmit a uniform mechanical load to a well-defined area of keratinized mucous membrane.

## MATERIAL AND METHODS

Esophagi were obtained from 50 young, adult male guinea pigs (720—950 gm body weight), 28 of which served as control material. In 22 animals the esophagi were dilated as subsequently described. All animals were killed by intraperitoneal injection of an overdose of Nembutal®. After making a ventromedian incision in the neck, the trachea and esophagus were freed at the level of the larynx and the tracheal bifurcation.

*Experimental material.* Dilation of the esophagi (22 animals) was carried out under deep Nembutal® anesthesia. Polyethylene tubes were passed carefully through the mouth into the esophagus until resistance was met at the cardia. Tubes of different caliber were used and it was found that tubes 3.0 mm and 4.3 mm in diameter gave a slight respectively a strong dilation of the organ. In seven animals, tubes 3.0 mm in diameter, and in 15 animals, tubes 4.3 mm in diameter were used. After ten minutes of dilation, the animals were killed with an overdose of Nembutal®. The trachea-esophagus tissue block was removed leaving the polyethylene tubes *in situ* and fixed as described for the controls. At a distance of about 10 mm from the inferior border of the larynx, pieces of esophageal tissue were removed and embedded perpendicularly to the long axis of the organ. The tissue was sectioned and stained as described for the controls. The difference between the variations of the length of the esophageal basement membrane of the control material and the length of the distended basement membrane expressed as the circumference of a tube with a diameter of 4.3 mm were calculated.

*Control material.* The esophagi of four animals were cut lengthwise, opened to expose the mucous membrane and pinned onto corkboard. The mounted mucosa was washed to remove food particles and fixed immediately in 10 % buffered formalin for 24 hours. After two hours in 70 % alcohol, it was painted with a 5 % alcoholic solution of iodine to increase contrast in the mucosal surface relief. The tissue was examined macroscopically at various magnifications with stereomicroscope using oblique, reflected and transmitted light.

For light microscopy (paraffin sections) trachea-esophagus blocks from 20 animals were fixed in 10 % buffered formalin for

three days. 18 blocks were cut perpendicularly to the long axis of the esophagus, approximately 1, 10, 15 and 20 mm from the inferior border of the larynx. From two tissue blocks the esophagi were examined in longitudinal sections. Measurements of the length of the epithelio-mesenchymal border (basement membrane) in sections cut perpendicular to the long axis of the esophagus were carried out as follows. Paraffin blocks from six animals (760—800 gm) were sectioned serially, 15 and 20 mm from the larynx. In each of these series the length of the basement membrane was measured in ten consecutive sections at intervals of 40 microns. By means of a projector (250 X magnification), the basement membrane of the cross-sectioned esophagus was traced and its length determined with a map measurer. In order to establish the error of the method (S(i)), measurements were duplicated in the ten sections and S(i) calculated according to the formula $\sqrt{\frac{\Sigma (x-y)^2}{2n}}$, to 14.2. An analysis of variance was carried out both for variation between animals and for variation between sections at varying distances from the larynx in the same animal.

Seven to eight micron thick sections were stained with hematoxylin-eosin, van Gieson-Hansen's connective tissue stain, periodic acid-Schiff (PAS) phloxin-tartrazine (*Lendrum*, 1947) and a modified Mallory's connective tissue stain (*Weinmann & Meyer*, 1959).

Esophageal tissue from four animals was excised 10 mm from the larynx and fixed in a modified paraformaldehyde-glutaraldehyde solution (*Karnovsky*, 1965). The tissue was embedded in Epon and one micron thick sections were stained with toluidine blue and paraphenylenediamine (*Estable-Puig, Bauer & Blumberg*, 1965).

