MICROVASCULARIZATION OF THE ESOPHAGUS IN THE COMMON TREE SHREW *(Tupaia glis)*

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Thesis
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MICROVASCULARIZATION OF THE ESOPHAGUS IN THE COMMOM TREE SHREW *(Tupaia glis)*

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บทคัดย่อ

การศึกษาหลอดอาหารในกระแตด้วยกล้องจุลทรรศน์ธรรมดา (LM) กล้องจุลทรรศน์อิเลกตรอนแบบส่องกราด (SEM) และศึกษาโครงหลอดเลือดด้วย Corrosion cast technique/SEM พบว่าชั้นกล้ามเนื้อของหลอดอาหารส่วนบนมีเฉพาะกล้ามเนื้อลาย ส่วนกลางประกอบด้วยกล้ามเนื้อลายและกล้ามเนื้อเรียบ ส่วนล่างมีเฉพาะกล้ามเนื้อเรียบเพียงอย่างเดียว และยังพบส่วนหลอดอาหารในลำคอ ได้รับเลือดมาเลี้ยงจาก superior และ inferior thyroid arteries หลอดอาหารในทรวงอกส่วนบนได้รับจากแขนงของ bronchial arteries และ thoracic aorta สำหรับหลอดอาหารส่วนล่างทรวงอกเลี้ยงด้วยแขนงโดยตรงจาก thoracic aorta จำนวน 1 - 2 แขนง ในระดับกระดูกสันหลังทรวงอกที่ 4 - 8 ส่วนเส้นสุดท้ายทอดไปตลอดความยาวของหลอดอาหาร ซึ่งไปเชื่อมต่อกับหลอดเลือดที่มาจาก gastric artery ที่มาเลี้ยงส่วนล่างของหลอดอาหาร รวมทั้ง short gastric และ splenic arteries ในการศึกษาการกระจายตัวของหลอดเลือดในชั้นต่างๆของหลอดอาหาร พบว่าเมื่อหลอดเลือดแดงใหญ่มาถึงผนังจะผ่านเข้าไปและวิ่งอยู่ในชั้นเยื่อบุผิวชั้นนอก ซึ่งจะให้แขนงของหลอดเลือดฝอยเลี้ยงชั้นนี้ รวมทั้งกล้ามเนื้อชั้นนอกที่วิ่งตามแนวยาว หลังจากนั้นเข้าไปเลี้ยงในชั้นใต้เยื่อบุผิวและชั้นเยื่อบุผิว เมื่อสังเกตจากทางด้านชั้นเยื่อบุผิว การกระจายของหลอดเลือดแดงฝอยมีทั้งแบบรังผึ้งและแบบสัน หลังจากนั้นเลือดดำจะออกจากหลอดอาหารโดยผ่านทางหลอดเลือดดำขนาดเล็กจนถึงขนาดใหญ่ ซึ่งวิ่งคู่กับหลอดเลือดแดง เลือดดำจากหลอดอาหารส่วนต้นเทสู่ laryngeal vein จากบริเวณหลอดอาหารส่วนกลางจะไหลเข้าสู่ azygos

สู่ azygos vein และจากส่วนล่างเข้าสู่ portal system นอกจากนี้ยังพบว่าบริเวณก่อนถึงกระเพาะอาหาร 5 - 10 มม จะมีหลอดเลือดมาเลี้ยงน้อยเมื่อเปรียบเทียบกับบริเวณอื่นของหลอดอาหาร

Thesis Title Microvascularization of the Esophagus in the Common Tree Shrew *(Tupaia glis)*

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Abstract

The esophagus from each of 15 common tree shrews of both sexes weighing among 120-180 g was studied by light microscopic (LM) conventional SEM and vascular corrosion cast technique with SEM, respectively. With LM technique, it is found that the esophagus divides into three parts. The muscularis of cervical part consists of striated muscle, thoracic part has both striated and smooth muscles while the terminal part consists of only smooth muscle. The rudiment of the esophageal gland is found in submucosa of terminal part. With vascular corrosion cast technique/SEM. It is found that the cervical esophagus receives blood supply from branches of superior and inferior thyroid arteries. The thoracic esophagus is supplied by esophageal branches of bronchial artery and thoracic aorta. The thoracic aorta gives 1-2 branches to supply esophagus below arch of aorta. The last branch at T4-T8 vertebral levels with a long branch running caudally along the dorsolateral aspect of the organ. The caudal esophagus is supplied by branches of left gastric, short gastric and splenic arteries. After entering the

branches running on the left and right sides. The small branches give rise to adventitial plexus supplying the adventitia and outer longitudinal muscle. The larger branches penetrate the muscular coat into the submucosa and branching to supply the muscle before becoming the submucosal plexus. This plexus off arterioles branching into capillaries supplying the mucosa. At the luminal surface, the capillary plexuses connected each other to form ridge or honey-comb like structure. The venous blood is collected into small then large venules before joining the submucosal plexus which drains into the major veins. The major veins from the cervical esophagus drains into the laryngeal vein while those in thoracic cavity join the azygos vein and the veins from the distal end empty the blood into the portal system. It was also shown that there is considerably less blood supply in the area at about 5-10 mm above the gastroesophageal junction when compared to that in other areas of the esophagus.

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LIST OF ABBREVIATIONS

A	=	artery
Ad	=	adventitia
Ao	=	aorta
AP	=	adventitial plexus
AV	=	azygos vein
a	=	arteriole
C	=	coeliac trunk
CA	=	circumferential artery
CM	=	inner circular muscle
CV	=	circumferential vein
c	=	capillary
cv	=	collecting venule
D	=	duodenum
d	=	diaphragm
E	=	esophagus
EA	=	esophageal artery
EV	=	esophageal vein
GA	=	gastric artery
GV	=	gastric vein
I	=	intestine
ITA	=	inferior thyroid artery
IVC	=	inferior vana cava
JV	=	jugular vein
K	=	kidney
L	=	lumen
LA	=	longitudinal artery
Li	=	liver
Lm	=	outer longitudinal muscle
Lu	=	lung
LV	=	laryngeal vein
M	=	mucosa
MC	=	muscular coat
MF	=	mucosal fold

MM	=	muscularis mucosa
MP	=	Meissner's or submucosal plexus
PA	=	perforating artery
PS	=	portal system
PV	=	perforating vein
S	=	stomach
SM	=	submucosa
SEP	=	subepithelial plexus
St	=	striated muscle
SM	=	submucosa
SMA	=	submucosal artery
SMP	=	submucosal plexus
SMV	=	submucosal vein
STA	=	superior thyroid artery
Sm	=	smooth muscle
SA	=	splenic artery
T	=	thyroid gland
V	=	vein
v	=	venule
vv	=	vasa vasorum

CHAPTER I

INTRODUCTION

The esophagus is a tubular organ belonging to upper digestive tract. It conveys the food bolus from the laryngopharynx to the stomach by the aid of well-organized muscular wall. The lumen of the esophagus is lined with stratified squamous epithelium which is keratinised in some animals with coarse diets as of rodents (Wheater, 1989). Histologically, the esophagus is divided into upper, middle and lower parts. Various aspects concerning the esophagus have been studied for many centuries. The blood vessels supplying the gullet is first described by Vesalius (1543) to be from the esophageal branches of the left gastric vessels lying close to vagus nerve. By dissecting the cadavers, Bartholin (1673), Cauldwell et al. (1948) and Swigart (1950) could show that the posterior intercostal (intercostales posteriores), bronchial (rami bronchioles) and the esophageal arteries arising directly from aorta contribute the blood to this organ.

With india-ink injection into the vessels of human esophagus, Demel (1924) has demonstrated that the different segments of human esophagus are with different vascular patterns. His observations suggest that the vessels are most abundant in the esophageal segment behind the tracheal bifurcation. At the gastroesophageal junction, the left margin is supplied by the inferior phrenic artery and the right one receives blood supply from the left gastroepiploic artery. Additional information is provided by Kegaries et al. (1934) that esophageal submucosa is very rich with veins and submucosal arterial plexus.

With the injection of india-ink in gelatin into the vessels, Madzharova (1978), point out that the esophagus is with deep and superficial vascular plexuses locating in the lamina propria and under the epithelium, respectively.

In order to visualize the vascular organization in various organs of circulation pathways Batson (1955) has injected low viscosity methyl methacrylate plastic mixture into the vascular system of newborn and adult cadavers. The plastic mixture could fill up all parameters of blood vessels. The vascular corrosion casts were then obtained by digestion of tissues in KOH solution. Murakami (1971) is the first investigator who introduces the scanning electron microscope (SEM) to study the vascular corrosion casts. This vascular corrosion cast/SEM technique had been widely used to study the detail vascular supply of various organs in different animals (Lametschwandtner et al., 1990; Ackermann et al., 1991; Aharinejad et al., 1991, 1992; Gaudio et al., 1993; Bamroongwong et al., 1992).

The technique has also been employed for the visualize vascular pattern in human esophagus (Spence et al., 1983 (a); Spence et al., 1983 (b); Kitano et al., 1986; Hashizume et al., 1988) and found that the intraepithelial channels in the lower esophagus are dilated in esophageal varices. With the same technique, Aharinejad et al. (1989, 1991, 1992) describe that the blood supply of esophagus in quinea pig, rat, rabbit and human is organized into subepithelial, submucosal and advential plexuses.

As the vascular corrosion cast technique had been used to study of the vascular organization of various organs in the common tree shrew, the animal

regarded as lower primate (Napier, 1972; Fugita, 1973; Gannon, 1976) including kidney (Bulkusol et al., 1990), pituitary gland (Sadwan et al., 1991), spleen (Bamroongwong et al., 1991), thyroid gland (Rattanachaikunsopon et al., 1991), pancreas (Bamroongwong et al., 1991, 1992), intestine (Wachmanus et al., 1992), uterus (Sangshu, 1992), dorsal root ganglion (Mankhetwit et at., 1993), spinal cord (Lanlua et al., 1993), stomach (Mingsakul et al., 1993), superior cervical ganglion (Chunhabundit et al., 1993), but not the esophagus. It is of interest that the vascular pattern including general features of the tree shrew esophagus be investigated.

CHAPTER II

MATERIALS AND METHODS

Fifteen adults common tree shrews *(Tupaia glis)* of both sexes, weighing between 120 and 180 gm, were used. They were purchased from Chatuchuk weekend Market, Bangkok, Thailand, after being captured from the forest in Singburi province. The animals were divided into three groups. The first group, three animals, was prepared for histological study of the esophagus under light microscope. The second group, nine animals, was injected with Batson's # 17 plastic mixture for the study of esophageal vascular architecture by stereomicroscopic and scanning electron microscopic observations. The last group, three animals, was prepared for the study of the esophagus with conventional SEM technique.

Animals Preparation

Each animal was anesthetized with diethyl ether and laid down on the metal mesh placing in the stainless steel tray. The subcostal incision was performed to open the thoracic cavity and the chest flap was retracted to expose the heart. The 0.05 ml of heparin (Leo; 5,000 iu/ml) was immediately injected into the left ventricle and allowed to circulate for 1 to 2 min. A blunt needle (18 gauge) was cannulated into the ascending aorta through the left ventricle and securely clamped. Thereafter, the right atrium was cut open to serve as efferent port of the blood and injected fluid. The animal was then perfused with approximately 150 to 200 ml of 0.9% NaCl solution through the cannula to wash the blood out from the blood vessels since the remaining

of blood cells could obstruct the blood vessels during the injection of the plastic mixture.

Conventional Light Microscopic Study (Group 1)

Following the 0.9% NaCl perfusion, the 100 ml of Bouin's solution was perfused into the left ventricle to fix the tissues. The esophagus was then removed, fixed in the same fixative overnight, rinsed several times in 50% alcohol until the solution was free from yellow color. The specimen was then dehydrated in a graded series of ethanol, cleared in xylene, embedded in paraffin, sectioned at 4 to 5 μm thick in coronal and horizontal planes, and stained with hematoxylin and eosin as described in the Appendix IV before being examined and photographed under the light microscope.

