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ESOPHAGEAL VASCULATURE IN THE GUINEA PIG:
A SCANNING ELECTRON MICROSCOPE STUDY OF VASCULAR CORROSION CASTS.

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Abstract

The esophageal vascularization of adult male and female albinotic Guinea pigs (Cavia porcellus) is studied by means of light microscopically evaluated serial sections and by scanning electron microscopy (SEM) of vascular corrosion casts. Bronchoesophageal artery (cervical portion), direct branches of tha aorta, recurrent branches of the intercostal arteries (thoracic portion) as well as of the left gastric artery (abdominal portion) supply the esophagus; internal jugular vein, inferior thyroid vein (cervical portion), azygos vein, intercostal veins (thoracic portion) and portal vein, gastroepiploic vein and cranial pancreatoduodenal vein (abdominal portion) drain it. Longitudinally arranged arterioles, venules and capillaries lying at the level of the lamina propria of the esophageal mucosa around the whole circumference of the organ are the most striking vascular features, whereby the venules are considered as those vessels from which esophageal varices arise under pathological conditions.

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Introduction

Pathological changes of the esophagus, particularly the formation of varices by portal hypertension as well as the direction esophageal carcinoma tend to metastasize (Dormanns (1949) stimulated the study of esophageal vasculature very early. So extra- and intraparietal blood supply of the esophagus of man are described (Demel, 1924) as well as the longitudinal network of arteries and veins of the esophageal mucosa and submucosa (Schumacher, 1927). Experimental studies focus upon changes of veins of the lower portion of the esophagus in portal hypertension (Kegaries, 1934; Kitano et al., 1986) and the connection of the continuous longitudinal arterial network of the submucosa with the adventitia vascular bed become elucidated (Potter and Holyoke, 1950). Stark (1966) describes that in the Rhesus monkey and rabbit large esophageal vessels follow closely the apolar muscular system, while Günther and Lierse (1968) claim them- with the exception of those of the lower esophageal segment - to follow the myoarchitecture. Esophageal varices and possible bleedings are studied by McCormack et al., (1983).

The present study intends: 1) to study main supplying and draining routes as well as course and branching patterns of intrinsic esophageal blood vessels in detail by scanning electron microscopy of vascular corrosion casts (Murakami, 1971; Hodde and Nowell, 1980; Lametschwandtner et al., 1984) and 2) to correlate the latter with tissue layers by comparing vascular cast anatomy with that of serial tissue sections.

