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

SOMLUK KONGSTAPONKIT

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

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Somluk Kongstaponkit

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บทคัดย่อ

จากการศึกษาปมประสาทสมองไทรเยมมินัลของกระแต ด้วยกล้องจุลทรรศน์แบบธรรมดา (LM), กล้องจุลทรรศน์อิเลคตรอนแบบส่องผ่าน (TEM) และด้วย vascular corrosion cast technique ร่วมกับกล้องจุลทรรศน์อิเลคตรอนแบบส่องกราด (SEM) พบว่า ปมประสาทสมองไทรเยมมินัลประกอบด้วยกลุ่มเส้นใยประสาทที่มีกลุ่มของเซลล์ประสาทล้อมรอบ เซลล์ประสาทแต่ละตัวมีนิวเคลียสอยู่ตรงกลางของเซลล์และ เซลล์ประสาทแต่ละตัวยังมีเซลล์พี่เลี้ยง (satellite cells) ล้อมรอบอีกทีหนึ่ง เป็นที่น่าสังเกตว่า มีหลอดเลือดกระจายอยู่หนาแน่นกว่าในบริเวณที่มีเซลล์ประสาท เมื่อเทียบกับในบริเวณที่มีเส้นใยประสาท จากการศึกษาด้วย TEM ไม่พบรูเล็กๆ (fenestration) ในผนังของหลอดเลือดฝอย ปมประสาทสมองคู่ที่ 5 ของกระแต ได้รับเลือดมาจาก 3 แหล่ง คือ แขนงของ pontine artery, แขนงของ stapedial artery หรือ บางครั้งมาจาก supraorbital artery, แขนงสุดท้าย คือ แขนงของ maxillary artery ที่ผ่านทาง foramen ovale ซึ่งหลอดเลือดนี้มีชื่อว่า accessory meningeal artery เมื่อหลอดเลือดเหล่านี้มาถึงบริเวณปมประสาทไทรเยมมินัล จะแตกแขนงออกเป็นหลอดเลือดฝอยล้อมรอบตัวเซลล์ประสาทและส่งแขนงเพื่อไปเลี้ยงเส้นใยประสาท หลอดเลือดฝอยที่เลี้ยงปมประสาทไทรเยมมินัลแล้วจะรวมกันเป็นหลอดเลือดดำขนาดเล็ก ซึ่งต่อมาจะรวมกันเป็นหลอดเลือดดำ ที่มีขนาดใหญ่ขึ้น ซึ่งมักพบที่ บริเวณพื้นผิวของปมประสาทนี้ หลอดเลือดดำที่อยู่ทางด้านในของปมประสาทไทรเยมมินัลจะเทเลือดเข้าสู่ cavernous sinus โดยตรง หรือ รวมกับหลอดเลือดดำที่มีชื่อว่า inferior hypophyseal vein ก่อนที่จะไหลเข้าสู่ cavernous sinus หลอดเลือดดำจากบริเวณส่วนท้ายของปมประสาท จะเทเลือดเข้าสู่ส่วนท้ายของ carvernous sinus ส่วนหลอดเลือดดำที่อยู่ทางด้านนอกของปมประสาทจะนำเลือดผ่านทาง accessory meningeal vein ไปยัง pterygoid plexus

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

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ABSTRACT

Sixteen adult common tree shrews of both sexes weighing 110-170g were divided into 3 groups for the study of trigeminal ganglion (TG) with LM, TEM and with corrosion cast technique/SEM, respectively. It was found that the TG was with clusters of cell bodies of neurons in the peripheral region surrounding the bundles of nerve fibers. Each ganglionic neuron was ensheated by the satellite cell and contained concentric nucleus. It was noted that there was higher density of blood vessels in the area where the neurons were predominated than that in the area occupied by nerve fibers. With TEM, it was shown that the TG contained mostly large round neurons with big nuclei and prominent nucleoli. The capillaries scattering in the TG were continuous type. The blood supply of the TG was from three sources. The first branch was from the most rostral branch of pontine artery. The second branch arise from the stapedial artery and sometimes from the supraorbital artery. The third branch was accessory meningeal artery which is a branch from maxillary artery passing through the foramen ovale. These arteries gave off branches to become capillaries network in the ganglion before draining the blood to the peripheral region. The veins at medial border drained the blood into the cavernous sinus (CS) directly or through the inferior hypophyseal vein. Those at the lateral side drained into pterygoid plexus via accessory meningeal vein. While the vein at the trigeminal nerve root joined the posterior part of the CS.

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LIST OF ABBREVIATIONS

A	=	artery
AMA	=	accessory meningeal artery
AMV	=	accessory meningeal vein
a	=	arteriol
BA	=	basilar artery
CB	=	cerebrum
Cb	=	cerebellum
Cp	=	capillary
CS	=	cavernous sinus
CV	=	collecting vein
DRG	=	dorsal root ganglion
En	=	endothelium
G	=	Golgi complex
GSA	=	ganglionic branch of stapedial artery
ICA	=	internal carotid artery
IHV	=	inferior hypophyseal vein
M	=	mast cell
Mi	=	mitochondria
MM	=	middle meningeal artery
MV	=	medial vein
My	=	myelinated nerve fiber
N	=	neuron
NF	=	nerve fiber
Nu	=	nucleus
P	=	pons
PA	=	pontine artery
Pe	=	pericyte
Pit	=	pituitary gland
rER	=	rough endoplasmic reticulum
SC	=	Schwann cell
SCG	=	superior cervical ganglion
SO	=	supraorbital artery

STA	=	stapedial artery
TG	=	trigeminal ganglion
V	=	vein
V1	=	ophthalmic branch
V2	=	maxillary branch
V3	=	mandibular branch
v	=	venule

CHAPTER I

INTRODUCTION

The trigeminal ganglion belongs to peripheral nervous system. It contains pseudounipolar sensory neurons which are derived from the most anterior part of neural crest cells in the embryo (Moore, 1988). The trigeminal ganglion is first appearing in the supraorbital region under the epidermis (Nichols, 1986; Kuratani, 1990). It is irregular and somewhat ovoid shape and could be divided into ophthalmic and maxillo-mandibular parts at stage 14 of human embryo. From these parts of the ganglion, the main branches of the fifth cranial nerve arise (Brusla and Wazniak, 1989). The trigeminal ganglion is being sandwiched by the dura mater and the greater wing of the sphenoid bone in the middle cranial fossa. Peripheral processes of these cells become the ophthalmic, maxillary and mandibular divisions of the trigeminal nerve innervating distinctive regions of the face, head and intraoral structures. Its central processes give rise to the sensory root of this cranial nerve traversing the lateral part of the pons to enter the pontine tegmentum and terminate upon sensory relay nuclei distributed in the pons and medulla oblongata (Carpenter, 1945). Although the trigeminal ganglion is a small organ, it is also a common site of diseases such as trigeminal neuralgia (tic douloureaux), herpes zoster, trigeminal neuritis (Earl, 1986; Rowland, 1989). Trigeminal neuralgia is the commonest disorder of the trigeminal nerve and the most frequent of all neuralgia which is mostly affected by distortion and compression of artery on trigeminal ganglion and its root (Haines et al., 1980). It is surprising, however, the information concerning the blood supply of the trigeminal ganglion is very limited. It is likely that Bergmann (1942) is the first investigator who describes the vascular supply of the trigeminal ganglion in human cadaver after making autopsy. According to him, the trigeminal ganglion in man recieves the blood supply from a twig of the internal carotid artery which crosses over the abducent nerve to supply the medial side of the ganglion.

The lower end of the ganglion is supplied by a medial branch of the middle meningeal artery or by the small meningeal artery. The arteries enter the ganglion, forming capillaries plexus and also distributes blood to the rootlets of the trigeminal nerve. They sometimes anastomose each other before giving off branches to the ganglion. The capillary plexus drains the blood into venules which join each other being the larger vascular channels around the ganglion before emptying the blood into the veins of the triangular plexus. Then the venous blood passes through the basal net of veins connecting with the cavernous sinus, the middle meningeal vein and the emissaries of the foramina lacerum and ovale. The root of ganglion carries small veins to the veins of posterior cranial fossa. Recently, Meana and Ballesteros (1989) have studied the arterial supply from the meningeal vessels to trigeminal ganglion in human cadaver by dissecting under microscope. They find that the arterial supply of the ganglion comes from middle meningeal artery, accessory meningeal artery and a branch from petrosal artery. Again Paullus et al. (1977) reported that the petrosal artery arises from middle meningeal artery and send branches to the trigeminal ganglion. In addition, Meckel (1748) has shown that the ganglion also receives blood from a very small branch of the internal maxillary artery entering the cranium through the foramen rotundum. After methyl methacrylate plastic mixture has been shown by Batson (1955) to have very good property to fill cavities of hallow organs including capillaries, Murakami (1971) injected the plastic mixture in combination with the application of the scanning electron microscope. He could cleary demonstrate the distributions of blood vessels including delicate capillaries in three dimentions. Okuda (1979) and Ohtsuki (1984) use this technique to studied blood supply in the trigeminal ganglion of the dog and monkey, respectively. In the dog, the blood supply of the trigeminal ganglion are from 6 sources. They are the petrosal branch, the anastomotic ramus, the anastomotic artery, the middle meningeal artery, the accessory meningeal artery, and the internal carotid artery. All of these arteries ramified into small arterial vessels. Some of them form rete in the capsule of the ganglion. The other spread into the ganglion becoming the capillary

networks. In the monkey, however, ganglionic blood supply is from the internal carotid artery, the accessory meningeal artery and the basilar artery. All of branches from these vessels become a network in the triangular plexus whereas the capillary network in the trigeminal ganglion is hardly seen. During the years 1978 and 1979, this technique (vascular corrosion cast) has been improved and widely used (Nopanitaya et al., 1979). In the later date, the technique has been employed to demonstrate the vascular patterns in various organs of the common tree shrew (Ritonga, 1988; Bulkusol, 1990; Ckunhabundit, 1991; Rattanachaikunsopon, 1991; Sudwan, 1991; Bamroongwong, 1992; Mingsakul, 1992; Sangchu, 1992; Chunhabundit, 1993; Lanlua, 1993; Mankhetwit, 1993), the animal regarded as primitive primate (Walker et al., 1964; Valen, 1965; Lekagul, 1977; Palley, 1984). As the blood supply of the trigeminal ganglion in this animal and other animals has not been investigated with the vascular corrosion cast technique, it is of interest to elucidate the vascular pattern in the trigeminal ganglion of tree shrew.

CHAPTER II

MATERIALS AND METHODS

Animal Preparation

Sixteen adult common tree shrews (Tupaia glis) of both sexes (Fig. 1) weighing between 110-170g were used and divided into 3 groups. The first group two animals, was for histological study of the trigeminal ganglion (TG) under light microscope (LM). The second group, two animals, was for the study of TG with light and transmission electron microscopy (TEM). The third group, twelve animals, was for the study of vascular corrosion cast of the TG in conjunction with SEM.

Animal was anesthetized with diethyl ether before the thoracic cavity was opened. Heparin, 0.05 ml (Leo, 5,000 iu/ml), was injected into the left ventricle and alloweded to circulate for about 1 min. A blunt needle (18 gauge) was inserted into the ascending aorta through the left ventricle. The vessel was clamped to the shaft of the needle while the right atrium was being cut open. The animal was perfused with 0.9% NaCl solution until the effluent was clear.

Routine Histological Preparation of the TG (Group 1)

Immediately after the perfusion with 0.9% NaCl solution, 250 ml of bouin solution was injected manually through the ascending aorta to fix the animal. The TG was carefully dissected *in situ* and immersed in the same fixative over night. After rinsing with distilled water, the specimen was dehydrated in a graded series of ethanal, infiltrated and embedded in paraffin. The ganglion was transversely sectioned at 5 to 7 μm thick, stained with thionine, cresyl violet before being viewed and photographed with the Olympus Vanox light microscope.

Preparation of the TG for LM and TEM (group 2)

Following the perfusion with 0.9% NaCl solution, 250 ml of iced cold 2.5% glutaraldehyde in 0.1 M phosphate buffer solution (PBS), pH 7.4, was slowly injected through the ascending aorta. The TG were carefully dissected, excised, minced into small cubes and immersed in the same fixative for 2 hr at 4°C. After transferring into 0.1 M PBS for 2 hr at 4°C, the tissue cubes were dehydrated in a graded series of ethanol, infiltrated and embedded in Araldite 502 resin (Electron Microscopy Sciences) and sectioned with Sorvall Porter-Blum MT-2 ultramicrotome. The 1 um thick of sections were stained with 1% toluidine blue, viewed and photographed with the Olympus Vanox light microscope. Ultrathin sections of silver-gray interference were collected on copper grids and stained with uranyl acetate and lead citrate before viewing and photographing under TEM (Hitachi H-300) at an accelerating voltage of 75 kV.

Preparation of the TG Vascular (Group3)

After being perfused with 0.9% NaCl solution, each animal was manually injected with 20 ml of freshly prepared Batson's #17 plastic mixture at the rate of 8 ml/min through the ascending aorta (Chunhabundit and Somana, 1988; Chunhabundit et al., 1988). The ascending aorta and the superior vena cava were then ligated. The animal was left for 30 min at room temperature to let the casting medium set. The head was removed and the cavarium was opened to exposed the brain (Fig. 2) before immersing in warm water (80°C) for 3 hr to ensure the complete polymerization of the casting medium. The temporal lobes from six heads were removed and digested in 40% KOH solution at room temperature for 24-48 hr. The other, six heads with intact brains were digested in 40% KOH solution at room temperature for 48-72 hr and transfered into 10% formic acid at room temperature for 24-48 hr. The vascular casts from all animals were carefully and slowly rinsed in distilled water to remove tissue debries. The vascular casts of TG were left air-dried and adhered on brass stubs with carbon paint,

coated with gold/palladium (Hummer VII sputter coater), viewed and photographed under SEM (Hitachi S-2500) at an accelerating voltage of 30 kV.

