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THE MICROVASCULATURE OF THE LARYNX: A SCANNING ELECTRON MICROSCOPIC STUDY

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Abstract

In ten adult guinea pigs, the microvascular architecture of the larynx was evaluated using microvascular corrosion casts and scanning electron microscopy. The vocal cords were provided with a subepithelial capillary network. The capillaries, freely anastomosing with each other, were supplied and drained via strongly undulating arteries and veins. The undulation of the latter vessels may explain their adaptability to volume changes of the larynx during phonation. The vasculature of the internal perichondrium of the thyroid cartilage was interrupted at the anterior commissure where an avascular zone was present at the origin of the vocal cords. This avascular area is common to both guinea pigs and humans and may explain the particular mode of tumor spreading, i.e., that the tumors remain unilateral for a long time. The rich vascular supply of the laryngeal mucosa prevents the organ from ischemic complications during surgical procedures. Our results show that the guinea pig may serve as a model for study of laryngeal disorders.

Key Words: Larynx, microcirculation, corrosion casting, scanning electron microscopy, guinea pig.

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Introduction

The microvasculature of the larynx is important to understand many clinical problems. It may determine how tumors extend and inflammatory diseases spread (Miodonski *et al.*, 1980; Olszewski, 1976). In this context, the glottic area plays an important role where two kinds of epithelia come together. It is well known that incipient tumor lesions of the vocal fold remain unilateral for a certain period of time, suggesting the existence of a barrier that prevents invasion to the contralateral side (Andrea, 1981; Bagatella and Bignardi, 1981; Freeland, 1975; Guerrier and Andrea, 1977, 1980; Pearson, 1975). Hence, one is tempted to speculate that this barrier function may be caused by a particular microvascular pattern. Also the rapid spreading of edema and inflammatory disorders suggests a significant involvement of the microvasculature. To the best of our knowledge, the study by Nakai *et al.* (1991) is the only one which focuses on the microcirculation of the larynx in guinea pigs, rabbits and humans using scanning electron microscopy of vascular corrosion casts; in that paper, some clinically relevant aspects of the larynx microvasculature are dealt with, however, there are some questions which should be still addressed. For instance, it is worth determining whether microvascular patterns have any bearing on the way in which tumors enlarge locally within the larynx. Therefore, in the present study we used guinea pigs as a model for studying the larynx microcirculation. Scanning electron microscopy (SEM) of microvascular corrosion casts (Aharinejad and Lametschwandtner, 1992; Aharinejad *et al.*, 1991; Lametschwandtner *et al.*, 1980) was felt to be an ideal technique for this approach.

Material and Methods

In ten guinea pigs (*Cavia porcellus*) of both sexes, weighing 200-250 g, the aortic arch was exposed under deep anesthesia (pentobarbital, 40 mg/kg body weight, intra peritoneally). A plastic cannula (Argyle 0.8 x 19 mm; Sherwood Medical, St. Louis, MO) was introduced

into the aortic arch via the left ventricle, ligated into place, and connected to a two-way connector (LS-2; B. Braun-Melsungen). The thoracic aorta was clamped distal to the branching site of the left carotid artery. Then the circulatory system was rinsed with 40 ml, prewarmed (42°C), heparinized (5,000 IU/L), physiologic saline solution, diluted with dextran (Macrodex; v:v/ 4:1). The efflux was drained via the inferior vena cava. Then 10 ml Mercox (CL-2B, Dainippon Ink & Chemicals, Tokyo, Japan) diluted with 2 ml methylmethacrylic acid and 0.375 g catalyst was injected through the aortic arch. The animal bodies were left at room temperature for 2 hours, before the neck was isolated from the trunk just above the clavicle and the specimens were transferred into beakers containing tap water, where they were incubated in a 60°C water bath overnight. Thereafter, the larynx was exposed under the dissecting microscope and obtained casts were macerated in a 5% KOH solution at 40°C for 12 hours, before the maceration solution was renewed and tissue digestion was allowed to proceed for another two days (Aharinejad *et al.*, 1993). Afterwards, the specimens were observed under a dissecting microscope and if the tissue maceration was complete, the specimens were transferred into beakers containing tap water where they remained for 30 minutes, followed by rinsing in a 5% formic acid solution for another 30 minutes. The rinsing procedure was completed with several rinsing passages with distilled water, where casts were frozen and then freeze-dried (Aharinejad and Lametschwandtner, 1992). In order to get insight into the larynx microvasculature, some casts were frozen in distilled water and cut with a specially adapted circular saw, both median-sagittally and horizontally at -20°C. The specimens were then mounted onto specimen stubs using silver paste and conductive bridges according to Lametschwandtner *et al.* (1980). The specimens were coated with evaporated carbon and gold for 3 seconds, then sputtered with gold for 600 seconds (Aharinejad *et al.*, 1992a, b) and examined with a Cambridge Stereoscan 90 B SEM operated at an accelerating voltage of 10 kV.

