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Microvasculature of the harderian gland in the common tree shrew *(Tupaia glis)*

Somneuk Nilbu-nga✉ , Wisuit Pradidarcheep
Department of Anatomy, Faculty of Medicine, Srinakharinwirot University

Abstract

The blood supply of the Harderian gland in common tree shrew of both sexes was studied by using vascular corrosion cast technique in conjunction with scanning electron microscope. It was found that the gland received the arterial blood from a branch of ophthalmic artery, which gave rise to arterioles located in each glandular lobule. and each arteriole then further branched into capillary plexuses that were organized into lobular appearance. Two capillary types were observed in this Harderian gland, which are small round and large irregular sinusoidal capillaries with fenestrations. The venous blood from this gland first drained into the orbital venous sinus locating between the glandular convex surface and the wall of the orbit, and finally to the ophthalmic vein. The presence of the particular vascularization together with electron dense granules and numerous stubby microvilli at the basal portion of the acinar cells suggest that the Harderian gland in the common tree shrews may possibly involve in endocrine function.
Key words: Harderian gland, Angioarchitecture, Vascular cast, Common tree shrew

Somneuk Nilbu-nga✉
Department of Anatomy, Faculty of Medicine Srinakhrinwirot University
Sukhumvit 23 Rd., Bangkok 10110, Thailand.
Tel. (662) 02-6641000 Ext. 4508 Fax. (662) 02-2601532
Email: chengnil@hotmail.com

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โครงหลอดเลือดละเอียดในต่อมฮาร์เดอร์เรี่ยนของกระแต

สมนึก นิลบุหงา, วิสุทธิ์ ประดิษฐ์อาชีพ
ภาควิชากายวิภาคศาสตร์ คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ

บทคัดย่อ

หลอดเลือดที่มาเลี้ยงต่อมฮาร์เดอร์เรี่ยน (Harderian) ของกระแต (common tree shrew) แสดงโดยใช้เทคนิค vascular corrosion cast ร่วมกับกล้องจุลทรรศน์อิเลคตรอนแบบส่องกราด (SEM) ผลการศึกษาพบว่าต่อมฮาร์เดอร์เรี่ยนของกระแตได้รับเลือดแดงมาจากหลอดเลือดออปทาลมิค (ophthalmic artery) ซึ่งแตกแขนงเป็นหลอดเลือดแดงขนาดเล็กแทรกเข้าไปในแต่ละ lobule และจะแตกแขนงย่อยต่อเป็นหลอดเลือดแดงฝอยแบบมีรูพรุน (fenestrae) ที่ผนัง สามารถแบ่งตามลักษณะและขนาดของหลอดเลือดแดงฝอยออกเป็น 2 ลักษณะ คือ ชนิดที่เป็นท่อกลมขนาดเล็กและชนิดที่รูปร่างไม่แน่นอนขนาดใหญ่ ซึ่งหลอดเลือดแดงฝอยทั้งสองลักษณะดังกล่าวก่อรูปเป็นโครงร่างแหตามรูปร่างของหน่วยสร้างสารของต่อมฮาร์เดอร์เรี่ยน สำหรับเลือดดำจากต่อมฮาร์เดอร์เรี่ยนจะไหลไปยังแอ่งเลือดดำที่อยู่บริเวณตาและไหลต่อไปยังหลอดเลือดดำออปทาลมิค (ophthalmic vein) จากลักษณะที่มีหลอดเลือดแดงฝอยชนิดที่เป็นรูพรุนจำนวนมากร่วมกับโครงสร้างของเซลล์ที่มีแกรนูลและกลุ่มของไมโครวิลไลที่ฐานล่าง จึงเป็นไปได้ว่า ต่อมฮาร์เดอร์เรี่ยนของกระแตอาจจะทำหน้าที่เกี่ยวกับการสร้างฮอร์โมน หรือสารบางชนิดเพื่อส่งเข้าไปยังหลอดเลือดแดง

