A CONTRIBUTION TO THE MICROSCOPIC ANATOMY OF THE POSTNATAL EPIGLOTTIS OF THE DOMESTIC ANIMALS

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A CONTRIBUTION TO THE MICROSCOPIC ANATOMY OF THE POSTNATAL EPIGLOTTIS OF THE DOMESTIC ANIMALS

By

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INTRODUCTION

Although the larynx of man and many animals has been thoroughly investigated, relatively little is revealed concerning the detailed histology of the epiglottis of the domestic animals. Several investigations concerning gross anatomy (Chauveau 1873, Sisson and Grossman 1953, Prodinger 1940, Nickel et al. 1960), function and origin in relation to primitive needs of the epiglottis (Negus 1927, 1929, 1949) in the animal kingdom are excellent.

While all of the current histology texts refer to the histology of the epiglottis of man, only a few (Carleton and Leach 1949, Krölling and Grau 1960) illustrated the discussion with an epiglottis of the domestic animals (cat, dog). The purpose of this report is to contribute to the literature concerning the normal microscopic anatomy of the postnatal epiglottis of the seven domestic animals, with the hope that it may be useful in the fields of comparative histology and physiology.

The gross anatomy in this report is limited, as this study was confined to the microscopic anatomy.

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REVIEW OF LITERATURE

The epiglottis is the most anterior single cartilage which forms the cartilaginous framework of the larynx (Chauveau 1873, Dubois 1886, Negus 1949, Sicher 1952, Sisson and Grossman 1953). Nickel et al. (1960) defined the epiglottis as the epiglottic cartilage covered by its mucosa. Since the epiglottis is a part of the larynx, we can learn about it in the literature concerning the larynx or the respiratory system.

Generally the larynx is called "voice box" (Nelsen 1953, Montagna 1960, Leach 1961, Greisheimer 1963). According to Negus (1949) this is somewhat of a misnomer, since the production of the voice is but one of the functions of the larynx. Jackson and Jackson (1942), and Boies (1955) listed the various functions of the larynx as respiratory, circulatory, fixative, protective, deglutitory, tussive, expectorative, and emotional. Negus (1924, 1927, 1929, 1949) reported that the larynx in certain species, but not in man, also played an important role in olfaction. Last (1954) also mentioned the olfactory function of the epiglottis. Negus (1949), Kaplan (1960) and Leonard (1960) agreed that removal of the epiglottis did not interfere with deglutition. Leonard noted that as long as the basal one-third of the epiglottis remained intact, the normal physiologic functions of the larynx still exist. In a recent review of the physiology of the larynx Pressman and Kelemen (1955) stressed the importance of the gross and microscopic anatomy of the various components of the larynx.

If the mammalian larynx is compared with that of the lower vertebrates, several changes appear, the most significant of which is the presence of the epiglottis (Kingsbury 1943, Negus 1949, Walter and Sayles 1949, Nelsen 1953, Montagna 1960). In amphibia (Wilder 1892), reptiles and birds (Negus 1929, 1949) there is almost a universal absence of the epiglottis.

Gegenbaur (1892) reported a paired organ and a hyaline type cartilage of the Monotreme epiglottis. According to Walander (1950) the epiglottic cartilage in the rat is developed from a region related to the fourth visceral arch. Walander reviewed various opinions of the derivation of epiglottic cartilage by several workers, namely: as a derivative of the third visceral arch (Rathke 1861, Kolliker 1861, Ganghofner 1880, Roth 1880, Born 1883, Steffenson 1932); as a derivative of the fourth visceral arch (His 1880, Soulie and Bonne 1907); as a derivative of the sixth visceral arch (Gegenbaur 1892); not as a derivative of the visceral skeleton, but had the same histological nature as the tracheal rings (Balfour 1881); and other workers (Schaffer 1907, Patzelt

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1924) thought that the epiglottic cartilage is formed secondarily in the mucous membrane.

Gross Anatomy of the Epiglottis

Position

Terminology:

Prevelar: The epiglottis lies below the soft palate; syn., contrapalatine.

Postvelar: The epiglottis lies above the soft palate; syn., intranarial.

Sisson and Grossman (1953) mentioned that the prevelar epiglottis is situated below the soft palate and the postvelar epiglottis is above the soft palate. According to Negus (1957) in most keen-scented mammals the epiglottis assumed an intranarial position, lying on the upper surface of the soft palate.

Howes (1889a) reported two cases of intranarial epiglottis in rabbits. Later Howes (1889b) detected an intranarial condition in the human fetus of five months and at birth. He listed mammals in which an intranarial epiglottis has been observed postmortem.

Bowles (1889) investigated the suckling pig soon after birth and found the epiglottis in the mouth, below and considerably anterior to the soft palate. In the adult pig, as in the horse, the epiglottis lies above the soft palate. Bowles suggested that probably the pig epiglottis

becomes intranarial within the fifth and sixth month of extra-uterine life. According to Negus (1929) the epiglottis is intranarial in cats, oxen, sheep, goats and horses and contrapalatine in dogs.

Shape

Chauveau (1873) described the epiglottis of the ruminants as wider but less pointed than in solipeds. In carnivores it is shorter, wider at the base and more triangular than in other species. The pig epiglottis is well developed with an omega shape.

Prodinger (1940) noted that the epiglottis is triangular in the horse, oval in ruminants, rounded in swine, and quadrilateral in carnivores. According to Nickel et al. (1960) the oral end of the epiglottis is pointed in carnivores, small ruminants and horses, and rounded in swine and cattle.

Miller (1952), Sisson and Grossman (1953) and Nickel et al. (1960) described a small projection on the base of the dog epiglottis, the so-called Petiolus epiglottidis. Nickel et al. (1960) and Vacirca (1961) mentioned this peduncle in the bovine epiglottis.

In the horse the innerpart of the cartilage base has lateral projections, called Cartilage cuneiformis Wrisberg (Sisson and Grossman 1953). Goppert (1894) recognized the Wrisberg cartilage as a derivative of the epiglottic artila

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cartilage. The cuneiform cartilage is a cartilage rod enclosed in the connective tissue of the aryepiglottic fold (Negus 1949).

Chauveau (1873) and Sisson and Grossman (1953) described two surfaces, two borders, a base and an apex on the epiglottis. The two surfaces are named differently by several authors. These are: (1) the lingual, anterior, ventral, oral, or pharyngeal surface. (2) the laryngeal, posterior, tracheal, or dorsal surface.

Hyoepiglottic muscle

Howes (1889a) described a well developed hyoepiglottic muscle in mammals having an intranarial epiglottis. Negus (1927) reported that the hyoepiglottic muscle is large and powerful in all animals whose epiglottis is large and mobile. Sisson and Grossman (1953) mentioned that the action of the hyoepiglottic muscle is to draw the epiglottis toward the root of the tongue. It connects the lower part of the oral surface of the epiglottis and the body of the hyoid bone. This muscle is enclosed by an elastic sheath, called the hyoepiglottic ligament. According to Sutton (1889) the hyoepiglottic ligament in man is the fibrous representative of a well formed muscle in many mammals. Another ligament which is attached to the base of the epiglottis is the thyroepiglottic ligament. It is composed of elastic tissue (Sisson and Grossman (1953).

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Nerves and vessels

Vogel (1952) stated that the internal laryngeal nerves of man have a motor component, but they are purely sensory in the dog. According to Sisson and Grossman (1953) the larynx is supplied by the laryngeal and ascending pharyngeal arteries and corresponding veins and the vagus nerve. The lymph vessels enter the anterior cervical and pharyngeal lymph nodes.

Microscopic Anatomy of the Epiglottis

The epithelial covering

Hopp (1955) reviewed the literature concerning the differences of opinion among histologists about the nature of the epithelium of the larynx including the epiglottis.

Hopp (1955), Gregg (1959), Finerty and Cowdry (1960) agreed that the laryngeal mucosa is metaplastic. There is always a differentiation of the epithelium of larynx. According to Gregg (1959) this metaplasia may be physiological or a precursor of malignant changes. In lower animals a primary neoplasm in the larynx is rare.

Patzelt (1921) studied the human epiglottis in prenatal and postnatal life. Different types of epithelial linings are related to different age periods. In the first week of life the epithelium of the epiglottis has no definite character. In the seven-week-old embryo the entire surface

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is still covered by a uniform epithelium, consisting of two or three layers of cylindrical epithelium. In later development the two surfaces of the epiglottis undergo different changes. At about eight weeks of age the laryngeal surface begins to have cilia. At nine months of embryonal age the lower half of the laryngeal surface is covered by stratified ciliated epithelium. The upper half of the laryngeal surface has a few cilia. The cilia gradually disappear and definite stratified pavement epithelium is formed on the upper half of the laryngeal surface. At the beginning of the second half of the pregnancy the pavement epithelium of the upper half of the laryngeal surface is 70 microns in thickness. At birth the cilia from the upper half have completely disappeared.

In the adult the lower part of the laryngeal surface is covered by stratified ciliated cells with goblet cells and is approximately 60-70 microns in thickness. Usually between the stratified pavement epithelium of the upper half and the stratified ciliated epithelium of the lower half of the laryngeal surface there is a stratified epithelium without goblet cells, the "intermediate" epithelium.

At nine months of embryonal age the lingual surface is covered by stratified columnar epithelium with very few cilia, and its lower half is arranged in many layers of cells. At birth the cilia disappear. In the adult the greater part of the lingual surface is covered by stratified pavement ithel

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epithelium. The thickness is different in different areas --at the anterior or upper part, 70-90 microns, and at the posterior or lower part, 90-150 microns.

Dawson (1948) noted a stratified squamous epithelium on the pharyngeal surface and pseudostratified epithelium on the laryngeal surface. Some authors (Greep 1954, Garven 1957, Bargmann 1959, Ham and Leeson 1961, Bloom and Fawcett 1962) mentioned a stratified squamous epithelium on the anterior and upper posterior surface and a pseudostratified ciliated epithelium on the lower part of the posterior surface. In contrast to these views most authors (Ellenberger and Schumacher 1914, Bremer 1944, Hewer 1947, Carleton and Leach 1949, Nusshag 1952, Trautmann and Fiebiger 1957, Ruedi 1959, Krölling and Grau 1960) mentioned only stratified squamous epithelium in relation to the epiglottis.

The only research on the epithelial covering of the free border of the epiglottis encountered by the author was by Bazzana (1953). This separate study was based on the localization of diseases in the three surfaces of the epiglottis. According to Bazzana the stratified squamous epithelium of the free border in man is constantly present from the first month of life. It remains unaltered in its structure up to old age. Only at about ninety years of age do senile changes begin. No particular microscopic changes were noted in the free border of the epiglottis of man (15-17 years) and young cats (one year).

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Submucosa

Sobotta (1930) mentioned that in the larynx a true tunica submucosa is lacking. Copenhaver and Johnson (1958) described a poorly defined submucosa in the larynx. According to Trautmann and Fiebiger (1957) the submucosa is thin, except at the base of the epiglottis.

Taste buds

Sussdorf (1911) reported the observation of "taste-goblets" on the laryngeal surface of the epiglottis by Verson (1868, 1872--man, sheep, dog, and cat), Honigschmied (1873, 1877, 1880--calf), and Davis (1877--swine). Michelson (1891) was the one who proved that these "taste-goblets" were true taste buds. He used Schroetter's larynx detector and mirror to introduce concentrated saccharin into the larynx on the inner surface of the epiglottis, in such a way that no drop fell on the tongue. The result was that the person could taste the introduced fluid. Many other authors (Rabl 1896, Patzelt 1921, Stohr 1949, Koizumi 1953b, Trautmann and Fiebiger 1957, Krolling and Grau 1960) reported taste buds on the laryngeal surface of the epiglottis,

Tuckerman (1889) found one taste bud on the anterior or lingual surface of a human epiglottis. Of 949 taste buds in the human newborn, LaLonde and Eglitis (1961) found three on the lingual surface with the remainder scattered on the laryngeal surface.

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Glands

Chauveau (1873) described the glands of the larynx as racemose and numerous on the posterior surface of the epiglottis. The glands are lodged in minute depressions of the cartilage. The propria mucosa of the larynx contains glands of serous, mucous and mixed type gland acini (Sussdorf 1911, Heiss 1936, Trautmann and Fiebiger 1957). According to Heiss, Von Ebner divided the glands of the larynx in three groups. These were (1) anterior, (2) middle and (3) posterior groups of glands. The anterior group includes the glands of the lingual and the laryngeal surface of the epiglottis. The ducts of the glands of the lingual side also open on the laryngeal surface. These ducts penetrate the epiglottic cartilage.

The epiglottic cartilage

Symington (1900) investigated elastic fibers in the epiglottic cartilage of Echidna and also noticed patches of hyaline cartilage in the Cetacean epiglottis. Most authors (Chauveau 1873, Dubois 1886, Heiss 1936, Trautmann and Fiebiger 1957, Copenhaver and Johnson 1958, Bargmann 1959, Bloom and Fawcett 1962) considered the epiglottic cartilage an elastic cartilage. According to Finerty and Cowdry (1960) it is hyaline at the beginning and becomes elastic in adult life. Carleton and Leach (1949) mentioned a parenchymatous epiglottic cartilage in many animals.

It contains an abundance of cells and very little matrix.

Schaffer (1907) investigated two different parts of the rat epiglottis: the small axial hyaline cartilage a continuation of the thyroid cartilage, and a large peripheral portion which is elastic.

Pressman and Kelemen (1955) stated that ossification and calcification take place in the various cartilages of the larynx, as part of the process of aging. However, there is no strict parallelism between the degree of ossification and age. According to Negus (1949), Carleton and Leach (1949), Sisson and Grossman (1953) the epiglottis never ossifies.

Johnson _et al._ (1958) and Gardner _et al_. (1960) described pits and foramina in the epiglottic cartilage. In the examination of the human, dog, cat and rabbit epiglottic cartilage Montagna (1949) reported that the chondrocytes contained glycogen granules.

Nerves and vessels

Many histologic studies on the innervation of the mucous membrane of the larynx and eventually of the epiglottis have been reported (Ploschko 1897, Koizumi 1953a b, Koizumi and Mikami 1953, Feindel 1956, Zufarov 1956, Lee 1959, Jabonero 1959, Voloshchenko 1960, Konig and Leden 1961). The findings of Koizumi (1953 a, dog), Feindel (1956, rabbit and monkey), and Lee (1959, goat) have shown

that the sensory nerve distribution on the laryngeal side is more numerous than on the lingual surface. There are a variety of sensory end organs in the epiglottis (Ploschko 1897, Feindel 1956, Jabonero 1959). Ploschko divided these nerve endings into two main groups, subepithelial and intra-epithelial.