Modified Batson's # 17 Plastic Mixture Injection (Group 2)

The modified Batson's # 17 plastic mixture was prepared according to the method described by Chunhabundit and Somana (1988). When the circulatory system of the animal was clear from the blood, approximately 23 ml of the freshly prepared plastic mixture was injected into the aorta through the cannula at the rate of 8 ml/min. Most vascular bed, should be completely filled with the plastic mixture when it flowed out from the right atrium. The animal was left for 15 to 30 min at room temperature to let the casting medium set partially before submerging into the warm water (80 °C) for 2 hr to accelerate the complete polymerization of the plastic. The large arterial supply and venous drainage of the plastic injected esophagi were

observed under stereoscope either in situ or the removed specimen. The esophagus was separated into 3 segments; cervical, thoracic, and abdominal parts, placed in 40% KOH solution at room temperature for 24 hr to corrode the tissue. The esophagus microvascular casts were rinsed in slow running tap water and gently washed with distilled water for further removal of the remaining tissues. The casts were left air-dried in the dust-free container at room temperature, mounted onto the metal stub with double faced sticky tape and conductive silver paint, coated with gold/palladium at 60 nm thick using Hummer-VII sputter coater. The vascular casts were examined under a Hitachi S-2500 scanning electron microscope at an accelerating voltage of 30 kV. The SEM micrograph were taken with the Kodak Verichrome Pan-120 film.

Conventional Scanning Electron Microscopic Study (Group 3)

After rinsing the blood with 0.9% NaCl solution, 150-200 ml of 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (PBS), pH 7.4, was perfused manually into the circulation through the same cannula. The esophagus was immediately removed, divided into 3 segments as being done in those of Group 2 and immerged in the same fixative for 2 to 3 hr at 4 °C. The specimens were flushed with 0.1% KOH solution several times to remove the remaining mucus on the esophageal luminal surface, rinsed in PBS, postfixed with 1% osmium tetroxide (OsO_4) for 2 hr, dehydrated in graded series of ethanol, and critical point dried in a Hitachi HCP-2 critical point dryer. The dried specimens were cracked or etched, mounted onto metal stubs, coated with gold/palladium in Hummer-VII sputtering system (ANATECH, Alexandria, VA.). Finally, the specimens were viewed and

photographed under a Hitachi S-2500 scanning electron microscope, developed and printed as described in the Appendix V.

CHAPTER III

RESULTS

General Morphology of Esophagus : Stereomicroscopic Observation

The esophagus in common tree shrew begins at the lower border of the cricoid cartilage in the neck. It lies along and anterior the vertebral column, passes through the diaphragm into the abdomen and ends at the cardiac orifice of the stomach opposite the twelfth thoracic vertebra. The cervical esophagus situates behind the trachea and it shifts slightly to the left at the 4^{th} to 5^{th} thoracic vertebral levels (Fig. 2). The diameter of this organ is 3 to 5 mm and is approximately 5 to 6 cm long (Fig. 3).

Blood Supply of the Esophagus : Stereomicroscopic Observation

As shown in Fig. 4, the cervical part of the tree shrew esophagus is supplied by branches of the superior and inferior thyroid arteries. Its thoracic part is nourished by the esophageal branches of the bronchial artery and direct branches from the thoracic aorta at the 4^{th} to 8^{th} thoracic levels. The left and right bronchial arteries give off 1 to 2 branches to supply the lateral side of the esophagus behind the trachea. While 1 to 2 branches from the thoracic aorta supply its thoracic part below the arch of aorta (Fig. 5). At the 6^{th} to 8^{th} thoracic levels, the aorta usually gives off the last esophageal branch running caudally along the esophagus on anterolateral side. The terminal part of the esophagus is supplied by gastric, short gastric and splenic arteries (Fig. 6). They approach this organ on the dorsolateral aspect before giving off branches to both left and right sides surrounding the organ in the adventitia and then in

the submucosa. These arteries anastomose with the last esophageal branch of the aorta approximately 5 to 10 mm above gastroesophageal junction.

The major esophageal veins accompany the arteries. These veins from the cervical part drain the blood into laryngeal vein which joins the external jugular vein. Those from the thoracic part join the azygos vein and from the terminal part empty the blood into gastric veins which become tributaries of the portal veins (Figs. 4, 5, 6).

Histology of the Esophagus: Light Microscopic Observation

As shown in Fig. 7, the esophagus of the common tree shrew consists of three layers, namely, mucosa, muscular coat and adventitia. It is obvious that the esophagus could be divided into three parts as in man basing on the different types of muscular coat. They are upper or rostral or cervical part, middle or thoracic part and lower or distal part. It is shown that, the muscular coat of the upper part contains only striated muscle (Fig. 8). The main artery are observed in the adventitia. It becomes the perforating artery by penetrating the muscular coat into submucosa (Fig. 9). The muscular coat of the middle esophagus consists of both smooth and striated muscles (Figs. 10, 11). In this part, many blood vessels are observed between circular and longitudinal muscle forming the plexus to supply muscle fibers and myenteric (Auerbach's) plexuses. The morphology of the terminal part of the esophagus is similar to that in other parts (Fig. 12). The Auerbach's plexus situated between inner circular and outer longitudinal muscles is more frequently found in this part than in other parts (Fig. 13). The submucosal plexuses are much fewer and smaller than myenteric plexuses (Fig. 14). Those capillaries are found

surrounding these plexuses than in the connective tissue. It should be noted here that, only rudiments of the esophageal gland are found in the submucosa near cardiac region of the stomach (Fig.15). In addition, the area of less blood supply is evident at approximately 5 to 10 mm above the gastroesophageal junction (Fig. 16).

Luminal Surface of Esophagus : Conventional SEM Observation

With the conventional SEM, the luminal surface is shown to cover with the stratified squamous epithelium (Fig. 17). Among the epithelial cells, the intercellular ridges at the cell margins can be easily identified (Fig. 18). Furthermore, the epithelial cell surface is not smooth as it appears with many small ridges (Fig. 18).

Vascular Corrosion Cast of Esophagus : SEM Observation

With vascular corrosion cast technique in conjunction with SEM, it is confirmed that the main blood supply of the cervical part of the esophagus is from the superior and inferior thyroid arteries (Figs 19, 20, 21). These blood vessels send off delicate vascular network supplying the adventitia as well as branching off capillaries running in parallel with muscular fibers to supply the lower laryngeal and upper esophageal muscle (Fig. 22).

The upper or rostral thoracic esophagus receives blood supply from the esophageal branches of bronchial artery while the lower or caudal thoracic esophagus below the bifurcation of the trachea is supplied by esophageal branches of aorta from 4^{th} to 8^{th} thoracic levels. These arteries and

accompanying veins are in the adventitia (Fig. 23) and give off adventitial capillary plexus (Fig. 24) to supply it.

The terminal or distal esophagus receives the arterial supply from the esophageal artery of left gastric, splenic and short gastric arteries. The esophageal vein with vasa vasorum and runs along the anteromedial side of the organ (Fig. 25).

After these main arteries give off branches to form adventitial plexus, they run along the organ before penetrating the muscular coat to become submucosal arteries forming the submucosal plexus (Figs. 26, 27). The submucosal plexus gives off arterioles and capillary forming submucosal or subepithelial plexus supplying lamina propria and epithelium (Fig. 28). The venous blood from the mucosa is collected into venules, submucosal vein, perforating vein and main vein, respectively (Figs. 28, 29, 30). It is noted that the perforating vein in the terminal part of the esophagus has valves (Fig. 30). When viewing from the luminal side, the subepithelial plexuses in different portions of the esophagus exhibit different capillary patterns. There are ridge-like and honey comb-like subepithelial network in the distal esophagus while the cervical and thoracic parts of the esophagus are with only honey-comb type of vascular arrangement (Figs 31, 32, 33, 34, 35, 36, 37). Furthermore, it is noted that the diameters of arterioles and venules in the cervical part are the largest (11-15 μm and 47-67 μm), shorter in the thoracic part (11-19 μm and 22-52 μm) and the shortest in the terminal part (5-11 μm and 8-19 μm).

Figure 1. Photograph of the adult common tree shrew *(Tupaia glis)*.

Figure 2. Photograph of the tree shrew esophagus, dorsal view, in relation with other visceral organs. d = diaphragm; E = esophagus; I = intestine; K = kidney; Li = liver; Lu = lung; S = stomach. X 3.

Figure 3. Photograph of tree shrew upper gastrointestinal tract. Note esophagus is about 5-6 cm long. D = duodenum; E = esophagus; S = stomach. X 1.3.

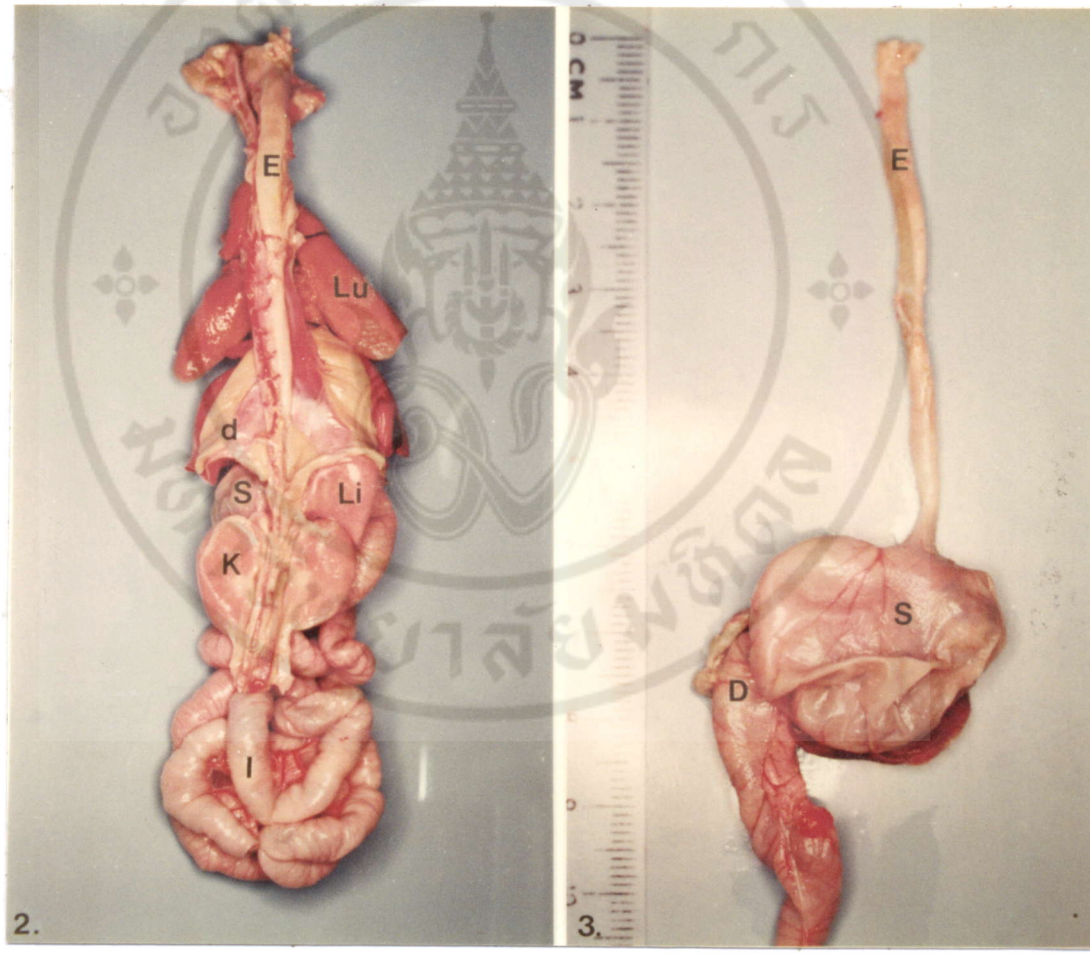
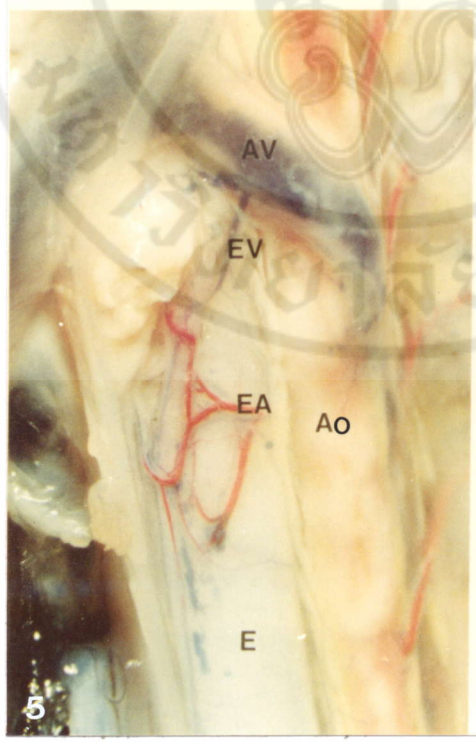


Figure 4. Photograph of the cervical esophagus and related structures, dorsal view. E = esophagus; ITA = inferior thyroid artery; JV= jugular vein; STA = superior thyroid artery; T = thyroid gland. X 7.5.