CHAPTER III

RESULTS

General Morphology of the TG: Stereomicroscopic Observation

The shape of the common tree shrew trigeminal ganglion (TG) is semilunar. Its average dimensions are 1 mm long, 2 mm wide and 0.5 mm thick (Fig. 3). The ganglion situates in middle cranial fossa just postero-lateral and inferior to the pituitary gland (Fig. 3). It lies on the greater wing of sphenoid under the dura mater.

Histology of the TG: Light and TEM Examination

Light microscopic observation of the paraffin sections stained with thionine and cresyl violet as well as of semithin sections stained with toluidine blue reveals that the central processes of the TG form the sensory root which traverses the lateral part of the pons (Fig. 4). Neurons organizing as clusters are located primarily in the peripheral region and few of them are found scattering in the central area of the ganglion (Figs. 5-6). The ganglion is covered with thin connective tissue sheath (Fig. 7). It contains both large and small round neurons with concentric nuclei and large amount of Nissl substance in their neuroplasm (Figs. 6,8). Moreover, some neurons are with 2 or 3 nucleoli in their nuclei (Figs. 6,8). It is observed that there are a large number of small blood vessels, predominantly capillaries, in the peripheral portion where the neurons situate while only few of them are seen among nerve fibers in the central portion of the ganglion (Figs. 7-9). The larger vessels are also occasionally found in the central region. (Fig. 7). In addition, each ganglionic neuron is surrounded by satellite cells (Fig. 10). The myelinated and nonmyelinated nerve fibers are usually found in the central region of the TG. The nuclei of Schwann cells are oftenly found in association with the myelins heath (Fig. 10). In addition, with TEM, it is shown that the TG contains round neurons with large nuclei and prominent

nucleoli (Fig. 11). A large amount of rough endoplasmic reticulum (rER), Golgi complex, and mitochondria are observed in the neuroplasm (Figs. 12-13). In addition, the supporting or satellite cells are found to ensheath the neuronal soma (Fig. 11) and the Schwann cells is closely associated with the myelinated nerve fibers (Figs. 12,15).

The capillaries scattering in the TG are continuous type of which endothelium is with rich micropinocytotic vessicles (Figs. 13-14). Small microvillous processes are present on the luminal surface of some vessels (Fig. 13). Occasionally, mast cells are also seen scattering in the intraganglionic connective tissue (Figs. 7, 16).

Stereomicroscopic Observation of Intracranial Blood Supply

In common tree shrew, the brain is supplied by vertebro-basilar and internal carotid systems (Figs. 17-18). The two vertebral arteries join together to form the basilar artery at the upper cervical-lower cranial region (Fig. 17). The basilar artery is straight, and gives off many branches. These branches are anterior inferior cerebellar, labyrinthine, pontine, and superior cerebellar arteries. The terminal basilar trunk at its rostral end anastomoses with the caudal branch of the internal carotid artery to form the posterior cerebral artery. Before two vertebral arteries joining togethers to be the basilar artery, they give off posterior inferior cerebellar arteries and branches to form the anterior spinal artery. The internal carotid artery in tree shrew gives off the stapedial artery upon entering the base of the skull (Figs. 17,19). This branch enters the tympanic cavity, and passes through the stapes before proceeding into middle cranial fossa. The stapedial artery also supplies the meninges via middle meningeal artery, and extrabulbar part of the orbit via the supraorbital branch (Fig. 19).

Blood Supply of the Trigeminal Ganglion

Stereomicroscopic observation of the TG vascular cast reveals that each TG recieves arterial supply from branches of three arteries. The first branch is from one of the pontine arteries. The second branch arises from the stapedial artery and sometimes from the supraorbital artery. The third branch is from the maxillary artery passing from the infratemporal region through the foramen ovale (Figs. 17-19).

With SEM of vascular corrosion casts, it is obvious that there are numerous blood vessels, especially, the capillaries forming network in the TG. However, there are less blood vessels in the area occupying by the nerve roots and major divisions of the trigeminal nerve (Fig. 20). The shape of the cast conforms to the TG appearance (Figs. 21-23). Again, it is confirmed that the TG are supplied by branches of three arteries. The first branch is from the last (most rostral) branch of pontine artery runing along the trigeminal nerve root before penetrating the posterior part of the ganglion (Fig. 24). Almost immediately after the pontine artery emerging into the ganglion, it divides into many arterioles before giving rise to capillaries (Figs. 25-26). The second branch is the ganglionic branch of stapedial artery. This branch is occasionally arises from the supraorbital artery. It courses medially across base of the skull in the middle cranial fossa to enter the TG (Figs. 27-28). The third branch arises from the maxillary artery and passes through the foramen ovale to supply the ganglion. This artery is known as accessory meningeal artery (Fig. 29). It is obvious that when the ganglionic branch of stapedial artery is large, the accessory meningeal artery is small or absent (Fig. 20). Nevertheless, the accessory meningeal artery is more oftenly present when compare with the ganglionic branch of stapedial artery. All of these arteries penetrate into the ganglion (Fig. 30) and branch off to form intraganglionic capillaries and anastomose with each other. It is noteworthy that the surface of the intraganglionic capillaries is without knobs which represent the fenestrations (Fig. 26). This indicates that these capillaries are of continuous type. The capillaries usually form loops around neurons before joining together

to form venules which drain the blood to the peripheral region of the ganglion (Fig. 31). The small venules drain the blood into larger venules called collecting venules which travel to the ganglionic border (Fig. 32). These collecting venules at the lateral border of ganglion empties the blood into pterygoid plexus via accessory meningeal vein or the vein along the maxillary branch of TG (Figs. 33-34), while those at the medial border of ganglion join the cavernous sinus directly (Figs. 35-37) or join with the inferior hypophyseal vein before emptying into cavernous sinus (Figs. 38-39). In addition, the collecting venules at the trigeminal nerve root course caudally and finally drains the blood into posterior part of the cavernous sinus (Fig. 40).

Figure 1. Photograph of the young adult common tree shrew.

Figure 2 Photograph of the intact brain of common tree shrew, dorsal aspect, showing the cerebrum (CB) and cerebellum (Cb). X = 2.5

1

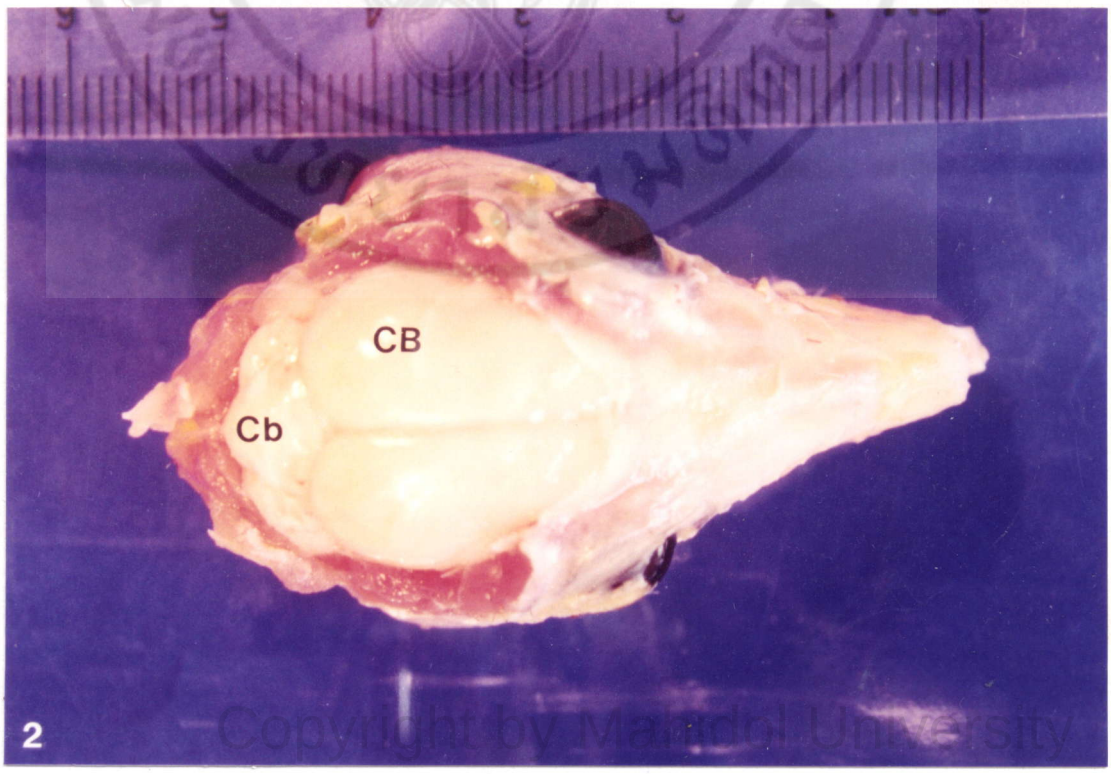

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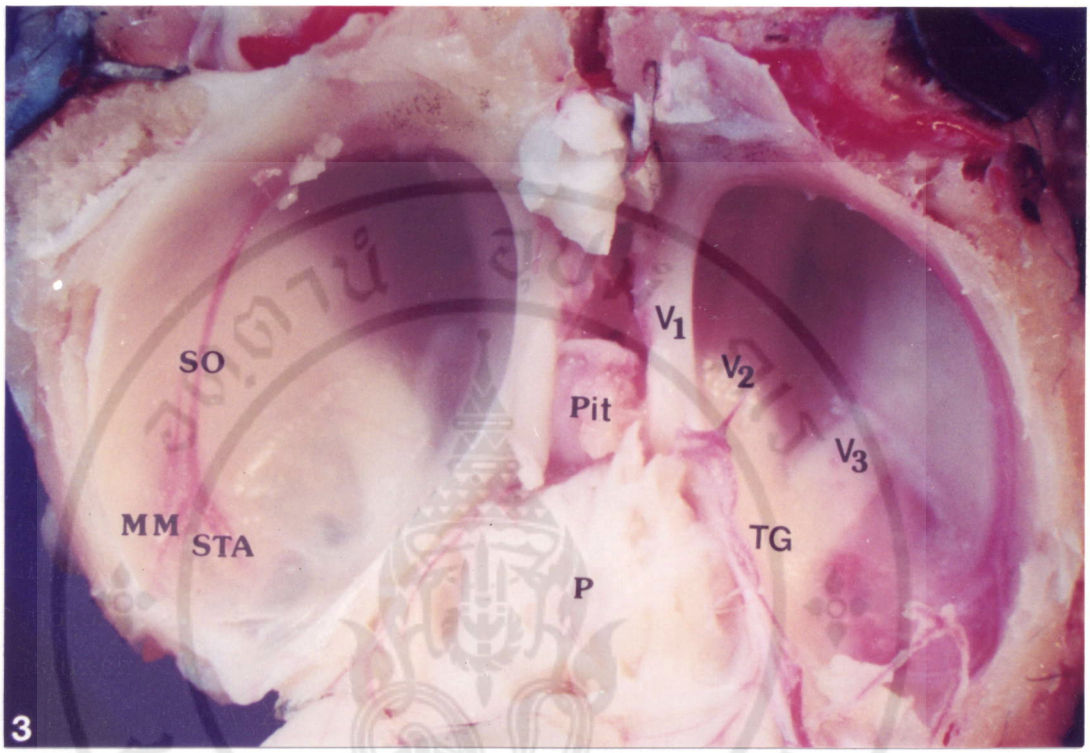
Figure 3. Photomicrograph of the common tree shrew middle cranial fossa, dorsal aspect, showing the TG situating just postero-lateral and somewhat inferior to pituitary gland (Pit). Note the stapedial artery (STA) approaching lateral side of TG. MM = middle meningeal artery; P = pons; SO = supraorbital artery; V1 = ophthalmic branch; V2 = maxillary branch; V3 = mandibular branch. X 7.

Figure 4. Photomicrograph of the common tree shrew TG, transverse section, stained with thionine, showing the root of the trigeminal nerve (*) traverses the lateral part of the pons (P). X 18.