Introduction

The Harderian gland was first discovered by Jacob Harder[1] in 1694. It was described as a large structure located inferomedially of orbital cavity and was found in most terrestrial vertebrates. The gland is well developed in rodents[2,3] for example, rat, mouse, hamster and guinea pig and also rabbit[4,5] but not in fish, bat, cow, horse, terrestrial carnivores and higher primates.[6] In adult human, the Harderian gland was absent, but not in fetus and neonate.[7-9] The secretions of mammalian Harderian gland are primarily composed of lipid, porphyrin, melatonin and inoleamine[10-13] which function in exocrine and endocrine manners. It had been proposed that the lubrication of the nictitans and cornea were considered to be the primary function of the gland. However, the actual function of this gland is still poorly understood. Even though several studies regarding Harderian gland morphology had been carried out in various species,[14-21] the detailed information concerning its blood supply is very limited. In birds, arterial blood supply of the Harderian gland comes from ophthalmotemporal branch of the external ophthalmic artery[22,23] whereas in rats, it comes from the inferior branch of the ophthalmic artery.[24] The characteristic of fenestration has been shown in the capillaries of the gland in armadillo,[25] mouse,[26] woodmouse[27] and golden hamster.[28] Its venous drainage was either via ophthalmic vein,[23] orbital venous plexus[29] or orbital venous sinus.[20,30]

Three-dimensional study of the vascular pattern in the Harderian gland by the corrosion cast technique in conjunction with scanning electron microscope had not previously been performed in any animal including the common tree shrew which was considered to be a primitive primate.[31,32] This study aimed to carry out two-and three-dimensional analyses of the vascular pattern of the Harderian gland in this animal.

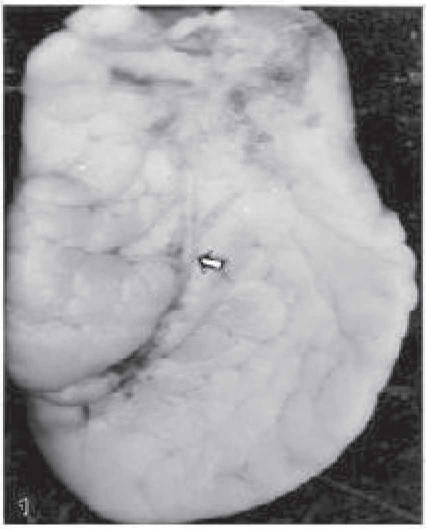
Materials and Methods

Ten common tree shrews *(Tupaia glis)*, five of each sex, weighing between 120-180 g were used. The preparation of the animals, the injection of Batson's #17 plastic mixture (casting medium) and the preparation of the Harderian gland vascular casts were performed according to the method previously described.[33,34] Each vascular cast of the Harderian gland was air-dried, adhered on a brass stub with silver paint, and coated with gold/palladium prior to being examined and photographed under a scanning electron microscope (SEM) at the accelerating voltage of 30 kV. For investigating by transmission electron microscopy (TEM), the Harderian glands from three females and two males were fixed by perfusion through the ascending aorta with 2.5% glutaraldehyde in phosphate buffer, pH 7.4 and left overnight in the same fixative agent at 4°C. The samples were then postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, infiltrated and embedded in Araldite 502 resin. Semithin sections (1μm) were stained with toluidine blue and examined by light microscopy (LM). Thin sections (70-100 nm) were stained with 1% uranyl acetate followed by lead citrate and examined under a TEM at an accelerating voltage of 75 kV.

Results

The Harderian gland of *Tupaia glis* appeared to receive blood supply from a branch of ophthalmic artery (Fig. 1). The average diameter of the artery was 0.1 mm. The vascular corrosion cast technique in combination with SEM at low magnification revealed that the Harderian gland of common tree shrew was highly vascularized. The morphology of the cast conforms to the appearance of the Harderian gland (Fig. 2). When approaching the gland from the posterior end, the artery ran in the middle sulcus along the whole length of the gland (Figs. 1, 3). This sulcus was also observed on the glandular surface adjacent to eyeball and eye muscles. While running through the sulcus, the artery gave off many smaller branches, which almost ran in perpendicular to the main stem (Fig. 3). Each of these small branches, thereafter, gave rise to 1-2 arteriole(s) to end in each glandular lobule (Fig. 4). Each arteriole further divided into plexus of true capillaries (5-7μm) as well as sinusoidal capillaries (10-18 μm) (Figs. 5, 6) being organized into lobular appearance (Fig. 7). It was observed that some of the capillaries display blind ends characteristic while others continue to form the small venules (5-8 μm). These venules collected the blood into large venules (10-14 μm) which then merged into collecting vein (17-22 μm) (Fig. 8). Approximately 3-4 collecting veins were found in each lobule and they were quite flat and ran in parallel to the surface of the gland which then emptied the blood into orbital venous sinus (Fig. 8). It was also observed that the venous blood in some venules at the glandular surface in adjacent to the orbital bones flowed directly into the orbital venous sinus (Fig. 9). This venous sinus was a broad and flat vascular space, and was seen only at the glandular surface contacting to the orbital wall (Figs. 9, 10). Moreover, the venous blood from the deep intraglandular portion drained into the collecting vein which projected directly into orbital venous sinus (Fig. 10). There were approximately 5-7 of such collecting veins in one gland.