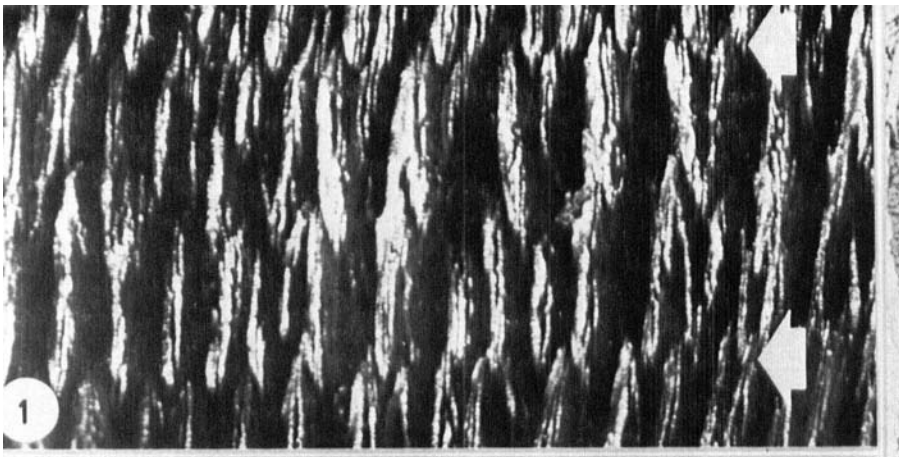
## RESULTS

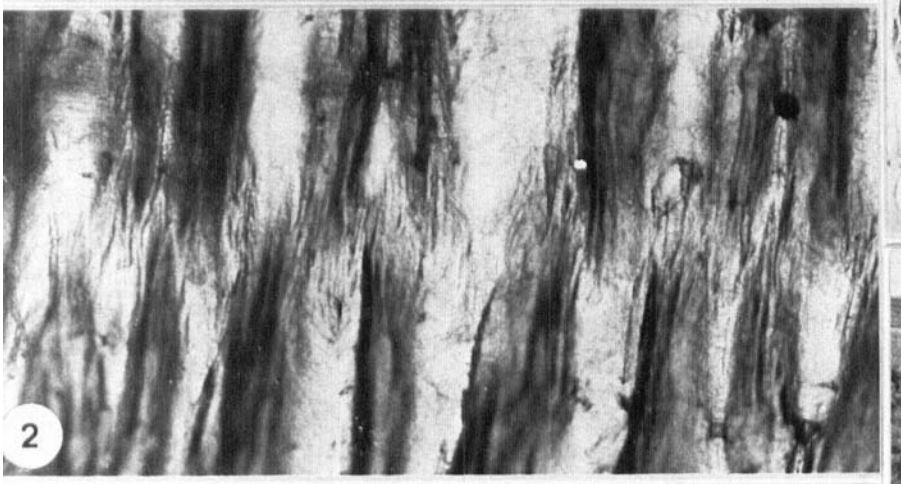
*Macroscopic examination.* The mucosal surface of the esophagus shows a regular pattern of circularly arranged crests separated by furrows. The height and width of both crests and furrows are very uniform. The crests often bifurcate. The system of crests can be resolved into longitudinal segments, separated by grooves Fig. 1). Each segment is displaced in relation to its neighbours. At the top of the individual crests and at the bottom of the grooves a microrelief can be discerned (Fig. 2).