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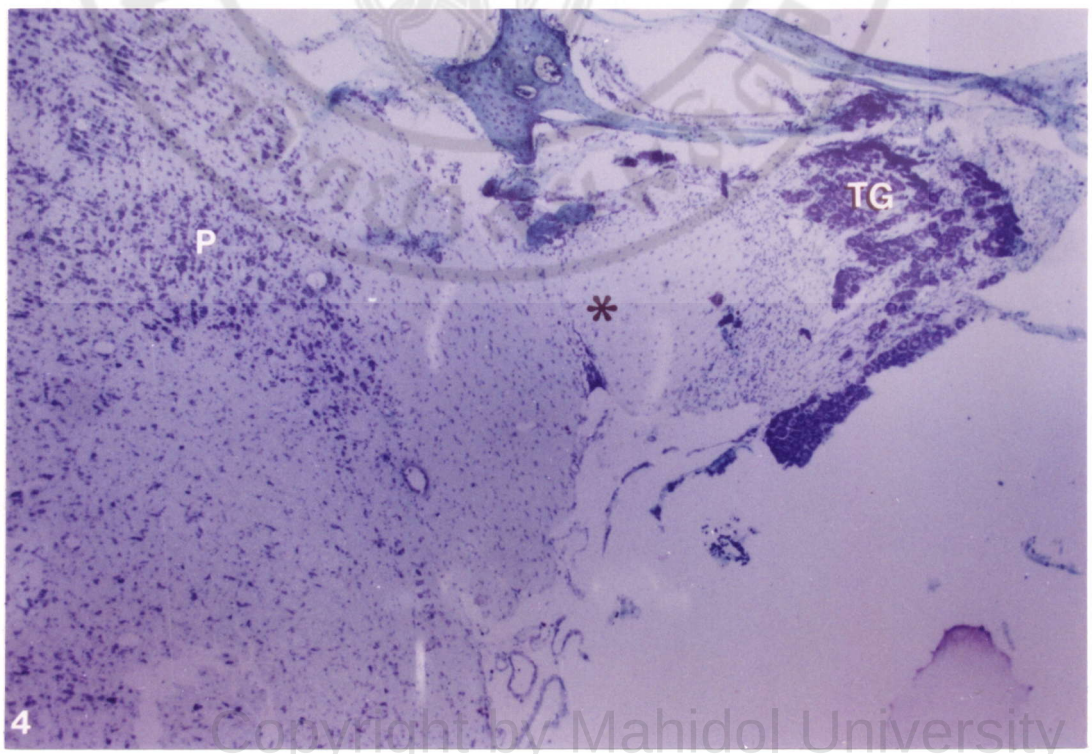
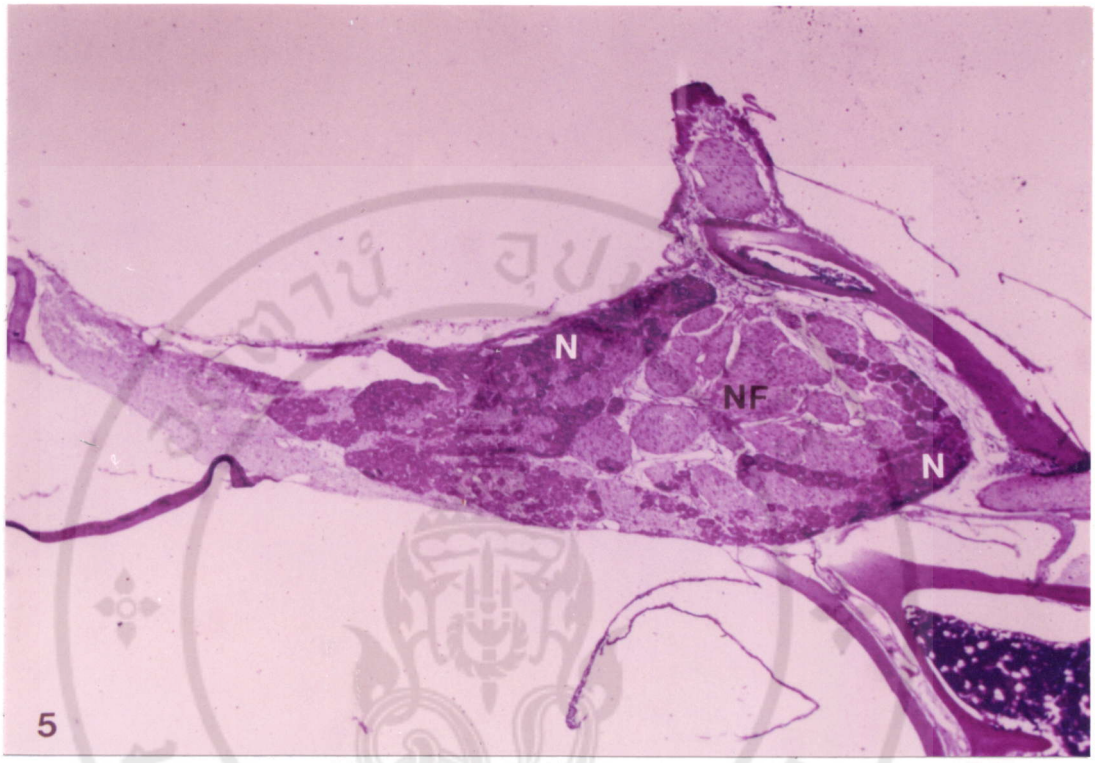


4

Figure 5. Photomicrograph of the common tree shrew TG, transverse section, stained with thionine, showing clusters of cell bodies of neurons (N) in the peripheral region of the ganglion and the nerve fibers (NF) in the central part. X 18.

Figure 6. Photomicrograph of the common tree shrew TG, stained with cresyl violet, showing large and small neurons. Note some of neurons having 2 or 3 nucleoli (arrowhead). NF = nerve fibers. X 184.



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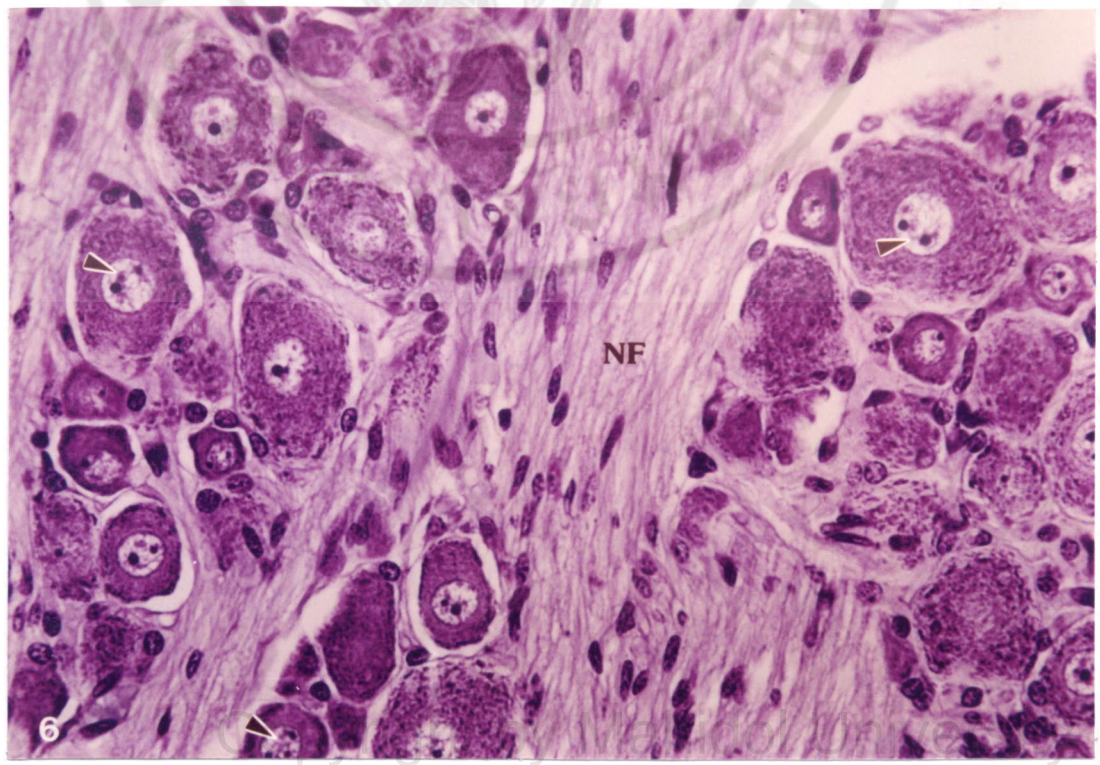

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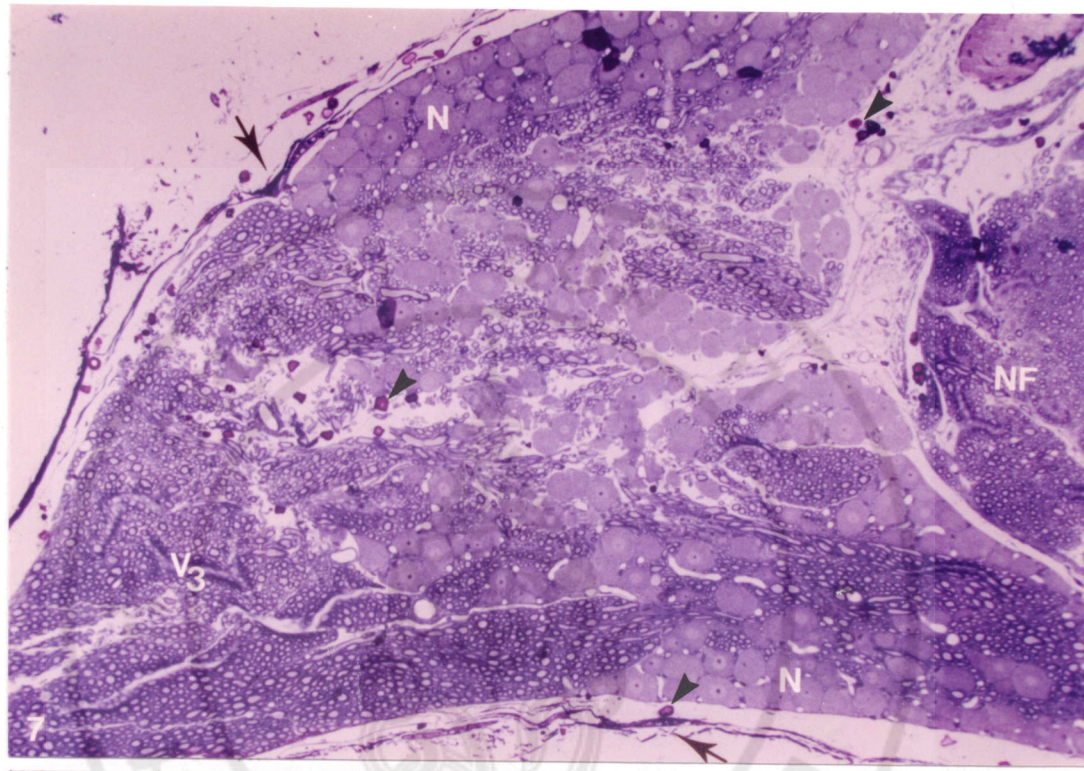
Figure 7. Photomicrograph of common tree shrew TG, transverse section, stained with toluidine blue, showing thin connective tissue capsule (arrow), clusters of cell bodies of neurons (N) as well as nerve fibers (NF). Note mast cells are scaterring in the loose areas of connective tissue (arrowhead). V3 = root of mandibular nerve. X 45.

Figure 8. Photomicrograph of common tree shrew TG, transverse section, stained with toluidine blue, showing higher density of capillaries (Cp) in the area where neurons (N) predominate. NF = nerve fibers. X 180.


N
NF
V_3
N
7

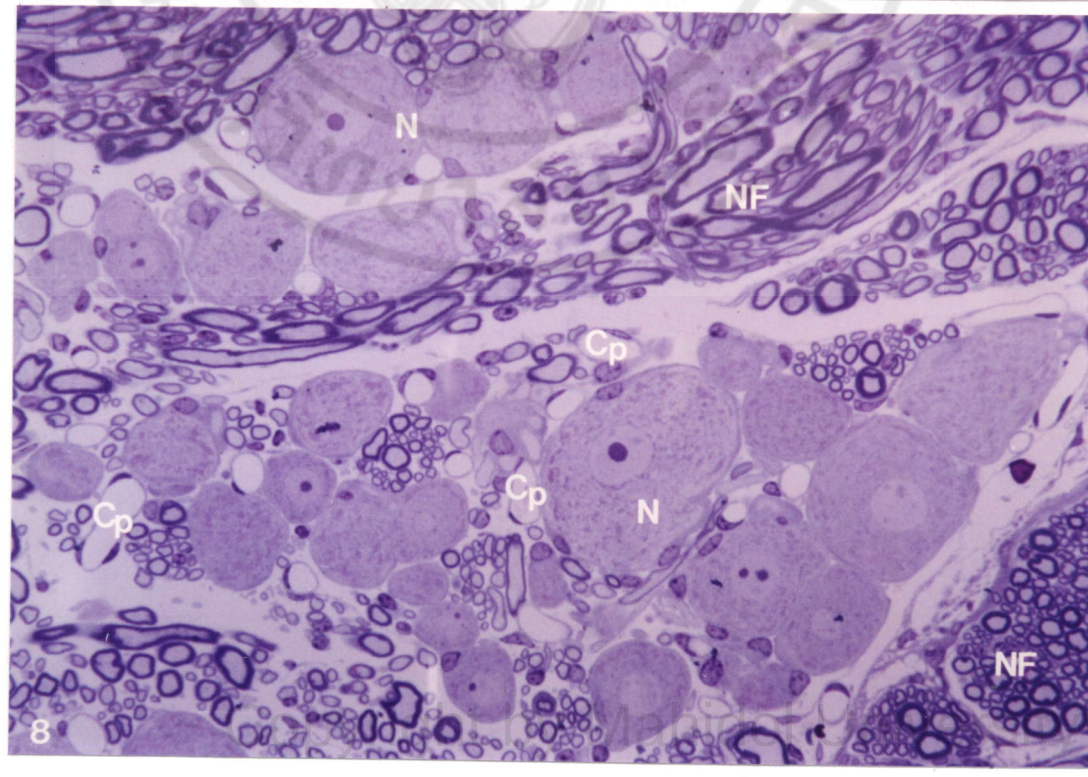
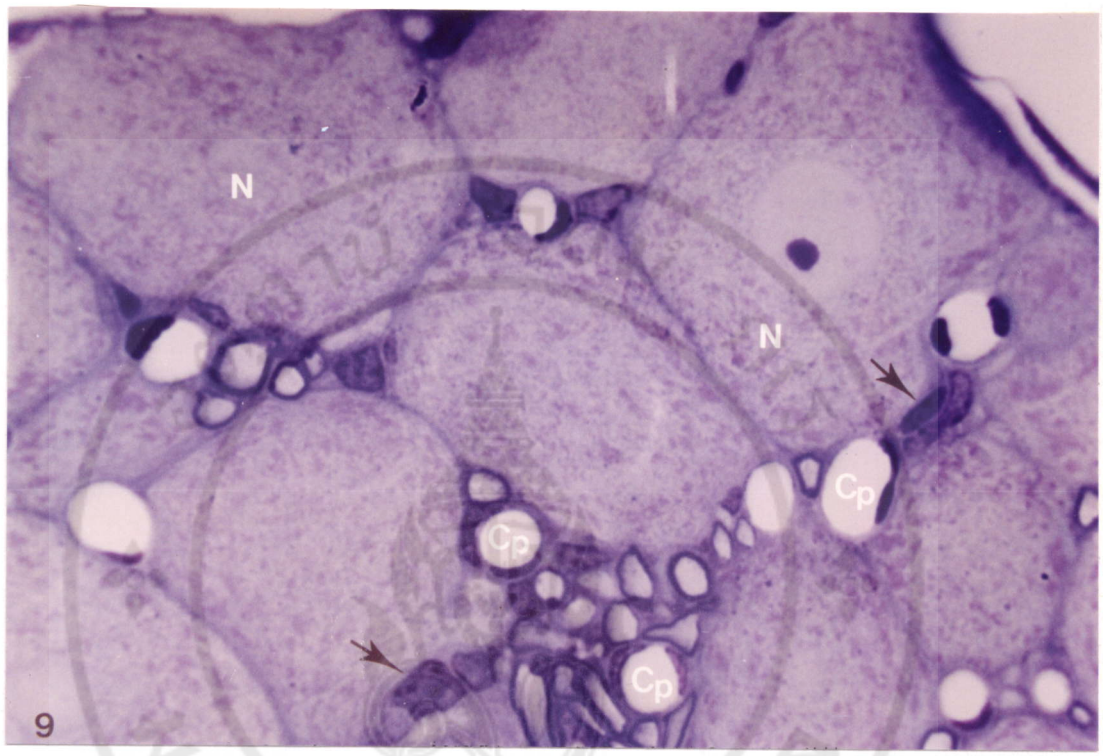