Materials and Methods

Vascular Corrosion Casting

40 albinotic Guinea pigs (Cavia porcellus) of both sexes with a body-weight ranging from 200 to 250 grams were used for casting studies. 24 specimens were sufficiently casted to be studied in
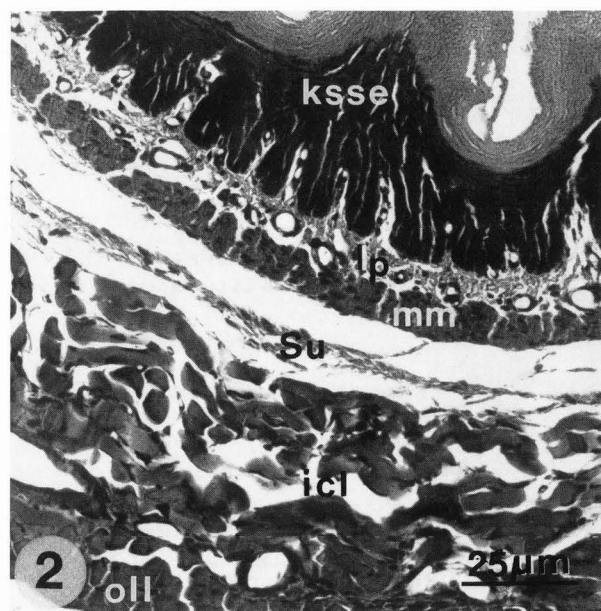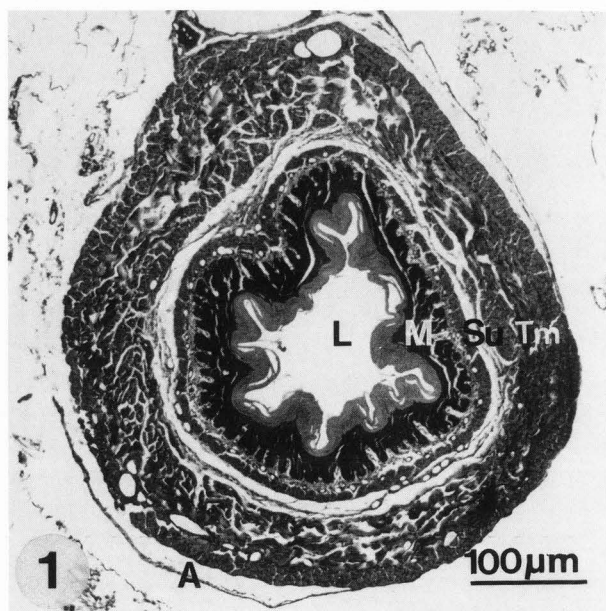
Fig. 1. Esophagus of the Guinea pig. Transverse section. 7 μm. Hematoxyline-Eosine. L = lumen, M = mucosa, Su = submucosa, Tm = tunica muscularis, A = adventitia (serosa).
Fig. 2. Detail from Fig. 1. ksse = keratinized stratified squamous epithelium, lp = lamina propria, mm = muscularis mucosa, Su = submucosa, icl = inner circular layer of striated muscle fibers of the tunica muscularis. Note the lamellae of the lamina propria projecting radially into the base of the mucosal epithelium and its abundant vessels.
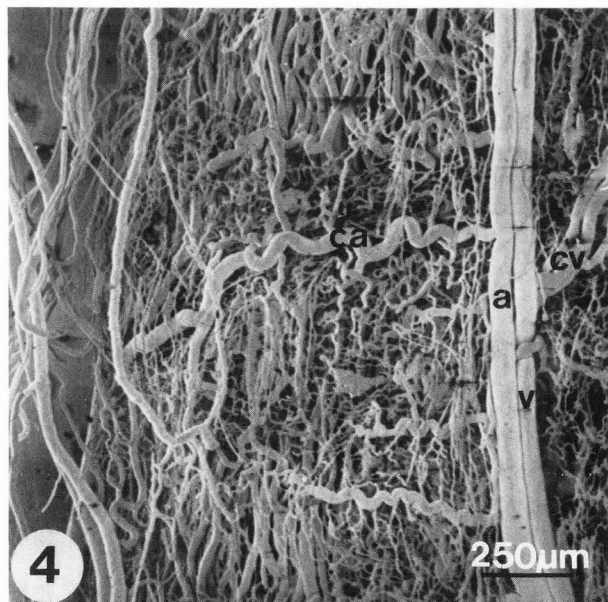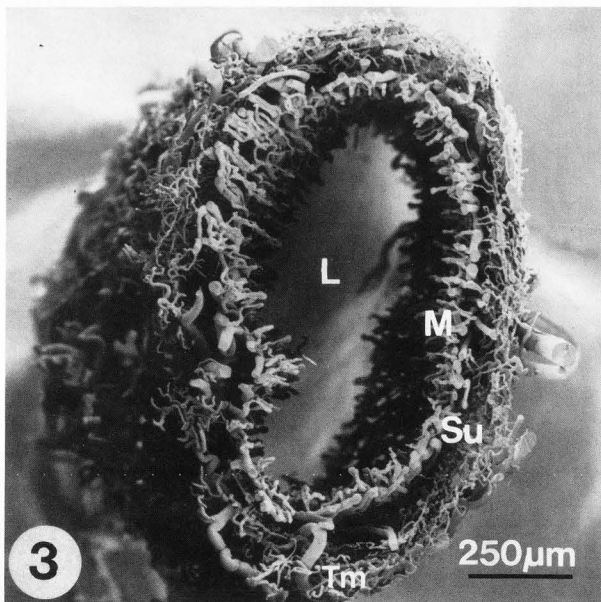

Fig. 3. Corrosion cast of the Guinea pig esophageal vascular bed. Transverse section. Compare with Fig. 1. For abbreviations see Fig. 1.
Fig. 4. Esophageal segment. Corrosion cast. a = artery, ca = circumferential arteriole, cv = circumferential venule, v = vein.