The Harderian gland of the common tree shrew, particularly at the convex surface, was covered with the endothelial cells of the orbital venous sinus (Fig. 11).



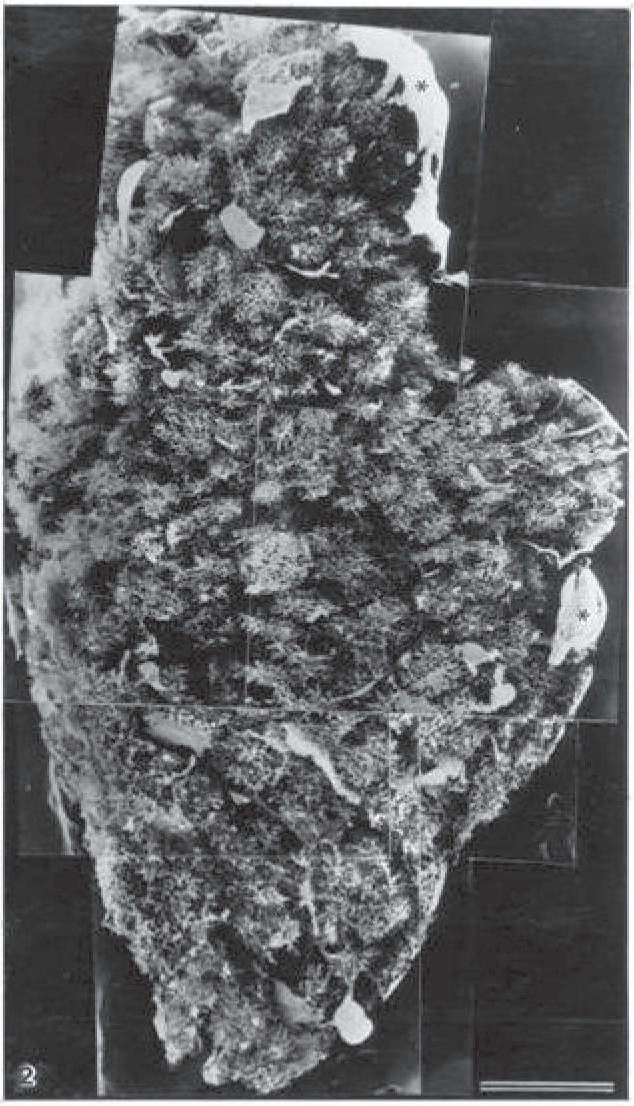


Figure 1. Picture taken from stereomicroscope of left common tree shrew Harderian gland. This surface is adjacent to the eye sclera. Note a branch of ophthalmic artery in the middle of the gland (arrow) and the glandular lobulations. x10.

Figure 2. SEM micrograph, at low magnification, of the whole vascular cast of the common tree shrew Harderian gland at convex side. This surface is covered with orbital venous sinus (stars). The cast of orbital venous sinus, in this case, is not completely filled with plastic mixture. Bar, 1 mm.

Figure 3. SEM micrograph showing a branch of ophthalmic artery (A) running along the sulcus on the concave surface of the common tree shrew Harderian gland. The artery branches in to many small arteries (stars). Bar, 500 µm.