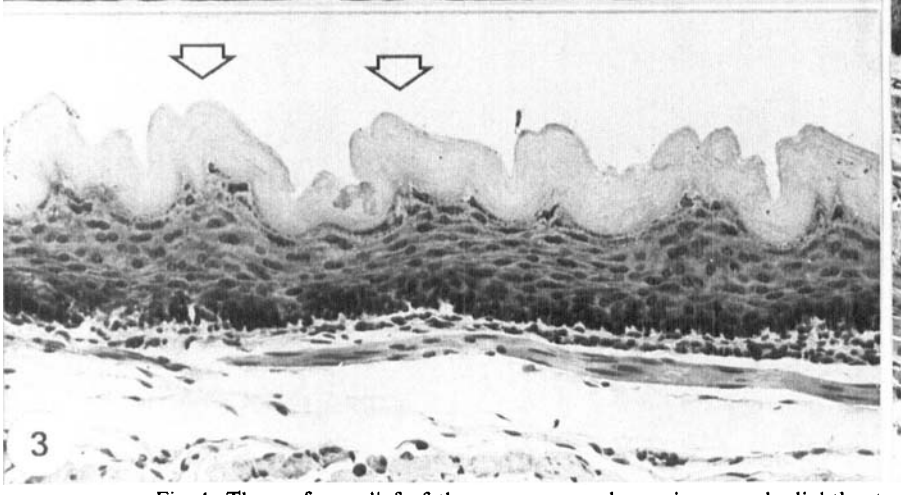
*Microscopic examination.* The esophagus of the guinea-pig consists of six layers of tissue (Fig. 4). The histology of the esophageal epithelium is uniform showing a festoon-shaped epithelium-connective tissue boundary with slender connective tissue papillae separating blunt epithelial ridges (Figs. 4 and 5). In longitudinal sections, the epithelium-connective tissue boundary is, however, almost level with only a few short connective tissue papillae (Fig. 3).

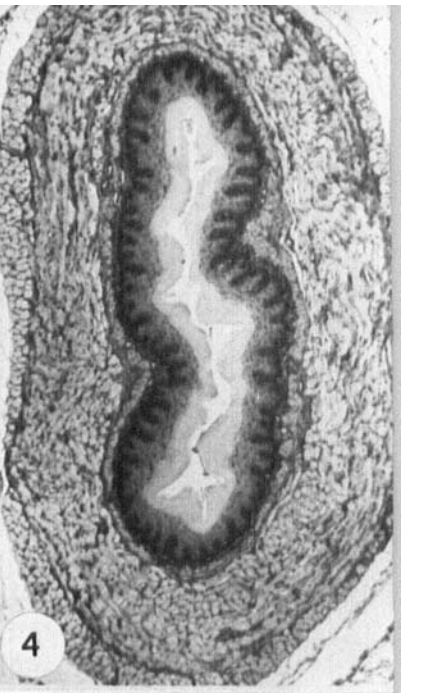
The stratum basale consists of a row of cuboidal cells dominated by deeply stained nuclei (Figs. 6 and 8). In 1 micron thick plastic sections the cytoplasm of the majority of basal cells is weakly stained. An elongated fusiform cell-type with deeply stained cytoplasm is often seen between the basal cells (Fig. 9).

The stratum spinosum consists of eight to ten layers of cells. In the center of the epithelial ridges, the cells become flattened with their long axes roughly parallel to the epithelium-connective tissue boundary (Fig. 9). The cells in the middle of the stratum spinosum are arranged in arcades, arching from epithelial ridge to epithelial ridge over the connective tissue papillae. This orientation of the cells be-