Results

The vocal cord, which is covered by stratified epithelium, was provided with a subepithelial capillary network which clearly followed parallel orientation to its longitudinal axis (Fig. 1). Capillaries were smoothly outlined and anastomosed frequently with each other. Draining veins were located under the subepithelial capillary network of the vocal cord (Fig. 2). These veins penetrated the vocal muscle, where they joined the convoluted capillaries forming the characteristic network of striated muscle (Fig. 3). In doing so, these veins accepted tributaries of muscular vasculature. Collecting veins accompanied the corresponding artery which supplied the subepithelial capillary network. All these vessels took an undulating course. The blood vessels in the free edge of the lamina propria ran longitudinally and arose from the anterior and posterior ends of the vocal fold, thereby having no contact with the underlying muscular layer. Remnants of elastic material, due to abundance of elastic fibers in the vocal cord and elastic conus, withstood KOH digestion procedure in part, and were seen beneath subepithelial capillaries (Fig. 2).

The vasculature of the internal perichondrium of the thyroid cartilage was interrupted at the anterior commissure, where an avascular zone appeared to emerge at the origin of the vocal cords (Fig. 4). At this location, the capillaries of the inner perichondrial layer of the thyroid cartilage were missing. Evidently, the superficial microvascular systems of the vocal cords were separated by the described avascular zone. However, the superficial capillary networks of both vocal cords were connected to each other by capillaries of the subglottic area.

The subepithelial capillary network of the vocal cord which showed axial orientation continued toward the ventral wall of the laryngeal ventricle, into irregular capillary nets. The same was seen dorsally, where these irregular capillary loops covered the arytenoid cartilages (Fig. 5). Here, remarkably wide veins were constantly seen under the subepithelial capillaries (Fig. 5). Subepithelial capillaries of the false cord were arranged similarly as did those capillaries covering the arytenoid cartilages (Fig. 6). This was not surprising, because both these structures are covered by ciliated epithelium. Thus, the subepithelial capillary network of the false cord was clearly different from corresponding networks of the vocal cord, which showed longitudinal orientation. The pharyngeal surface of the epiglottis showed a delicate and tightly-packed capillary meshwork (Fig. 7). These capillaries often formed ring-shaped anastomoses, which reflected the orifices of the mucous glands in the mucosa of the epiglottis (Fig. 8). A venous plexus was present beneath the mucosa. These veins traversed the perforations of the epiglottic cartilage, thereby connecting the subepithelial capillary networks located on both sides of the organ (Fig. 9).

To observe the inner surface of the laryngeal cavity, the specimens were cut sagittally. Subepithelial capillary patterns of the subglottic regions exactly corresponded to the described capillary networks which underlaid ciliated epithelium. Toward the tracheal mucosa, this irregular pattern changed to a highly ordered one.

The subepithelial capillary network which is underlying the pharyngeal mucosa of pirifom sinus took the form of an irregular meshwork (Fig. 10). Capillaries of this meshwork were drained toward the lamina propria