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the scanning electron microscope (SEM). Briefly animals were anesthetized with sodium pentabarbital (ip., 40 mg per kg body weight) and thoracotomized. A plastic catheter was introduced into the aortic arch via the left ventricle. The

Fig. 5. Esophageal segment with circumferential vessels. Corrosion cast. Note the circumferential venule (cv) draining into the inferior thyroid vein (itv). ac = adventitial capillaries.
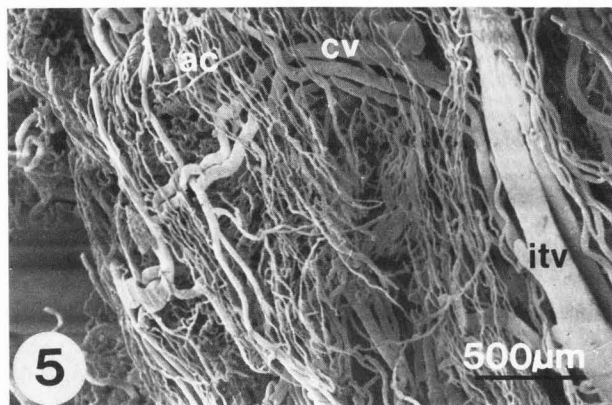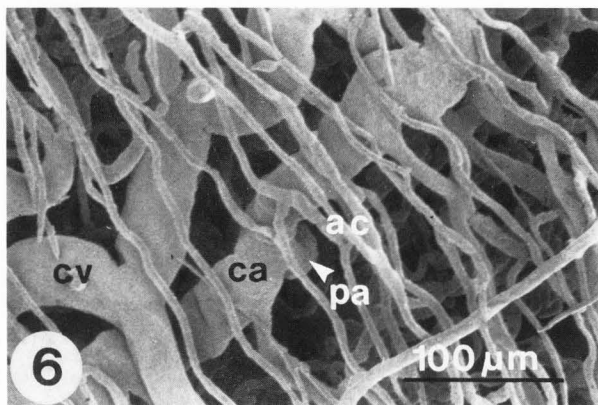

Fig. 6. Detail from Fig. 5. ac = adventitial capillaries, ca = circumferential arteriole, cv = circumferential venule, pa = perforating arteriole.
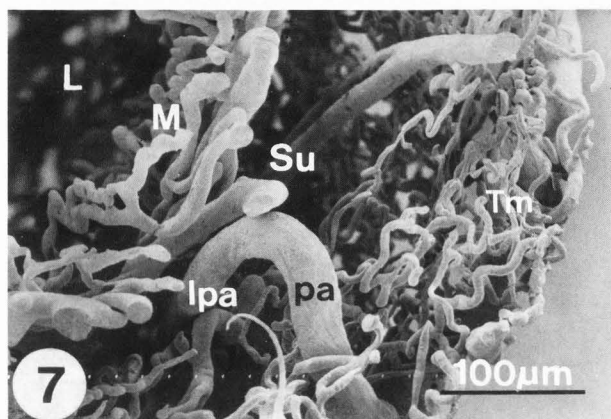

Fig. 7. Vascular layers within the esophagus wall. Detail from Fig. 3 revealing vessels of the esophageal mucosa (M), submucosa (Su) and tunica muscularis (Tm). pa = perforating arteriole, L = lumen, lpa longitudinally orientated continuation of pa.