Figure 5. Photograph of the thoracic esophagus, ventrolateral view, showing the aorta sending off branch to supply the thoracic part and the esophageal vein draining into azygos vein (AV). Ao = aorta; E = esophagus; EA = esophageal artery; EV = esophageal vein. X 7.5.

Figure 6. Photograph of the terminal esophagus, ventral view, showing its arterial blood supply from the gastric artery (GA). E = esophagus; GV = gastric vein; Li = liver. X 7.5.



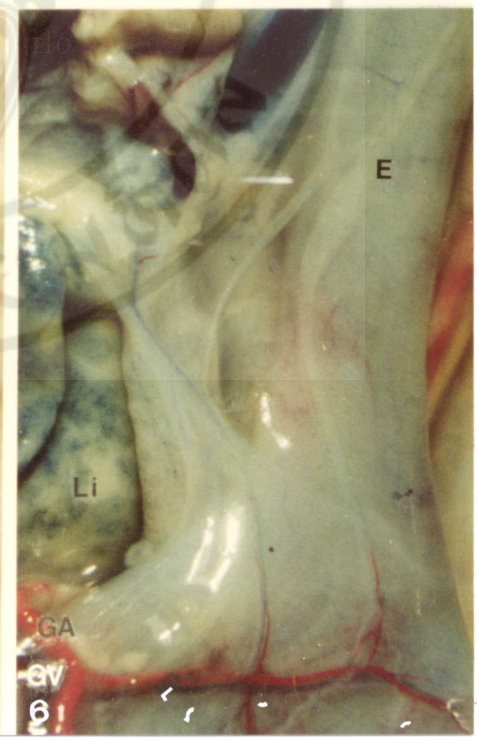
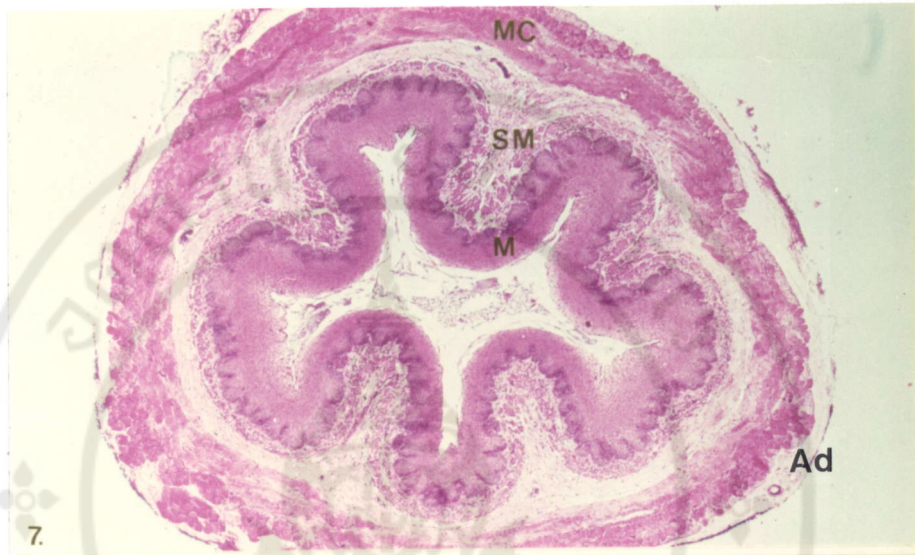


Figure 7. Photomicrograph of the cervical esophagus, in cross section, showing mucosa (M), submucosa (SM), muscular coat (MC), adventitial layers (Ad). Note the muscular coat consists of the striated muscle. Hematoxylin and eosin. X 40.

Figure 8. Photograph of the cervical esophagus showing main artery (MA) penetrating the muscular coat into the submucosa (SM). M = mucosa. Asterisk (*) = perforating vessel. Hematoxylin and eosin. X 200.

Figure 9. Photomicrograph, high magnification, of cervical esophagus showing the blood vessels in the submucosal plexus (*) sending branches to supply the submucosa (SM) and mucosa (M) being the subepithelial plexus beneath the basement membrane of the stratified squamous epithelium (arrowhead). Hematoxylin and eosin. X 400.



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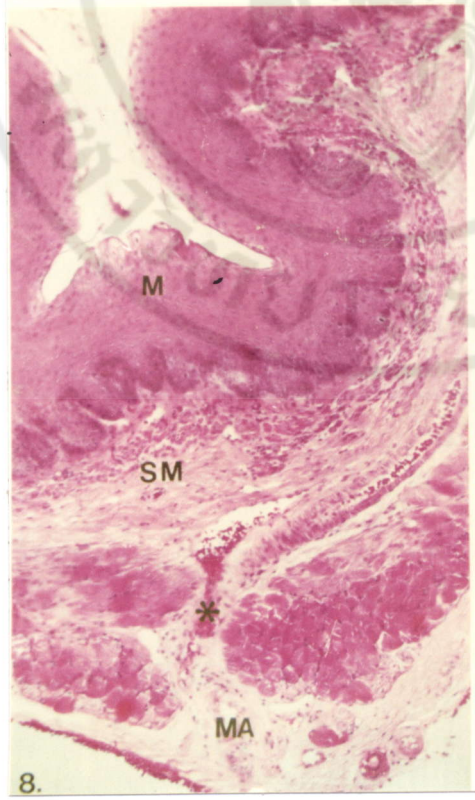

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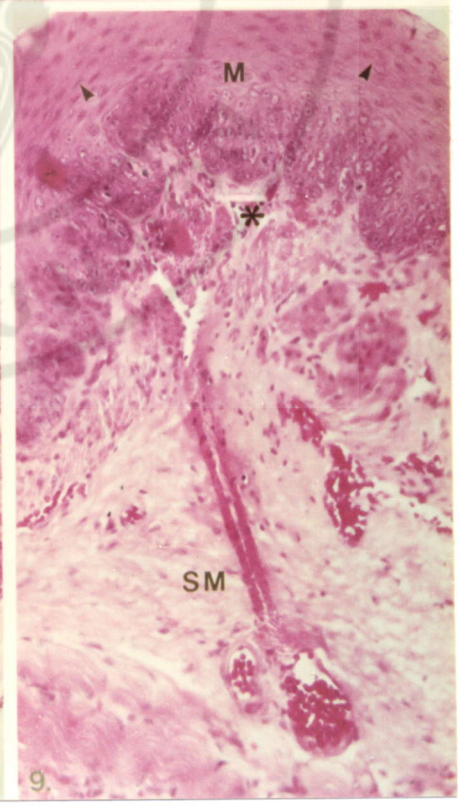

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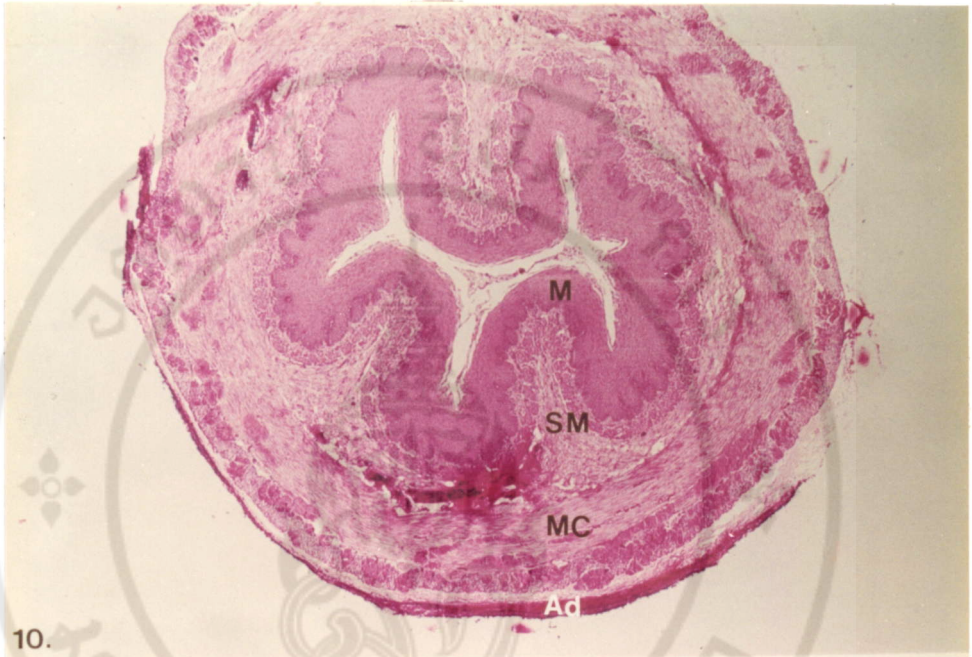
Figure 10. Photomicrograph of the thoracic esophagus in cross section showing its general appearance. Ad = adventitia; M = mucosa; MC = muscular coat; SM = submucosa. Hematoxylin and eosin. X 40.

Figure 11. Photomicrograph of the thoracic at higher magnification in cross section showing the muscular coat consists of both striated muscle (St) and smooth muscle (Sm). Hematoxylin and eosin. X 400.



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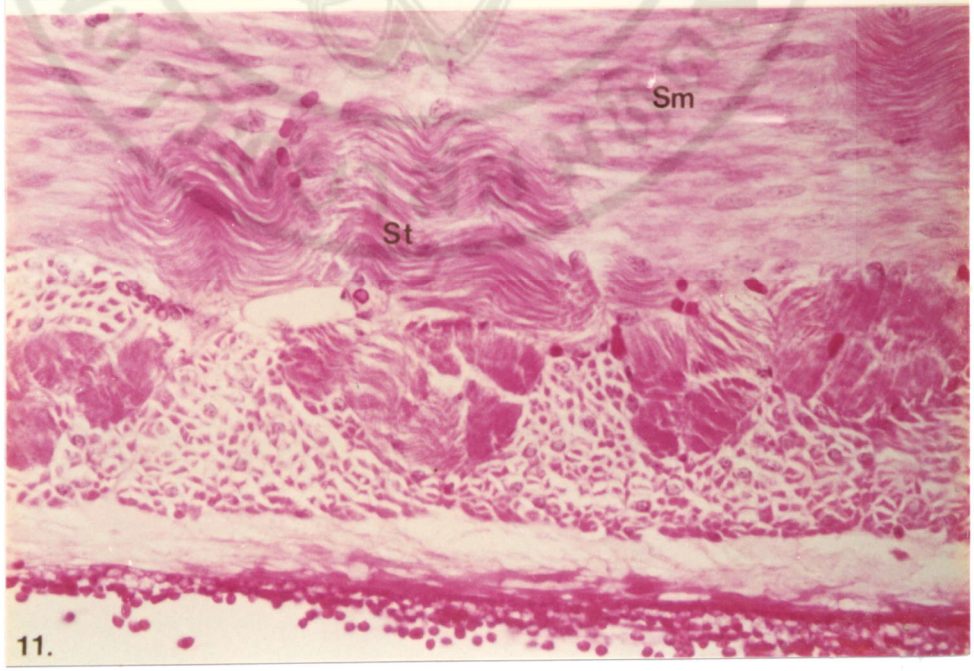

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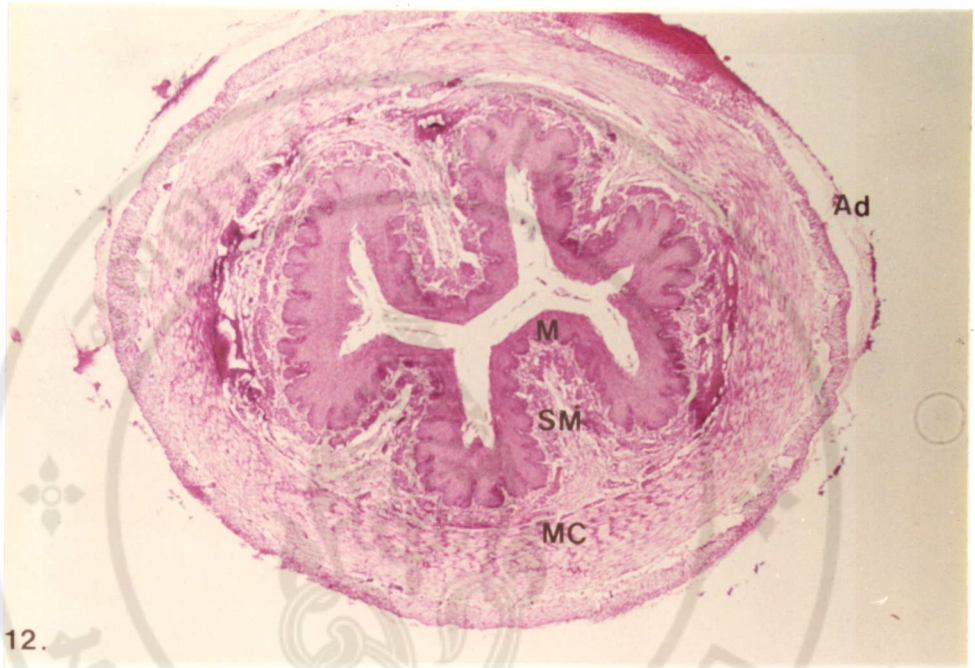
Figure 12. Photomicrograph of the terminal esophagus in cross section showing the muscular coat (MC) consisting of only the smooth muscle. Ad = adventitia; M = mucosa; SM = submucosa. Hematoxylin and eosin. X 40.