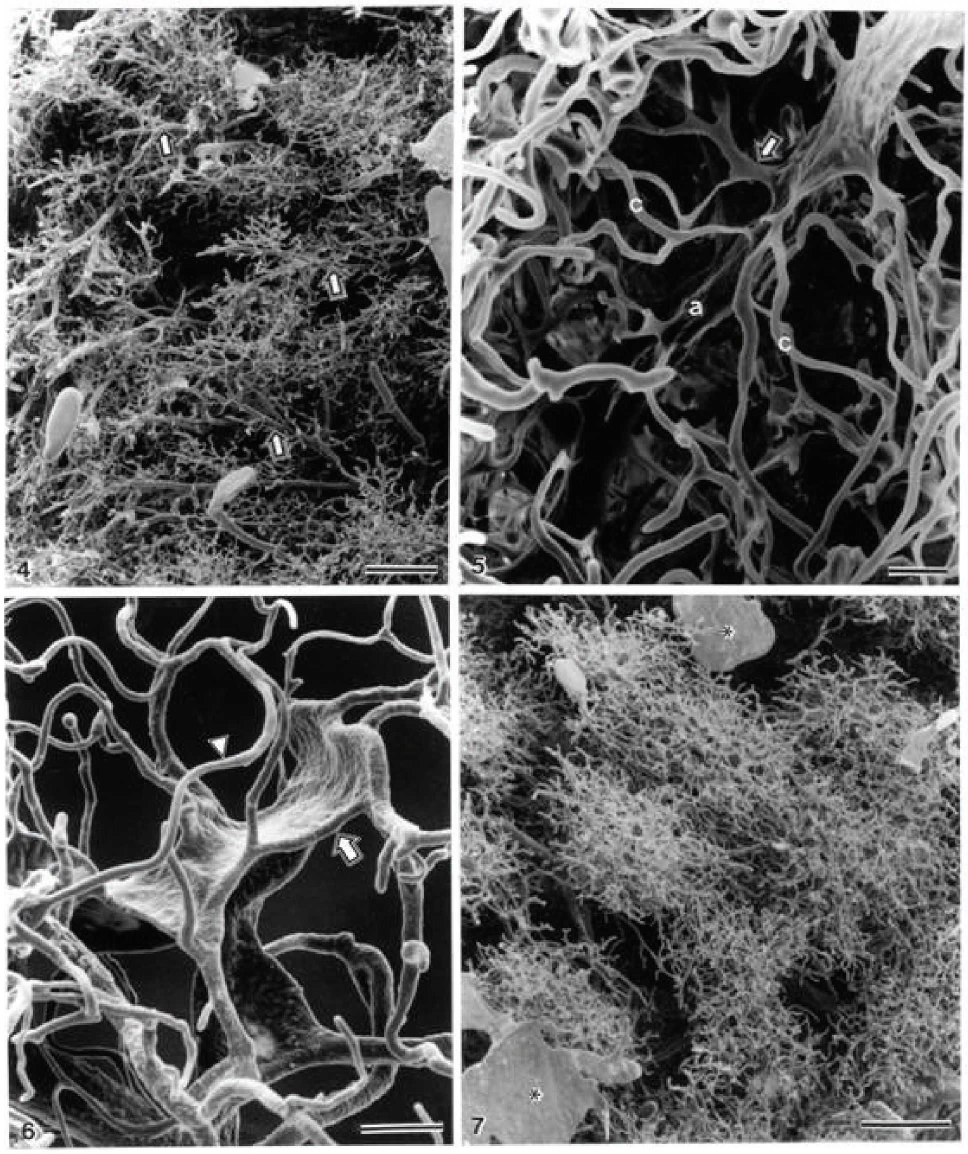


Figure 4. SEM micrograph of the vascular cast at convex surface of the common tree shrew Harderian gland after partial injection with plastic mixture, showing arterioles (arrows) branching into capillaries in each lobule. Bar, 200 μm.

Figure 5. SEM micrograph of an arteriole (a) branching into capillaries (c) and sinusoid (arrow) in the glandular lobule. Bar, 100 μm.

Figure 6. SEM micrograph demonstrating the casts of capillaries (arrowhead) and sinusoids (arrow) in the common tree shrew Harderian gland obtained from the razor cut specimen. Bar, 50 μm.

Figure 7. SEM micrograph showing the lobular organization of the capillaries in the common tree shrew Harderian gland at convex surface. Asterisk, part of the cast of the orbital venous sinus. Bar, 200 μm.