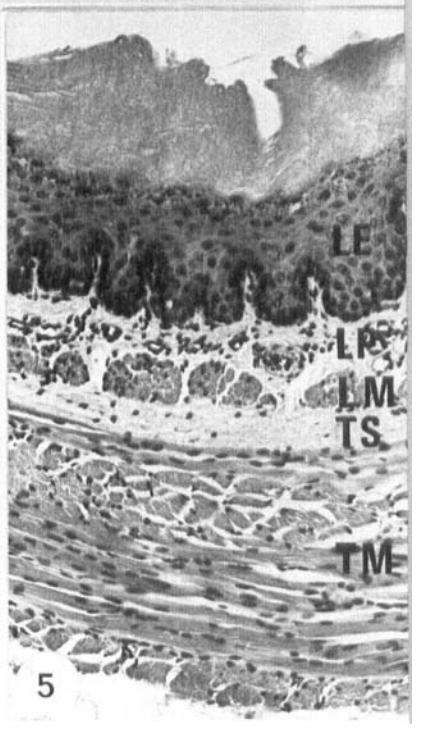



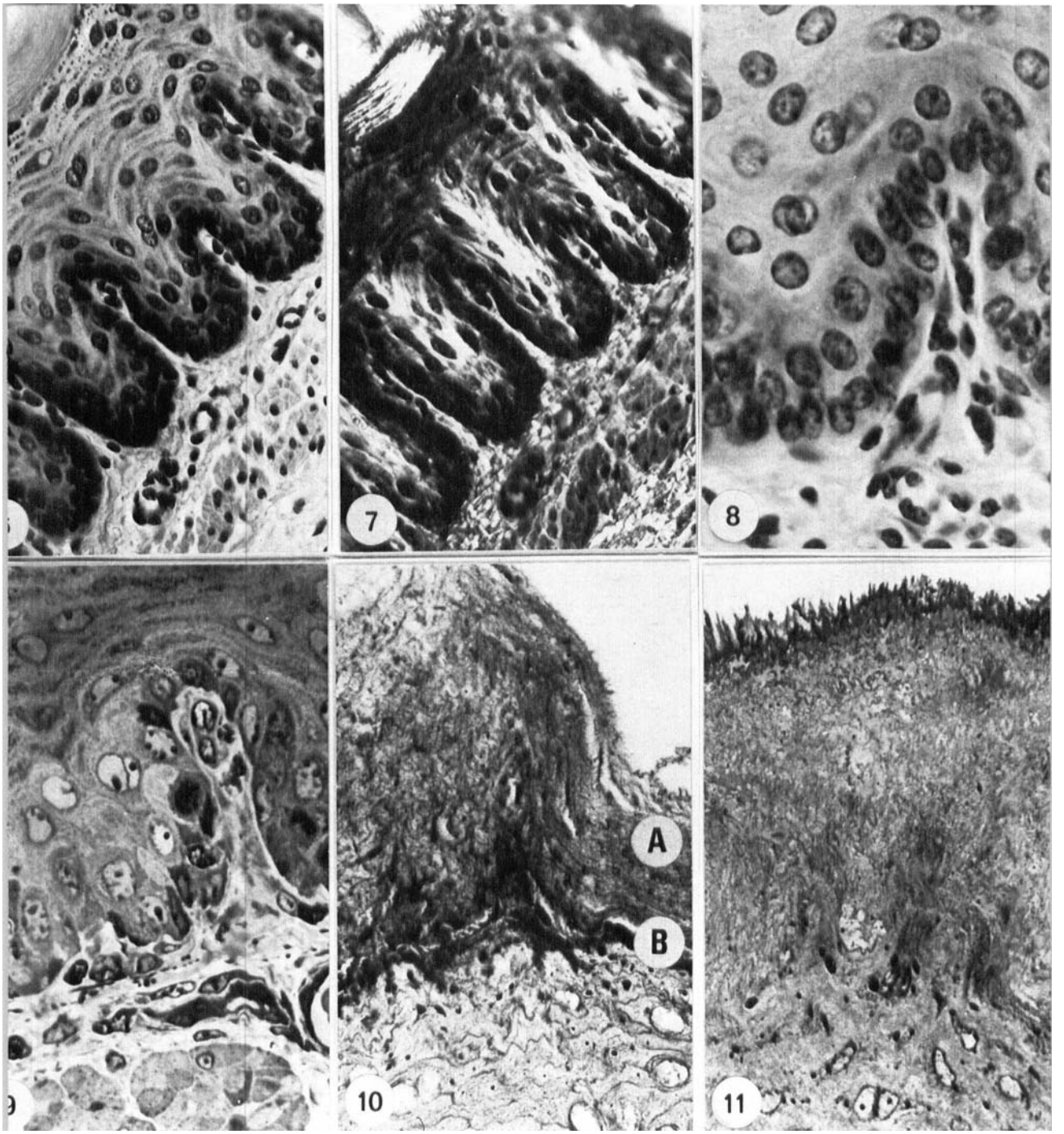

Fig. 1. The surface relief of the mucous membrane in opened, slightly stretched esophagus, photographed in oblique, reflected light. The short, circularly arranged keratin crests are separated by furrows and arranged in longitudinal segments. The crests overlap across longitudinal grooves (arrows). Treated with alcoholic iodine solution. ×40.