Sussdorf (1911) mentioned that the blood, lymph vessels and nerves of the larynx and eventually the epiglottis had been investigated by Boldyrew (1871) and Spiefs (1894). There is a rich lymph vessel network on the oral side of the epiglottis. The numerous blood vessels form a network and also penetrate the cartilage.

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MATERIALS AND METHODS

For this study a total of forty-six animals were used. This group of animals included:

Dogs : eighteen (five females and thirteen males); one day to five years.

Cats : six (one female and five males); eight months to five years.

Cattle: six (four females and two males); six weeks to eight months.

Sheep : four (one female and three males); one week to two years.

Goats : four (one female and three males); two weeks to seventeen months.

Swine : six (two females and four males); one week to one year.

Horses: two (male); aged.

The animals were obtained from the Departments of Anatomy and Veterinary Pathology at Michigan State University, and Van Alstine's Meat Processing Plant in East Lansing. Whenever possible the relation of the epiglottis to the soft palate was observed grossly.

Epiglottises were removed from the freshly killed animals and cut in half sagittally (see Figure I). One-half of each section was boiled. The mucosa was removed and the epiglottic cartilage was stored in 100 per cent alcohol. Observations were made on the shape of the cartilage and the presence of pits and foramina.

The other half of each section was placed in F. A. A. (Lavdowsky's mixture, Guyer 1953) for fixation. After fixation the dehydrating and infiltrating method of Johnson _et al_. (1943) was followed using Tissuemat* as the embedding medium. Blocks of tissue from young dogs and cats were cut at 7 microns and from the rest of the animals were cut at ten microns.

Three stains were used (1) Harris' hematoxylin and eosin as a routine stain, (2) Weigert's and Van Gieson's connective tissue stain to identify elastic and collagenous tissue, and (3) half per cent of Toluidine blue (Lillie 1954) for determination of mucus.

Using slides of sagittal sections, the following observations and measurements were made:

1. Epithelial height
2. Subepithelial connective tissue thickness
3. Taste bud location
4. Distribution of gland acini
5. The structure of the cartilage

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*Fischer Scientific Company, Pittsburg, Pennsylvania.

The measurements were taken at four different levels (see Figure II) on each epithelial surface of the sagittal section of the epiglottis. The measurements were made in microns with the aid of an ocular micrometer.

At each level individual measurements were made on four slides and a level average calculated. From these data a total average for each species was obtained. These are recorded in Table I.

The number of cells in the epithelial layers was counted at the typical lowest and highest areas of the epithelium. The subepithelial thickness was measured from the epithelium to the edge of the epiglottic cartilage. Taste buds were recorded as (0), (1), (2), and (3) depending on the number of taste buds observed. The gland acini were recorded as (1), (2), and (3) depending on the population of the acini. These are recorded in Table I.

RESULTS AND DISCUSSIONS

Gross Anatomy

<u>Position</u>

Howes (1889a) mentioned that in many quadrupedal animals the relationship between the soft palate and the epiglottis was different than those of the human subject.

Bowles (1889) found that in adult man the soft palate did not reach the tongue. Negus (1929, 1949) stated that the gap between the soft palate and the epiglottis was partially due to the degeneration of either one of these structures.

Postmortem examination of several animals in this study revealed the epiglottis lying in contact with the soft palate to varying degrees.

<u>Dogs</u>.--Of 4 newborn puppies from the same litter, the epiglottis was above the soft palate in one female, and below the soft palate in the other three (2 females and one male). Of the adult dogs, two (one female and one male) had an epiglottis above the soft palate, and five (2 females and 3 males) below the soft palate.

<u>Cats</u>.--In all five male cats and the one female cat the epiglottis was below the soft palate.

Cattle.--The epiglottis was above the soft palate in five adult cattle (4 females and one male), and below the soft palate in one female and one male calf.

Sheep.--Four male adult sheep and one male lamb were observed. One adult and the lamb had an epiglottis above the soft palate, but it was located below the soft palate in the other three.

Goats.--Two male goats showed an epiglottis below the soft palate and one male goat had an epiglottis above the soft palate. The one female was not observed grossly.

Swine.--The epiglottis was below the soft palate in one female and one male pig, and above the soft palate in the two adults (one female and male).

Horses.--In a limited number of horses (two male adults) the tip of the epiglottis was lying on the upper part of the soft palate. Sisson and Grossman (1953) noted that the epiglottis may project into the isthmus faucium, or on the pharyngeal side of the soft palate.

In agreement with the reports of Howes (1889a, b), Bowles (1889), Negus (1929, 1949) and Bhargava (1957) this study has shown that the epiglottis of the domestic animals is in contact with the soft palate to some extent. The epiglottis may be either below or above the soft palate (see Figure III).

Shape

Negus (1929, 1949) and Cleary (1954) described the aryepiglottic fold. This is a fold of mucous membrane which extends from the lateral side of the epiglottis to the arytenoid cartilage.

To determine the shape of the epiglottis in this study the epiglottis was viewed dorsally at the entrance of the larynx (see Figure IV).

Dogs.--The epiglottis of the dog attached to the whole larynx was in a unique position, not seen in other animals. The mucosal foldings from the lateral sides were prominent and formed two free sheets with a small slit in between. The epiglottis formed a triangle. The two borders measured from the pointed apex to the point where the mucosa folded were the same length. The angle at the apex ranged from 60 to 90 degrees in the observed adult dogs.

Cats.--From the dorsal view the epiglottis of the cat resembled that of the dog. However, in the cat the mucosa of the lateral borders did not show a distinct free folding as in the dog. The angle of the apex was approximately 45 degrees.

Cattle.--In agreement with Nickel et al. (1960) the edge of the apex was rounded. Generally the apex resembled a lip which curved to the lingual side. The mucosa of the lateral borders extended to the lateral margin of the arytenoid eminence. The epiglottis was thick.

Sheep.--The epiglottis of the sheep was triangular as in the dogs and cats. The arytenoid eminence extended to the lateral sides. The extension of the mucosa from the lateral borders resembled that of the cattle. The tip of the apex was not as pointed as in the dogs or cats.

Goats.--The epiglottis of the goats resembled that of the sheep, but it was thicker and larger in size. Prodinger (1940) reported that sometimes the apex of the goats' epiglottis formed a lip-like structure.

Swine.--The epiglottis of swine and cattle resembled each other, but in the former it was thinner. There was a notch in the arytenoid mucosa of swine.

Horses.--The epiglottis of the horse was large and thick. The tip of the apex curved to the lingual side. In agreement with Nickel et al. (1960) the apex of the epiglottis of the horse, dog, cat and small ruminants was pointed.

The epiglottic cartilage

Removal of the mucous membrane of the epiglottis revealed an epiglottic cartilage which did not conform to the shape of the epiglottis.

Dogs.--The epiglottic cartilage had four sides. The thickness of the cartilage increased from the apex toward the base. No indentations and foramina were detected on the surface of the dog epiglottic cartilage.

Cats.--The epiglottic cartilage of the cat was small and thin. It had four sides. The anterior sides were longer than the posterior sides and the base was the thickest part. No indentations and foramina were detected on the surface of the cartilage.

Cattle.--The apex was round and curved to the lingual side. The base was thick and usually the cartilage from the base curved toward the lingual side. Pits and foramina were detected on the laryngeal surface of the cartilage.

Sheep.--The epiglottic cartilage of the sheep was triangular in outline. Small indentations and foramina were observed on the laryngeal side of the epiglottic cartilage.

Goats.--The epiglottic cartilage of the goats resembled the epiglottic cartilage of the sheep. However, the indentations and foramina of the cartilage were more numerous and larger in the goats.

Swine.--The shape of the epiglottic cartilage of swine was similar to that of the cattle. The pits and the foramina on the cartilage were small and not so widely scattered as in the cattle.

Horses.--The epiglottic cartilage was long and large. The base was thick. From the lateral parts of the base there were two projections, continuations of the epiglottic cartilage. The laryngeal surface of the epiglottic cartilage was indented by numerous pits.

Hyoepiglottic muscle

In sagittal section of the epiglottis (see Figure II) the posterior part of the epiglottis is surrounded by the tissue of the remainder of the larynx. This posterior part is in contrast with the anterior part of the epiglottis which is free. Negus (1949), and Sisson and Grossman (1953) described fully the components of the attached part of the epiglottis with the thyroid, hyoid and the arytenoid cartilage. These connections of the epiglottis with the various parts of the remainder of the larynx can be seen in the sagittal section of the whole larynx. The sagittal section of the epiglottis in this study demonstrated only the muscle which was attached on the posterior part of the lingual surface of the epiglottis. This hyo-epiglottic muscle was observed in all the animals studied.

Microscopic Anatomy

The base of the epiglottis blended with the tissues of the remainder of the larynx and was not covered with epithelium. In either cross or longitudinal section, the epiglottis demonstrated three epithelial surfaces enclosing the epiglottic cartilage and its surrounding tissue (see Figures II, V).

The epithelial surfaces were (1) the laryngeal surface, (2) the free border, and (3) the lingual surface.

The mucosa of the laryngeal surface of the epiglottis blended into the mucosa of the inner part of the larynx. The free border formed the connection between the laryngeal and the lingual surface. The epithelium of the lingual surface was continued with the epithelium of the mucosa of the tongue.

The epithelial covering

The type of epithelium covering the greater part of the epiglottis was typical stratified squamous. The lingual and the free border surfaces were covered exclusively by stratified squamous epithelium and were marked by prominent subepithelial papillae. The papillae of the laryngeal surface were distinct in the region near the free border and on the most posterior region. The laryngeal surface may lack papillae in young dogs and cats (see Figure VI).

The stratified squamous epithelium of the laryngeal surface sometimes showed patches of stratified columnar epithelium (see Figure VII). In some areas the laryngeal surface did not have either typical stratified squamous or a clear stratified columnar epithelium, but had a stratified layer of epithelium in which the nuclei of the superficial layer were not flat (see Figure VIII). This atypical epithelium was not observed in all the microscopic sections of the laryngeal surface of the epiglottis.

The number and the height of the cell layers were not uniform. In all the animals studied the average height of the laryngeal surface was less than that of the lingual or the free border surface. The average height of the free border was less than that of the lingual surface.

The average epithelial height of the laryngeal surface in descending order was goats (118.1), cattle (82.4), horses (82.2), dogs (74.2), cats (71.6), swine (69.4), and sheep (62.6). The average epithelial heights of the free border in descending order was: goats (118.1), cattle (102.1), horses (91.1), dogs (85.0), sheep (81.3), cats (75.3), and swine (73.8). The average epithelial heights of the lingual surface in descending order was goats (237.6), cattle (226.9), horses (225.9), swine (133.9), sheep (130.7), dogs (113.3), and cats (103.9).

Of the ruminant epiglottises, cattle and goats more nearly resembled each other as sheep was much thinner. Of the carnivores the cats had the thinnest epithelial covering of the epiglottis. The average heights of the epithelial covering of the horse epiglottis was higher than that of carnivores and resembled that of cattle and goats. The epithelial heights in swine and sheep were so variable that they did not fit a definite pattern (Table I).

Taste buds

Taste buds were found on the laryngeal surface of the epiglottis of all animals studied (see Figures IX, X).

They were present at irregular intervals. In some sections there were none observed. Comparing the number of taste buds observed, dogs and cats had the least (Table I). Taste buds were observed on the lingual surface of swine but apparently were absent in all the other species. Only in the swine and sheep slides were taste buds seen on the free border surface. In his observations on "taste-goblets" in the epiglottis of the dog and cat Schofield (1876) indicated that "The surface of the epithelium just over a goblet is invariably pitted: there is a shallow saucer-like depression with the tip of the goblet abutting its lowest point. With each goblet is associated the duct of a mucous gland, which opens either at the side of the depression above mentioned, or on the free surface of the epithelium at some little distance." In this study no relationships was observed between the taste bud and the duct of the gland.

Subepithelial tissue

The lamina propria of the epiglottis blended with the tissue surrounding the cartilage. There was no demarcation between the mucosa and the submucosa. In this study the region between the epithelium and the cartilage was called the subepithelial tissue. This area was composed of collagen, elastic fibers and fibroblasts. It also contained fat tissue, glands, mast cells, leucocytes, vessels and nerves. In general this layer was loose in dogs and cats, and dense in ruminants, swine and horses.

In all the animals studied the average thickness of the subepithelial layer on the laryngeal side was less than that of the lingual counterpart. The measurements of the thickness of the subepithelial layer of the epiglottis are shown in Table I. Except for the numbers of glands and the observation of ganglia no particular differences were observed in the subepithelial tissue of the various species.

<u>Glands</u>

The glands of the epiglottis were branched tubulo-acinar. The location of the glands was not confined to the subepithelial layer but also penetrated the cartilage area. The acini of the glands were mucous, serous or mixed. The mixed gland acini were mucous with serous crescents. There was no uniformity of the type of the gland acini. In some areas the gland acini were predominantly mucous and in others serous. The percentage of mucus secreting acini was larger than the serous type.

Very few glands were present in the epiglottis of dogs and cats. There were fewer glands in the lingual side than in the laryngeal mucosa. A few glands were present in the subepithelial layer of the free border. No glands were observed in the cartilage area of the dog. Glands were found in the perichondrium of the epiglottic cartilage of the cats (see Figure XI). Glands were also found at the base of the cartilage area. The subepithelial layer of the lingual surface of cattle, sheep, goats, swine and horses contained more glands than the laryngeal side. The glands in swine and horses were not as numerous as in the ruminants. In cattle the glands directly beneath the lingual surface epithelium consisted predominantly of mucous acini. The glands in the deeper layer were composed of mucous, serous and mixed gland acini. These were located in the cartilage area and penetrated it. A thick connective tissue band separated these two groups of glands. This band was also present in the sheep, goats and swine, but the superficial glands were not as numerous in cattle (see Figures XII, XIII).