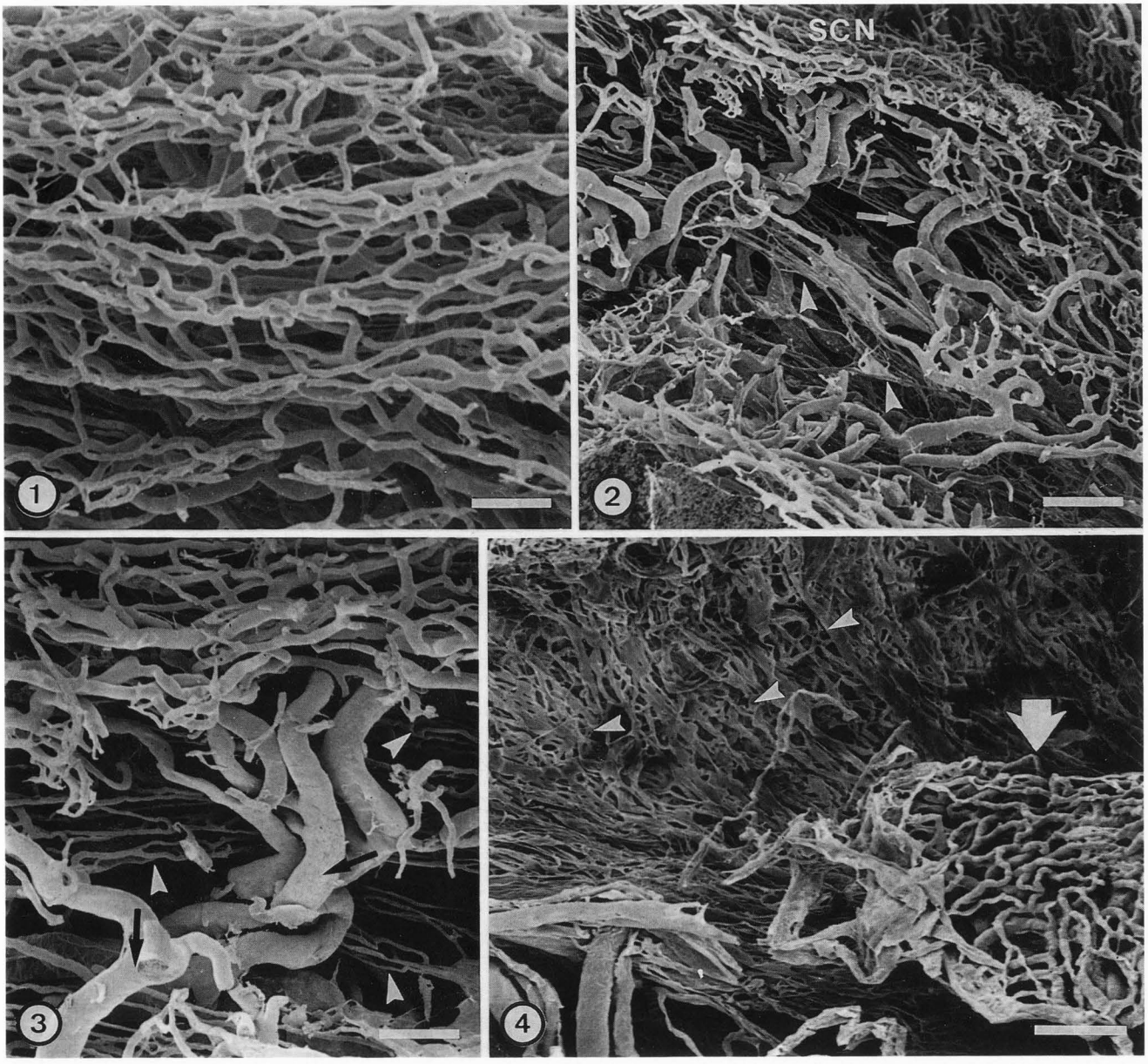


Figure 1. Subepithelial capillary network of the vocal cord, viewed from medial. Bar = 100 μm.

Figure 2. The interior of the vocal cord, the medial wall is partly dissected away. SCN: subepithelial capillary network; arrows indicate the undulating arteries and veins; arrowheads mark remnants of elastic material. Bar = 100 μm.

Figure 3. Higher magnification of Figure 2. Draining veins are marked by arrows. Note the cork screw-shaped capillaries of the muscular layer (arrowheads). Bar = 100 μm.