N
NF
Cp
Cp
Cp
N
NF
8

Figure 9. High magnification of TG section showing the numerous capillaries (Cp) surrounding the neurons. arrow = nucleus of satellite cell. X 450.

Figure 10. High magnification of the TG section showing myelinated nerve fibers (My), nuclei of Schwann cells (arrowhead), capillary (*) and neuron (N). Note the satellite cell (arrow) ensheaths the neuron. X 420.



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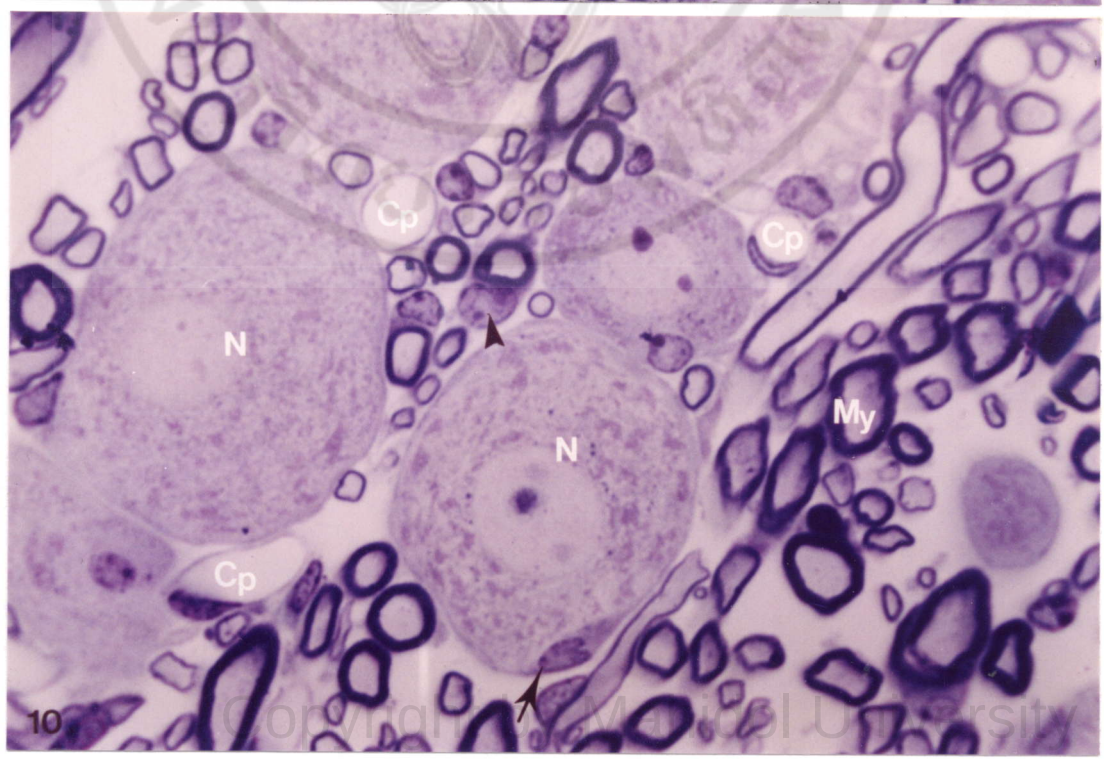
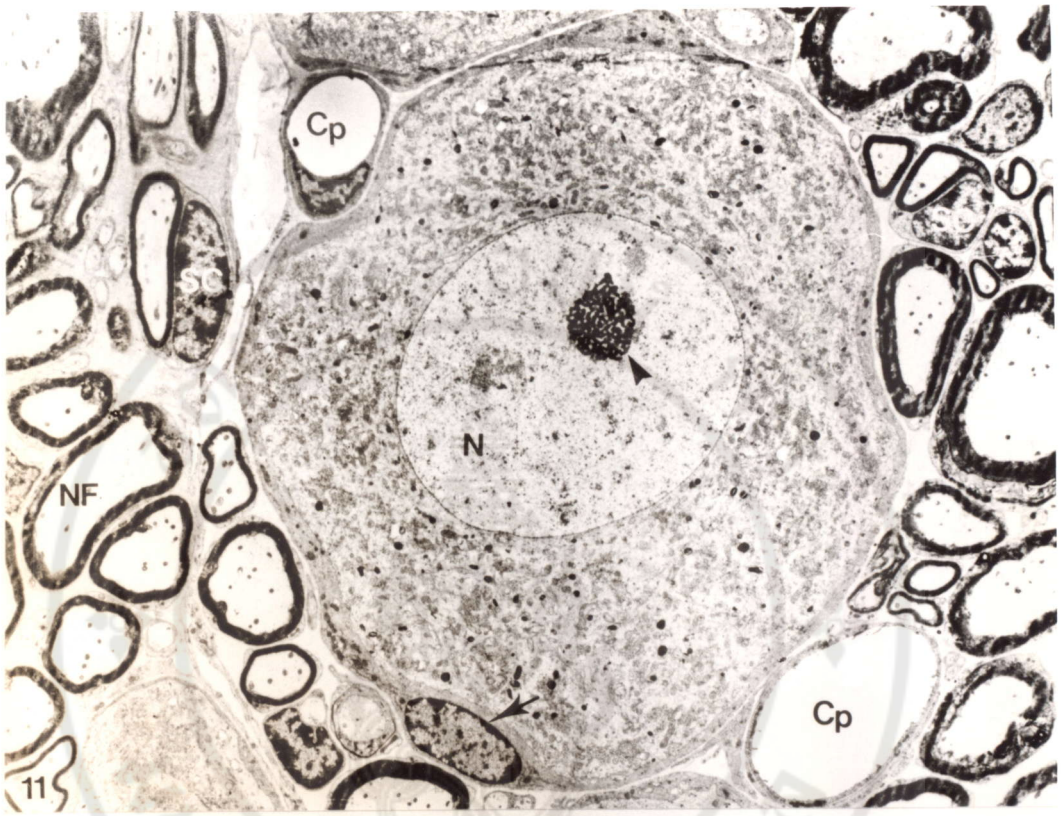


10

Figure 11. TEM micrograph of the common tree shrew TG showing cell body of neuron (N) completely surrounded by satellite cell (arrow). Note capillaries (Cp) are in adjacent to the neurons. NF = nerve fibers; arrowhead = nucleolus; SC = nucleus of Schwann cell. X 2,655.

Figure 12. TEM micrograph, high magnification of the TG showing the perikaryon with numeuous rough endoplasmic reticulum (rER). G = Golgi complex; Nu = nucleus. X 22,800.


Cp
N
NF
Cp
11

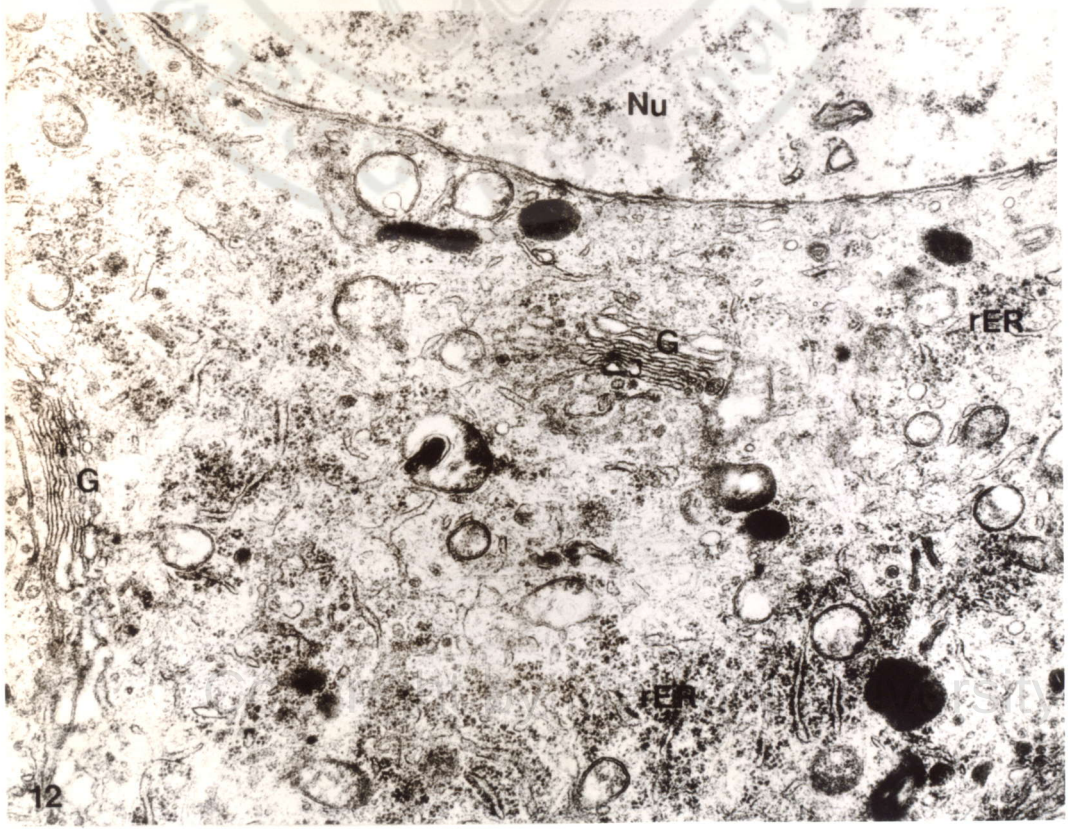
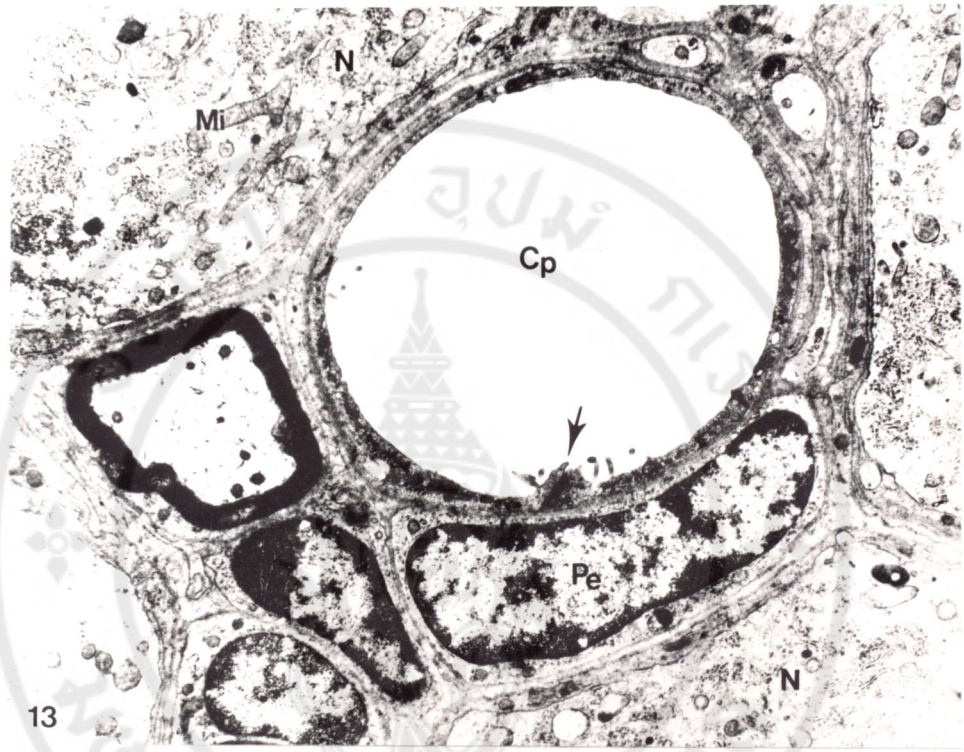

Nu
rER
G
G
rER
12

Figure 13. TEM micrograph of the TG showing continuous type of capillary (Cp) among the neurons (N). Note microvillous processes on the luminal surface of the endothelial cell (arrow). Mi = mitochondria; Pe = pericyte. X 9,300.