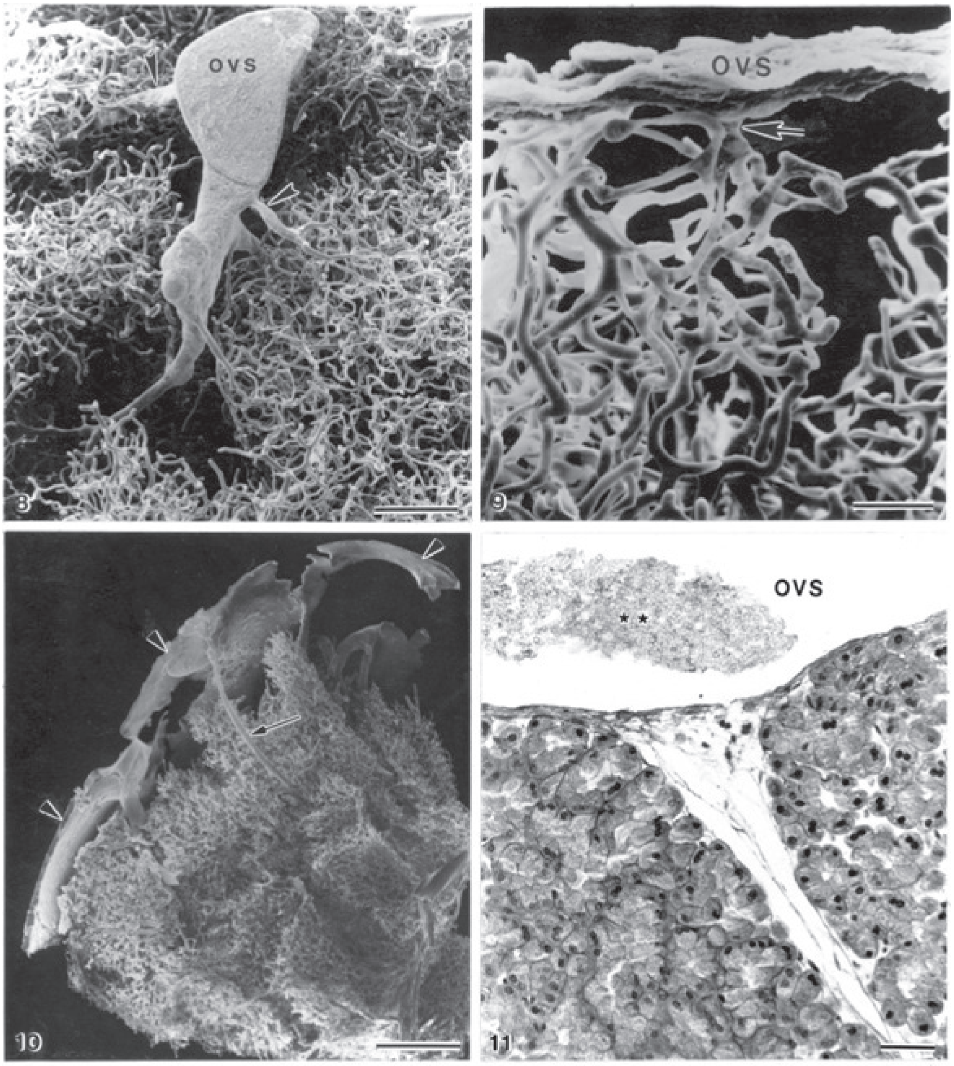


Figure 8. SEM micrograph illustrating the collecting veins (arrowheads) converge into the orbital venous sinus (OVS) at the glandular convex surface of the common tree shrew Harderian gland. Bar, 120 μm.

Figure 9. SEM micrograph showing the venule (arrow) of some lobule drain the blood directly into the orbital venous sinus (OVS) of the common tree shrew Harderian gland. Bar, 25 μm.

Figure 10. SEM micrograph, at low magnification, demonstrating an intraglandular vein (arrow) empties the blood into the orbital venous sinus (arrowheads) of the gland. Bar, 500 μm.

Figure 11. Light micrograph of thick section with H&E staining showing the orbital venous sinus (OVS) filled with blood cells (stars) covering the convex surface of the Harderian gland. X10.