Figure 4. The avascular zone at the origin of the vocal cord, viewed from medial. The elastic fibers of the elastic conus are marked by arrowheads. Part of the vocal cord (bold arrow) is seen at the right of the micrograph. Bar = 100 μm.

via descending venules which joined a dense venous plexus in the submucosa. Arteries which fed the capillary meshwork were located in the submucosa, where they also formed a dense plexus (Fig. 11). Supplying arterioles which ascended perpendicularly toward the surface, directly fed the subepithelial capillary plexuses.

Discussion

The supplying arteries and draining veins of the vocal cord take an undulating course. Such arrangement of these vessels may be of advantage when considering the length changes and excursions of the vocal cord during phonation. In other words, the wavy course of the mentioned vessels represents their adaptability to the volume changes of the organ during phonation.

Matsuo *et al.* (1987) observed that the oxygen partial pressure was decreased in the lamina propria and the vocal muscle during phonation with the ischemic response in the lamina propria being less than the muscular layer. These authors assumed that the marked ischemic change in the vocal muscle was due to the compression of vessels during contraction of the vocal muscle. Arnstein *et al.* (1989) state that total blood flow is increased during phonation and that the increase in flow is entirely due to the increase in flow to the muscularis layer, with even distribution throughout the layer. The blood flow to the lamina propria was unchanged during phonation, and no gross change in distribution was noted. He also claimed that the blood flow to the lamina propria and muscularis can change independently during phonation. During phonation, the lamina propria receives a constant blood supply despite the underlying muscular contraction with its greatly increased blood supply. It is suggested that the increased flow during phonation is caused by muscular contraction and not vocal fold vibration (Arnstein *et al.*, 1989). Our findings would favor that the blood flow to the vocal fold might be increased during phonation. But the abundance of anastomoses between the muscular layer and lamina propria would rather suggest that the blood flow could increase in the lamina propria at least in the mid portion of the vocal fold.

As mentioned in the **Introduction**, tumors of the vocal cord remain unilateral for a long period of time (Bridger and Nassar, 1972). The described dense fibrous avascular zone in the anterior commissure may represent a barrier for tumor spreading (Andrea, 1981; Freeland, 1975; Guerrier and Andrea, 1977, 1980; Pearson, 1975). Moreover, due to the special arrangement of the blood vessels of the anterior commissure, initially unilateral lesions of the vocal cord which grow in median direction, will primarily spread toward the subglottic regions and reach the contralateral vocal cord later on from there (Freeland, 1975). A U-shaped detour of tumor spreading results according to the arrangement of the subepithelial microvascular system in this area. In the supraglottis, we did not observe any vessels supplying the anterior commissure. This result indicates that the anterior commissure might be isolated from the supraglottis in the region of the anterior commissure and the thyroepiglottic ligament (Andrea, 1981). Bagatella and Bignardi (1981), however, contradict this hypothesis. They state that glandular and vascular tissue is present in the most cranial part of the anterior commissure beneath the mucosa. Such avascular zone in the midline at the anterior commissure is also present in monkeys and humans (Freeland, 1975). These facts have led to the hypothesis that there should be a small zone just above the anterior commissure in the midline larynx where the glands are absent and the mucosa is separated from the thyroid cartilage only by fibers (Andrea, 1981).

Figure 5. The right arytenoid cartilage with its capillary meshwork (arrowheads), covering large-sized veins (V). View from postero-cranial. Bar = 100 μm.

Figure 6. The capillaries of the false cord. Note that capillaries do not show a recognizable orientation, they rather form frequent anastomoses. The curved arrow points to the position of the laryngeal ventricle. Bar = 50 μm.