Figure 14. TEM micrograph, high magnification, of the TG showing continuous capillary (Cp) with numerous vesicles (arrowhead). En = endothelium; Mi = mitochondria. X 37,100.



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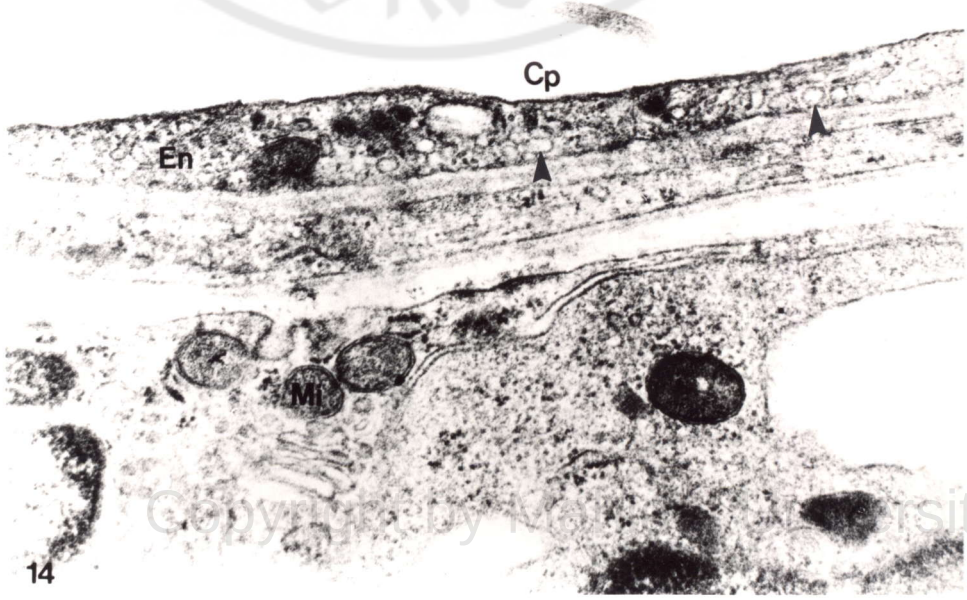

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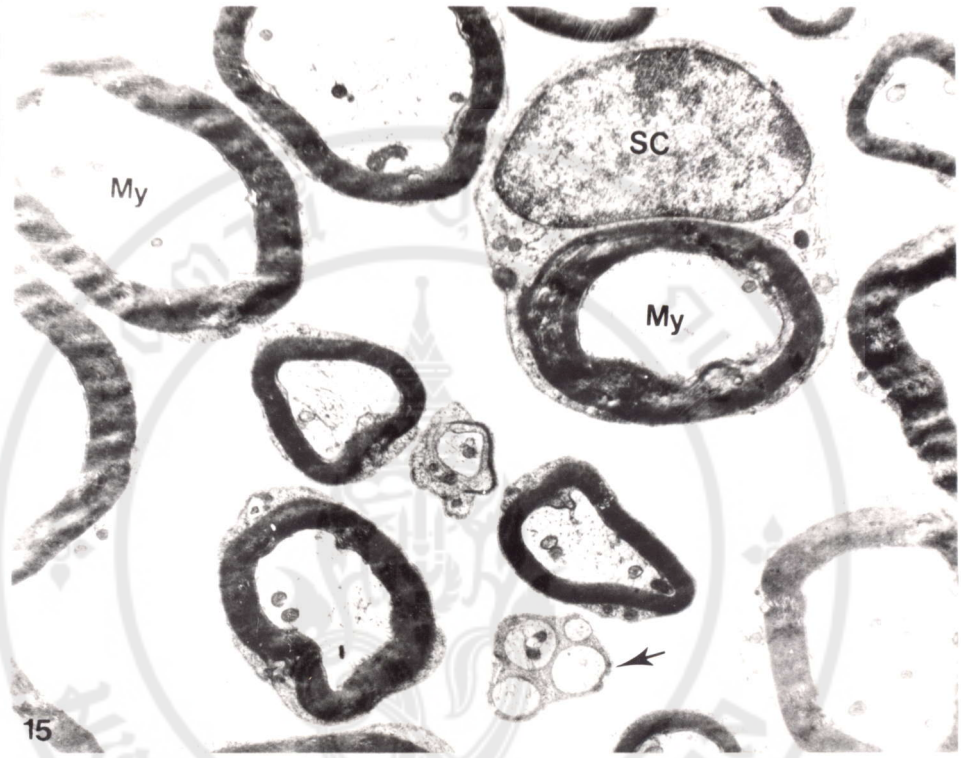
Figure 15. TEM micrograph of the nerve fibers at high magnification showing myelinated nerve fiber (My) associated with the Schwann cell (SC) and group of non-myelinated nerve fiber (arrow) surrounded by Schwann cell. SC = nucleus of Schwann cell. X 45,700.

Figure 16. TEM micrograph of the TG showing a mast cell (M) in association with the capillary (Cp). X 9,700.



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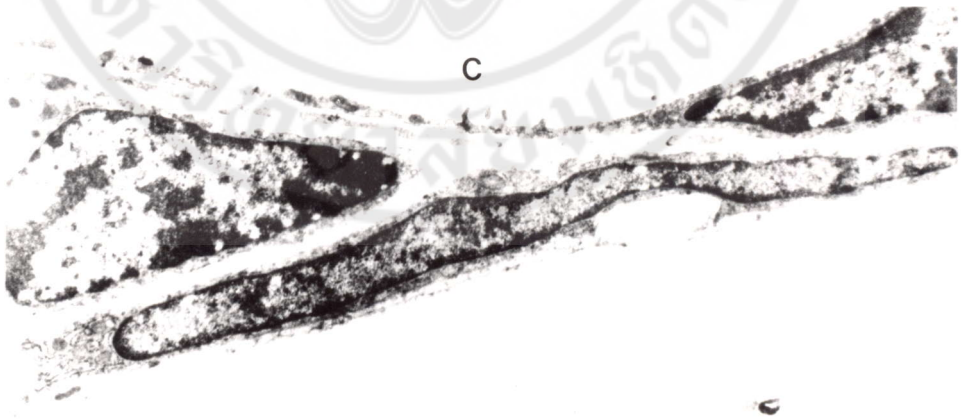
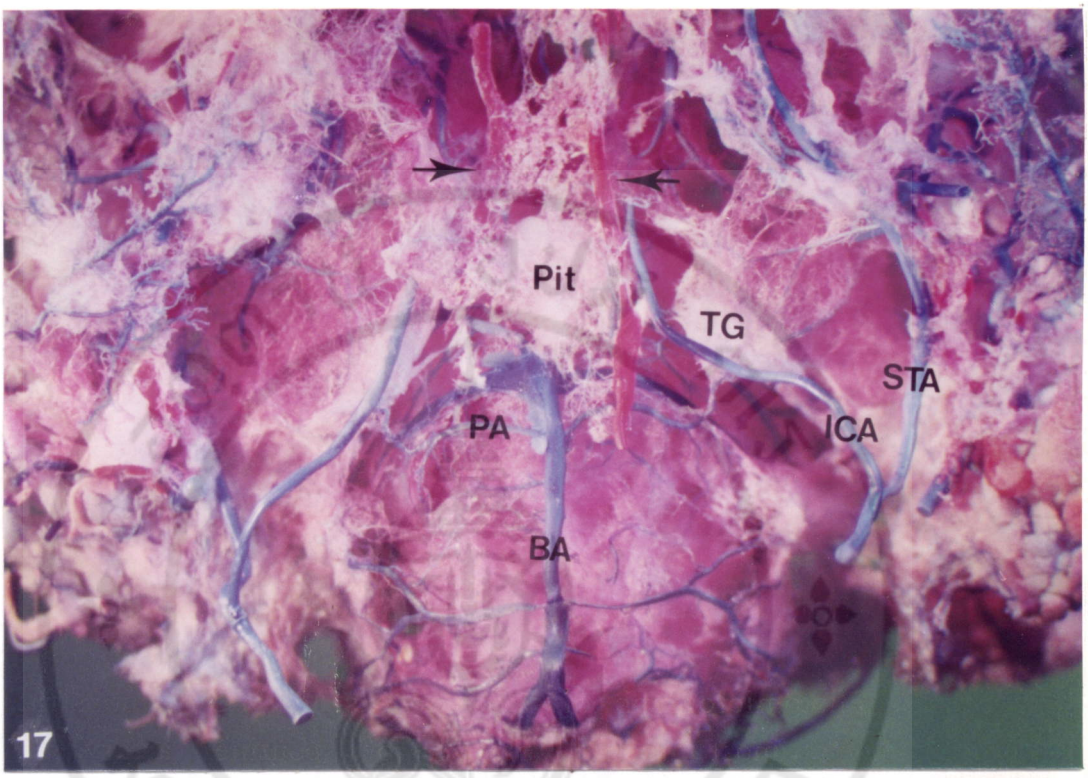


16

Figure 17. Photomicrograph of vascular cast in the head region of commom tree shrew, showing basilar artery (BA), internal carotid artery (ICA), pontine artery (PA), pituitary gland (Pit), stapedial artery (STA), and TG. X 7.

Figure 18. Photomicrograph of vascular cast of TG demonstrating the most rostral branch of pontine artery (PA) penetrating the root of TG. Note, the accessory meningeal artery (AMA) passing from infratemporal region to supply the TG. BA = basilar artery; ICA = internal carotid artery; IP = infundibular process; IS = infundibular stalk; PD = pars distalis; STA = stapedial artery. X 15.



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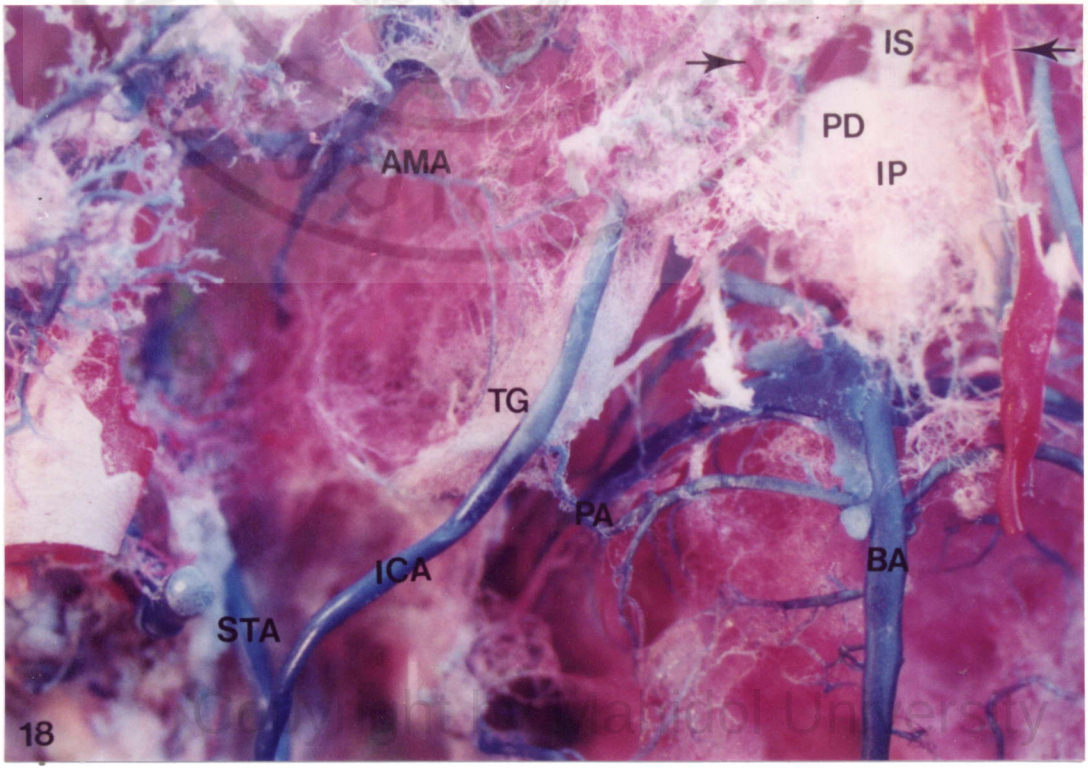

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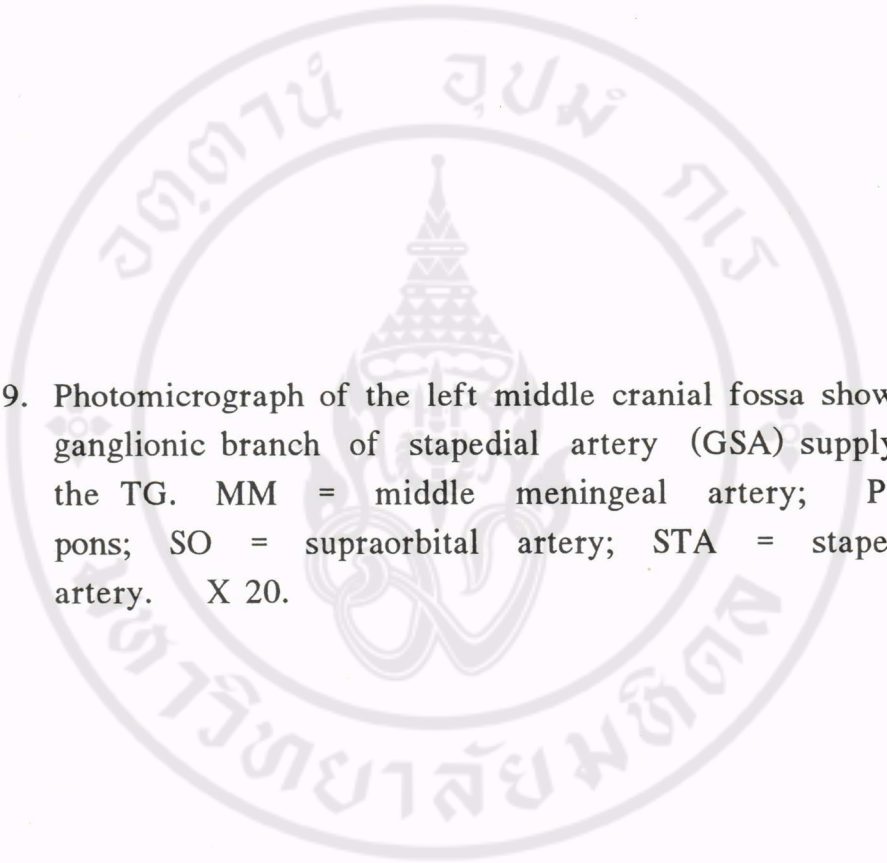


Figure 19. Photomicrograph of the left middle cranial fossa showing ganglionic branch of stapedial artery (GSA) supplying the TG. MM = middle meningeal artery; P = pons; SO = supraorbital artery; STA = stapedial artery. X 20.