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vena cava was opened and the circulatory system was rinsed with 60 ml of warm (37° C) Tyrode-solution using hand pressure. Then the abdominal aorta was clamped proximally to the renal arteries, the subclavian arteries distally to the costocervical trunk, and Mercox-Cl-2B (Dainippon-Ink & Chemicals, Tokyo, Japan) diluted with monomeric methylmethacrylate (v/v 4:1; Hodde, 1981) was injected manually. To allow solidification of injected resin animals were left for 2h at room temperature (20°C) before they were tempered overnight in the water bath at 60°C. Maceration was done in 15 % potassium hydroxide at 40°C for two days or longer; decalcification was in 2%
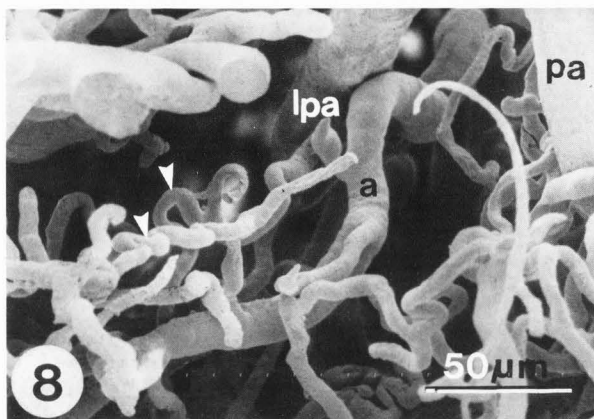

Fig. 8. Detail from Fig. 7. Circularly running arteriole (a) coming from a perforating arteriole (pa) supplying the circular arranged fibers (inner layer) of the tunica muscularis (Tm). Note the wavy course of the capillaries typically for striated muscle capillaries (arrowheads). lpa longitudinally orientated continuation of pa.

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hydrochloric acid at 20°C for another two days or longer. The esophageal and tracheal vasculature with their vascular surroundings were dissected free under the dissecting microscope, cleaned in 5 % formic acid, rinsed in several passages of distilled water and finally frozen in it. Freeze-dried casts were mounted onto copper foils fixed to specimen stubs with colloidal silver using the conductive bridges method of Lametschwandtner et al. (1980). Casts were sputtered with gold and examined with a scanning electron microscope Stereoscan 250 at 5 to 15 kV accelerating voltage.

Light Microscopy

Four animals aged 2 - 4 weeks were fixed by perfusion with 2.5 % buffered glutaraldehyde (sodium cacodylate, pH 7,35; 0.15 M). Dissected esophagi were dehydrated in a graded series of alcohol and embedded in paraffin. Serial sections (7µm) were stained with hematoxyline-eosine.

For studying the gross vascularization of injected specimens casts were examined under the dissecting microscope.

Terminology

For denomination of anatomical regions see Cooper and Schiller (1975). The classification of vessels as arteries, arterioles, capillaries or venules is according to Rhodin (1974) based upon their diameters in casts.

Results

Anatomy of the Guinea pig esophagus

From inside to outside the esophagus (Fig.1) is formed by: 1) the mucosa (M), which in the Guinea pig consists of a cornifying stratified squamous epithelium (ksse), a thin connective tissue lamina propria (lp) and a longitudinally arranged layer of smooth muscle cells, the muscularis mucosae (mm); 2) the submucosa (Su); 3) the tunica muscularis (Tm), which consists of an inner circular layer (icl) and an outer longitudinal layer (oll) of striated muscle fibers and 4) a thin connective tissue adventitia or serosa (A). The thin lamina propria of the mucosa projects with radially directed connective tissue lamellae deeply into the mucosal epithelium. While in the lamellae a two-dimensional vascular network formed by two or three capillaries lying upon each other is present, larger vessels lie adjacent to the muscularis mucosae (Fig. 2). A transverse sectioned cast of the esophageal vasculature (Fig. 3)-corresponding to the tissue sections shown in figures 1 and 2 -outline very clearly this arrangement.