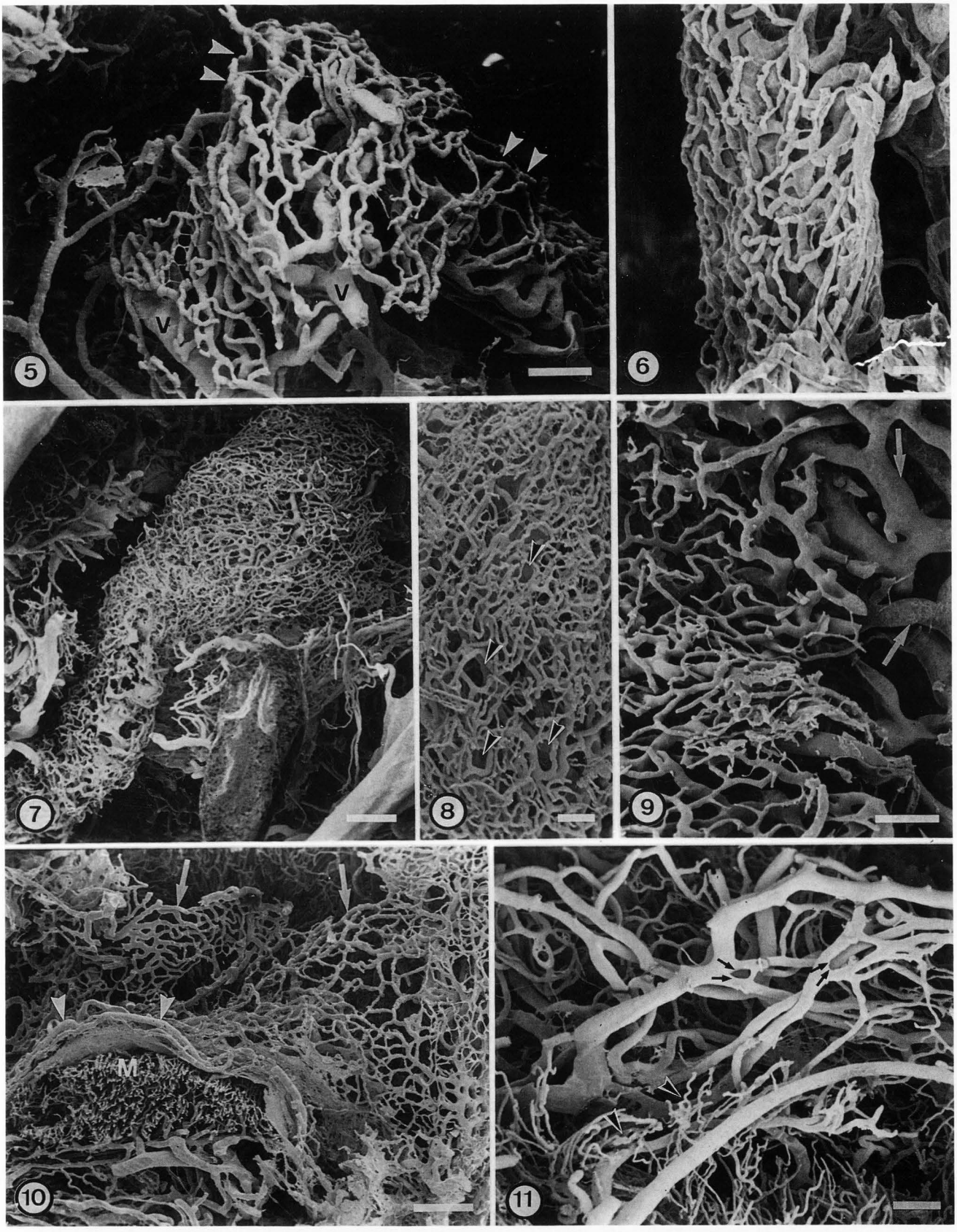
Figure 7. Overview of the pharyngeal surface of the epiglottis. Bar = 250 μm.

Figure 8. Higher magnification of Figure 7. Note the ring-shaped capillaries (arrowheads) mimicking the orifices of the glands. Bar = 100 μm.

Figure 9. A plexus of large-sized veins (arrows) underlies the capillaries of the pharyngeal portion of the epiglottis. Bar = 100 μm.

Figure 10. The vasculature of the piriform sinus (right half of the larynx) viewed from the mucosal side. An irregular capillary network (arrows) covers the relatively thick muscular layer (M). Arrowheads mark draining veins of the capillary network. Bar = 200 μm.

Figure 11. The vasculature of the piriform sinus viewed from the muscular side. Many supplying arteries are seen in the submucosa, which sometimes form loops (arrows). Cork screw-shaped capillaries in the bottom of the micrograph (arrowheads) belong to the muscular layer. Bar = 100 μm.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Considering the fact that the avascular zone occurs both in guinea pigs and humans, and because the general vascular architecture is also similar in both species, guinea pigs may well serve as a model for study of laryngeal lesions.