On the most posterior region the connective tissue band was mingled with skeletal muscle fibers (see Figure XIII). Glands were present in the perichondrium of the epiglottic cartilage of the horse and small group of glands penetrated the cartilage (see Figures XIV, XV). An estimation of the gland population in the epiglottis of domestic animals is shown in Table I.

The epiglottic cartilage

Although the epiglottic cartilage of all the animals studied was elastic in nature, the microscopic arrangement of this cartilage was not uniform. In all except the carnivores it was indented with pits or perforated by foramina.

As a result of these foramina, sections through the cartilage may present the appearance of being made up of several irregular plates. The indentations contained glands and the perforations were penetrated by glands, fat, blood vessels, and nerve trunks. The glands in the cartilage area of swine and horses were not as numerous as in the ruminants.

The microscopic structure of the Processus cuneiformis of the horse was similar to that of the epiglottic cartilage. Microscopically the epiglottic cartilage of a young dog and an adult one were not alike structurally. Figure XVI (a and b) show the epiglottic cartilage of a one-and-a-half-week-old dog. The cartilage area consisted of small cells resembling mesenchyme cells, elastic fibers, fat cells and a few blood capillaries. Figure XVII shows the epiglottic cartilage of a five-year-old dog in which the cartilaginous portion was confined to the periphery near the perichondrium. Its inner space was filled with fat tissue with a few blood vessels. Small islands of elastic cartilage could be seen within the fat tissue.

According to Schaffer (1907), Rheiner (1852) and Dekhuysen (1889) in an adult dog epiglottis, the cartilage was on the periphery and the center was filled with fat. Trautmann and Fiebiger (1957) mentioned that the cartilage of the epiglottis may be partly or entirely replaced by adipose tissue. According to Krolling and Grau (1960) histogenetically the fat cells in the epiglottic cartilage area were not a transformation product of the cartilage cells, but were developed from the undifferentiated mesenchyme cells.

In a section of the whole larynx of a very young dog, the perichondrium of the epiglottic cartilage was continuous with the perichondrium of the thyroid cartilage. In an adult cat a cartilage plate was present at the anterior end but its perichondrium was not distinct. Small islands of elastic cartilage lined the lingual and the laryngeal side and the inner part was filled with fat cells. The entire cartilaginous mass of the posterior region was replaced by fat tissue. It also contained blood capillaries, lymph nodules and glands. A few glands penetrated the perichondrium on the laryngeal side (see Figure XI, XXI). The perichondium of the epiglottic cartilage of all the animals studied was composed primarily of collagenous fibers and elastic fibers. Due to the penetration by the glands, small blood vessels, fat tissue and nerve trunks, the perichondrium was not a solid sheet surrounding the whole epiglottic cartilage.

Nerves and vessels

Very small vessels and nerve trunks were scattered throughout in the subepithelial layer. The vessels were cut in a cross or horizontal plane. The latter were parallel with the epithelial surface. Ganglia were observed in the subepithelial layer of the sheep, goats and swine (see Figures XVIII, XIX, XX).

Lymphocytes

The most predominant form of leucocytes present in the epiglottis were the lymphocytes. Lymphocytes were usually present in both the epithelial and the subepithelial layer. They were scattered or formed aggregated nodules. Aggregated lymphocytes were more frequent in the subepithelial layer of the laryngeal surface than in the lingual counterpart (see Figure IX).

Table I.--Comparative data on the epiglottis of domestic animals.

Animal/Location	Epithelium			Subepithelial Layer Thickness (mm)		Taste* buds (No. observed)	Gland** population
	Range in no. of cell layers	Height (u) Range	Height (u) Average	Range	Average		
Cat							
Lingual	3-17	30.6-249.9	103.9	0.10-0.60	0.2	0	1
Laryngeal	3-10	25.5-163.2	71.6	0.07-0.40	0.1	2	2
Free border	4-10	20.4-158.1	75.3	0.05-0.20	0.1	0	1
Dog							
Lingual	5-20	15.3-392.7	113.3	0.10-1.20	0.5	0	1
Laryngeal	3-12	15.3-198.9	74.2	0.04-0.60	0.2	2	2
Free border	4-12	10.2-382.5	85.0	0.02-0.90	0.3	0	1
Cattle							
Lingual	5-33	30.6-943.5	226.9	0.50-4.60	2.2	0	3
Laryngeal	4-15	25.5-173.6	82.4	0.20-2.10	0.6	3	2
Free border	4-17	25.5-204.0	102.1	0.40-2.10	1.1	0	2
Sheep							
Lingual	5-16	35.7-382.5	130.7	0.20-2.50	1.0	0	3
Laryngeal	4-10	30.6-153.0	62.6	0.30-1.50	0.5	3	2
Free border	6-12	30.6-306.0	81.3	0.10-2.00	0.6	1	2
Goat							
Lingual	6-34	51.0-744.6	237.6	0.40-6.10	1.8	0	3
Laryngeal	4-15	30.6-244.8	88.0	0.20-1.10	0.6	3	2
Free border	5-25	20.4-377.4	118.1	0.20-2.30	0.8	0	2
Swine							
Lingual	5-20	51.0-214.2	133-9	0.40-2.50	1.0	1	3
Laryngeal	4-10	25.5-153.0	69.4	0.30-1.20	0.7	3	2
Free border	4-13	20.4-122.4	73.8	0.20-1.10	0.5	2	2

Continued on next page

Table I.--Continued

Animal/Location	Epithelium: Range in no. of cell layers	Epithelium: Height (u) Range	Epithelium: Height (u) Average	Subepithelial Layer: Thickness (mm) Range	Subepithelial Layer: Thickness (mm) Average	Taste* buds (No. observed)	Gland** population
Horse							
Lingual	5-36	30.6-719.1	225.9	0.90-1.60	1.3	0	3
Laryngeal	3-13	30.6-102.0	82.2	0.50-1.20	0.8	3	2
Free border	4-20	25.5-102.0	91.1	0.10-0.50	0.3	0	2

*0 - None
1 - scarce
2 - few more
3 - most

**1 - sparse
2 - medium
3 - dense

SUMMARY AND CONCLUSIONS

Gross and histologic studies of the epiglottis were made on the seven domestic animals, including eighteen dogs, six cats, six cattle, four sheep, four goats, six swine, and two horses. No appreciable sex differences were noted. To some extent the tip of the epiglottis was overlapped by the soft palate. The position of the epiglottis of the cats was prevelar but in all other species it was either prevelar or postvelar. The apex of the epiglottis was pointed in dogs, cats, small ruminants and horses, rounded in cattle and swine. Indentations of the epiglottic cartilage were grossly visible on the laryngeal surface, and foramina were present in the thin part of the cartilage in ruminants, swine and horses. Neither indentations nor foramina were observed in the dogs and cats.

There were several factors that could be used to distinguish the laryngeal from the lingual surface. These were (1) epithelial height (2) the thickness of the subepithelial layer (3) taste buds (4) lymphocytes (5) skeletal muscle (6) subepithelial papillae and (7) glands. The average heights of the epithelial covering and the subepithelial layer of the laryngeal surface were less than that of the lingual counterparts. Taste buds were found on the laryngeal surface of the epiglottis of all animals studied but only swine had taste buds on the lingual surface. The free border which connected the lingual and laryngeal surfaces had the same characteristics as the lingual surface. Lymphocytes were usually present in both the epithelial and the subepithelial layer. They were scattered or formed aggregated nodules which were more frequent on the laryngeal surface. Hyoepiglottic muscle fibers were present on the most posterior part of the lingual side. The linqual surface was marked by prominent subepithelial papillae in all animals. Numerous glands were present on the lingual surface of ruminants, swine and horses. In contrast to this, dogs and cats had only a few glands on the lingual side. The glands were most numerous in the ruminants. Mucus secreting acini were more numerous than the mixed or serous type.

The type of epithelium covering the greater part of the epiglottis was typical stratified squamous. All animals studied had stratified columnar epithelium in several areas on the most posterior part of the laryngeal surface. The epiglottic cartilage was elastic in nature and except for the carnivores had indentations containing glands and perforations penetrated by glands, fat blood vessels, and nerve trunks. In older carnivores the cartilage was gradually replaced by fat beginning at the base.

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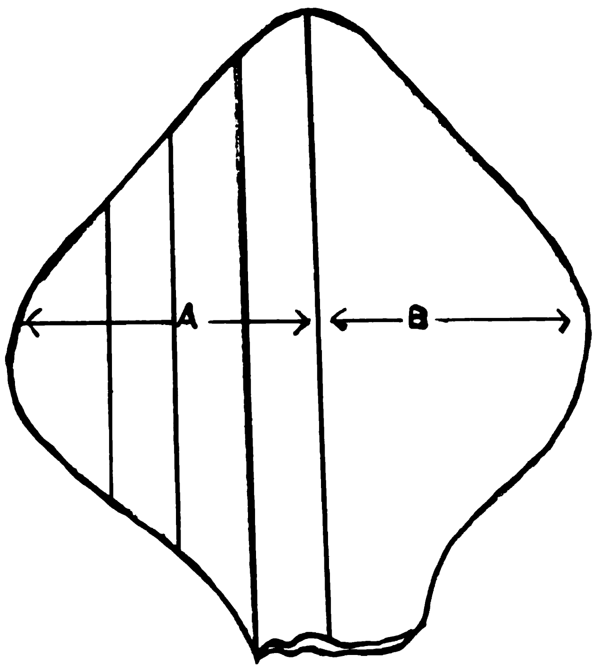


Figure I.--Outline of epiglottis to show the planes of sagittal sections.

A. One-half of the epiglottis was blocked at four levels for sagittal sections.

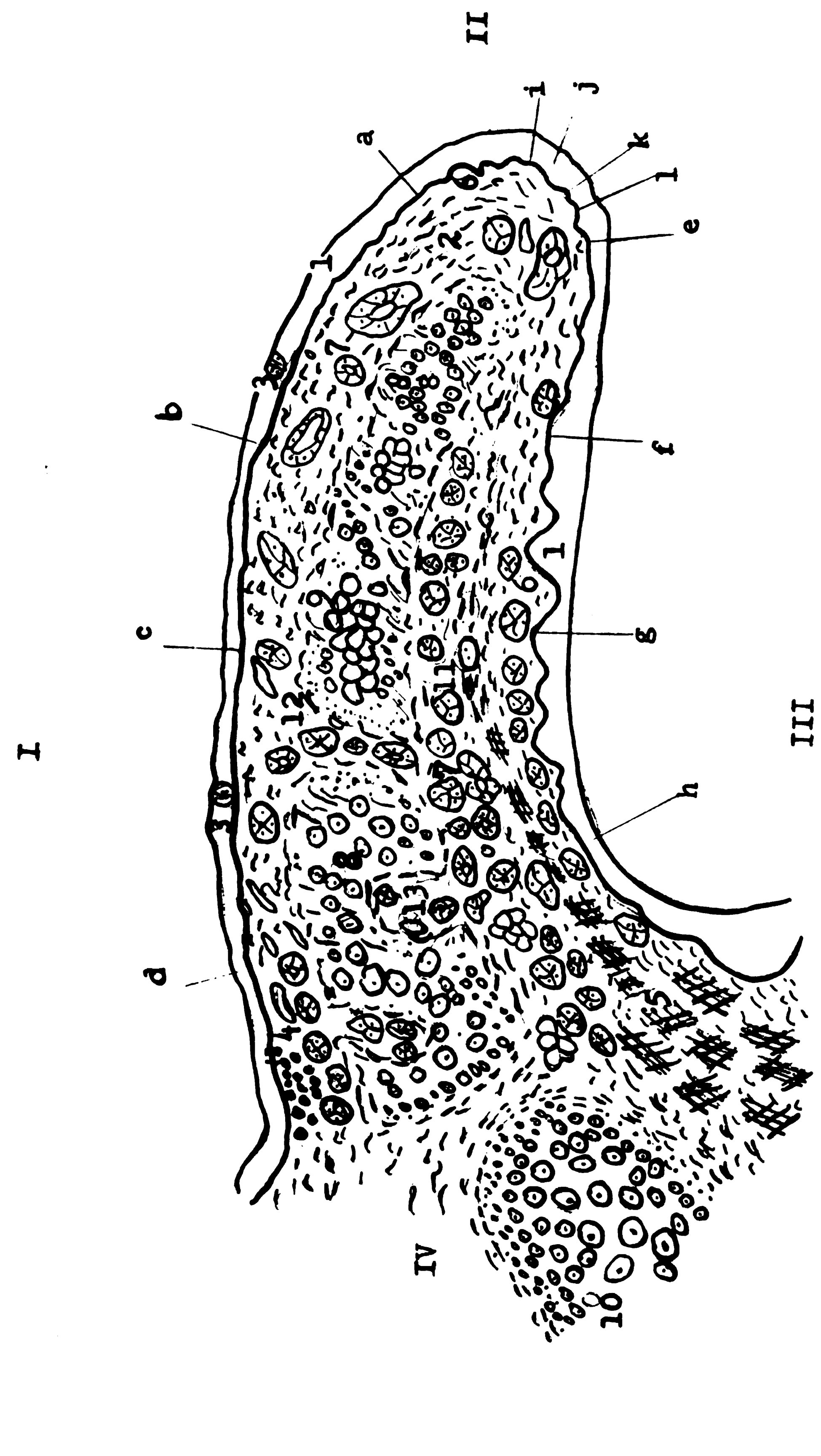
B. Remaining one-half of the epiglottis was boiled. The mucosa was removed and the epiglottic cartilage was stored in 100 per cent alcohol.

Figure II.--A schematic diagram of the sagittal section of the epiglottis.

I. Laryngeal surface.
II. Free border.
III. Lingual surface.
IV. Region of attachment to the remainder of the larynx.

1. Epithelial covering.
2. Subepithelial layer.
3. Taste buds.
4. Aggregated lymphocytes.
5. Hyoepiglottic muscle.
6. Papillae.
7. Glands; mucous, serous, or mixed.
8. Elastic cartilage.
9. Fat tissue in the cartilage area; it is confined to the epiglottic cartilage of the carnivores.
10. Thyroid cartilage.
11. Connective tissue band; it is confined to the sub-epithelial layer of the lingual side of the epiglottis of ruminants.
12. Glands penetrating a perforation in the cartilage; it is confined to the epiglottic cartilage of the ruminants and swine.
13. Depressions of cartilage containing glands in the ruminants, swine and horses.