Main blood supply and drainage

Cervical portion. This region is supplied via the bronchoesophageal artery which either originates from the aortic arch or from the subclavian artery, the inferior thyroid artery, the common carotid artery or the external carotid artery. The drainage is via the jugular vein and the inferior thyroid vein.

Thoracic portion. This region is supplied by direct branches of the aorta and recurrent branches of the intercostal arteries. The drainage is via azygos vein and intercostal veins.

Abdominal portion. This region is supplied by direct branches of the aorta and the left gastric artery. The venous drainage was found to be either via several (generally four) esophageal veins into the portal vein (in 25% of the specimens) or into the gastroepiploic vein and the cranial pancreatoduodenal vein.

Intrinsic esophageal vasculature

Arterioles (terminology according to Rhodin, 1974) with a diameter of less then 50 µm, in general, arise from main supplying vessels at angles below 45 degrees (Figs. 4 and 5). They initially run slightly oblique towards cranial, pierce the esophageal adventitia and bend to course along the circumference of the esophagus towards the ventral and dorsal midline (Fig. 5). We term these vessels circumferential arterioles.

Adventitial vasculature

The adventitita is supplied by 15 µm thick branches of the circumferential arterioles which abut at acute angles (Figs. 4,5 and 6). The arterioles branch and form the adventitia capillary bed (Figs. 5 and 6). Diameters of capillaries range from 4 µm to 10 µm with most capillaries being in the lower range (Fig. 6). There are very few interconnections between smooth appearing, longitudinally running capillaries in the adventitia (Fig. 6).

Vasculature of the tunica muscularis

There are only a few vessels interposed between the outer longitudinal running skeletal muscle fibers (Figs. 1,2, 4 and 5). The wavy course of the vessels allows one to attribute them to this layer (Fig. 4). Diameters of these vessels are around 10 µm. Larger vessels lying closest to these outer longitudinally arranged striated muscle fibers are in the border region to the inner circular arranged striated muscle fibers (Figs. 1 and 2).

The inner circular running muscle fibers have a rich capillary bed. Capillaries have a smooth surface with a uniform diameter of 5 µm and reveal an undulating course typically for capillaries of striated muscles (Fig. 7). Capillaries are fed by 15 µm to 20 µm thick arterioles running parallel to muscle fibers (Fig. 8). These arterioles arise at the level of the submucosa from longitudinally running arterioles with diameters around 30 µm and which are the continuation of perforating arterioles (Fig. 7, pa). Perforating arterioles again abut from the circumferential arterioles (Figs. 5 and 6, ca) and pierce the tunica muscularis radially before changing their course towards caudal on arrival at the submucosa.

Vessels of the submucosa

In the submucosa some larger arterioles and venules are present (Figs.1,2,7 and 8). These vessels primarily run longitudinally.

Vasculature of the muscularis mucosae

Like in the tunica muscularis very few vessels are interposed between the longitudinally arranged smooth muscle cells of this layer (Figs. 1 and 2). Closest larger vessels lie in the adjacent lamina propria.

Vasculature of the lamina propria

Because of the abundance of arterioles, venules and capillaries lying in this zone around the whole circumference of the esophagus we term this layer the main esophageal vascular layer. It actually consists of: 1) a tight plexus of longitudinally running vessels facing the muscularis mucosa and 2) the esophageal mucosa capillary bed lying within the longitudinally arranged connective tissue lamellae projecting radially into the esophageal epithelium (Figs. 1,2,3, 7-11).

Longitudinal vascular plexus. This plexus consists of many —more or less regularly spaced — longitudinally running venules and fewer arterioles (Figs. 1,2,9-11). Venules with diameters from 20 to 40 µm have numerous cross connections leading to a plexus (Figs. 9-11). Venules as well as arterioles do not lie in separate planes but are rather interwoven (Figs. 10 and 11).