The laryngeal surface of the epiglottis shows a delicate and tight meshwork as does the false cord, while its oral surface is provided with loosely arranged capillary loops. This clearly reflects the different types of overlying epithelium, which is stratified on the oral surface but ciliated on the laryngeal surface. Subepithelial capillary networks of both surfaces of the epiglottis are connected by thick draining veins which traverse the epiglottic cartilage via its perforations. This particular angioarchitecture may also explain clinical observations of tumor spreading into the pre-epiglottic space (Bridger and Nassar, 1972; Freeland, 1975).

In summary, corrosion casts demonstrate the presence of an avascular zone within the anterior commissure where the vocal ligaments insert into the thyroid cartilage. This avascular zone is a point of higher resistance and can thus explain the particular mode of tumor spreading. In addition, the region of the vocal ligament is loosely vascularized, a condition which accentuates the significance of subepithelial capillaries described. The undulating course of arteries and veins in the vocal cord can be interpreted as adaptation of the vascular system to its excursions during phonation. Finally, the rich vascular supply of the laryngeal mucosa prevents complications during surgical treatment. Undoubtedly, some morphological aspects still remain unclear which could contribute to a better understanding of the genesis of particular disorders in larynx. One of these aspects might be the lymphatic drainage of the organ. This topic should be addressed in forthcoming studies.

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Discussion with Reviewers

A. Miodonski: How wide (in vertical dimensions) is the avascular zone within the anterior commissure? You have implied that this may serve as a model for study of laryngeal lesions, particularly for evaluation of the mode of tumor spread?
Authors: The avascular zone is approximately 500 μm wide as measured in the vertical direction. As we mentioned in the **Discussion**, there is wide agreement on the role of the avascular area concerning tumor spreading in the larynx. However, evidence has yet to be provided. Hence, an experimental model can serve as a basis to conclusively shed light on this question.

A. Miodonski: On Fig. 8 you are presenting capillary loops mimicking the openings of the glands. Do you think that they are really caused by glands or they may represent the openings of the short "channels" piercing the cartilaginous skeleton of epiglottis, which are caused by blood vessels connecting mutually the vascular systems covering the pharyngeal and laryngeal surfaces of the epiglottis [such connections has been observed by Olszewski (1976) in human material obtained during surgery because of advanced carcinoma]?
Authors: The openings shown in Figure 8 are certainly not openings of any vascular routes piercing the cartilaginous skeleton of the epiglottis. This is because: **1.** if these openings were vessels, then they would be cast!; and **2.** if they were vessels, then we would deal with a very densely packed plexus of vessels, with the individual vessels having a luminal diameter of 400 μm in average. Such a vascular plexus would not be characteristic for cartilage.

A. Castenholz: The architecture of the subepithelial capillary network of the vocal cord and its connections to the vessels of its muscular layer may be important for blood flow dynamics during phonation. Did you find any structures such as precapillary or venular sphincters in the larynx which have been described by you previously in the lung and pancreas?
Authors: No. The drainage of vocal cord region into the neighboring skeletal muscle mimics the drainage pattern normally seen in osseous and muscular tissue. Remembering of the drainage pattern of the bone, for instance, where the supplying and draining vessels not only join routes far from the bone, but also directly the capillaries of the neighboring muscles, we are not surprised that the blood supply in the cartilage is reminiscent of that in the bone.

A. Castenholz: You have briefly mentioned the lymphatic vascularization of the larynx in the spread of tumor in your **Discussion**. They must be important and I suggest that you study them using appropriate techniques in the anterior commissure.
Authors: Our next studies will focus on this very basic and clinically relevant topic. We fully share your opinion.

D.E. Schraufnagel: Does the vasculature differ from that of other skeletal muscle?
Authors: The skeletal muscular part of the larynx does not differ from that seen in another parts of the body, in its microvascular pattern.

D.E. Schraufnagel: Does the vasculature of the muscle change if the covering epithelium changes?
Authors: Skeletal muscle microvasculature was not the major subject of the present study. But as far as we know, the microvascular pattern of the muscle changes along with the contraction state of the muscle, no matter which kind of epithelium covers its surface.