Figure 13. Photomicrograph of the terminal esophagus at high magnification showing Auerbach's or myenteric plexus (*) situating between the inner circular muscle (CM) and outer longitudinal muscle (LM). Hematoxylin and eosin. X 1,000.



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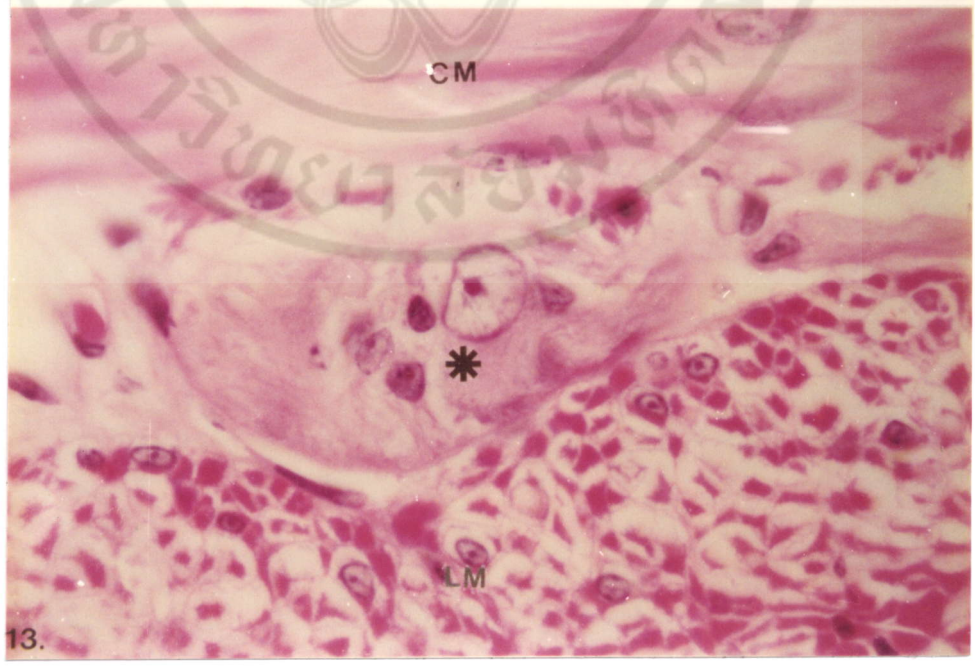

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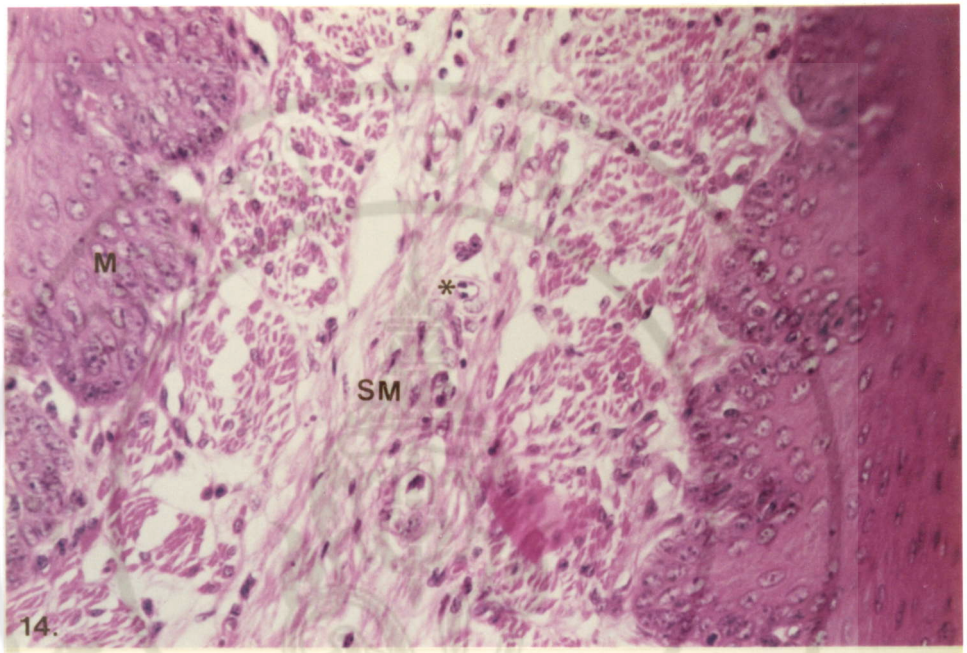
Figure 14. Photomicrograph of the terminal esophagus showing the submucosal plexus (*) in the submucosa. M = mucosa. Hematoxylin and eosin. X 1,000.

Figure 15. Photomicrograph of the terminal esophagus showing the rudiment of the esophageal gland (*) in submucosa (SM). MC = muscular coat. Hematoxylin and eosin. X 400.



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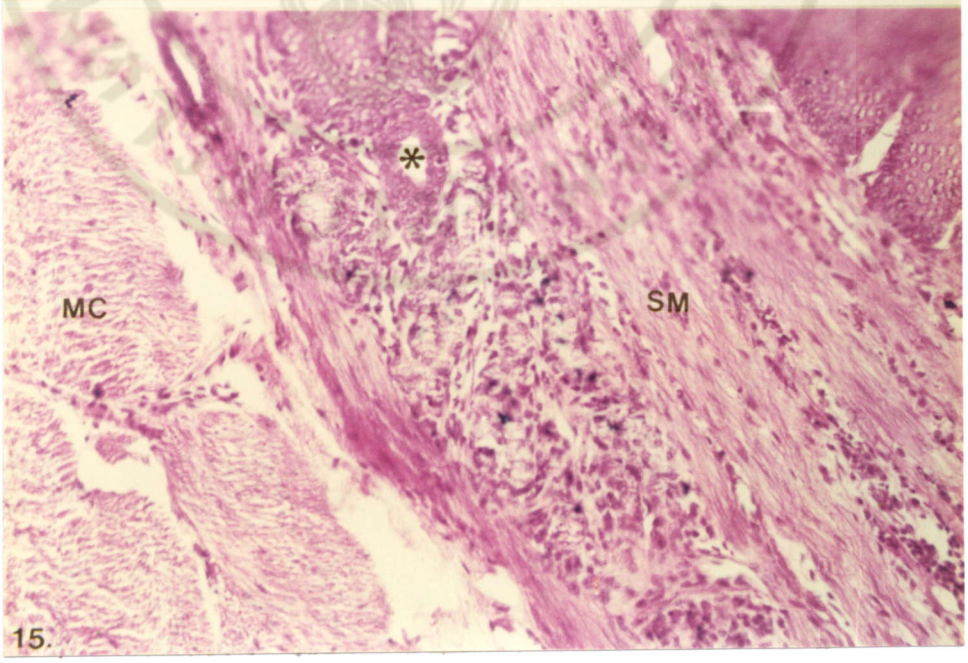

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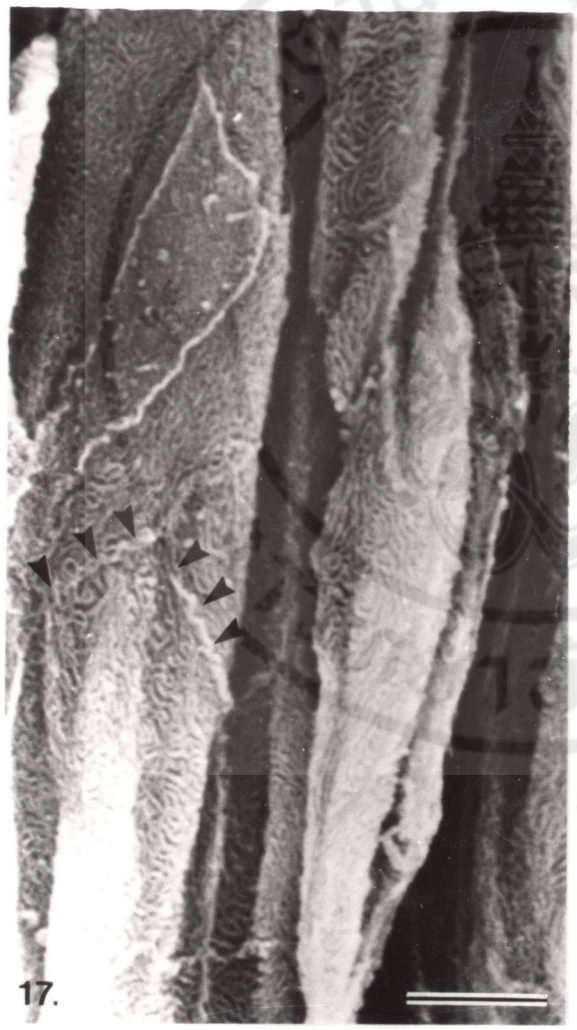
Figure 16. Stereomicrograph of the terminal esophageal vascular cast, ventral view during digested. Note area of less blood supply at 5 - 10 mm proximal to gastroesophageal junction. E = esophagus; S = stomach. X 7.5.

16.

Figure 17. SEM micrograph, luminal view, showing the stratified squamous epithelial cells lying on the luminal surface. Note the intercellular ridge (arrowhead) among the cells. Bar = 9 μm.

Figure 18. SEM micrograph at higher magnification, showing the luminal surface of the epithelium lining cells and intercellular ridge (arrowhead). Bar = 1 μm.



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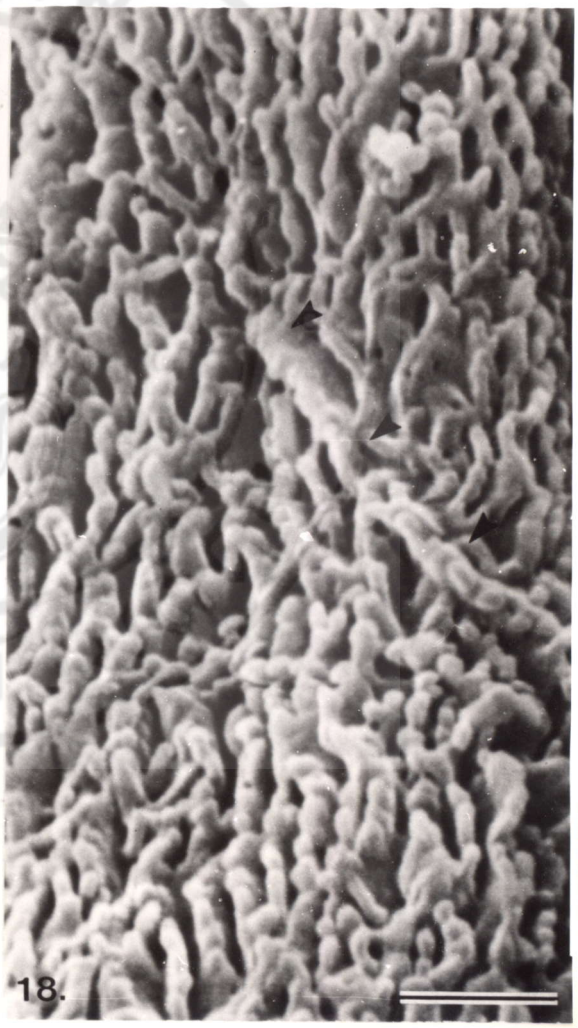


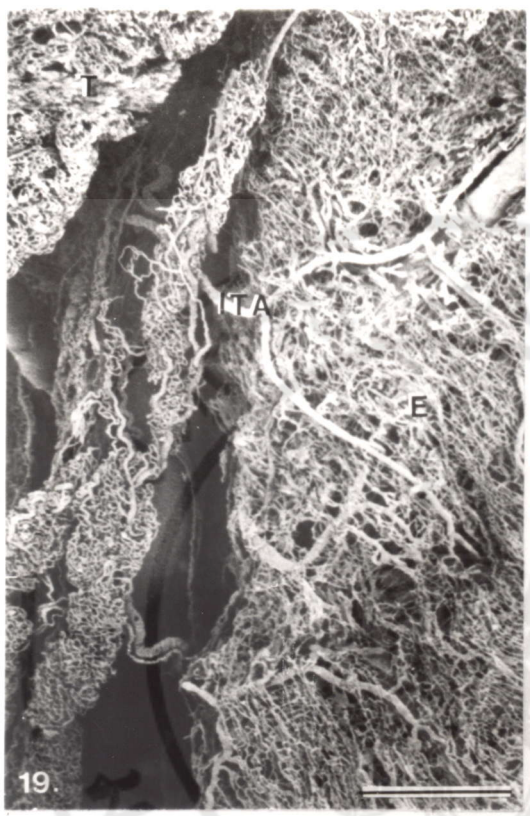
18.