Fig. 2. Detail of longitudinal (in the photograph horizontal) groove between two segments, illuminated by transmitted light. The surface of the individual keratin crests, and of the base of the groove, is characterized by a microrelief. Treated with alcoholic iodine solution. ×100.

Fig. 3. Microphotograph of longitudinally sectioned esophageal mucosa. The epithelium-connective tissue boundary is almost level. Along the epithelial surface keratin papillae are marked with arrows (compare with the surface relief in figs. 1 and 2). Note the numerous binucleate cells in the stratum granulosum and stratum spinosum. Hematoxylin-eosin. ×100.

Fig. 4. Cross-section of esophagus, 10 mm from the lower border of the larynx. Periodic acid-Schiff (PAS). ×30.

Fig. 5. Detail of the esophagus wall. Lamina epithelialis (LE), lamina propria (LP), lamina muscularis mucosae (LM), tela submucosa (TS), tunica muscularis (TM). Hematoxylin-eosin. ×130.




Figs. 6 & 7. Detail of the lamina epithelialis without and with polarized light. In Fig. 6, cells of the stratum spinosum are arranged in an arcade from epithelial ridge to epithelial ridge. This cell arrangement is also distinct in polarized light (Fig. 7). The stratum basale appears as a densely stained row of cells dominated by nuclei. In the stratum granulosum, many keratohyalin granules are visible, increasing in size towards the stratum corneum. Hematoxylin-eosin. ×300.

Figs. 8 & 9. Epithelial ridges in paraffin-embedded (Fig. 8) and plastic-embedded tissue (Fig. 9). In 7 to 8 $\mu$ thick paraffin sections, no differences are seen in the staining of the cytoplasm of the cells of the stratum spinosum and stratum basale, but in 1 $\mu$ thick plastic sections, differently stained cells are seen in both strata.

Fig. 8: Phloxin-tartrazine. ×700.

Fig. 9: Toluidine blue. ×700.

Figs. 10 & 11. The superficial layers of the lamina epithelialis in paraffin-embedded (Fig. 10) and plastic-embedded tissue (Fig. 11). The stratum corneum is composed of loosely structured, orthokeratinized cell layers, which in paraffin sections show a zonation in an upper (A) and deeper (B) part (Fig. 10). In the stratum granulosum numerous irregular keratohyalin granules are seen.

Fig. 10: Mallory stain. ×700.

Fig. 11: Toluidine blue. ×700.

comes especially distinct in polarized light, when the birefringent bundles of tonofibrils in the cells appear as light bands (Fig. 7). The uppermost cells of the stratum spinosum are flattened and arranged parallel to the boundary between the stratum granulosum and stratum corneum. In 1 micron plastic sections typical polygonal, lightly stained stratum spinosum cells occur only in the epithelial ridges. The flattened stratum spinosum cells have a deeply stained granulated cytoplasm and extend down into the epithelial ridges (Fig. 9). Binucleate stratum spinosum cells are often seen in considerable numbers (Fig. 3).

The stratum granulosum consists of four to six flattened cell layers, containing deeply stained keratohyalin granules (Figs. 10 and 11).

The stratum corneum consists of numerous loosely structured, orthokeratinized cell layers (Figs. 5, 10 and 11) with numerous keratin papillae, corresponding to the ridges described macroscopically (Fig. 1). In sections stained with phloxin-tartrazine and Mallory's connective tissue stain, the stratum corneum can be divided into a deeper, narrow, heavily stained zone and an upper, lightly stained, broad zone (Fig. 10). The cell borders are difficult to distinguish, except along the outermost cells where irregular, tongued cell membranes may be discerned (Figs. 10 and 11).