Four different levels of measurement in laryngeal (a-d), lingual (e-h), and in free border surface (i-l). These lines are drawn approximately at the anterior edge of the cartilage, one quarter, midway and three quarters of the sagittal length of the cartilage (a-d and e-h); and at the laryngeal edge, one quarter, three quarters, and lingual edge of the tip of the cartilage (k-l).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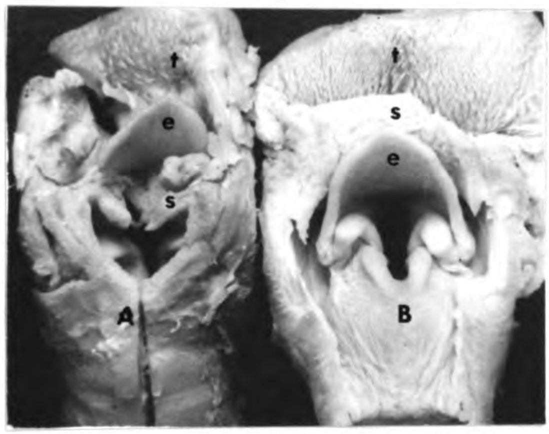


Figure III.--The relationship between the epiglottis and the soft palate (dogs).

A. Prevelar.
B. Postvelar.

t. tongue.
e. epiglottis.
s. soft palate.

Figure IV.--Dorsal view of epiglottis of the domestic animals.

A. Horse; aged.

B. Dog; five years.

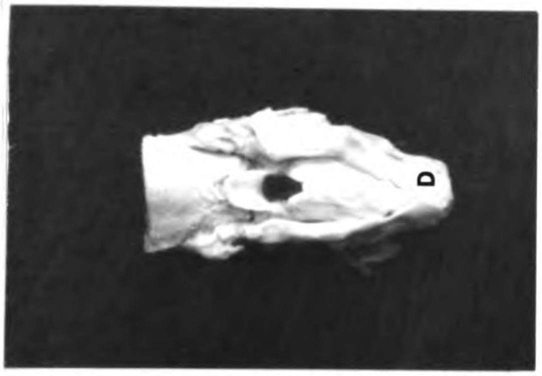
C. Sheep; eight months.

D. Cat; one year.

E. Cattle; three months.

F. Pig; one week.

G. Goat; seventeen months.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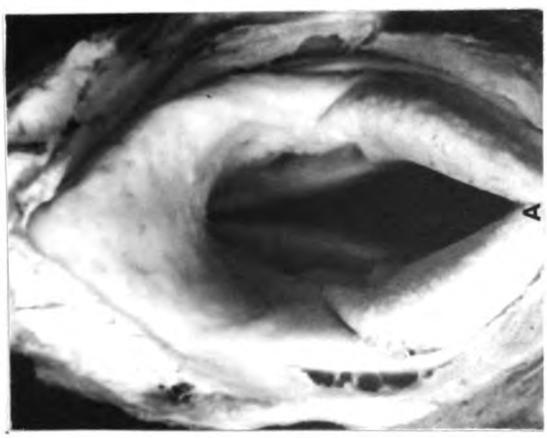
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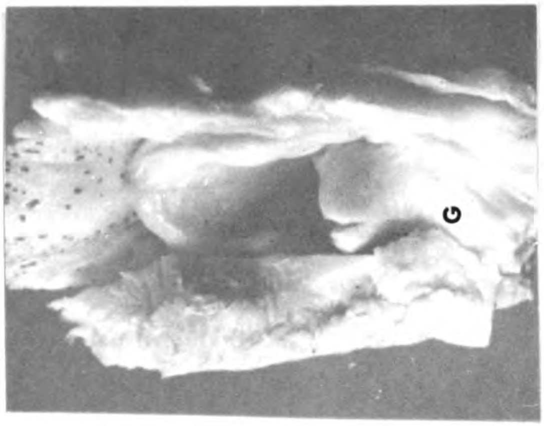
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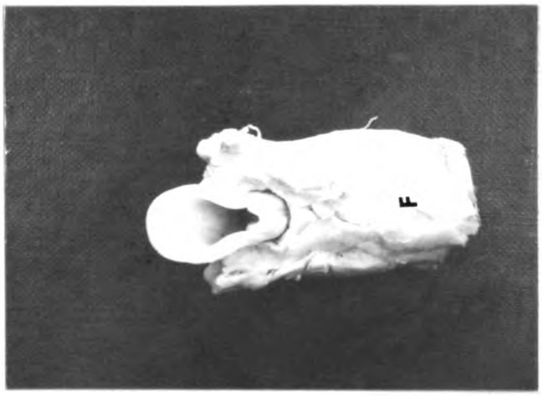
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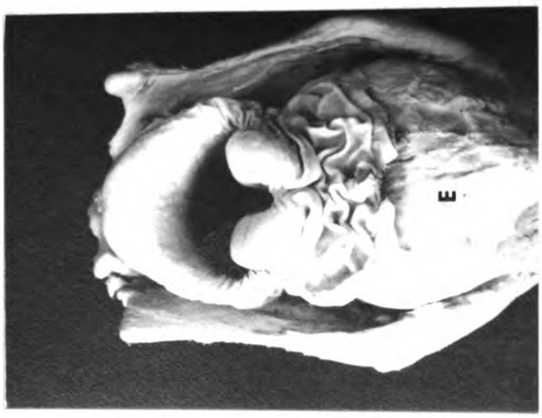
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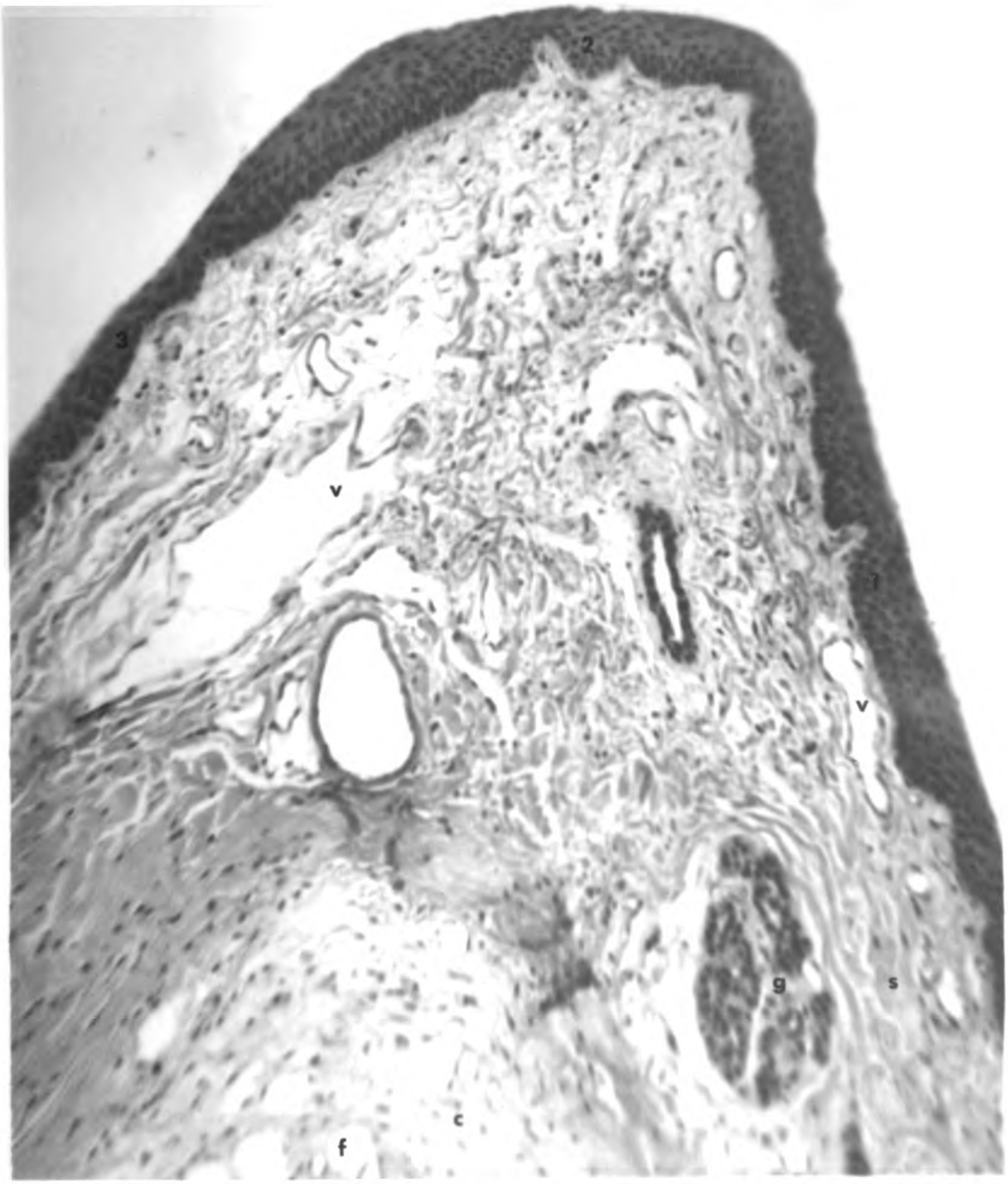

Figure V.--Sagittal section of the tip of the epiglottis of the dog.

(1) Laryngeal surface; (2) Free border; (3) Lingual surface; (c) Cartilage area; (f) Fat cells; (g) Glands; (s) Subepithelial layer; (v) Vessels. Hematoxylin and eosin stain; X257.

Figure VI.--Nonpapillated laryngeal surface of the dog epiglottis.

(e) Epithelial layer; (m) Mucous; (s) Serous; (x) Mixed glands. Hematoxylin and eosin stain; X277.

Figure VII.--Thin stratified squamous epithelium with patches of stratified columnar epithelium on the laryngeal surface of the horse epiglottis.

(c) Areas showing denudation of the flattened surface cells leaving a stratified columnar like epithelium; (d) Duct of the glands; (g) Glands; (s) Thin stratified squamous epithelium. Hematoxylin and eosin stain; X336.

Figure VIII.--Atypical stratified squamous epithelium on the laryngeal surface of the dog epiglottis.

(e) Epithelium; (s) Subepithelial connective tissue. Hematoxylin and eosin stain; X135.

Figure VIII.--Atypical stratified squamous epithelium on the laryngeal surface of the dog epiglottis.

(e) Epithelium; (s) Subepithelial connective tissue. Hematoxylin and eosin stain; X135.

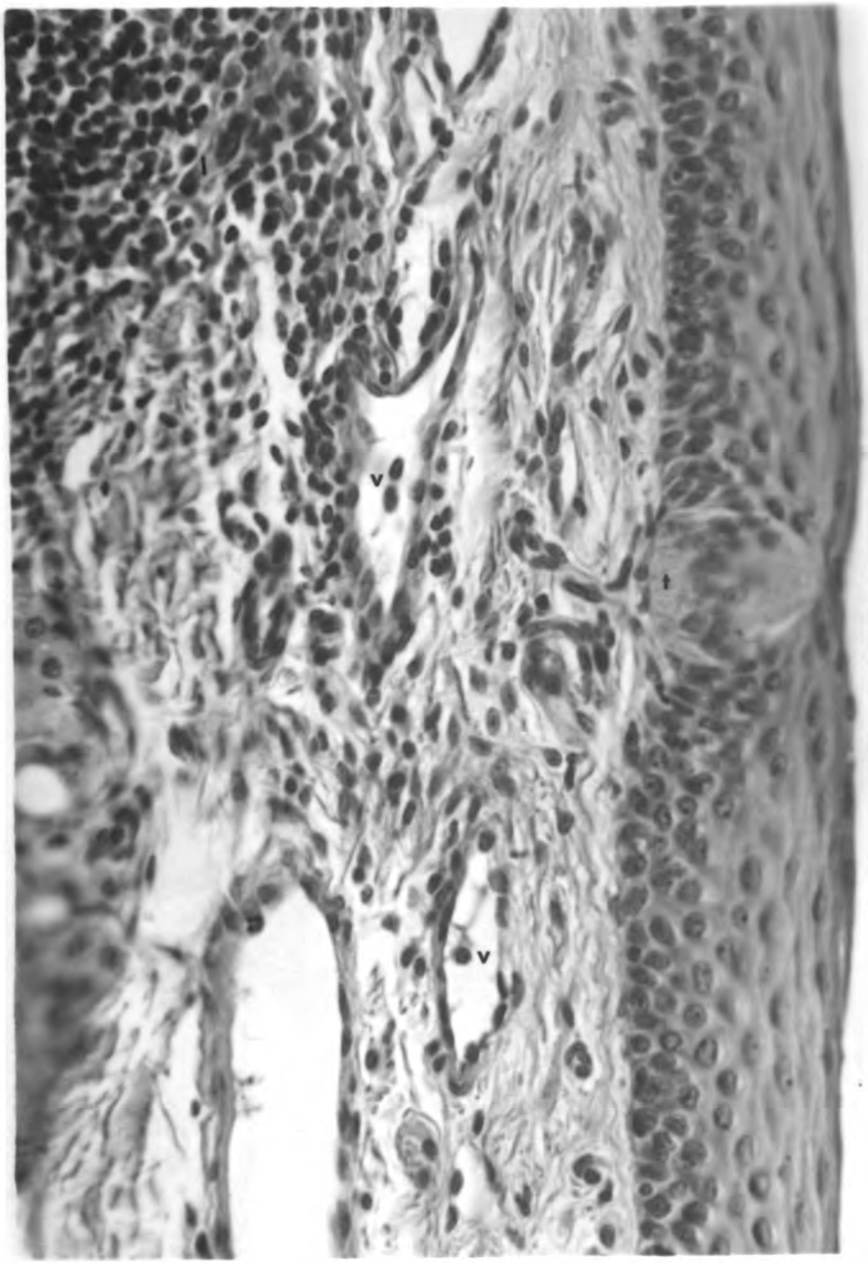

Figure IX.--Taste bud and aggregated **lymphocytes on** the **laryngeal surface** of the dog epiglottis.

(l) Lymphocytes; (t) Taste bud; (v) **Lymph vessel.** Hematoxylin and eosin stain; X250.