19

Figure 20. SEM micrograph of the left TG, vascular cast, dorsal view, showing denser capillaries in the TG than in major divisions of the trigeminal nerve. V1 = ophthalmic branch; V2 = maxillary branch; V3 = mandibular branch; * = ganglionic branch of stapedial artery. Bar = 250 μm.

Figure 21. SEM micrograph of the left TG vascular cast, ventral view, showing relationship between the TG and internal carotid artery (ICA). Note the ICA does not give off branch to supply the TG. PA = pontine artery. Bar = 200 μm.

Figure 22-23. SEM micrograph of left and right TG vascular casts, ventral view, after removal of ICA to exhibit TG vasculature. a = arteriole; PA = pontine artery. Fig.22 Bar = 250 μm, Fig.23 Bar = 200 μm.

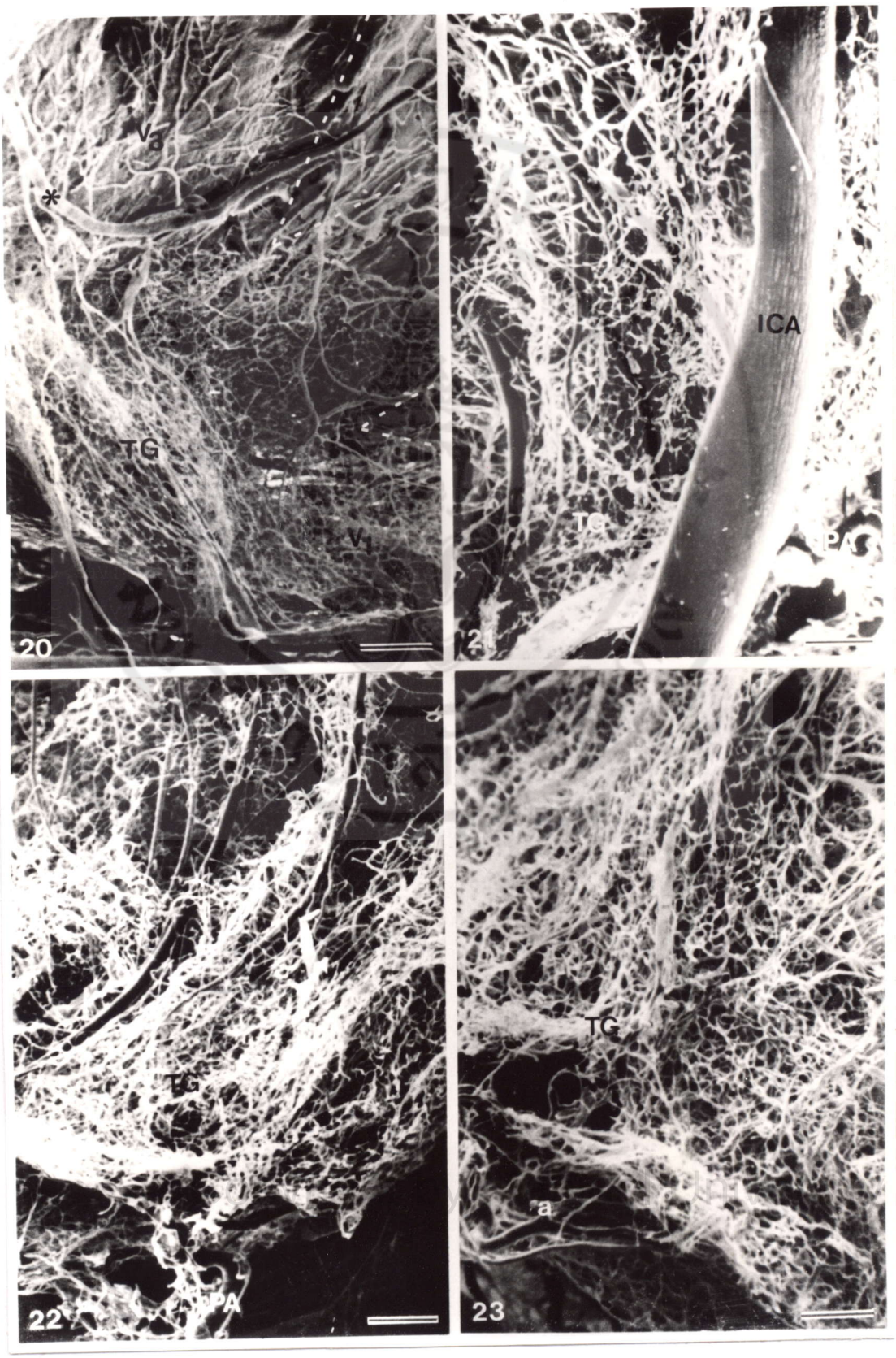
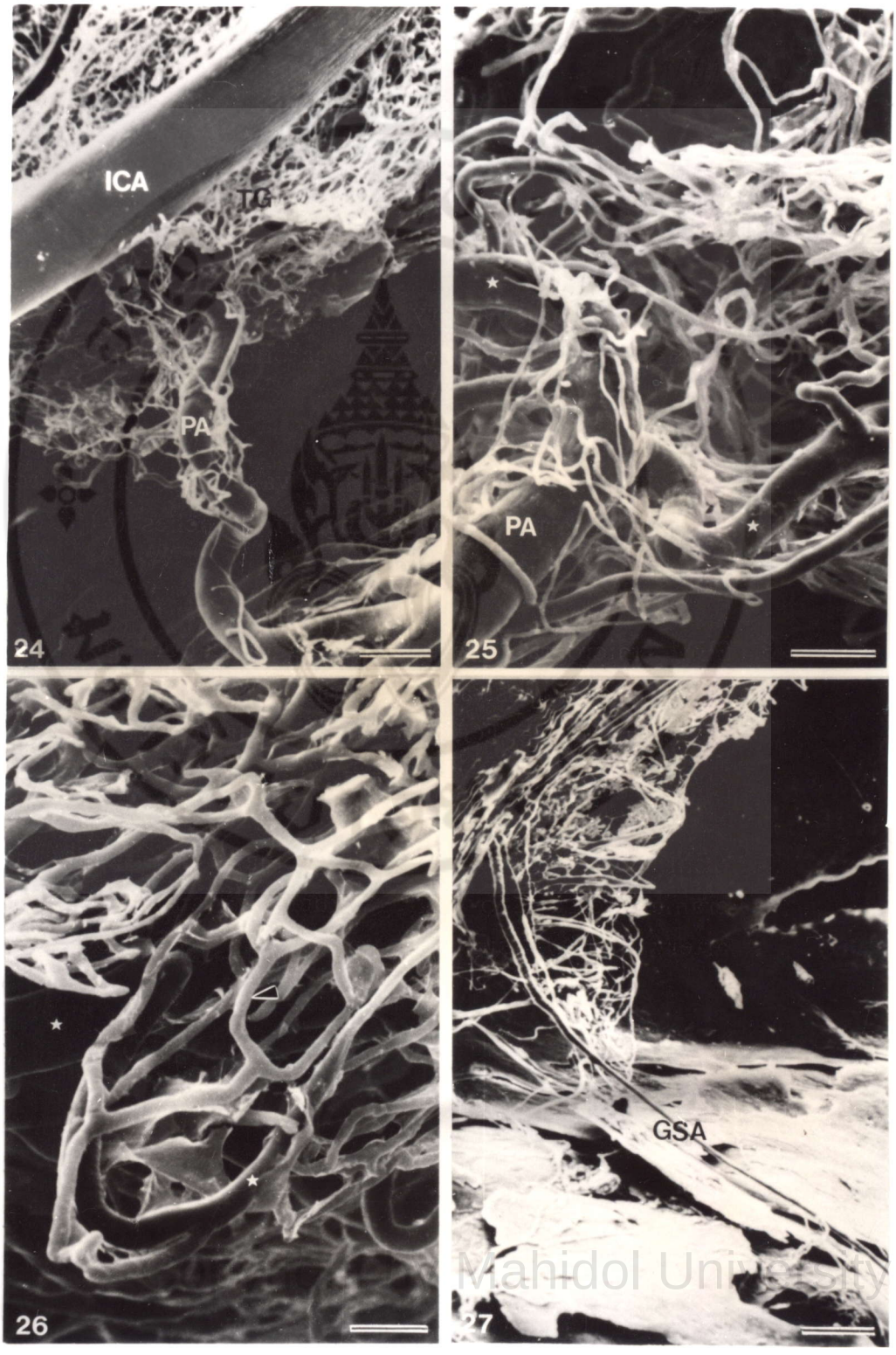

V3
*
TG
V1
20
ICA
TG
PA
22
TG
PA
TG
a
23

Figure 24. SEM micrograph of left TG vascular cast showing a branch of pontine artery (PA) entering the TG at the root of the TG. ICA = internal carotid artery. Bar = 200 μm.

Figure 25. SEM micrograph of the TG vascular cast illustrating the pontine artery (PA) giving off branches (*) to supply the TG. Bar = 75 μm.

Figure 26. SEM micrograph at high magnification of the TG vascular cast illustrating a branch of the pontine artery (*) entering the TG. Note the capillaries are without the knobs (arrowhead). Bar = 30 μm.

Figure 27. SEM micrograph of the vascular cast with middle cranial fossa showing ganglionic branch of stapedial artery (GSA) supplying the TG. Bar = 250 μm.



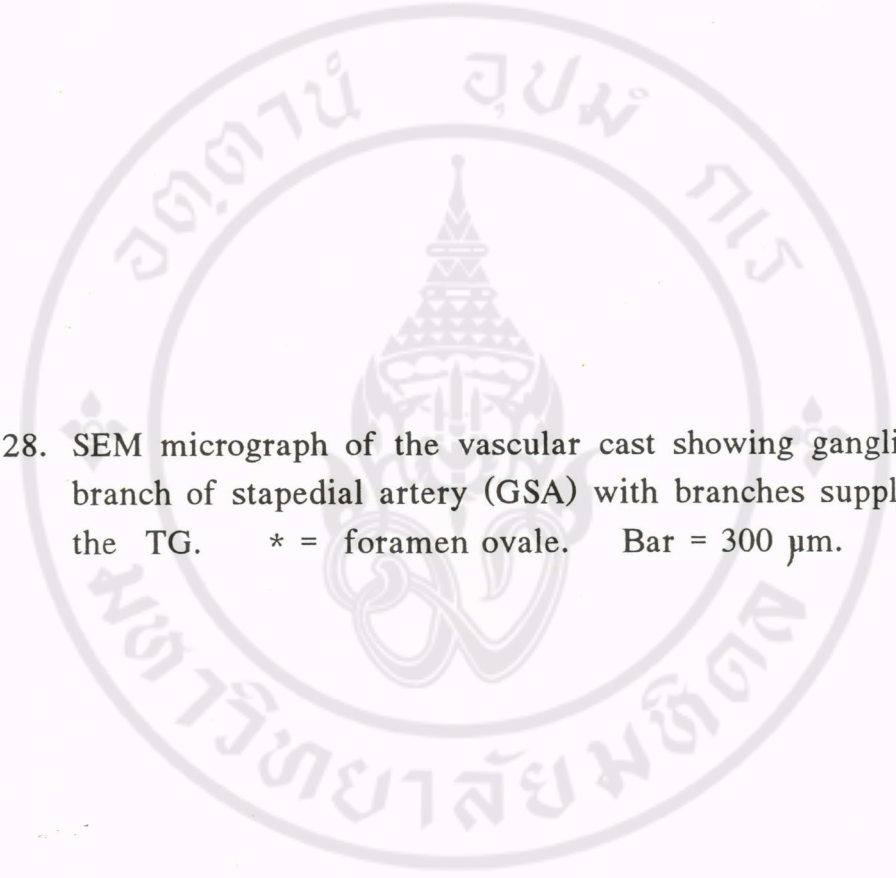


Figure 28. SEM micrograph of the vascular cast showing ganglionic branch of stapedial artery (GSA) with branches supplying the TG. * = foramen ovale. Bar = 300 µm.