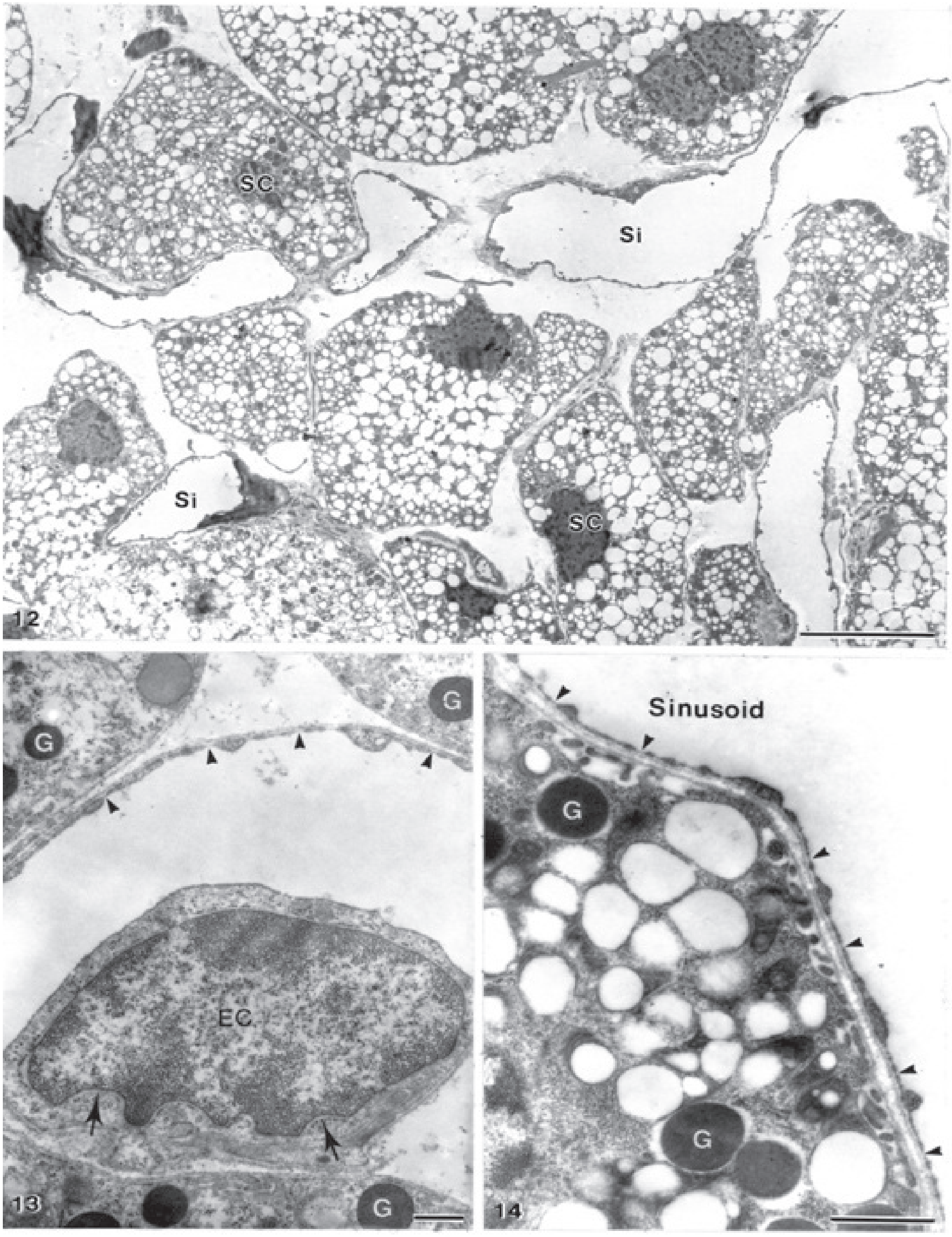


Figure 12. TEM micrograph, at low magnification, showing several fenestrated microvessels in the interstitium of the male common tree shrew Harderian gland. Note the close proximity between the sinusoids (Si) and the basal portion of secretory cells (SC). Bar, 10 μm.