Figure 19. SEM micrograph of the vascular cast, anterolateral view, showing the inferior thyroid artery (ITA) giving off branches supplying the cervical esophagus. E = esophagus; T = thyroid gland. Bar = 500 μm.

Figure 20. SEM micrograph of the cervical esophageal vascular cast showing the superior thyroid artery (STA) supplying upper posterolateral part of the thyroid gland (T). E = esophagus. Bar = 250 μm.

Figure 21. SEM micrograph of the cervical esophageal vascular cast, anterolateral view showing the laryngeal vein (LV) receives the venous blood from the anterior part of the cervical esophagus and thyroid gland (T). E = esophagus; STA = superior thyroid artery. Bar = 500 μm.

Figure 22. SEM micrograph of the cervical esophageal vascular cast showing small blood vessels running along the muscle fibers. A = artery; V = vein. Bar = 500 μm.



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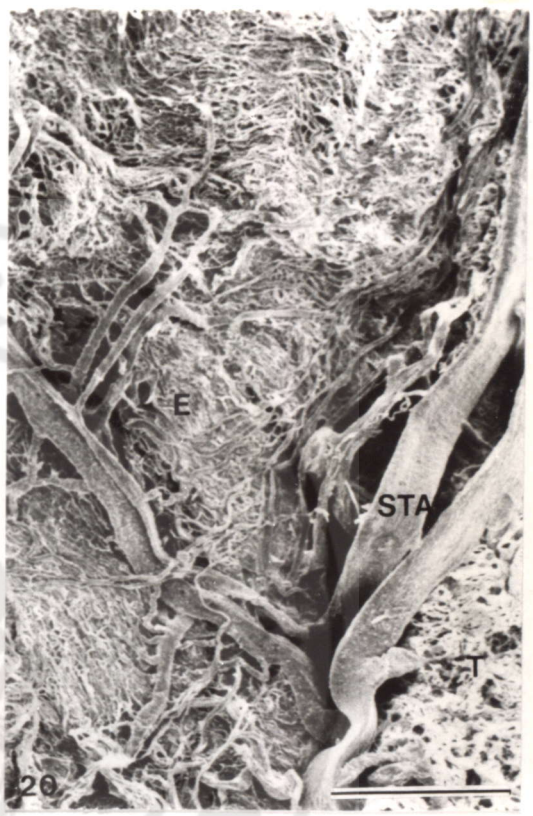

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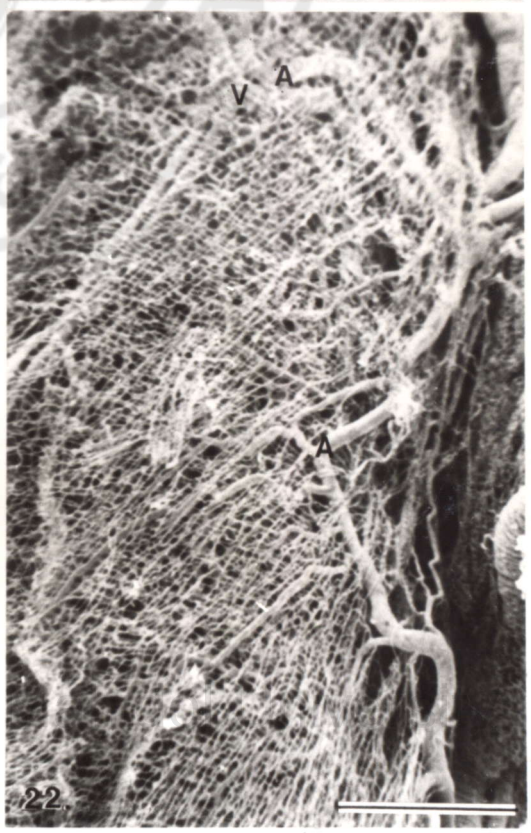

22.

Figure 23. SEM micrograph of the thoracic esophageal vascular cast showing the artery (A) and vein (V) running under adventitial plexus (AP). A = artery; V = vein. Bar = 150 μm.

Figure 24. SEM micrograph of thoracic esophageal vasculas cast showing the adventitial plexus. A = artery; c = capillary; V = vein. Bar = 50 μm.

Figure 25. SEM micrograph of the terminal esophageal vascular cast after removing the adventitial plexus, anterior aspect, showing the large vein with vasa vasorum (vv) and valve. EA = esophageal artery; EV = esophageal vein. Bar = 300 μm.

Figure 26. SEM micrograph of the terminal esophageal vascular cast after partial removing the adventitial plexus to show the submucosal blood vessels supplying the submucosa and mucosa. LA = longitudinal artery; PA = perforating artery; Asterisk (*) = submucosal vein. Bar = 450 μm.

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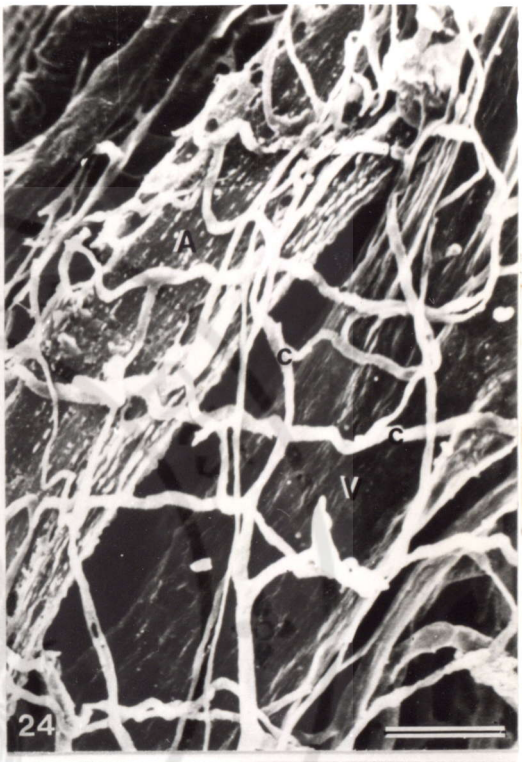

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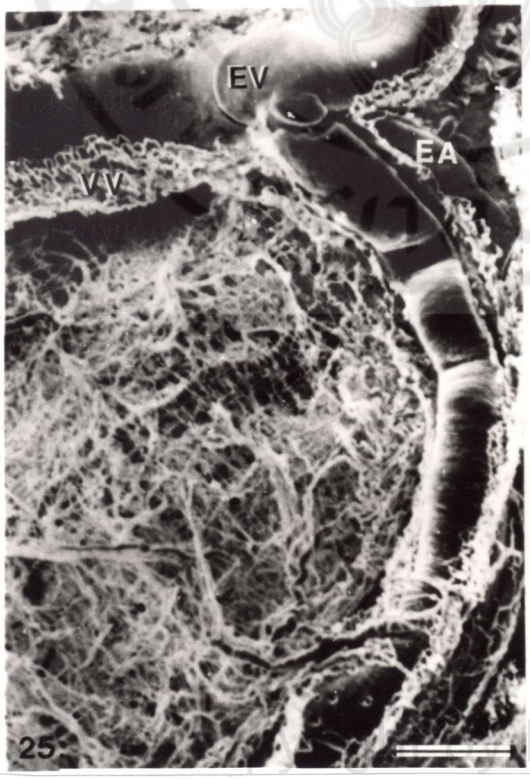

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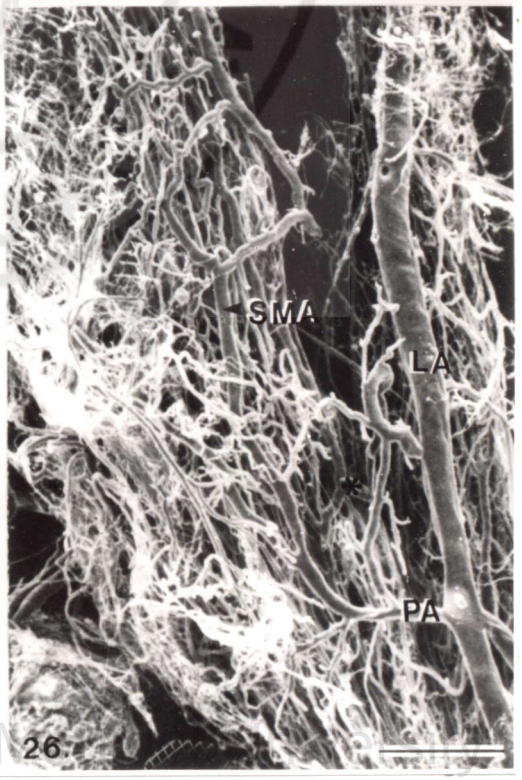

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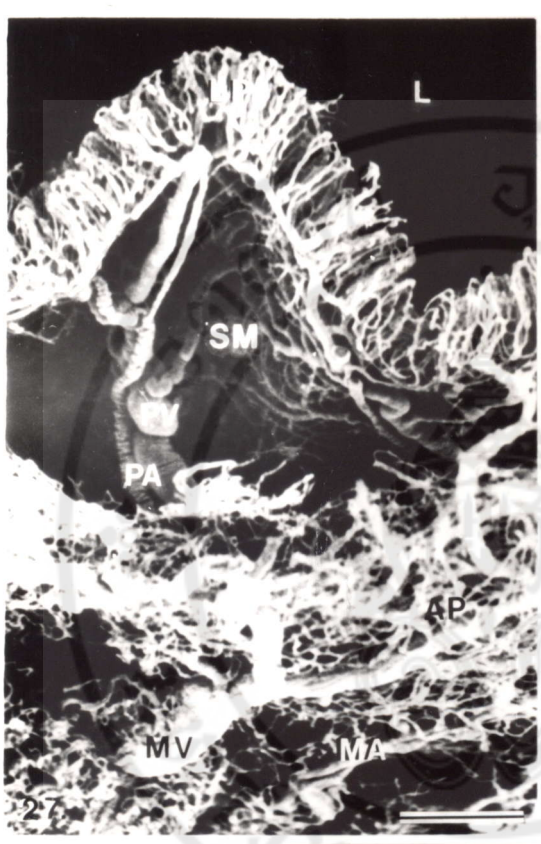
Figure 27. SEM micrograph of terminal esophageal vascular cast, cross section, showing the main artery (MA) giving off the perforating artery (PA) supplying muscular coat and submucosa (SM) before branching to form submucosal plexus and supplying the lamina propria (LP). The venous blood drains into the veins that run accompany with arteries. AP = adventitial plexus; L = lumen. Bar = 200 μm.

Figure 28. SEM micrograph of terminal esophageal vascular cast showing the arterial and venous blood flow (arrowhead), perforating artery (PA) giving off branches to supply lamina propria (LP) before branching into capillaries (c) supplying mucosa. Note the venous blood from the capillaries emptying into collecting venule (Cv), submucosal vein (SMV) and perforating vein (PV), respectively. L = lumen. Bar = 250 μm.

Figure 29. SEM micrograph at high magnification in luminal view, showing the capillaries (c) drain the venous blood into the collecting venule (cv) which locates in the center. Bar = 50 μm.

Figure 30. SEM micrograph of terminal esophageal vascular cast showing the venous valve (vv) of the perforating vein (PV) in submucosa (SM). Bar = 100 μm.