Glycogen could not be demonstrated in any of the epithelial strata.

The length of the basement membrane shows no significant difference ($p>0.05$) in the same animal between measurements made at a distance of 15 mm and those made at a distance of 20 mm from the larynx ($F_{\frac{1}{2}} = 1.476$), whereas the individual variation was significant, $p<0.01$ ($F_{\frac{5}{54}} = 29.96$).

The introduction into the esophagus of polyethylene tubes with external diameters of 3.0 mm and 4.3 mm respectively causes dilatation of the organ with compression of the wall (Figs. 12, 13 and 14). After dilatation with 3.0 mm tubes, a levelling of the corrugated epithelium-connective tissue boundary is seen, with only a few lamina propria papillae (Fig. 15). The number of nucleated layers in the epithelium is reduced from 15 to ten. The stratum corneum is compressed and of uniform thickness without keratin papillae. The nuclei of the basal cells are oval with their long axes parallel to the epithelium-connective tissue boundary, and in the upper layers, dense compressed nuclei are seen.

After dilatation with 4.3 mm tubes, the nucleated part of the epithelium consists of about seven cell layers with all nuclei flattened and densely stained (Fig. 16). The stratum corneum is strongly compressed. The connective tissue papillae are reduced in height to weak invaginations along an otherwise even epithelium-connective tissue border. A break in the continuity of the epithelium has not been observed in any of the dilated esophagi.

## DISCUSSION

The irregular keratin papillae observed in the histological preparations have shown to be part of a complex system of keratin ridges, which can be made very distinct when the mucous membrane is treated with an alcoholic solution of iodine. This relief or pattern of the mucosal surface can be smoothed by a stretching of the membrane along the long axes of the organ, indicating that the keratin layers possess a high degree of flexibility. The system of ridges presumably has a mechanical function in the process of swallowing.