Figure 13, 14. TEM micrographs, at high magnification, of the male common tree shrew Harderian gland illustrating the capillary (Figure 13) and sinusoid (Figure 14) with fenestrations (arrowheads). Endothelial cells (EC) are usually seen with indentations (arrows) at the basal portion. Stubby microvilli at the basal portion of the secretory cell. Bar, 1 μm.

TEM micrograph showed that the gland had profused vascularization (Fig. 12). The blood vessels in the gland were of particular interest as not only small round capillaries (5-7 μm), but also large sinusoidal capillaries (10-18 μm) were found in various shapes (Fig. 12). These two types of capillaries were seen with fenestration (Figs. 13, 14) all over the organ. However, the population of large sinusoidal capillaries was more numerous than small round capillaries.

The capillary and sinusoidal walls were lined with an extremely flattened endothelium (Fig. 12). Frequently, the irregular sinusoidal shape adapts to the interstitial space among secretory units (Fig. 12). Those sinusoidal vessels, were located in close proximity to the acinar cells and often wedge into the basolateral portion of the cells (Fig. 12). The secretory cells contained several small electron dense granules and numerous stubby microvilli at the basolateral portion (Figs. 13, 14).

The endothelial nuclei were normally flat with minimal protrusion into the capillary lumen. The nuclear contour was relatively smooth with occasional indentations (Fig. 13). Capillary endothelial nuclei were surrounded by a small amount of cytoplasm displaying few organelles. The features of cytoplasmic expansions of the capillary endothelial cells were similar to those observed in the most endothelia. The endothelial cell junctions could be observed and seemed to be of normal morphology. The capillary endothelium usually displayed pores intervening with thin diaphragms (Figs. 13, 14). Perforations similar to those in the hepatic sinusoids were rarely observed. It was worth noted that there was no remarkable sexual dimorphism in angioarchitecture of the common tree shrew Harderian gland.

Discussions

The Harderian gland of the common tree shew receives blood supply from a branch of ophthalmic artery similar to that of sparrow,[23] rat[24] and pig.[20] In order to illustrate the three-dimensional macro and microangioarchitectures of the Harderian gland, vascular corrosion cast technique in conjunction with SEM were used in this study. With these techniques, the potassium hydroxide (KOH) is required for tissue maceration. However, as the Harderian gland tissue is composed mostly of lipid, using only KOH alone is not quite effective in tissue digestion. Therefore, a mixture of 40% KOH and 15% triton X-100 at 2:1 ratio were used instead.

Two types of capillaries were found in the Harderian gland of the common tree shrew. They were small round and sinusoidal capillaries with fenestrations. The existence of fenestrated capillaries has been previously described in the Harderian glands of nine-banded armadillo,[25] mouse,[3,26,35] Sprague-Dawley rat,[21,36] wood-mouse[27] and guinea pig.[3] Sinusoidal vessels with fenestrations similar to those found in the common tree shrew have also been documented in the Harderian glands of mouse,[26] rat[36] and hamster.[28] However, the sinusoidal vessels in the Harderian gland of the rat, mouse and hamster appear to be less dense and smaller than those of the common tree shrew when compared with literatures. In this study, the high degree of development of vascular network and the large numbers of fenestrations of the capillaries in the interstitium of the common tree shrew Harderian gland are noteworthy. Although the precise role of sinusoids and capillaries with fenestrations found in Harderian gland of the common tree shrew are still unknown, these vessels have been generally considered as morphological evidence of an increased transport rate.[13,37] The fenestrated vessels are commonly found in endocrine organs and the liver.[37,38] Therefore, the presence of fenestrated capillaries/ sinusoids together with the morphological features of the basal portion of the epithelial cells and the presence of stubby basal microvilli of the common tree shrew Harderian gland suggests the possibility this gland to involve in endocrine function. In other words, it suggests that there is an active transfer between the bloodstream and gland cells even though the direction of the flow was still unknown.

The venous blood of the common tree shrew Harderian gland drains into the orbital venous sinus similar to that of mice[30] , whereas in the case of birds[23] and rats[29] or piglets[39], it is drained via ophthalmic veins and orbital venous plexus, respectively. Because of the difference in venous drainage, drawing the blood from a mouse through the orbital venous sinus is more practical than that of a rat. The differences between orbital venous sinus and orbital venous plexus are still controversy, especially in terms of function.

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