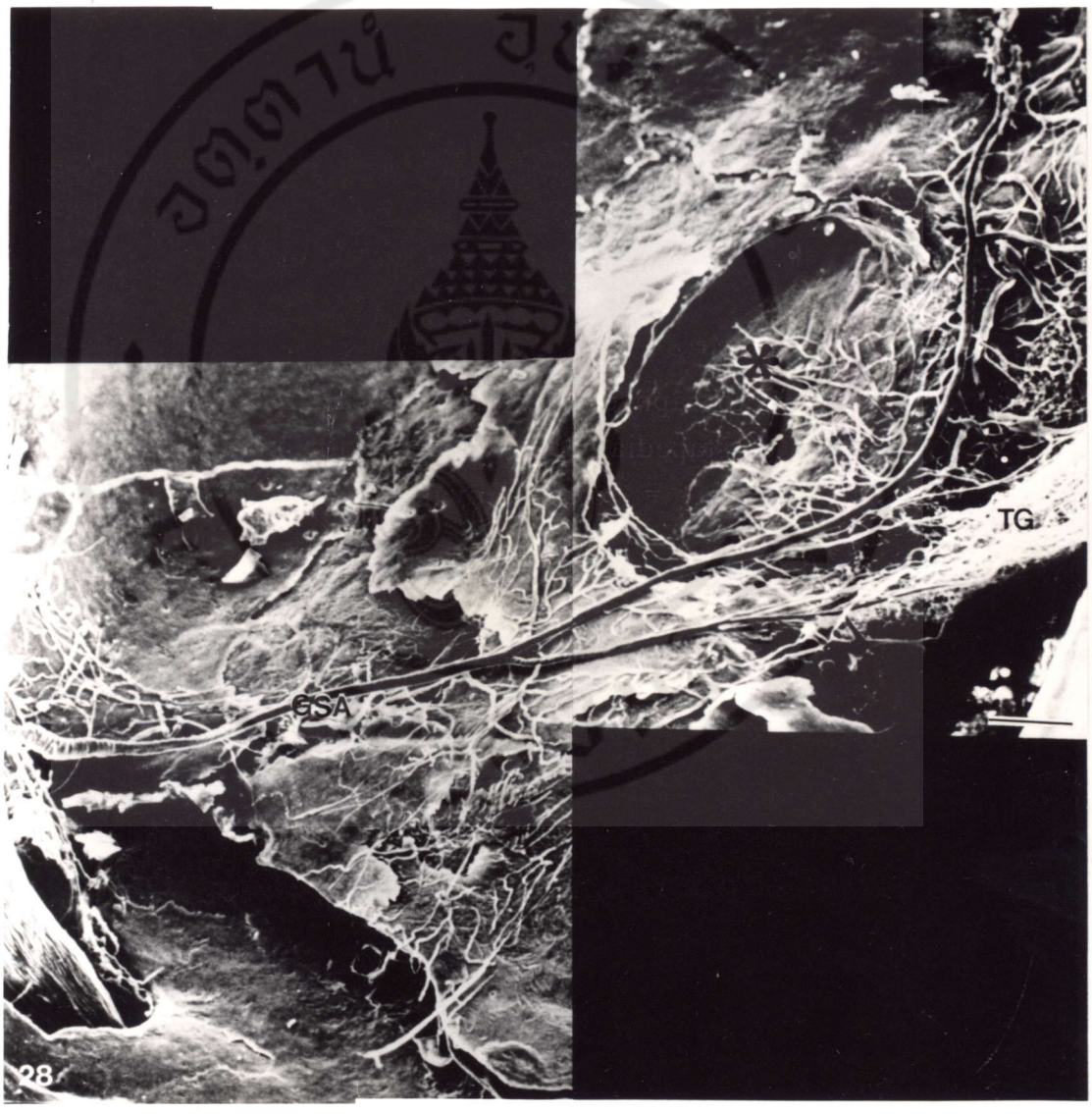


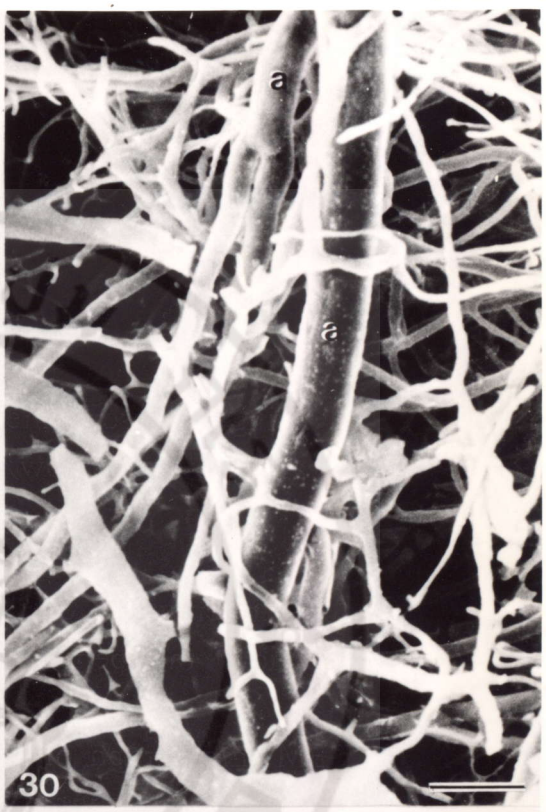
Figure 29. SEM micrograph of the vascular cast of the right TG showing accessory meningeal artery (AMA) entering the foramen ovale (arrow) to supply the ganglion. Bar = 250 µm.

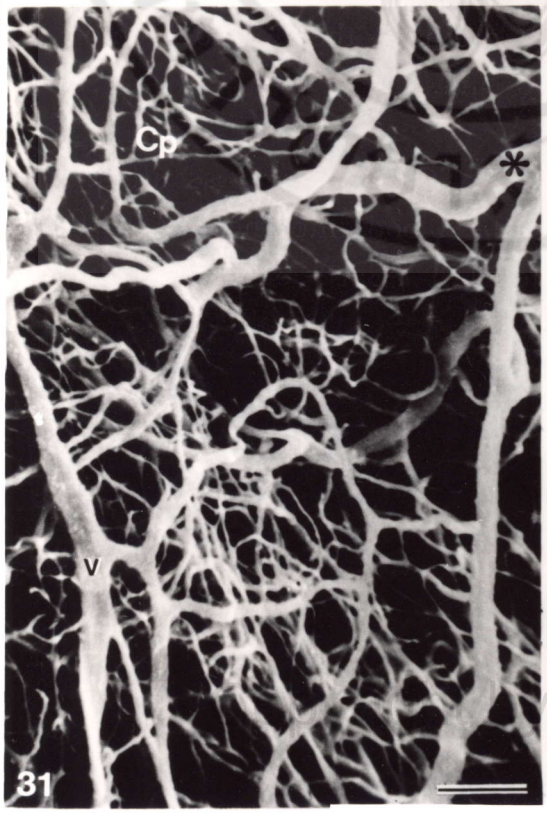
Figure 30. SEM micrograph of the TG vascular cast, illustrating the arteriole (a) penetrating into the organ. Bar = 50 µm.

Figure 31. SEM micrograph of the TG vascular cast illustrating the arteriole (*) giving off branches to become capillary plexus (Cp) which drains the blood into the venule (v). Bar = 50 µm.

Figure 32. SEM micrograph of the TG vascular cast illustrating the capillaries draining the blood into the venule (v) at the medial side of the TG. Bar = 35 µm.


AMA
TG
29


30


CP
V
31

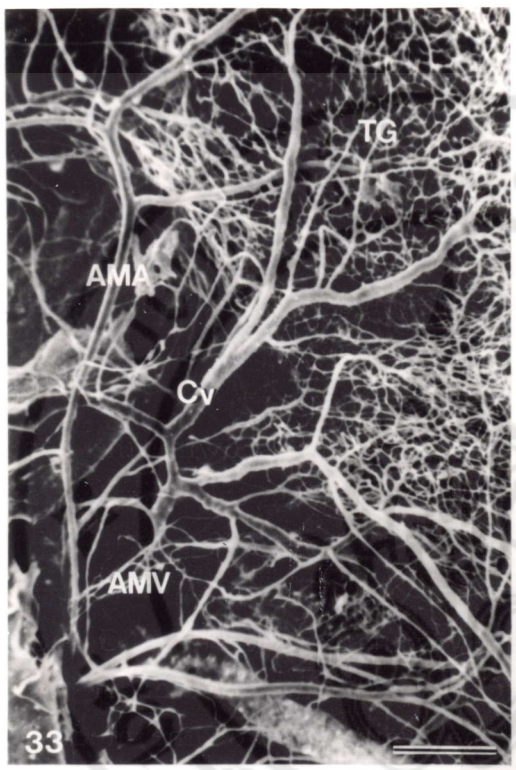

V
V
32

Figure 33. SEM micrograph of the left TG vascular cast showing a venule at the lateral side draining the blood into the collecting venule (Cv) and further into the pterygoid plexus via the accessory meningeal vein (AMV); AMA = accessory meningeal artery. Bar = 200 μm.

Figure 34. SEM micrograph of TG vascular cast showing the venules emptying into the pterygoid plexus via the vein (V) along the maxillary branch of TG. Bar = 200 μm.

Figure 35. SEM micrograph of the vascular cast of left TG showing the collecting venule (CV) on the medial side of the TG draining the blood into the vein (MV) on the medial side of the TG. Bar = 250 μm.

Figure 36. SEM micrograph of the vascular cast of TG showing the vein on the medial side (MV) of the TG directly emptying the blood into cavernous sinus (*). Bar = 100 μm.


TG
AMA
Cv
AMV
33


V
TG


Cv
V
Cv
TG
35

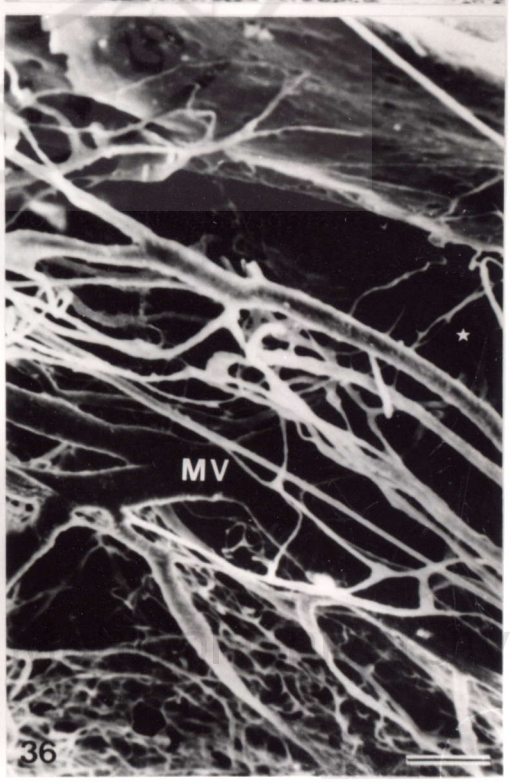
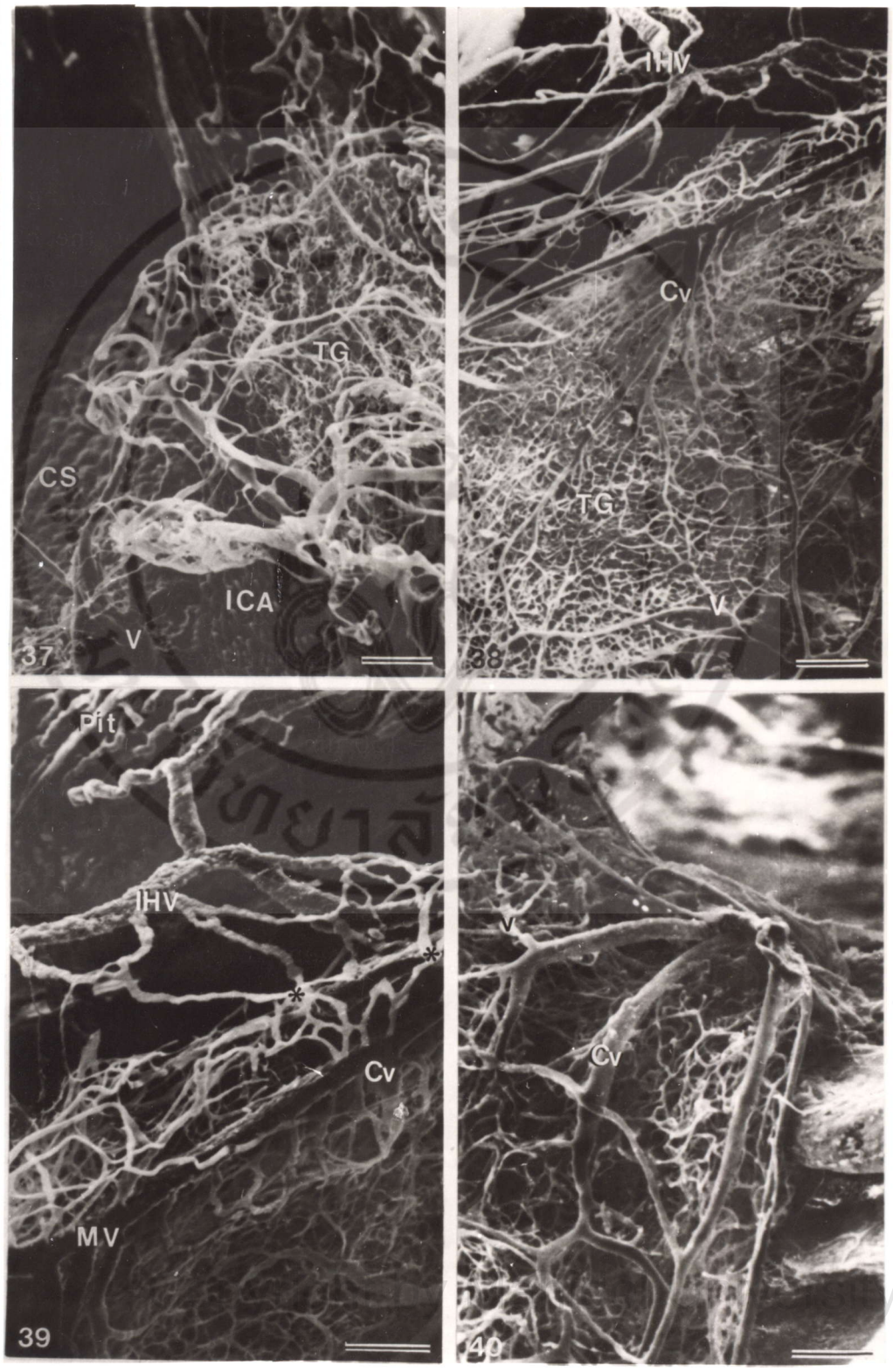

MV
36

Figure 37. SEM micrograph of the vascular cast of TG showing the vein (V) on the medial side of the TG draining the blood into cavernous sinus (CS). ICA = internal carotid artery. Bar = 125 µm.