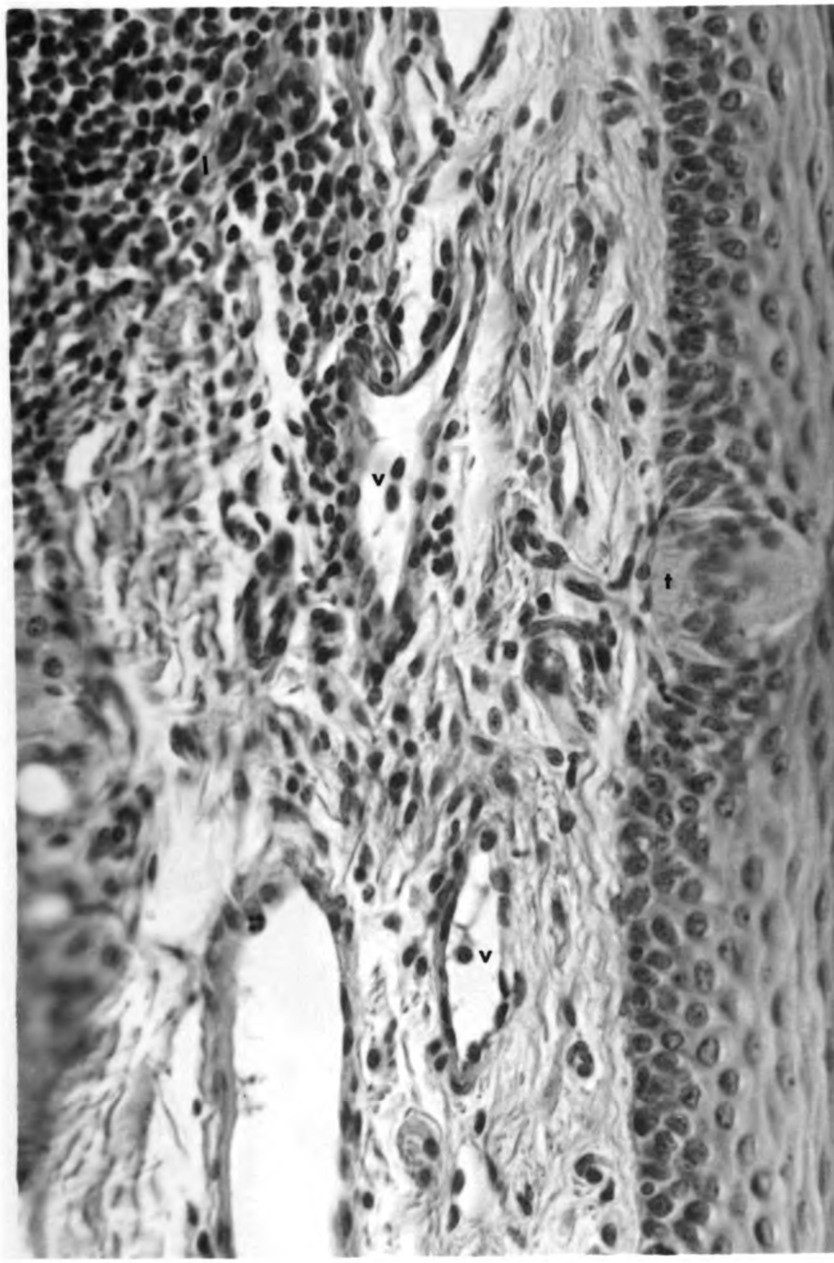

Figure IX.--Taste bud and aggregated lymphocytes on the laryngeal surface of the dog epiglottis.

(l) Lymphocytes; (t) Taste bud; (v) Lymph vessel. Hematoxylin and eosin stain; X250.

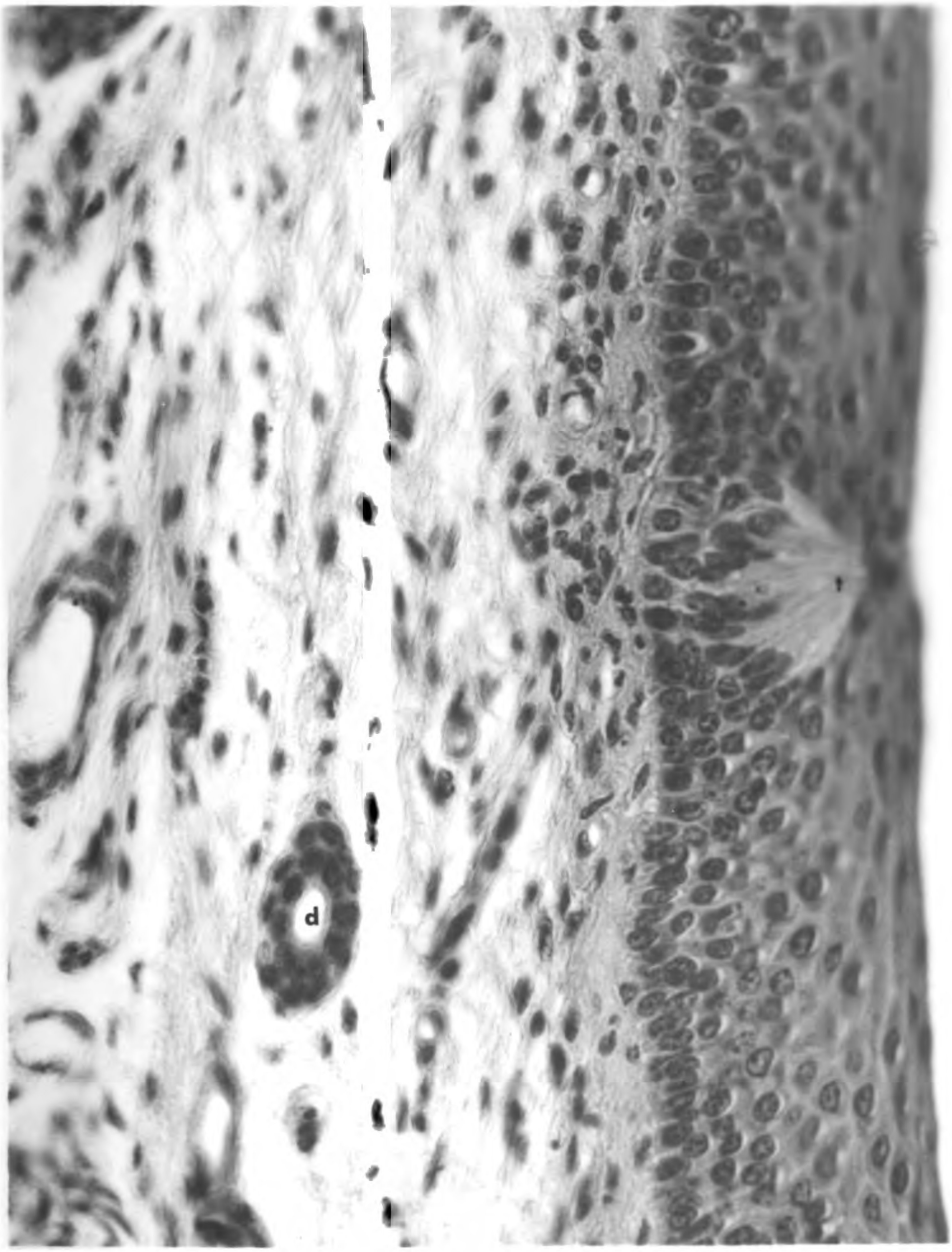

Figure X.--Taste bud on the laryngeal surface of the epiglottis of cattle.

(d) Gland duct; (t) Taste bud. Hematoxylin and eosin stain; X250.

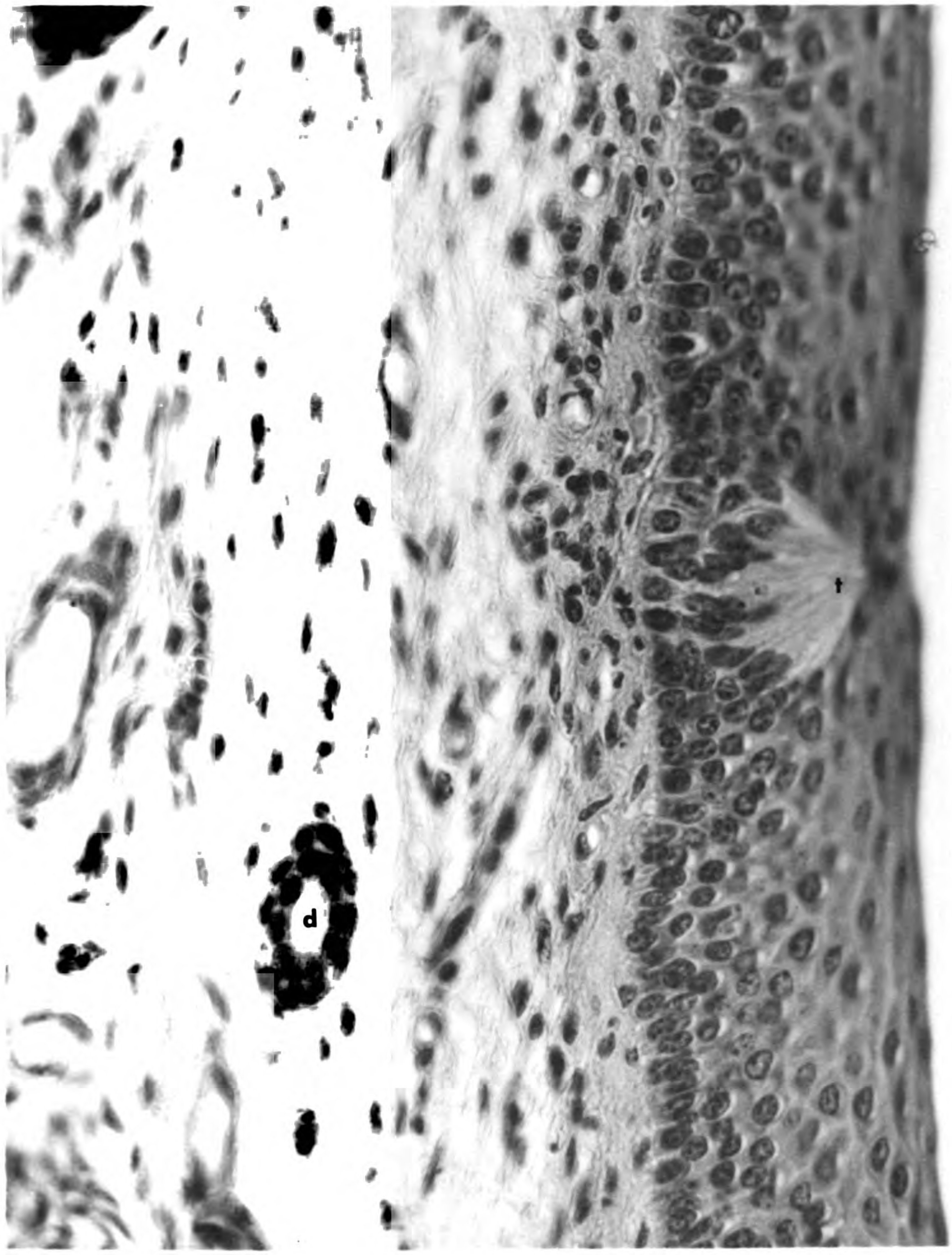

Figure X.--Taste bud on the laryngeal surface of the epiglottis of cattle.

(d) Gland duct; (t) Taste bud. Hematoxylin and eosin stain; X250.

Figure XI.--Glands in the perichondrium of the cat epiglottis.

(a) Laryngeal surface; (b) Subepithelial layer; (c) Cartilage area filled with fat cells; (d) Dorsal perichondrium with glands; (e) Ventral perichondrium. Hematoxylin and eosin stain; X379.

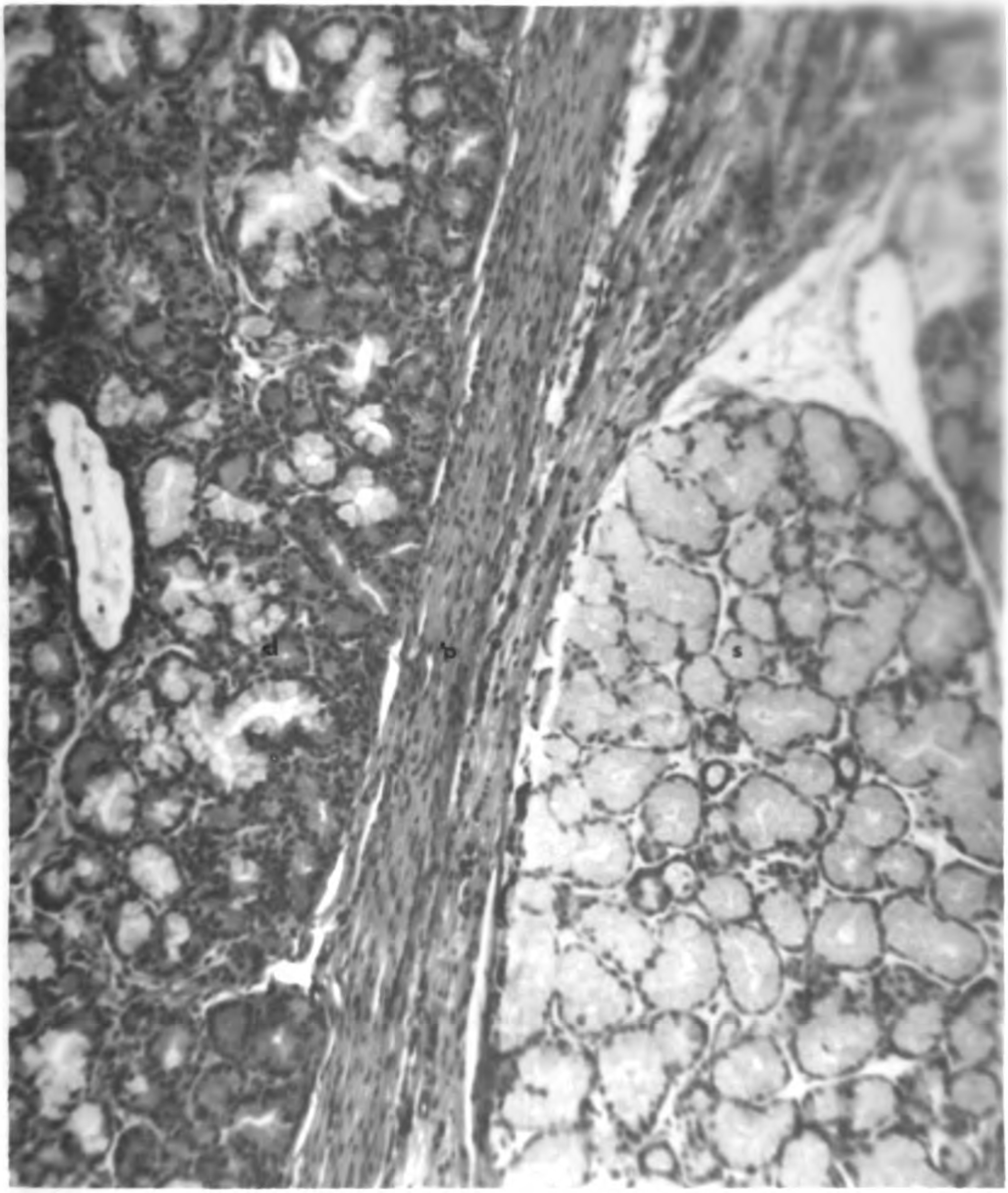

Figure XII.--Connective tissue band dividing the superficial glands and the deep layer glands in the sub-epithelial layer of the cow epiglottis.