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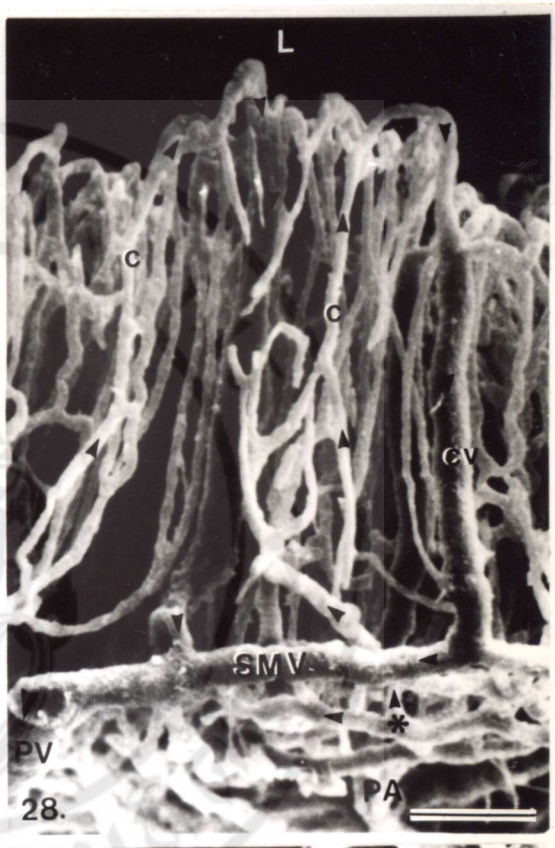

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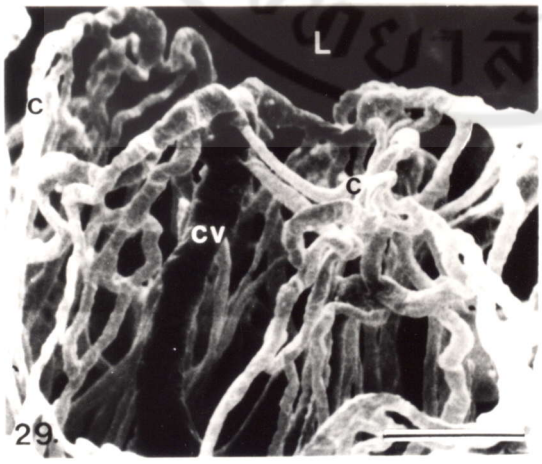

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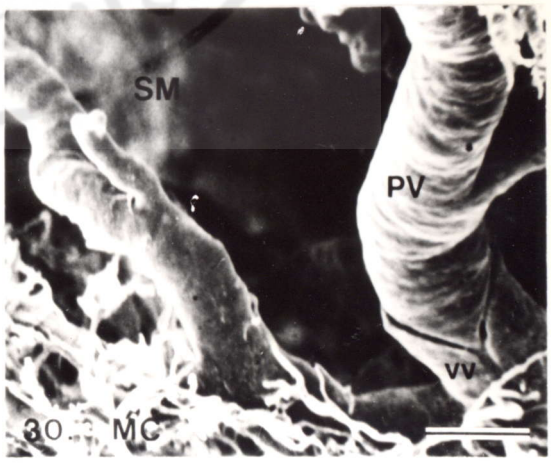

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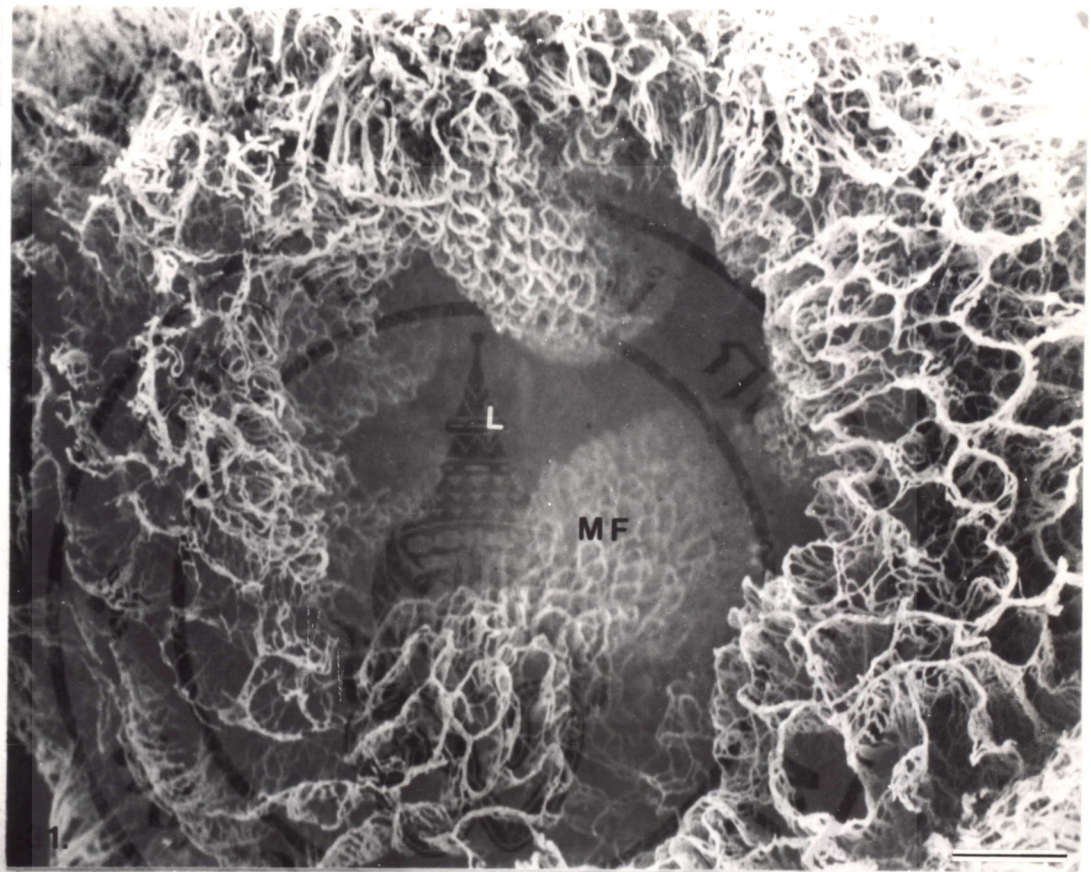
Figure 31. SEM micrograph of the terminal esophageal vascular cast showing the cast of mucosal fold (MF). Note the luminal surface consists of the ridge pattern. M = mucosa; L = lumen. Bar = 250 μm.

Figure 32. SEM micrograph of terminal esophageal vascular cast showing the capillaries forming ridge pattern. c = capillary; v = venule. Bar = 50 μm.

Figure 33. SEM micrograph, oblique view of luminal surface, showing the subepithelial plexus (SEP) being the honey-comb appearance. c = capillary; cv = collecting venule. Bar = 50 μm.



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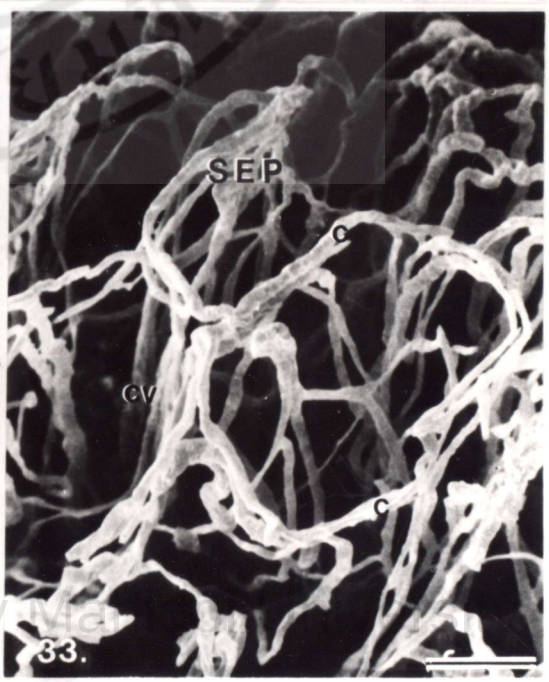

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Figure 34. SEM micrograph of cervical esophageal vascular cast in luminal view. Bar = 450 μm.

Figure 35. SEM micrograph at higher magnification of the cervical esophagus, showing diameter of submucosal artery (*) is approximately 11-15 μm and of the submucosal vein (SMV) is about 47-67 μm. c = capillary; v = venule. Bar = 50 μm.

Figure 36. SEM micrograph of thoracic esophageal vascular cast, luminal view. Bar = 200 μm.

Figure 37. SEM micrograph of the thoracic esophageal vascular cast, luminal view, at high magnification showing the diameter of submucosal artery (SMA) is about 11-19 μm and of the submucosal vein (SMV) is approximately 22-52 μm. Bar = 50 μm.

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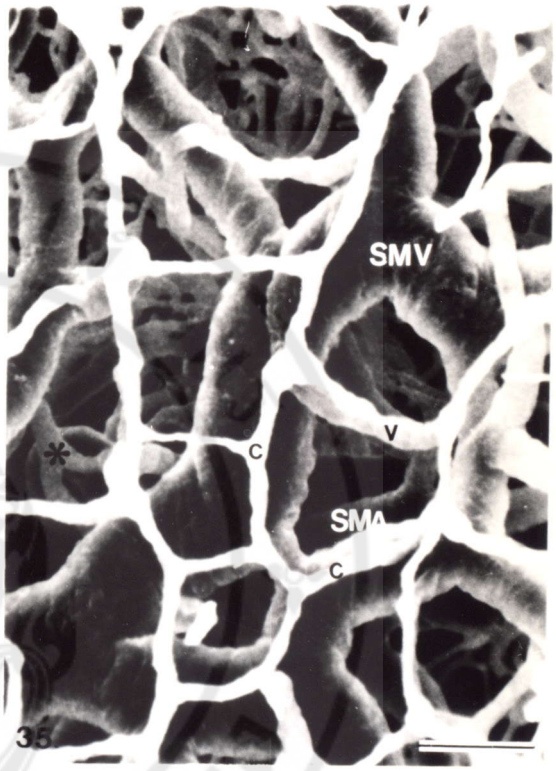

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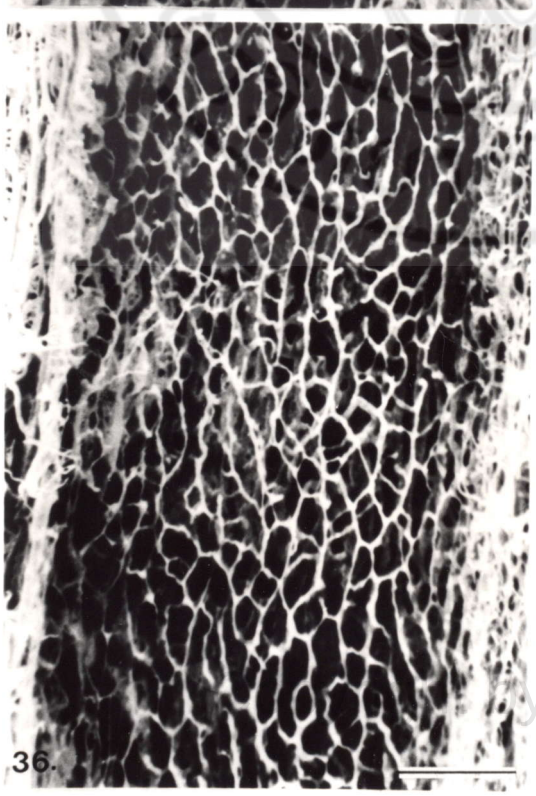