Esophageal mucosa capillary bed. The capillary bed lies within the radially towards the lumen projecting connective tissue lamellae (compare Figs. 1 and 2 with Fig. 3). It is fed by arterioles coming from the longitudinally running vessels at the base of the lamina propria and consists of a two dimensional network with two to three capillaries lying on top of each other (compare figs. 10 and 11). T-like branchings are very few (Figs. 11, arrows). Parallel running capillaries are by and by crosslinked (Fig. 10; arrows). In some esophageal areas the innermost running capillary has an undulating course with nodular thickenings (Fig. 9; arrows). Free capillary lengths range from 100 µm to several hundred µm. Capillaries in turn form postcapillary venules which then join to form the venous plexus which by means of perforating venules empty into circumferential venules (Figs. 4 and 5) draining into main draining vessels (see above). Fig. 12 summarizes the angioarchitecture found.

Discussion

In 1924 Demel described the esophageal blood supply in man by using gelatine injections for macroscopical inspection and "Teichmannsche Masse" (Teichmann, 1952) for macroscopical studies. In this excellent paper he states that the thoracic portion of the esophagus is supplied by branches of the descending aorta which he terms them dorsal and ventral esophagio-tracheal arteries. In the Guinea pig we find no such arteries, instead in this region the inferior thyroid artery sends individual branches towards the esophagus and trachea to supply both individually. These branches are often found to anastomose. Demel (1924) further points out large vessels running longitudinally in the tela submucosa. This study shows that these vessels are veins