(b) Connective tissue band; (s) Superficial glands, predominantly mucous; (d) Deep layer glands--mucous, serous, or mixed. Hematoxylin and eosin stain; X221.

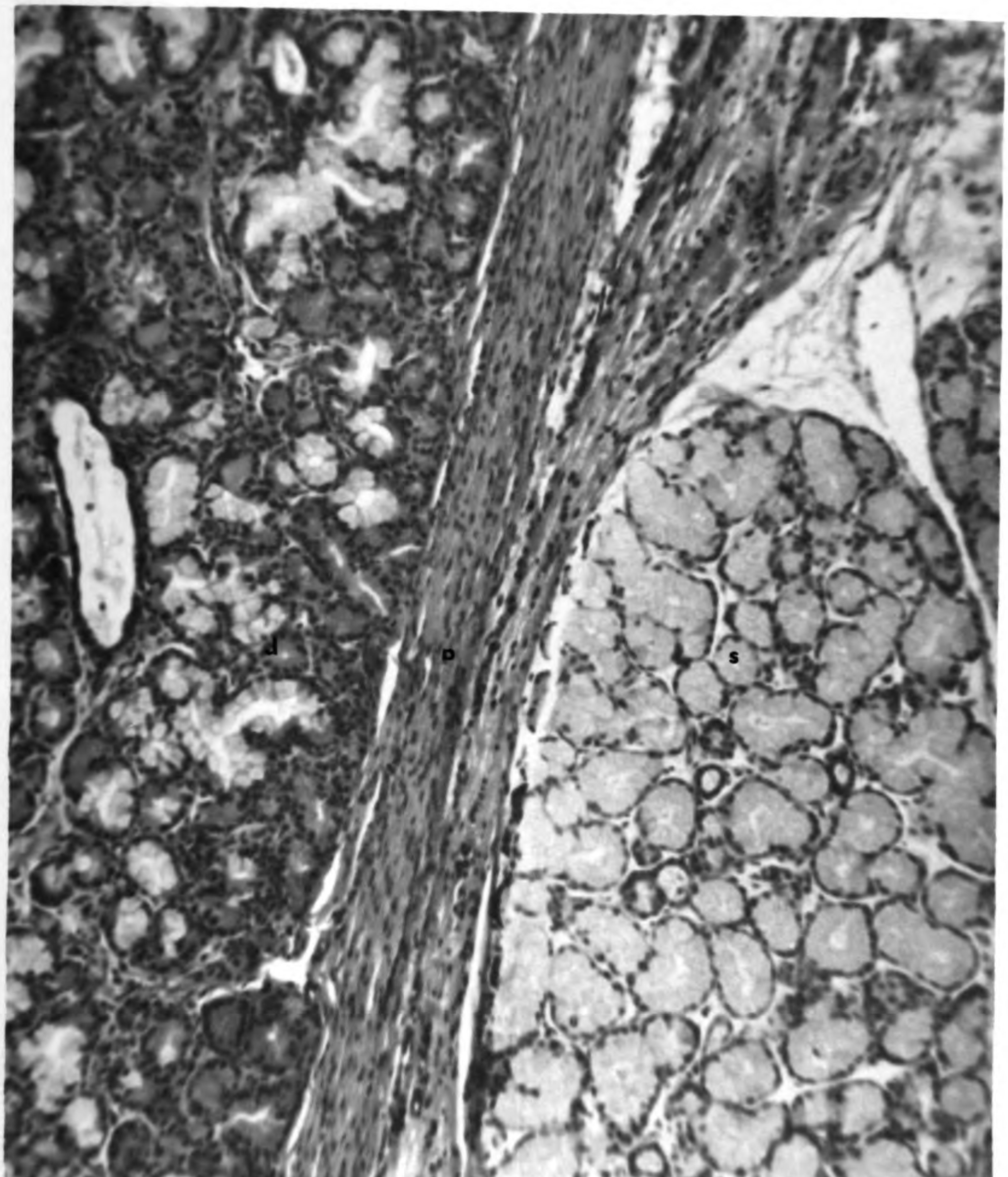

Figure XII.--Connective tissue band dividing the superficial glands and the deep layer glands in the sub-epithelial layer of the cow epiglottis.

(b) Connective tissue band; (s) Superficial glands, predominantly mucous; (d) Deep layer glands--mucous, serous, or mixed. Hematoxylin and eosin stain; X221.

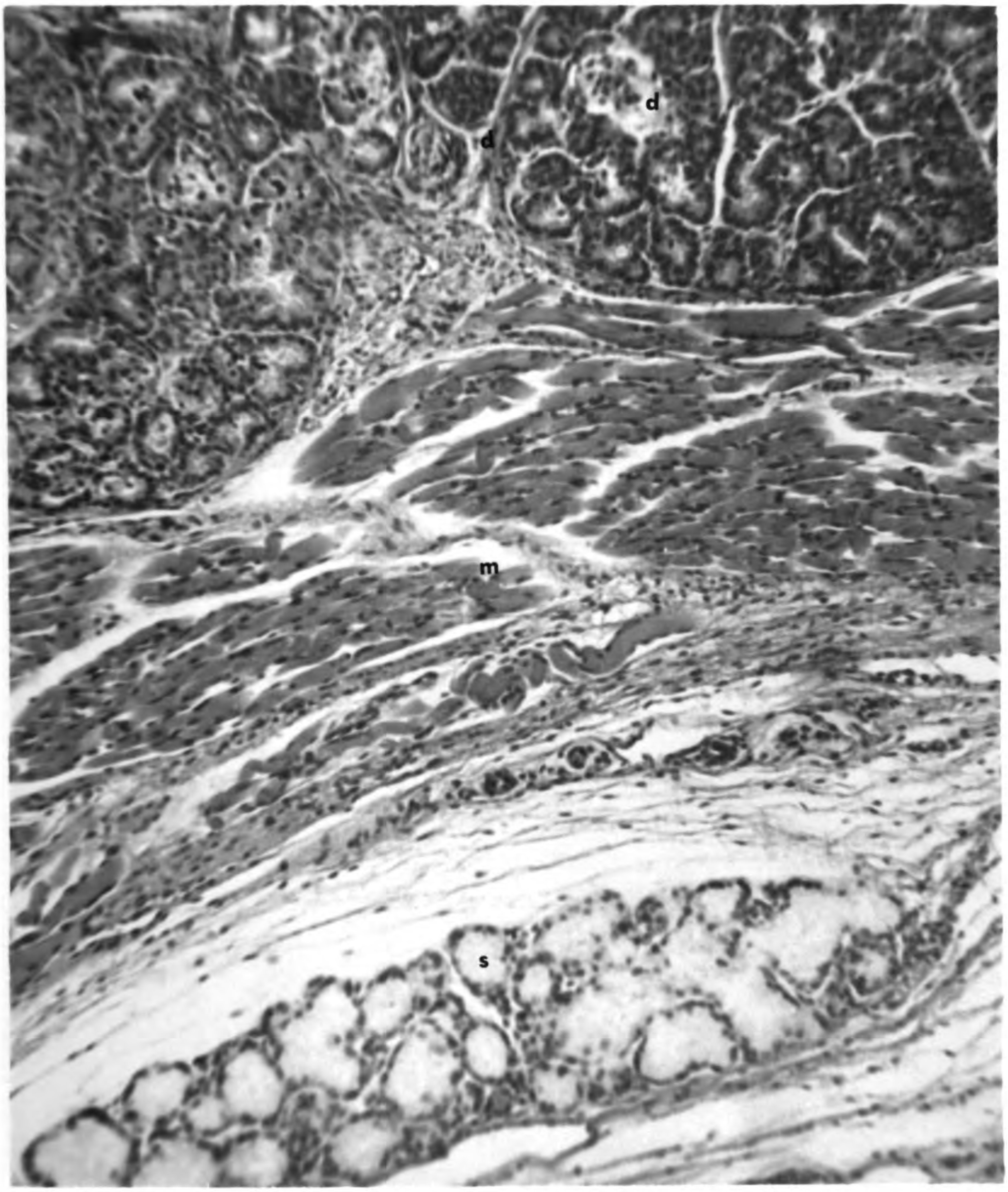

Figure XIII.--Hyoepiglottic muscle fibers with the connective tissue band separating the superficial glands and the glands in the deeper layer in the sheep epiglottis.

(d) Glands in the deeper layer--mucous, serous, or mixed; (m) Muscle fibers in the connective tissue band; (s) Small group of superficial glands--mucous and mixed. Hematoxylin and oesin stain; X254.

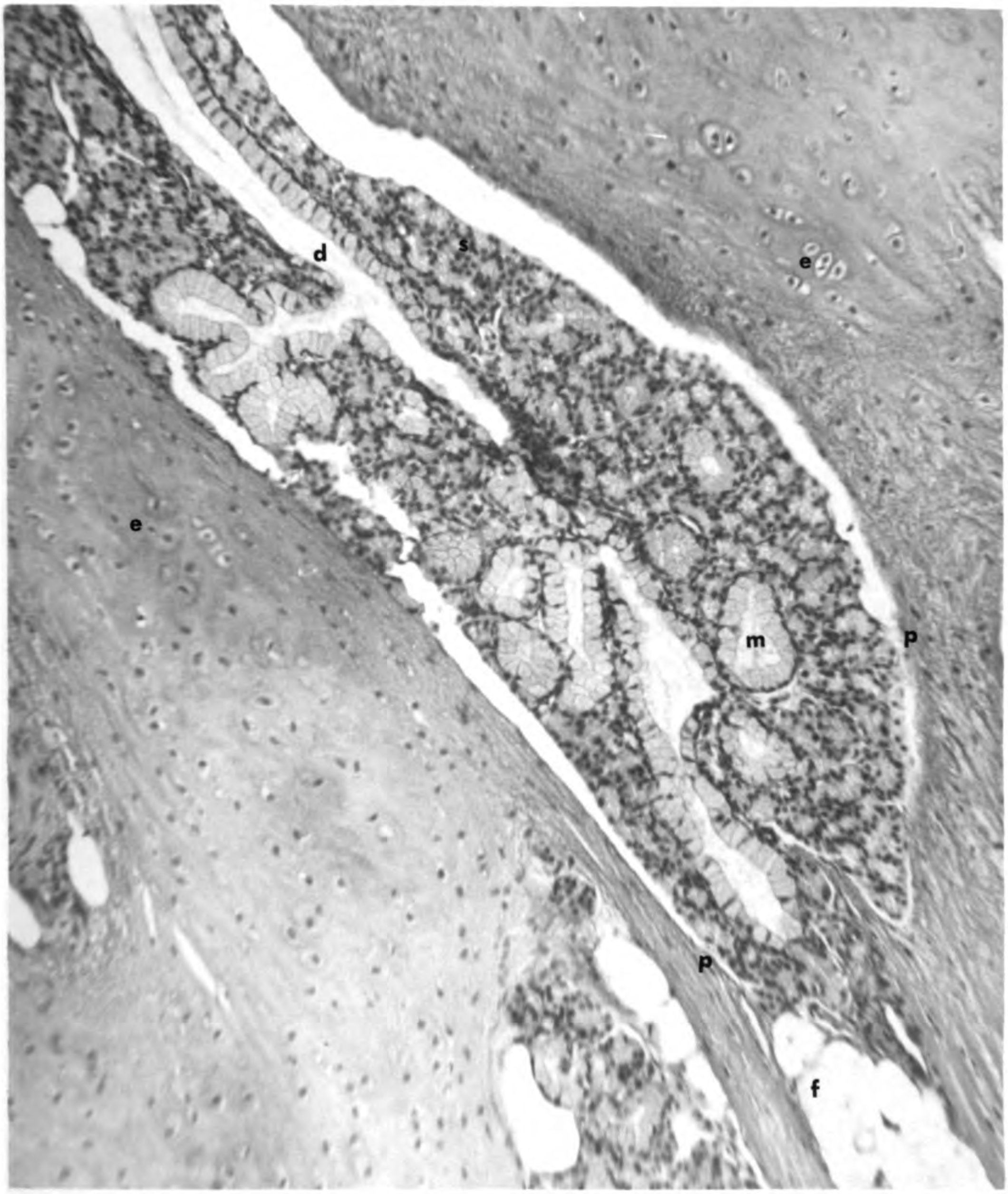

Figure XIV.--Glands within the epiglottic cartilage plate of the horse.

(e) Elastic cartilage; (d) Duct; (f) Fat; (m) Mucous gland; (p) Perichondrium; (s) Serous gland. Hematoxylin and eosin stain; X224.