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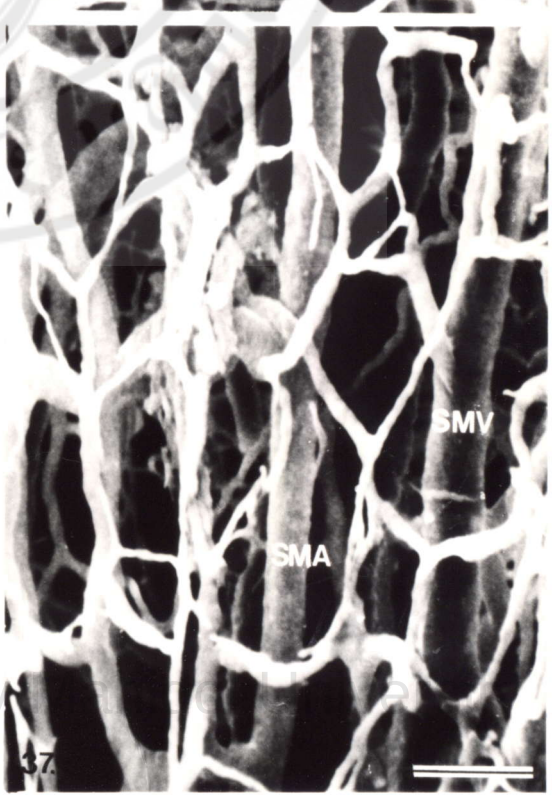
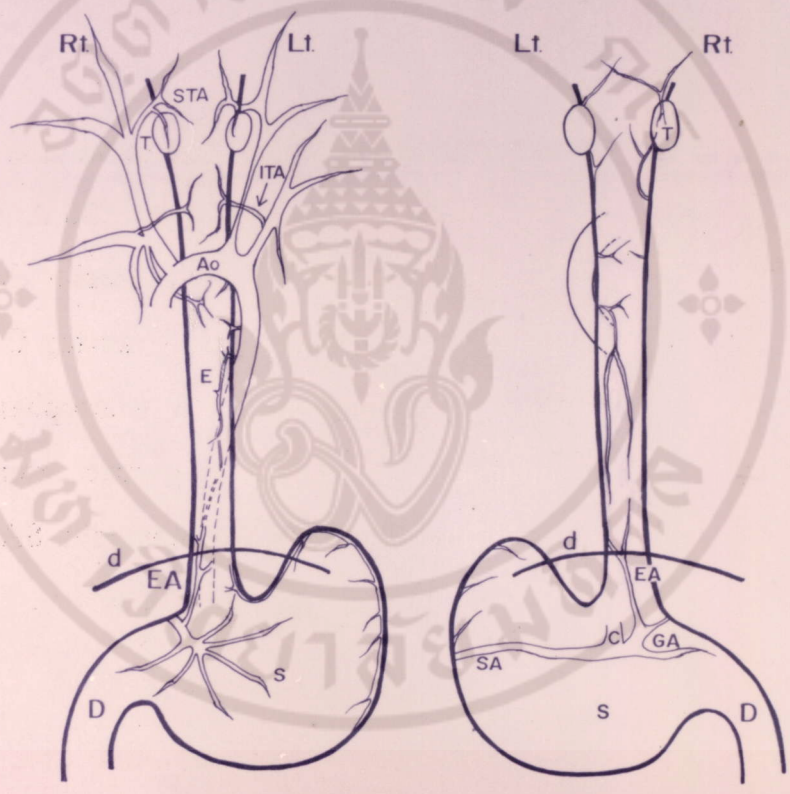


37.

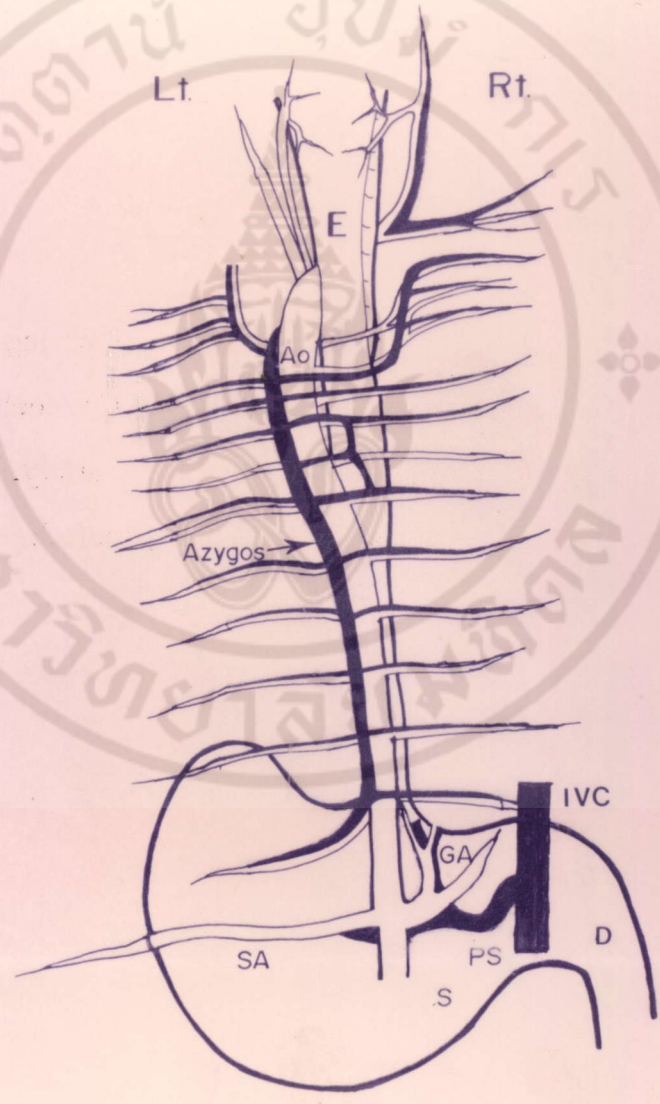
Figure 38(A, B). Diagram demonstrating the main arterial supply of the tree shrew esophagus. 38 A and 38 B showing the anterior and posterior esophageal aspects, respectively. Ao = aorta; C = coeliac trunk; D = duodenum; E = esophagus; EA = esophageal artery; GA = gastric artery; ITA = inferior thyroid artery; S = stomach; SA = splenic artery; STA = superior thyroid artery; T = thyroid gland.



38 (A). ANTERIOR ASPECT

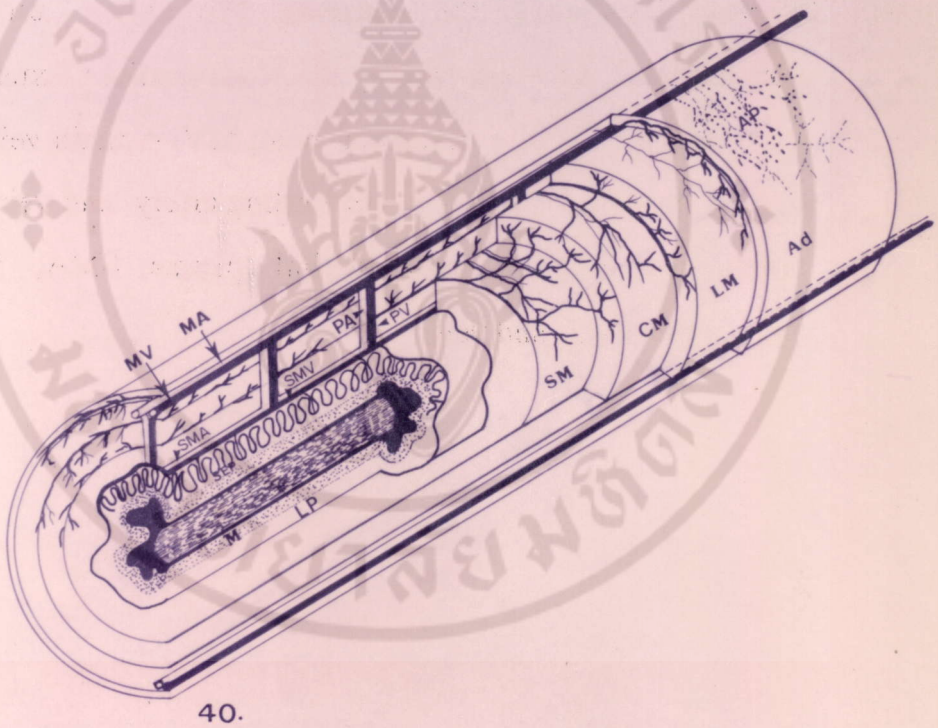
38 (B). POSTERIOR ASPECT

Figure 39. Diagram of the main blood vessels supplying the tree shrew esophagus, posterior view. A o= aorta; C = coeliac trunk; D = duodenum; E = esophagus; GA = gastric artery; IVC = inferior vana cava; PS = portal system; S = stomach.



39. POSTERIOR ASPECT

Figure 40. Diagram illustrating the common tree shrew esophageal vasculature. Ad = adventitia; AP = adventitial plexus; MA = main artery ; MC = muscular coat; MV = main vein; LP = lamina propria; PA, PV = perforating artery and vein; SM = submucosa; SEP = subepithelial plexus; SMA, SMV = submucosal artery and vein.



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CHAPTER IV
DISCUSSION

Since the tree shrew is considered as lower primate (Laven, 1965; Napier, 1972; Chiarell, 1972; John, 1988), it is expected that the general morphology and histology of its esophagus would be similar to those of higher primates. The speculation is quite true for it consists of adventitia, muscularis, submucosa, lamina propria and epithelium. Its muscular coat of the upper part comprises of only striated muscle while the middle part is with both striated and smooth muscles. That of the lower part consists of only smooth muscle. On the middle and lower parts of the esophagus, the muscularis is seen in two layers, namely, outer longitudinal and inner circular muscles. Between the two muscular layers, the myenteric plexuses of Auerbach are observed. This plexes is larger and easily found when compared with the submucosal plexus of Meissner. The tree shrew esophagus has very thick muscularis mucosa. These features are not different from those of man (Aharinejad et al., 1989). In addition, the mucosal epithelial of tree shrew esophagus is stratified squamous type forming longitudinal folds. When observing the cross section of the tree shrew esophagus, the longitudinal folds are arranged in Y shape which is quite characterized in the primate (Valen, 1965). The number of esophageal mucosal folds in tree shrew is lesser than in the dog (Miller, 1964; Roy, 1986) and rabbit (Aharinejad et al., 1991) but similar to that in man (Aharinejad et al., 1989, 1991).

One major difference between the human and tree shrew esophaguses is that the esophageal gland in man is very well developed while in the tree shrew the gland poorly developed and very hard to find. This gland in human is

seromucous type (Wheater et al., 1989) and could keep the esophagus moist. The moisture in the tree shrew esophagus may be compensated with the secretion from the salivary gland as its submandibular gland is quite large.

The application of vascular corrosion cast technique in conjunction with SEM for the study of esophageal microvascularization three dimensionally in this study is quite appropriate for all parameters of blood vessels could be visualized in detail under SEM (Murakami, 1971). It would take much more time to study the blood vessels distributing in any organ by making serial sections and looking through the light microscope or electron microscope. The vascular cast technique/SEM is also useful to employ in the study of the vascular pattern in human esophageal varices (Spence et al., 1883a; Spence et al., 1983b; Vianna et al., 1987).

When using the vascular corrosion cast/SEM for the study of the tree shrew esophagus, it is obvious that the general pattern of the esophageal blood supply is similar to that in the small intestine (Wachmanus, 1992) and large intestine (Waraklang, 1994) of the same animal. That is when the main blood vessels come into the organ, they break into small branches supplying the adventitia or serosa before having the large branches penetrating the muscular coat to become the submucosal plexus. While passing the muscularis, they gives off branches to supply the muscle. The submucosal plexus give rise to smaller arteries and arterioles which branch into capillaries in the lamina propria supplying the mucosal epithelium. Among the blood supply to the mucosa, submucosa, muscularis and adventitia, it is noted that to the mucosa is the richest indicating that the functional activity including the turn over rate of the epithelial cells are the highest. Moreover, the density of blood supply to the

mucosa in all parts of tree shrew esophagus is quite similar. While the esophageal blood supply of the tree shrew is richest in the mucosa, that of the rhesus monkey and baboon is richest in the submucosa (Napier, 1972; Chiarell, 1972; John, 1988). This could be due to the different types of food usually eaten by these animals for the tree shrew eats on fruits and insects (Valen, 1965) while the rhesus monkey and baboon like the eat animal meat (Napier, 1972; Chiarell, 1972; John, 1988).

When focusing on the blood supply of the muscular coat in different parts of the tree shrew esophagus, it is surprising that the density of blood vessels is the largest in the upper part where striated muscles are located. In addition, the capillaries run mainly in parallel with the muscular fibers. It is no doubt that the muscle in the upper part of the esophagus is active and under voluntary control in swallowing. However, the muscle cells in the esophagogastric sphinctor has also been shown to require higher oxygen and with increased mitochondrial mass when compared with the body of the esophagus (Christensen and Roberts, 1983). This brings about high metabolic rate necessary for maintaining tonic contraction. Eventrough, the blood supply in the esophageal mucosa is the highest, it is still lesser than that in the mucosa of the stomach and intestine is even highest. This would be due to the functional difference of the columnar and stratified squamous epithelia (Hollwarth et al., 1986; Prokopiw et al., 1989; Zulstra et al., 1989, 1990).

The present study also reveals that there is an area with strikingly less blood supply at 5 to 10 mm above the gastroesophageal junction. This area in the tree shrew is the region for the anastomoses among branches of esopahgeal artery from thoracic aorta and gastric artery from coeliac trunk. This seems to

correspond with the area of 2 to 5 cm above the gastroesophageal junction in human where the esopageal varices is usually evident (Spence, 1987; Vianna, 1987; Noda et al., 1989). Nearby this area valves are also found in the main esophageal veins directing the blood to flow rostrally. This is quite unique in the tree shrew as such valves are not demonstrated in the esophageal veins of guinea pig (Aharinejad et al., 1989), rat (Aharinejad et al., 1989), rabbit (Aharinejad et al., 1991) and human (Aharinejad et al., 1991, 1992). As the tree shrew and most mammals are standing with the belly down, the blood flowing cranially or caudally along the esophagus could be equally convenience. Furthermore, the venous valves of the tree shrew esophageal veins seem to facilitate most of the esophageal venous blood to show cranially; the stagnation of the esophageal venous blood is unlikely. Thus, the esophageal varices in most mammals, espectially, in the tree shrew would more or less never occurs. Moreover, the use of the tree shrew as a model for experimental esophageal varices would not be for better than that of other mammals.