and agrees with Schumacher (1927) who described them as becoming thinner the closer they approach the mucosa to finally form capillary loops within the mucosal papillae which in this study could be examined in great detail. Kegaries (1934) considers the submucosal plexus formed by veins as being of clinical importance. Kitano et al. (1986) also focus on these veins and report "deep veins" and "superficial veins", whereby the former form less anastomoses than the latter. Spence (1984) and Spence et al. (1983, 1984) in studying esophageal varices described the mucosal veins as becoming larger under these conditions and the submucosal ones as becoming thinner. Since in this study healthy esophagi were studied no comments upon these results can be made. But because of the finding that primarily the veins of the lamina propria and the submucosal veins were the largest we rather consider them as capacity vessels and the site of varices origin. Kegaries (1934) described also porto-caval anastomoses without giving further topographical details. This study reports for the lower third (abdominal portion of the esophagus) a direct portal drainage as well as an indirect one via the right epiploic vein and the cranial pancreatoduodenal vein.
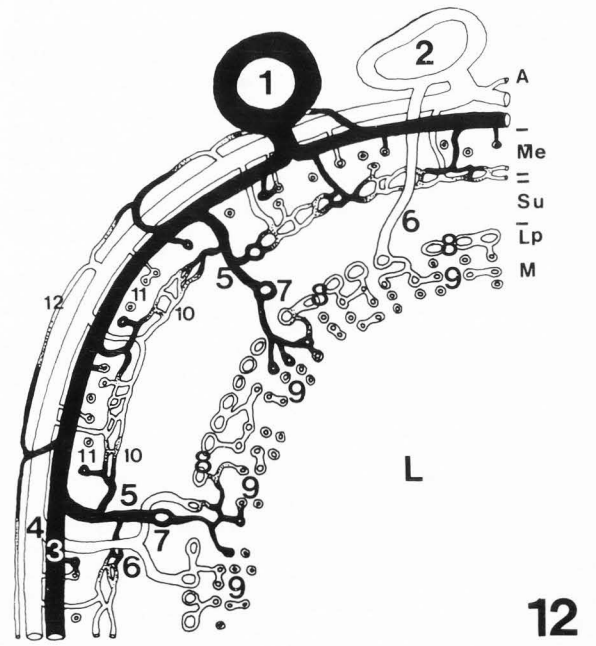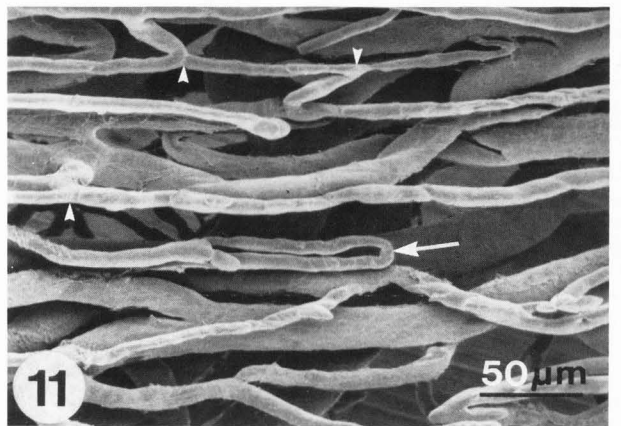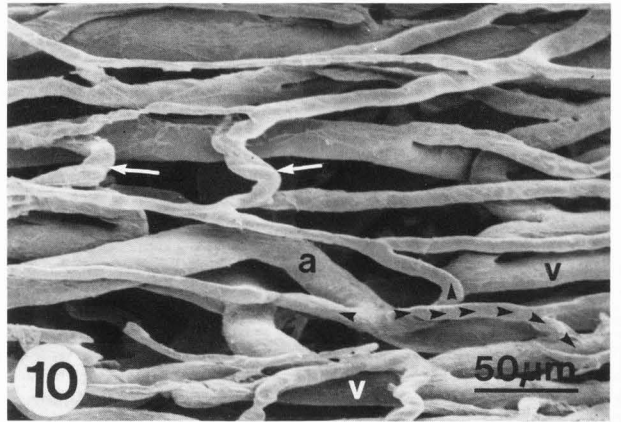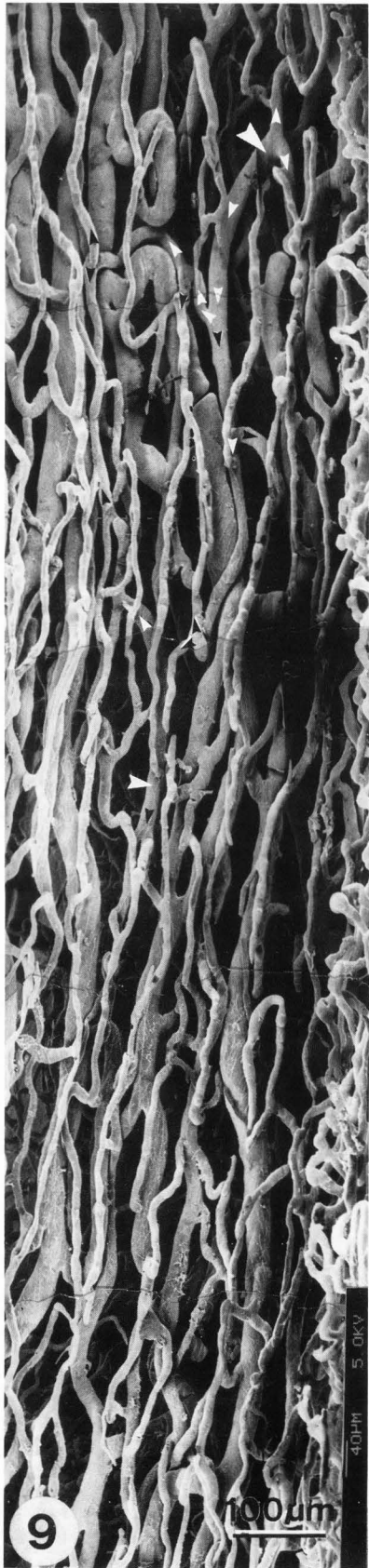
Günther and Lierse (1968) in the rat report longitudinally running arteries within the submucosa of the esophagus and describe the angioarchitecture of the muscular lamina of the mucosa.

Demel (1924) introduces the right lower portion of the esophagus of man as least vascularized. This is not the case in the Guinea pig (this study).