Figure 38. SEM micrograph of the TG vascular cast showing the collecting venule (CV) on medial side of the TG joining the inferior hypophyseal vein (IHV). MV = medial vein. Bar = 250 µm.

Figure 39. SEM micrograph of the vascular cast of TG illustrating the anastomoses between collecting venule (CV) and inferior hypophyseal vein (IHV); MV = medial vein; Pit = pituitary gland. Bar = 150 µm.

Figure 40. SEM micrograph of the vascular cast of TG showing the collecting venule (CV) at the root of ganglion emptying the blood into posterior part of the cavernous sinus. v, venule. Bar = 100 µm.


IHV
Cv
TG
CS
ICA
V
37
38
Pit
IHV
Cv
MV
39
40

CHAPTER IV

DISCUSSION

The findings of this study indicate that the location and general morphology of the TG in the common tree shrew are similar to those reported in human (Burr and Robinson, 1925), rat (Greene, 1963), and monkey (Gasser, 1971; Ohtsuki, 1984) for the TG situates in the middle cranial fossa between the skull and dura mater just posterolateral and somewhat inferior to the pituitary gland. It connects with the sensory nerve root which emerges from the lateral region of the pons. From the ganglion, the bundles of nerve fibers break up into the ophthalmic, maxillary, and mandibular divisions.

With LM and TEM, it is shown that the common tree shrew TG is surrounded by connective tissue as that in human (Burr and Robinson, 1925). The ganglionic neurons tend to aggregate in the peripheral part, whereas the nerve fibers are oftenly observed in the central region of the ganglion. Such findings are similar to those for the dorsal root ganglion (DRG) earlier reported in common tree shrew (Mankhetwit, 1993) but quite different from the features of the tree shrew superior cervical ganglion or SCG (Samritthong et al., 1992), monkey pterygopalatine ganglion (Wilson, 1984), and of cat ciliary ganglion (Zhang et al.,1993) as their postganglionic autonomic neurons distribute somewhat evenly throughout the ganglia. In addition, the TG consists of large neurons with concentric and prominent nuclei, densely packed rER and with a large amount of mitochondria (Mi) as those found in the DRG of the same animal (Mankhetwit, 1993) and of the ferret (Palmer and Holland, 1988). In contrast, the neurons of SCG in common tree shrew (Samritthong et al., 1992), of the pterygopalatine ganglion of the monkey (Wilson, 1984) and of the ciliary ganglion of the cat (Zhang et al., 1993) are small with eccentric nuclei.

Two types of the neuroglia, the satellite cell and Schwann cells, have also been found in the tree shrew TG as in human (Bruska and Wozniak, 1991; Janpueira et al., 1986; Ross and Romrell,

1989). In the TG, the satellite cells send off the cytoplasmic sheath enclosing the sensory neurons as has been demonstrated in the DRG (Mankhetwit, 1993). The satellite cells are also found covering the postganglionic sympathetic cells in the SC (Samritthong et al., 1992) and postganglionic parasympathetic cells of the pterygopalatine ganglion (Wilson, 1984) and of the ciliary ganglion (Zhang et al., 1993).

The satellite cells in the TG could perform similar function as those in the DRG, SCG and in other nervous tissues that they protect and support the neurons, and aid in the metabolic exchange processes of nerve cell membrane to the initiation and propagation of nerve impulses (Rhodin, 1974). The Schwann cell, however, is well ducumented to be the source of myelin sheath of the peripheral nerve fibers (Rhodin, 1974; Janqueira et al., 1986; Ross and Romrell, 1989).

As has been reported in the SCG, in the DRG of the tree shrew (Chunhabundit and Somana, 1991; Samritthong et al., 1992), and in the TG of the mouse (Wechbanjong, 1988), the mast cell has been also found in the TG of the tree shrew. The role of the mast cell in SCG has been posturated to participate in cathecholamine transportation to the capillary endothelium (Samritthong et al., 1992). The mast cell could usually be found in most of the connective tissues and its secretory granules contain heparin, histamine, and serotonin (Williams et al., 1989). It has been well documented that the cell releases these mediators to alter the capillary permeability (Williams et al., 1989; Samritthong et al., 1992). It is likely, therefore, that the mast cell in the TG could facilitate the molecular exchange between blood and neurons.

With vascular cast/SEM, it is clearly demonstrated that the blood supply of the tree shrew TG are from three sources. They are the ganglionic branch of stapedial artery, the most rostral branch of pontine artery, and the accessory meningeal artery. In the tree shrew, the stapedial artery is a branch of internal carotid artery which gives off the ganglionic branch running across the middle cranial fossa to supply the TG. This is different from what has been reported in human, dog and monkey that the TG receive blood supply directly from a twig branch of internal carotid artery (Bergmann, 1942;

Okuda, 1979; Ohtsuki, 1984). However, the ganglia of human, monkey and tree shrew receive the blood supply through the most rostral branch of pontine artery which is a branch of basilar artery (Bergmann, 1942; Ohtsuki, 1984). This branch is called trigeminal artery (Roger and Donald, 1969). In addition, the accessory meningeal artery which is a branch of maxillary artery entering the middle cranial fossa via foramen ovale to supply the TG in the tree shrew could also be demonstrated in dog, monkey and human TG (Okuda, 1979; Ohtsuki, 1984; Meana and Ballesteros, 1989). With corrosion cast technique, as well as LM and TEM, it is clearly shown that the TG as well as the DRG (Mankhetwit, 1993) contains a lot of blood vessels distributing among the neurons. The dense capillary plexus is also found throughout the SCG of the tree shrew (Chunhabundit et al., 1993) and rat (Chunhabundit et al., 1992). This indicates that both sensory and autonomic ganglia are with high metabolic activity.

In spite of, fenestrated capillaries have been reported in the DRG of rat (Anzil et al., 1976) and of monkey (Yoshizawa et al., 1991), such feature could not be demonstrated in the tree shrew TG nor in the tree shrew DRG (Mankhetwit, 1993). Moreover, the fenestrated capillaries could be shown in the rat SCG (DePace, 1981), but not in the tree shrew SCG (Samritthong et al., 1992). The findings indicate that there are some basic anatomical and functional differences of blood vessels among animal species. In other words, the blood ganglion barrier may be tighter in the tree shrew when compared with the rat.

The intracranial blood supply in the tree shrew are similar to that in the higher mammals and primates (Bugge, 1974a) for it is from internal carotid and vertebral arteries. In tree shrew the stapedial artery branches off from internal carotid artery at the base of the skull. This branch is well developed as seen in hamster, new world mice, voles, squirrel, birch mouse, garden dormouse which are rodents (Bugge, 1970, 1971a, 1971b, 1974a), asiatic black bear which is canivore (Bugge, 1974b), and in insectivores (Bugge, 1974a). In other mammals including higher primates, portion of stapedial system disappears or connects with branches of the internal

and external carotid arteries (Bugge, 1974a). In human, the stapedial artery is under developed and disappears before birth (Diamond, 1987).

The feature of vertebro-basilar system in tree shrew is similar to human for it gives off posterior inferior cerebellar, anterior spinal, anterior inferior cerebellar, labyrinthine, pontine, superior cerebellar, and posterior cerebral arteries (Martin, 1991). The latter formed by the terminal of basilar trunk at its rostral end anastomoses with the caudal branch of the internal carotid artery. In the dog and the cat, however, only 2 cerebellar arteries are present (Gillilan, 1976). They are inferior cerebellar and superior cerebellar arteries. It is obvious that the vertebro-basilar artery system in tree shrew is similar to that of higher primate while the internal carotid system is similar to that of insectivore.

What has been mentioned above is one of many examples that the tree shrew posses dubious features to be accepted as a member of the Order Primates. Thus the animal has been once placed in the Order Insectivora and the Family Tupaidae (Gregory, 1910; Hill, 1953; Grasse, 1955; Findley, 1967; and Lekagul and McNeely, 1977). On the other hand, the animal is classified to be a lower primate (Simpson, 1945; Saban, 1954; Linnaeus, 1958; and Bugge, 1974) for its cephalic arterial pattern is considerably more advanced than that in the insectivores. The pattern of orbital blood supply is different from insectivores that it has internal ophthalmic artery branching from the distal end of internal carotid artery compatible with ophthalmic artery in higher primates.

CHAPTER V

CONCLUSION

The present study reveals that:

1. The trigeminal ganglion (TG) has common features as of other sensory ganglia for:
   - 1.1 It contains predominantly large neurons with concentric and prominent nuclei.
   - 1.2 The neurons aggregate in the peripheral region.
   - 1.3 Each neuron is ensheathed by satellite cells.
2. The blood supply of the TG are from 3 sources:
   - 2.1 From the most rostral branch of pontine artery (trigeminal artery) which enters the TG via the root of trigeminal nerve.
   - 2.2 From the ganglionic branch of stapedial artery and runs across the middle cranial fossa to enter the TG.
   - 2.3 From the accessory meningeal branch of the maxillary artery entering the skull through foramen ovale to supply the TG.
3. There is richer blood supply in the area occupied by neurons than that by nerve fibers.
4. The venous blood from the TG drains into the veins at the borders of the TG:
   - 4.1 The vein on the medial side drains directly into cavernous sinus or to the inferior hypophyseal vein before joining the cavernous sinus.
   - 4.2 The vein on the lateral side drains into accessory meningeal vein or the vein along the maxillary branch of the TG.
   - 4.3 The vein along the trigeminal nerve root joins the posterior part of cavernous sinus.
5. The fenestrated capillaries could not be demonstrated in the TG.

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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 modesta 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	mm
		tail	: 148-198	mm
		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 a 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 fully opposible, well-marke dpads 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 in medial orbital wall, enlarged braincase, advanced form of auditory ossicles
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 trigeminal ganglion 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 temperture) but more soluble in alcohol (about 5%) and benzine (10%). Picric acid precipitates nucleoproteins and causes little shrinkage. In general, this fixative can rapidly penetrate 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

Thionine Staining Method

Fixation

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

Thionine Stain

Thionine	0.5	g
Distilled water	100	cc
Acetic acid	2	drops

Differentiating Solution

Equal parts of absolute alcohol and dioxane (histologic). This solution is stable.

Dissolve the thionine in distilled water and then added acetic acid into solution.

Staining Procedure

1.Xylene, dioxane, distilled water.

2.Stain in thionine solution for 5 minutes.

3.Differentiate in alcohol-dioxane solution until practically colorless. Experience alone will determine the time of optimum differentiation. Never place stained sections in water or alcohol after differentiation as both will remove stain cells.

4.Dioxane, 2 changes.

5.Xylene, 2 changes.

6.Mount in Permount.

Results: Nerve cells - bright blue.
Background - colorless.

Cresyl Echt Violet for Nissl Substance Staining Method

Cresyl echt violet solution

Cresyl echt violet	0.5	g
Distilled water	100	cc

Disslove the cresyl echt violet in distilled water then ripened 24 to 48 hours, thereafter filter it before used.

Staining procedure

1. Xylene, absolute alcohol, 95% alcohol, distilled water.
2. Stain for 3 to 5 minutes in cresyl echt violet solution.
3. Rinse in 2 ghanges of distilled water.
4. Alcohol 95% for 30 seconds.
5. Absolute alcohol for 30 seconds.
6. Xylene , several changes.
7. Mount in permount.

Results: Nissl substance - purple.

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.
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 print is obtained.

The scanning electron micrograph is now ready for evaluation.

APPENDIX VI

Solutions and Embedding Media for TEM

Fixatives

1. Glutaraldehyde solution (2.5%) in phosphate buffer
2. Osmium tetroxide (1%) in phosphate buffer

Buffer Solution pH 7.4

0.2 M Phosphate buffer

Solution A: Monobasic sodium phosphate ($NaH_2PO_4 \cdot H_2O$)
2.76 g in 100 ml H_2O 19 ml

Solution B: Dibasic sodium phosphate ($Na_2HPO_4 \cdot 7H_2O$)
5.66 g in 100 ml H_2O 81 ml

Staining Solutions

1. 5% Uranyl acetate

uranyl acetate	2 g
distilled water	40 ml

2. Lead citrate

NaOH	2 g
Lead citrate	0.25 g
distilled water	50 ml

Embedding Media

Araldite 502	27 ml
DDSA	23 ml
Accelerator DMP-30	1 g