CHAPTER V
CONCLUSIONS

The histological study reveals that the proximal or cervical part of common tree shrew esophagus consists of striated muscle which the middle part consists of both striated and smooth muscles and terminal part consists of only smooth muscle. Only the rudiment of the esophageal gland in submucosa of the terminal part is observed. The submucosal (Meissner's) plexus is smaller less frequently found than the myenteric (Auerbach's) plexus.

The cervical part of the esophagus is supplied by superior thyroid and inferior thyroid arteries, the thoracic part is supplied by esophageal branch of bronchial artery and of thoracic aorta. The terminal part is supply by esophageal branch of gastric, short gastric and splenic arteries.

The main artery gives off branches running on left and right side of the organ before giving rises to smaller and larger arteries. The small arteries give rise to adventitial plexus supplying the adventitia and outer longitudinal muscle. The larger arteries penetrate the muscular coat into the submucosa being the perforating arteries which give off branches to supply muscular coat before becoming the submucosal plexus. This plexus sends off arterioles branching into capillaries supplying the mucosa. The venous blood is collected into small then large venules before joining the submucosal plexus which drains into the major veins. These major veins from the cervical part of the esophagus join the laryngeal vein (jugular system), the thoracic parts join azygos vein while those from the terminal part join the portal system.

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APPENDIX

APPENDIX I

The common tree shrew

Taxonomy

Kingdom	:	Animal
Phylum	:	Chrodata
Subphylum	:	Vertebrata
Class	:	Mammalia
Subclass	:	Eutheria
Order	:	Primate
Suborder	:	Prosimii
Family	:	Tupaiidae
Subfamily	:	Tupaiinae
Genus	:	Tupaia
Species	:	glis

Synonyms

Sorex glis Diard 1820

Tupaia ferruginea Raffes 1821

Cladobates belangeri Wegner 1841

Tupaia peguanus Lesson 1842

Sciurus dissimilis Ellis 1860

Tupaia chineusis Anderson 1879

Tupaia picta Thomas 1892

Tupaia phaeura Miller 1902

Tupaia chrysogaster Miller 1903

Tupaia carimatae Miller 1906

Tupaia esta Allen 1906

Tupaia discolor Lyon 1906

Tupaia concolor Bonhote 1907

Tupaia lacernata Thomas and Wroughton 1909

Tupaia obscura Kloss 1911

Tupaia natunae Lyon 1911

Tupaia belangeri Thomas 1914

Tupaia conedor Kloss 1916

Tupaia clarissa Thomas 191

Vernacular Names

Spitzhornchen

Common tree shrew

Tupaie ferrugineux

Painted tree shrew

Mill's tree shrew

Diagnosis

"Tupaia" is derived from the Malay "tupai", meaning squirrel-like animal. Tree shrew can be distinguished from squirrels in the field by their long, pointed muzzle and behavior less arboreal than their common name would suggest.

The common tree shrew, is semi-arboreal and feed extensively on the ground. The characteristics of the tree shrew are:

Size	:	head to body	:	160-230	cm
		tail	:	148-198	cm
		weight	:	85-185	gm
Fur	:	greybrown, finely speckled with black			
Cranium	:	primate-like, rounder, elongated muzzle and small brain case, laterally directed orbits but show a post orbital bar			
Eye	:	relatively big, completely encircled by bone			
Ear	:	thick, small and quite human in form			
Nose	:	elongated shrew-like nose terminating in naked moist snout			
Body	:	squirrel- like animals			
Hand and Feet	:	5 fully-formed digits on each hand and foot, all digits bear claws, no nails and not full opposible, well-marked pads on palm			
Tail	:	long and slender, well long hair on dorsal surface with undersurface lacking long hairs			
Mamme	:	two, four or six mamme			

Distribution

From Napal and Sikkim east to South China and throughout Southeast Asia to Indonesia but not in Philippines. There are 8 mainland Thai subspecies: *Tupaia glis ferrugonea, Tupaia glis wilkensoni, Tupaia glis clarissa, Tupaia glis belangeri, Tupaia glis chinensis, Tupaia glis laotum, Tupaia glis olivacea and Tupaia glis concolor.*

Ecology and Behavior

The common tree shrew actually spend much of their time on the ground, foraging on the forest floor and generally omnivorous, eating anything they come across, including ants, termites, beetles, fruit, spiders, seeds, bugs and even lizards and small rodents. There is no evidence that they shovel through the forest litter as shrews do.

Tree shrews are nervous, aggressive animals and males will not tolerate the presence of other males, though there seems to be little fighting between the sexes. They typically form pairs which are strongly territorail, and follow the same pathways within their territory. They are very fond of water, and often bathe in water-filled hollows of trees.

There is no fixed birth season in captivity, pregnancies have been observed to be associated with the period of low rainfall; June, July and August. Nests are built in holes in fallen trees, hollow bamboos or similar sites. The gestation period is approximately 41-50 days. The numbers of young are 1-4 (usually 2). Newborn is pink, hairless and has closed eyes.

Pigmentation appears on the fourth day; hair begins to grow on the fifth day. Teeth begin to appear about the eleventh day and the eyes open on the twenty fifth day. At 6 months, they are sexually mature. Longevity is 2-3 years, with maximum of 5.5 years in captivity.

The main similarities between tree shrews and primates

Skull	:	snout relatively short, enlarged, forward facing orbits, postorbital bar present, pattern of bones imedial orbital wall, enlarged braincase, advanced form of auditory
Dentition	:	tooth-comb present in front of lower jaw
Limbs	:	highly mobile, ridged skin on palms and soles
Brain and sense organ	:	olfactory apparatus reduced, visual apparatus enhanced, central avascular area of retina, neocortex expanded, calcarine sulcus present
Reproductive	:	pendulous penis, scrotal testes, discoidal placenta, small number of teats
Miscellaneous	:	caecum present

APPENDIX II

Batson's # 17 Plastic Mixture for Vascular Casting

Plastic Mixture Preperation

Batson's # 17 Monomer base solution *	12.5	ml
Batson's # 17 Catalyst *	3.5	ml
Batson's # 17 Promoter *	0.5	ml
QR liquid for quick repair **	6.5	ml

Prepared in an ice bath. The solutions are mixed throughly and used immediately.

* Batson's corrosion kit can be obtained from :

Polyscience, Inc.
Paul Valley Industrial Park
Warrington, Pa. 18976 USA.

* QR liquid for quick repair can be obtained from :

S.D. Dental suppliers
Bangkok, Thailand

APPENDIX III

Bouin's Solution

For histological study, the Bouin's solution was used to preserve the esophageal tissue.

This fixative contains :

Picric acid (saturated aqueous)	75	ml
Formaldehyde 40%	25	ml
Glacial acetic acid	5	ml

Picric acid is a bright yellow crystalline substance, usually suppied damped with water because of its explosive property if heated. It is slightly water soluble (about 1% at room temperature) but more soluble in alcohol (about 5%) and benzine (10%). Picric acid precipitates nucleoproteins and causes little shrinkage. In general, this fixative can rapidly penitrate to provide well preservation of the tissues. The yellow staining enables the tissues to be seen more easily in embedding step. But Bouin causes partial lysis of red blood cell, and may result in swelling of collagen fiber.

Formaldehyde is a gas which is water soluble extends to the maximum of 40 % formalin. This solution is nearly acid and becomes formic acid in stock solution. The magnesium and calcium carbonate are usually allowed to neutralize the solution. Formalin is diluted with tap water, normal saline or buffer-salt solution, commonly in the proportions of 10 parts formalin to 90 parts diluent. Therefore in practice, 10% formalin in normal saline means

10 parts formalin in 90 parts physiological saline or mean 4% formaldehyde in physiological saline. Formalin fixes protein by forming additive compound with little shrinkage. It provides the staining of acidic stuctures (nuclei) with basic dyes and diminishes effect on basic structures (cytoplasm) with acid dyes.

Glacial acetic acid, pure acetic acid is called glacial because it hardens at very low temperature, 17 °C. It is used in many fixatives to provide the action of other ingredients. It helps to reduce the swelling of collagen fibers and precipitates nucleoprotein.

APPENDIX IV

Hematoxylin and Eosin Stainning Method

Fixation

Any fixative could be used, but in this case Bouin's solution was employed to preserve the esophageal tissue by perfusion and immersion.

Harris's Hematoxylin solution

Hematoxylin	5	g
Ethyl alcohol, 95%	50	ml
Potussium or Ammonium alum	100	g
Distilled water	1,000	ml
Mercuric oxide	2.5	g

Dissolve the hematoxylin in ethanol and dissolve the potussium alum in distilled water with frequent stirring and boil. Adding the mercuric oxide during the mixture is still hot. The container is quickly moved to the cool water bath and filter.

Eosin

Eosin-Y	10	g
Distilled water	50	g
Ethyl alcohol	940	ml

Dissolve Eosin-Y in the distilled water, then added the ethanol. In order

to prevent mould growing, either a little drop of formalin or crytal of phenol may be added or filter prior to use.

Stainning Procedure

1. Deparaffinize in xylene before hydrating in alcohol and water.
2. Stain with a hematoxylin solution for 6 min and wash in tap water.
3. Transfer to acid alcohol 5 to 6 quick dips.
4. Wash well in tap water. At this stage check microscopically; the nuclei should be blue while the background is very light or colourless.
5. Dip in lithium carbonate for 10 sec to condense the hematoxylin and remove the picric acid.
6. Wash in water and stain with the eosin solution for 20-30 sec.
7. Dehydrate in graded series of ethanol.
8. Clear with xylene and mount in permount.

APPENDIX V

Film Processing and Printing for SEM Photograph

Negative Film Processing

1. Load the exposed film in the rack or film holder in the total darkness.
2. Wash the film in the cool distilled water (21-22 °C) for 1 min to clear the distilled water prevents excessive softening of the emulsion.
3. Develop with Microdol-x (22 °C) with continuously stirred for 12.5 min to activated silver halide grains turn to silver metallic under alkaline condition.
4. Wash in the distilled water for 1 min.
5. Place in stop bath (acetic acid) for 1 min to stop the reaction of the developer.
6. After washing in the distilled water for 1 min, the film is immerged in a fixer to convert silver halide into silver thiocyanate which is water soluble. This step is under acidic condition which will neutralize excess alkaline developer that preventing film fog.
7. Wash in distilled water before placing in hypoclearing agent for 2 min.
8. The well developed film can be visualized under the room light. It is rinsed in the running tap water for 10-15 min before dipping in the water-repellent fluid (photo flo) for 1-2 min to prevent water spots.
9. Finally, the film is dried in the film dryer.

Enlargement and Printing

The quality of the negative film should be evaluated prior enlarging. To classify the quality according to the film contrast are three groups. The low contrast negative film requires the high contrast paper (BH paper), while the medium to high contrast negative film should require the normal to low contrast paper (BN to BS papers).

The process of enlargement and printing is done under the safe light as follows:

1. Place the negative film on the negative holder by emulsion down.
2. Turn on the light source of enlarger and open the aperture widely.
3. Adjust the enlarger by either raising up or lowing down for the appropiated size as need. The image is projected on the easel.
4. The sharp grains on the image, seeing with the image focuser, is adjusted by focusing knob.
5. Adjust the easel until the image fills on the paper.
6. Set the appropiated aperture.
7. Turn off the light of the enlarger, and set the proper exposured time.
8. Place the photographic paper in the easel and turn on the light of the enlarger and allow to expose it for the desired time.
9. Take the exposed paper and submerged in developer (Dextol : water = 1 : 2).
10. Tranfer the developed paper to stop bath for 20 sec to 30 sec.

10. Tranfer the developed paper to stop bath for 20 sec to 30 sec.
11. Rinse in running tap water and dip in fixer for 15 min.
12. Wash the photograph in running tap water for 30 min to 1 hr.
13. Submerge in a dilute water-repellent agent (Photo flo) for 15 min.
14. Dry with the print dryer, place the emulsion side up against a drying drum so the grossy prin it is obtained.

The scanning electron micrograph is now ready for evaluation