Fig. 9. Esophageal mucosal capillary bed. Corrosion cast. Luminal view at a longitudinally sectioned esophagus segment. Note two supplying arterioles marked by arrowheads.

Fig. 10. Mucosal capillaries. Detail. Longitudinally running capillaries are interconnected by bridging vessels (arrows). Note the short arterio-venous transition route (arrowheads). Y-like branchings of capillaries and venules dominate.

Fig. 11. Mucosal capillaries reveal a loop (arrow) and T-like branchings (arrowheads).

Fig. 12. Scheme of esophageal vascular architecture. Transverse section. Black vessels = arteries and arterioles, white vessels = veins, venules and capillaries. 1 = main supplying artery, 2 = main draining vein, 3 = circumferential arteriole, 4 = circumferential venule, 5 = perforating arteriole, 6 = perforating venule, 7 = longitudinally orientated continuation of the perforating arteriole (5), 8 = longitudinally running venules of the lamina propria, 9 = mucosal capillaries, 10 = vessels of the internal layer of the tunica muscularis running circularly, 11 = vessels of the external layer of the tunica muscularis running longitudinally, 12 = vessels of the adventitia (serosa). L = lumen.

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Discussion with Reviewers

D.F. Schraufnagel: What are the advantages and disadvantages of serial reconstruction compared to scanning electron microscopy of casts?
Authors: The advantage of serial reconstruction is that this technique enables to demonstrate relations between several structures; for example between blood vessels, nerve cells and glial cells. Serial reconstruction in any case is time consuming and laborious. Modern image reconstruction systems again need high data storage capacities. Plotted graphs are confusing, lack the structural resolution and to not give the clear "3-D-impression" SEM micrographs of corrosion casts do so convincingly.

D.F. Schraufnagel: How are the bronchial and esophageal circulation interrelated?
Authors: We have not studied detailed bronchoesophageal vascular interrelations.

M.T. Hull: Did the authors identify any system of vertically orientated capillaries in the mucosal system that is in any way similar to the one seen in the human esophagus within connective tissue papillae?
Authors: Apart from the quantitative aspect we consider the capillaries within the lamina propria of the Guinea pig esophagus as the equivalent of the capillaries within the human esophagus connective tissue papillae.

Reviewer III: In the description main blood supply and drainage no pictures are mentioned. Has it been only written out from the literature?
Authors: No. The main blood supply and drainage was studied by examining corrosion casts with the dissecting microscope. Because of the low depth of focus of this instrument we did not take pictures.

Reviewer IV: According to our experience decalcification with HCl and formic acid wash out the nucleic impressions. Please comment.
Authors: In our experience neither the application of 2% HCl (up to 23 hours at 20°C) nor that of 5% formic acid (up to 23 hours at 20°C) wash out endothelial cell nuclei imprints (see Fig.7, perforating arteriole (pa)). In a previous study (Weiger T, Lametschwandtner A, Simonsberger P (1982) Methylmethacrylat und Mercox Cl in der Rasterelektronenmikroskopie von Korrosionspräparaten. Methylmethacrylate and Mercox Cl in the Scanning Electron Microscopy of Corrosion Casts. Mikros-kopie (Vienna) 39, 187-197) we tested the effects of different concentrations of sodium and potassium hydroxid (20% and 30%) over 2 and 23 hours at 20°C), and of sulphuric, hydrochloric and formic acid (2%, 5%, 10% and 20%) upon the surface structure of test probes made from Mercox Cl (M) and Methylmethacrylate (MMA). The application of 3% and 5% hydrochloric acid up to 23 hours did not result in any corrosion of the surface of probes made from M. In probes made from MMA (original mixture of Murakami, 1971) the application of 3% hydrochloric acid for 30 minutes and of 5% formic acid for 60 minutes resulted in first corrosion effects. Higher concentrations together with longer periods of application (23 hours) resulted in surface corrosion of probes made from M and MMA. The wash out of nucleic impressions as you have experienced might result from such conditions.