The epithealial lining of the esophagus

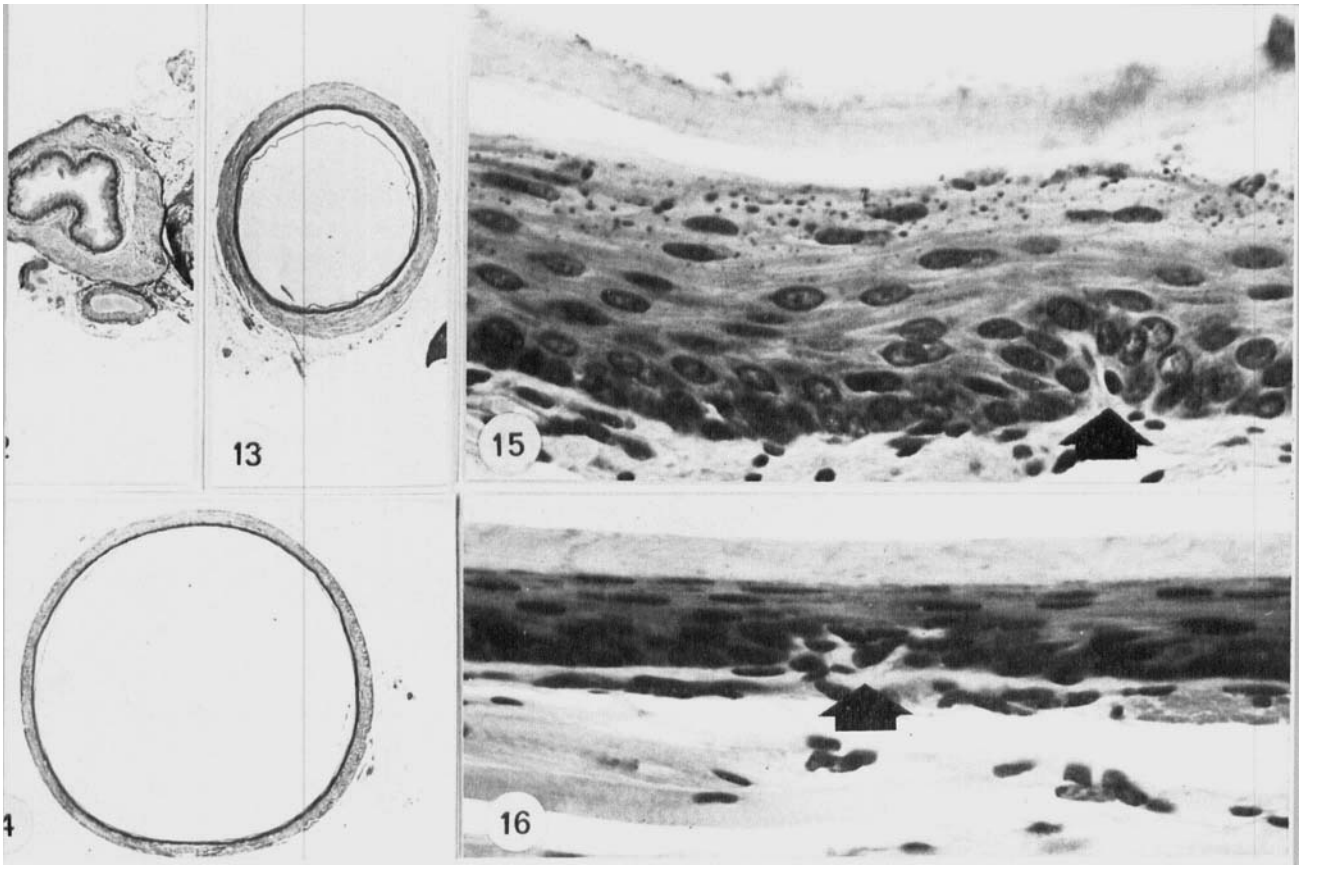



Fig. 12. Orientation section of a normal esophagus. Hematoxylin-eosin. ×8.
Figs. 13 & 14. Esophagi dilated with tubes 3.0 mm and 4.3 mm in diameter. Hematoxylin-eosin. ×8.
Fig. 15. Detail of the lamina epithelialis of an esophagus dilated with a 3.0 mm tube. Note flattening of all the cell layers, parallel to the surface. Partial smoothing of the epithelium-connective tissue relief, with a small lamina propria papilla (arrow). Hematoxylin-eosin. ×540.
Fig. 16. Detail of the lamina epithelialis of an esophagus dilated with a 4.3 mm tube. Strong flattening of all cell layers. The epithelium-connective tissue boundary is parallel to the surface except for the few lamina propria papillae (arrow). Hematoxylin-eosin. ×540.

in the guinea-pig has been studied histologically by *Arcangeli* (1908), *Goetsch* (1910), *Kollman & Papin* (1914), *Kullman* (1931), *Török* (1966) and *Oláh & Török* (1966). The result of the present investigation agrees with these studies. The high degree of uniformity in the histology of the epithelium both between individual animals and between the different examined parts of the esophagus makes this mucous membrane an ideal test object when studying experimental pathology of keratinized squamous epithelium. The regular interface relief between the epithelium and the connective tissue is a feature in which the guinea pig differs from other rodents (*Figdor*, 1957).

The difference in stainability of the cytoplasm of the stratum spinosum cells has not been described in previous light and electron microscopic examinations of esophageal epithelium of young guinea pigs (*Oláh & Török*, 1966). The deeply stained cells in the stratum spinosum correspond to the arcading cell layers where a distinct birefringence of tonofibrils is found in paraffin embedded material. The present authors interpret this cellular arrangement as an expression of an adaptation to the mechanical stresses to which the flexible esophageal mucosa is constantly subjected. A comparison with the firmly bound, immovable palatal mucosa of the guinea-pig has shown that the stratum spinosum

of the epithelium does not exhibit a similar arcade arrangement in the very tall and slender epithelial ridges (*Philipsen*, 1971).