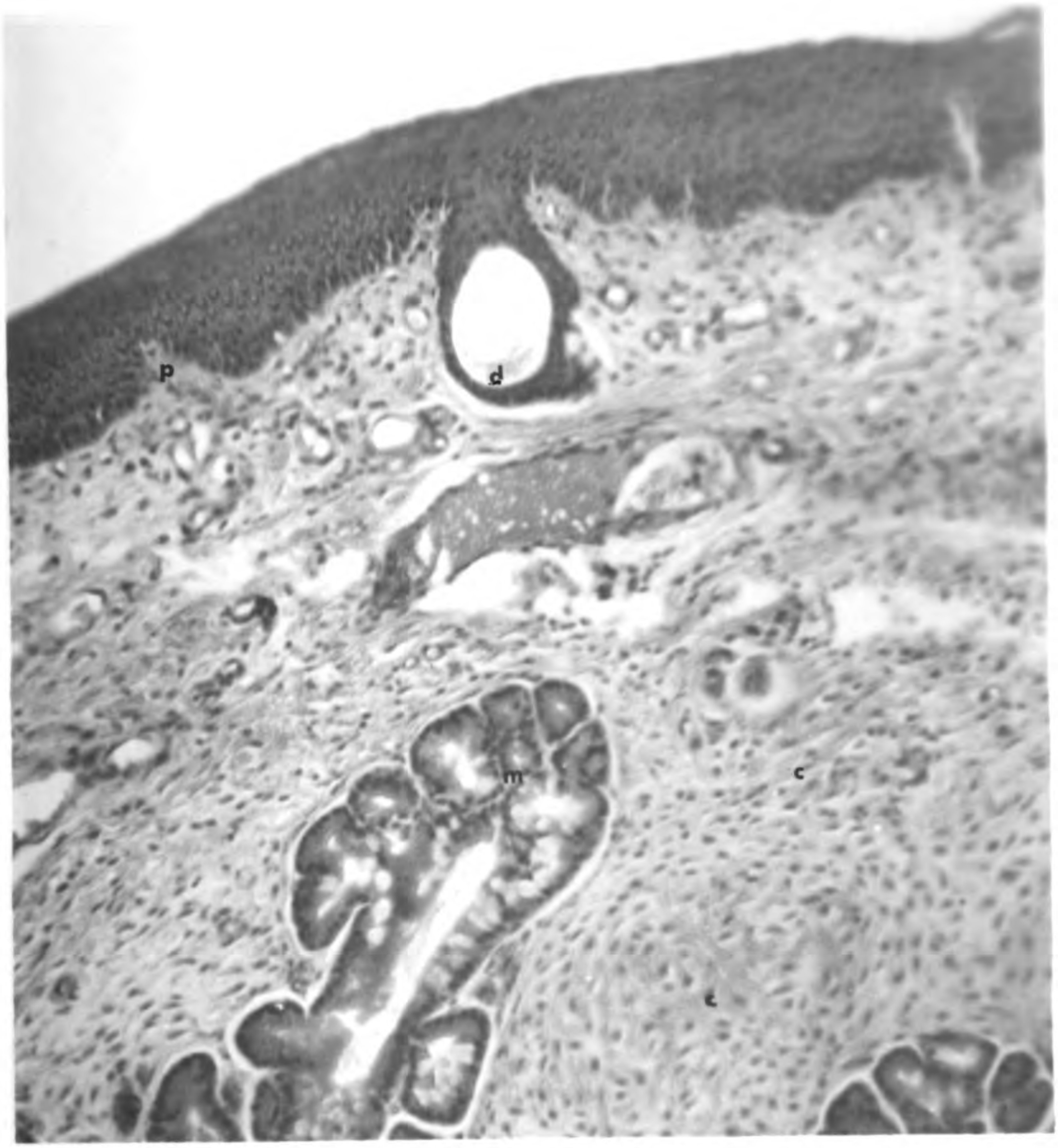

Figure XV.--Glands in the epiglottic cartilage of cattle.

(c) Remnant of elastic cartilage plate (note absence of a perichondrium); (d) Duct of glands; (m) Mixed glands; (p) Papilla. Hematoxylin and eosin stain; X260.

Figure XV.--Glands in the epiglottic cartilage of cattle.

(c) Remnant of elastic cartilage plate (note absence of a perichondrium); (d) Duct of glands; (m) Mixed glands; (p) Papilla. Hematoxylin and eosin stain; X260.

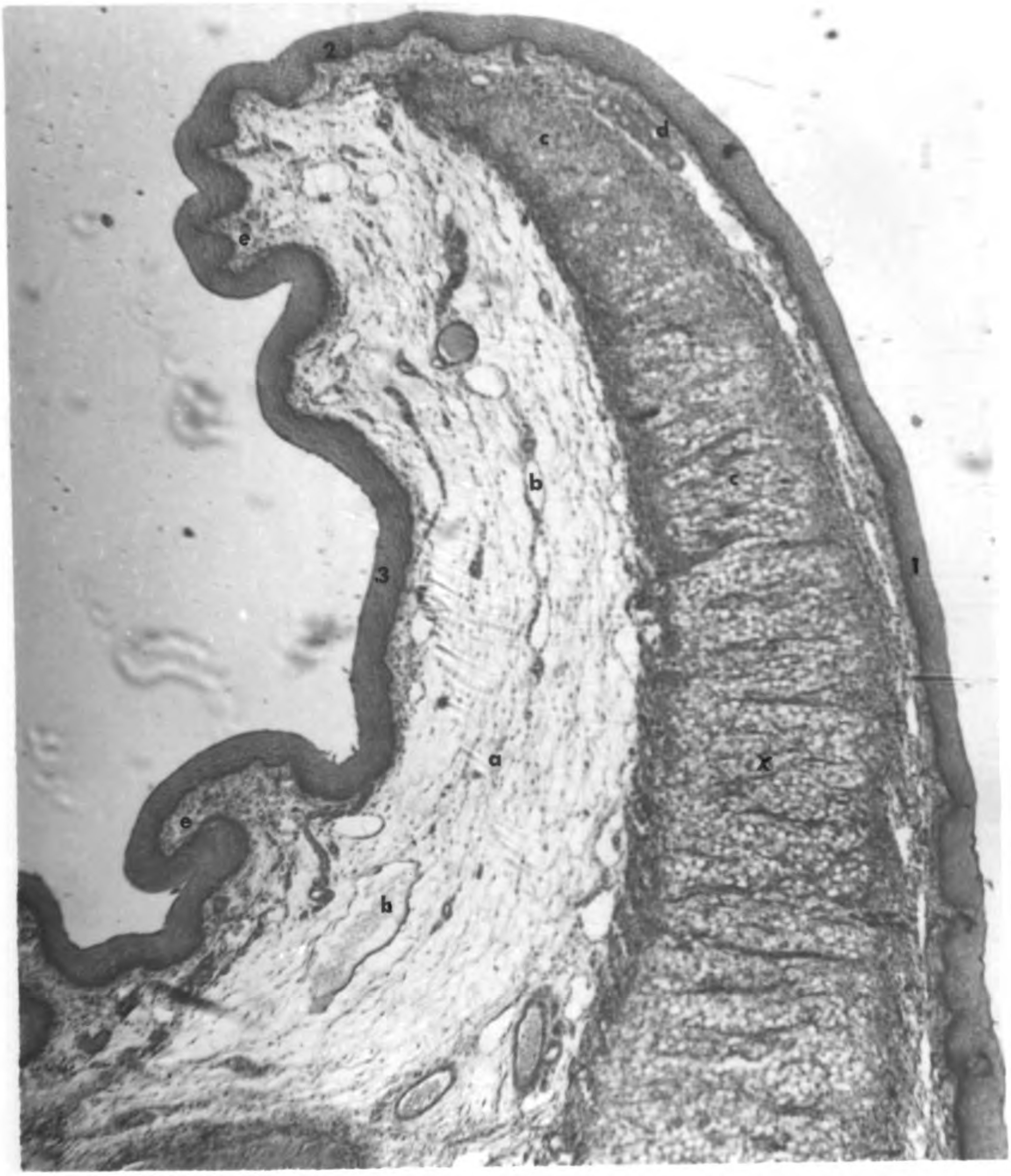

Figure XVI (a).--Sagittal section of the epiglottis of one-and-a-half-week old dog.

(1) Laryngeal surface; (2) Free border, (3) Lingual surface, (a) Subepithelial layer, (b) Vessels, (c) Cartilage area, (d) Glands, (e) Papillae, (X) Area which is enlarged in Figure XVI (b). Hematoxylin and eosin stain.

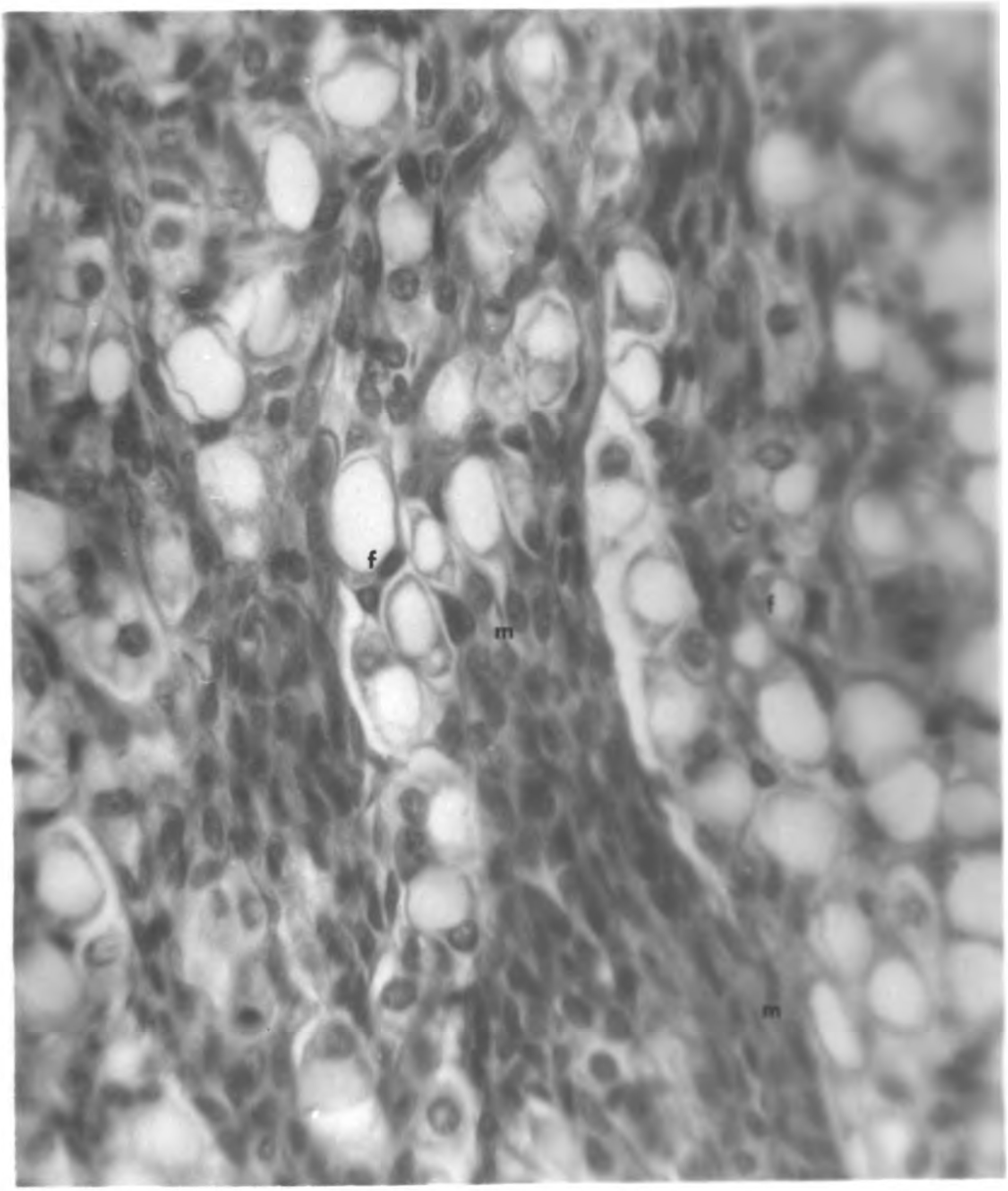

Figure XVI (b).--Epiglottic cartilage of one-and-a-half-week old dog.

(f) Fat cells; (m) Mesenchyme cells. Hematoxylin and eosin stain; X915.

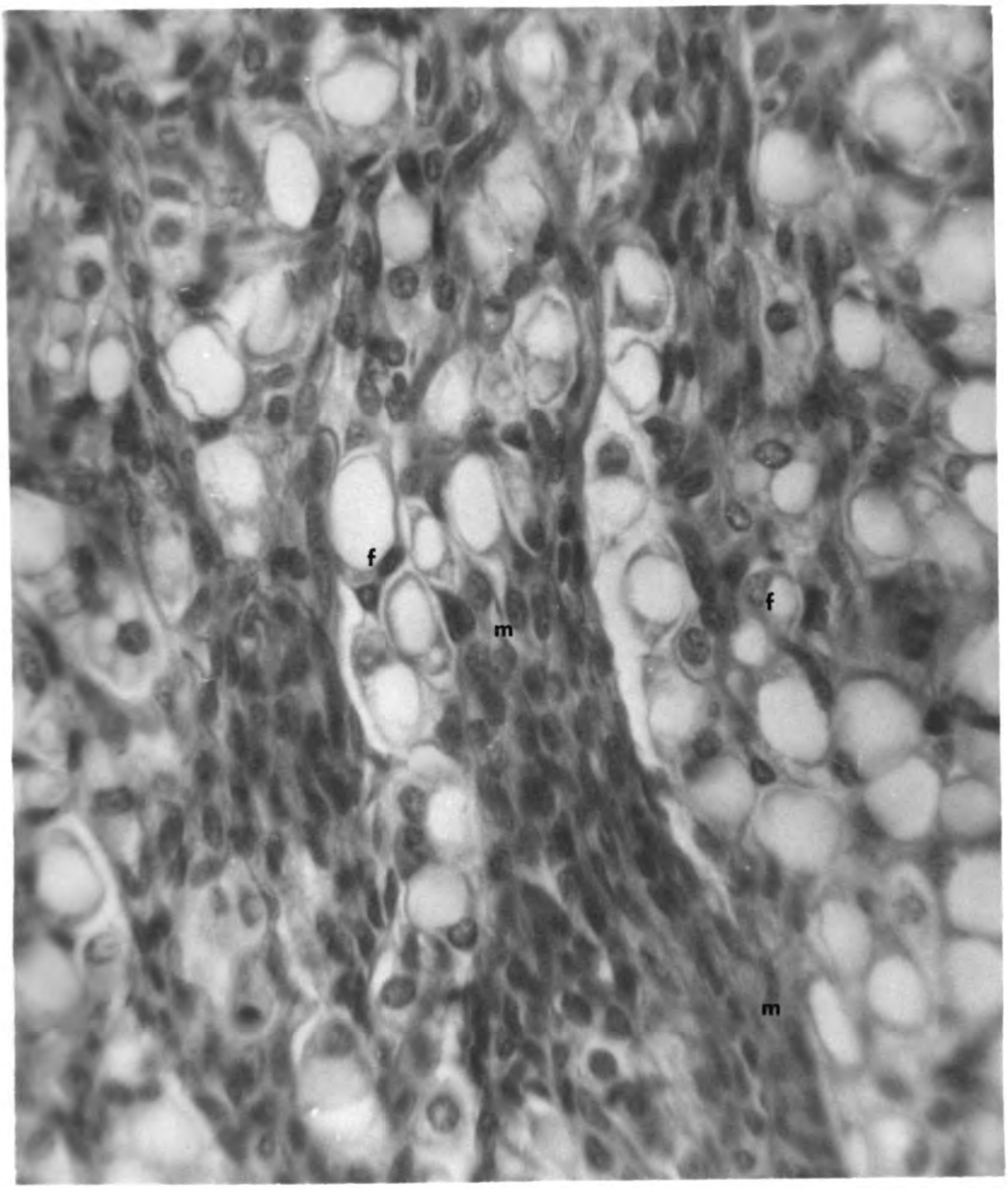

Figure XVI (b).--Epiglottic cartilage of one-and-a-half-week old dog.