The presence of binucleate cells in the stratum spinosum has been described earlier by *Pacaut* (1905, 1909), *Arcangeli* (1908), *Ditlevsen* (1911), and *Kollman & Papin* (1914). The nuclear arrangement within the cells corresponds to that described in detail in the stratified squamous epithelium of the hard palate of the guinea-pig (*Philipsen*, 1971).

The division of the stratum corneum into a deeper and an upper zone of different stainability conflicts with the observations of *Arcangeli* (1908), who could demonstrate only one layer. It corresponds closely, however, to what *Kollmann & Papin* (1914) have described and termed »couche cornée inferieur et superieur». In the rat, *Marques-Pereira & Leblond* (1965) have likewise observed a deeply stained deeper zone, termed the »glassy layer» or stratum lucidum. *Weinmann et al.* (1960) have observed in the cheek mucosa of the mouse a zonation of the stratum corneum corresponding in the staining properties in all respects to that found in the esophagus of the guinea pig. They consider this zonation to be an expression of an »incomplete orthokeratinization». It is only seen in connection with a stratum granulosum containing coarse keratohyalin granules, as in esophageal epithelium.

Glycogen has not been demonstrated in the epithelium of the esophageal mucosa. This agrees with observations in the esophageal mucosa of adult mice (*Parakkal*, 1967), but conflicts with *Török's* observation (1966) in the esophagus of the guinea pig, as he finds a slight accumulation of glycogen in the stratum basale with a strong increase in amount in the stratum spinosum. The material in the latter study stems from »embryonic, newborn and sexually mature» animals, without the author stating in which group glycogen was observed.

With the introduction of tubes with a circumference larger than the periphery of the esophagus lumen, a combined compression and stretching of the mucosa occurs. This results in a smoothing of the epithelium-connective tissue boundary with a strong flattening of all cell layers. *Scapino* (1967) and *Kydd et al.* (1969) have studied the effect of mechanical stress on firmly bound oral mucosa (mucoperiosteum) in dogs. Their experiments show that the mucous membrane is not smoothed out but instead an irregular distortion of the epithelial ridges and connective tissue papillae occurs. The extent of this alteration varied with the force and the duration of the applied force. *Kydd et al.* (1969) found no sign of cytological changes in the epithelium subjected to a 5 g/mm$^2$ force until the 4 hr duration was reached. At this stage vacuolation, cellular swelling, increased nuclear size and intercellular oedema were found. The present authors interpret these changes as being a combined mechanical and metabolic (ischaemic) effect on the living epithelium. When cellular changes of this type have not been observed in the present study it is most likely due to the short duration of the applied force (10 min.). Within this period no effect of pressure induced circulatory insufficiency can be expected to manifest itself cytologically.

During acute moderate dilation of the esophagus, no signs of break in tissue continuity have been found. This shows that the epithelium of the esophagus is highly flexible. The marked reduction in thickness of the stratum corneum, with the complete loss of the surface pattern, in-

dicates that the keratinized cell layers are easily extended and very deformable.

In the strongly dilated esophagi, the epithelium-connective tissue interdigitation pattern is completely flattened and only occasional invaginations in the epithelium can be interpreted as connective tissue papillae. It has not proved technically feasible to produce perfect serial sections of the dilated esophagi, to allow direct measurements of the length of the basement membrane.

Instead, the circumference of the tubes has been regarded as the approximate length of the stretched basement membrane. The true length of the basement membrane in the dilated esophagus is nevertheless greater, as it constitutes a circle concentric with the tubes, but of greater diameter. The circumference of the largest tube has been found to be considerably greater than the average length of the basement membrane in the normal esophagus ($p<0.05$), and therefore a pronounced stretching of the nucleated epithelial cells and the basement membrane must have taken place.

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