(f) Fat cells; (m) Mesenchyme cells. Hematoxylin and eosin stain; X915.

Figure XVII.--Epiglottic cartilage of five-year-old dog.

(a) Laryngeal surface; (b) Lingual surface; (c) Cartilage plates; (d) Mixed glands, more numerous on the laryngeal side; (e) Fat in center of epiglottic plate. Hematoxylin and eosin stain; X35.

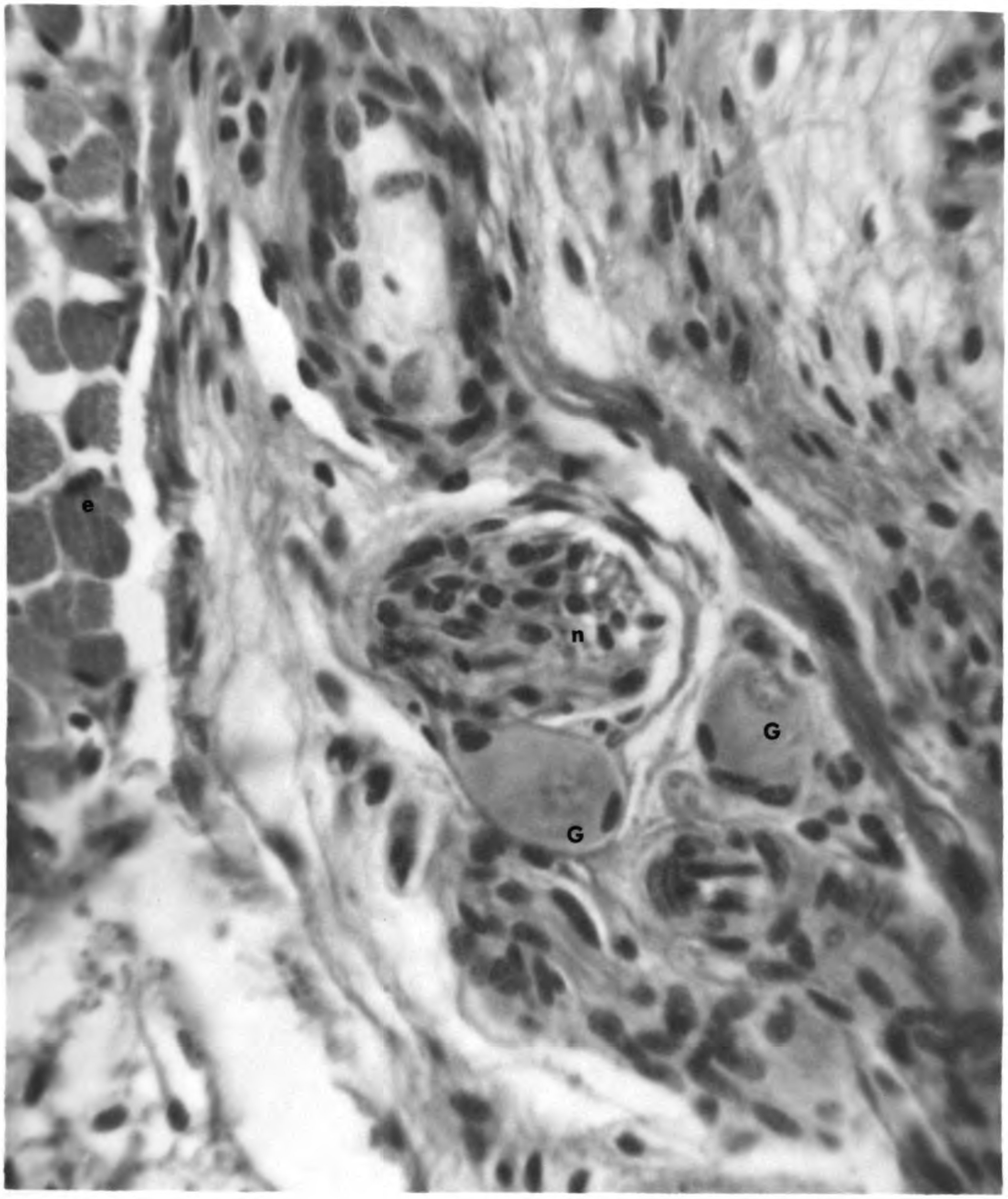

Figure XVIII.--Ganglion in the lingual subepithelial layer of sheep epiglottis.

(e) Skeletal muscle; (G) Ganglion cells; (n) Nerve trunks. Hematoxylin and eosin stain; X84.

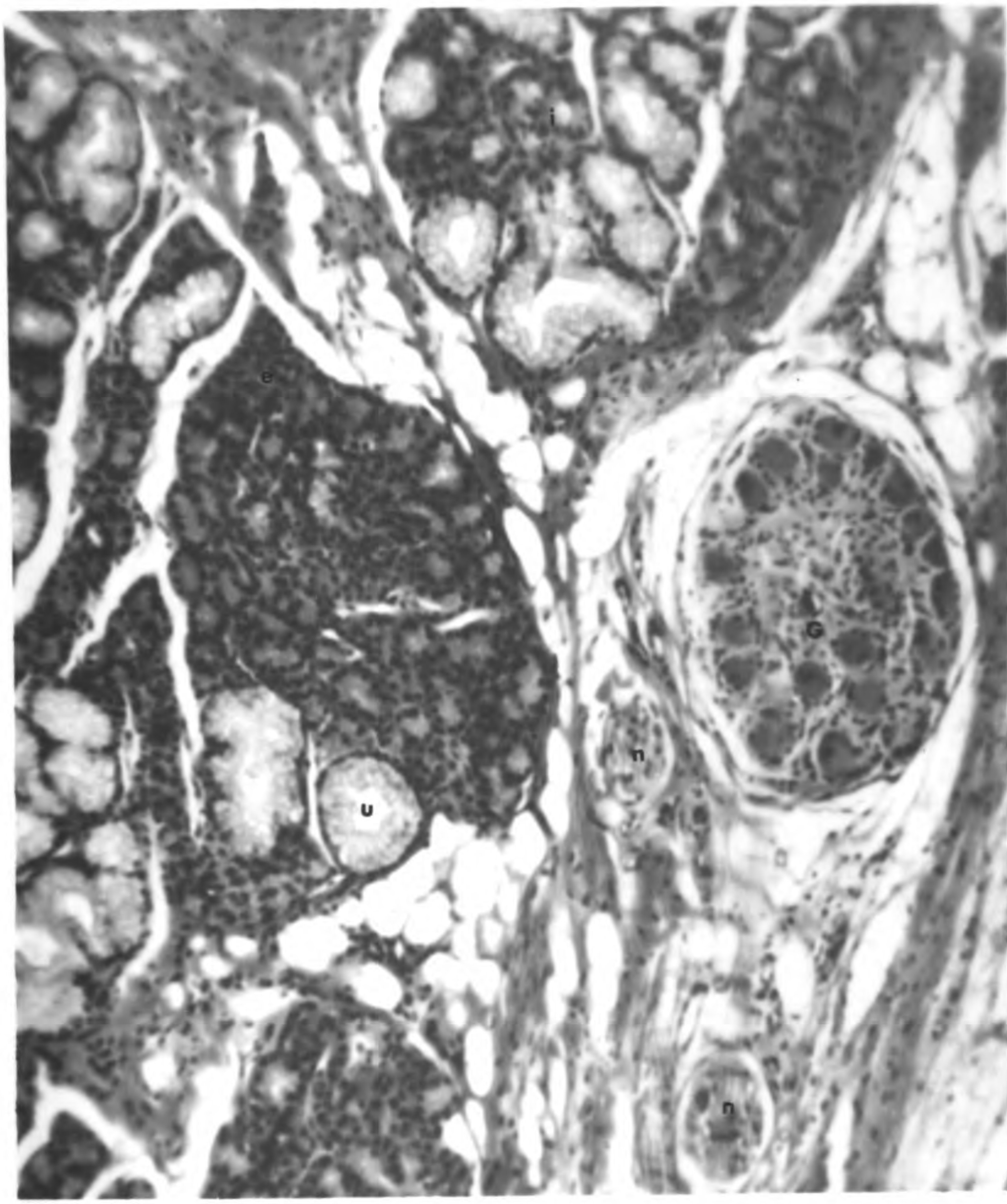

Figure XIX.--Ganglion in the lingual subepithelial layer of the goat epiglottis.

(e) Serous glands; (G) Ganglion; (i) Mixed glands; (n) Nerve trunks; (u) Mucous glands. Hematoxylin and eosin stain; X214.

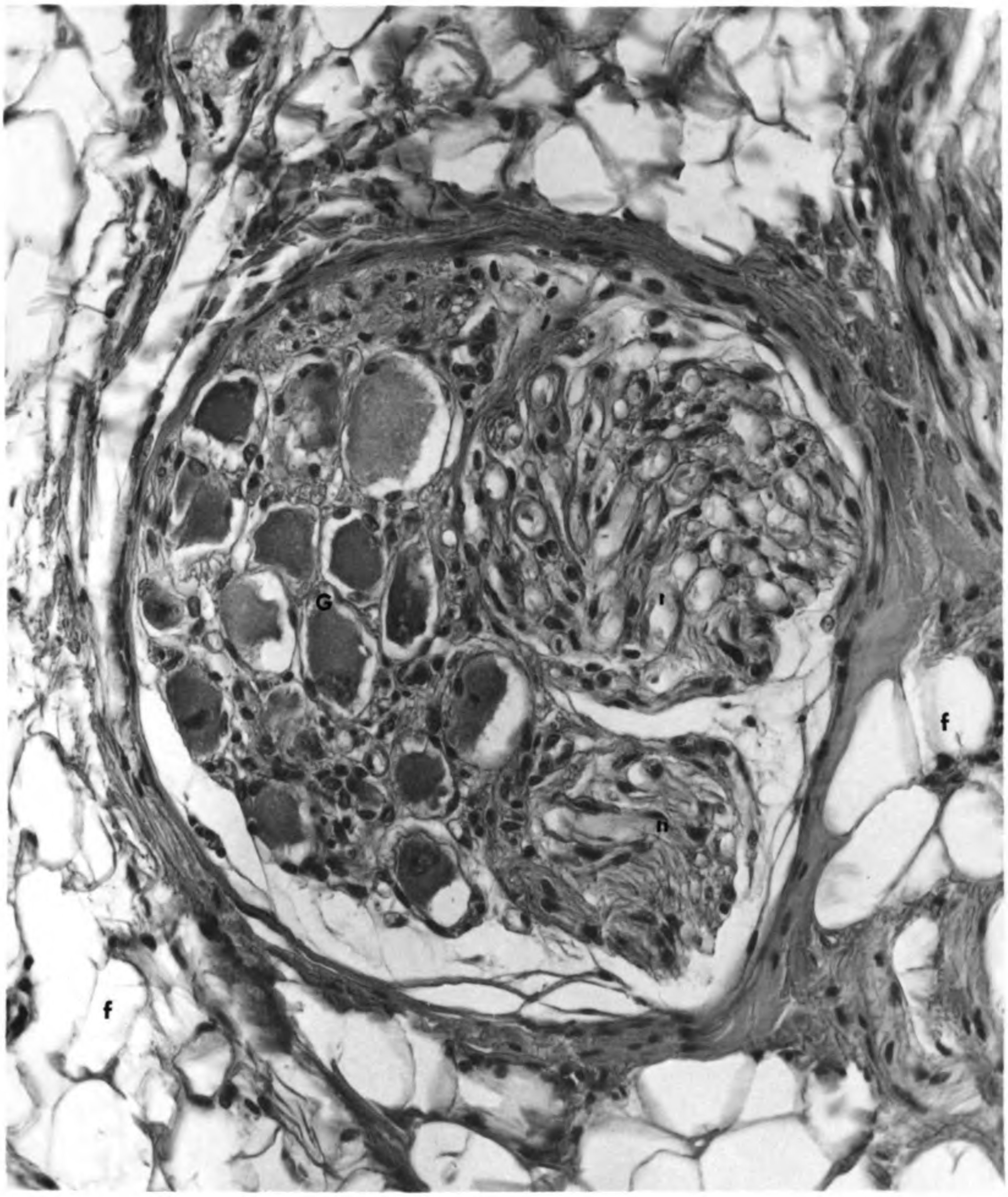

Figure XX.--Ganglion in the subepithelial layer of the swine epiglottis.

(f) Fat cells; (G) Ganglion cell; (n) Nerve trunks. Hematoxylin and eosin stain; X185.

Figure XXI.--The prevelar epiglottis of the cat.

(1) Laryngeal surface; (2) Free border; (3) Lingual surface; (e) Epiglottic cartilage; (g) Aggregated lymphocytes; (m) Skeletal muscle; (s) Soft palate; (T) Thyroid cartilage; (H) Hyoid. Weigert and Van Gieson stain.

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