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MICROVASCULAR SYSTEM OF THE HUMAN FETAL INNER EAR: A SCANNING ELECTRON MICROSCOPIC STUDY OF CORROSION CASTS

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Abstract

The vascular system of the inner ear was investigated in 18-21 weeks old human fetuses, using the corrosion cast technique in scanning electron microscopy. At that developmental stage, vascularization of the cochlea and semicircular canals shows a pattern very similar to that described for adults. The most important differences which can be regarded as fetal features include: (1) denser limbus vessels, (2) the marginal vessels of the spiral lamina appearing as irregular network which shows a less clear arcade-like arrangement, (3) some radiating arterioles of the spiral lamina and marginal vessels possessing connections with the vascular system of the external wall, and (4) a dense, sinusoidal network of draining venules at scala tympani. These features apparently disappear during the final remodelling of the inner ear microvasculature in the last trimester.

Key Words: Microvasculature, Corrosion Casting, Fetal Inner Ear, Cochlea, Vestibule, Semicircular Canal, Scanning Electron Microscopy.

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Introduction

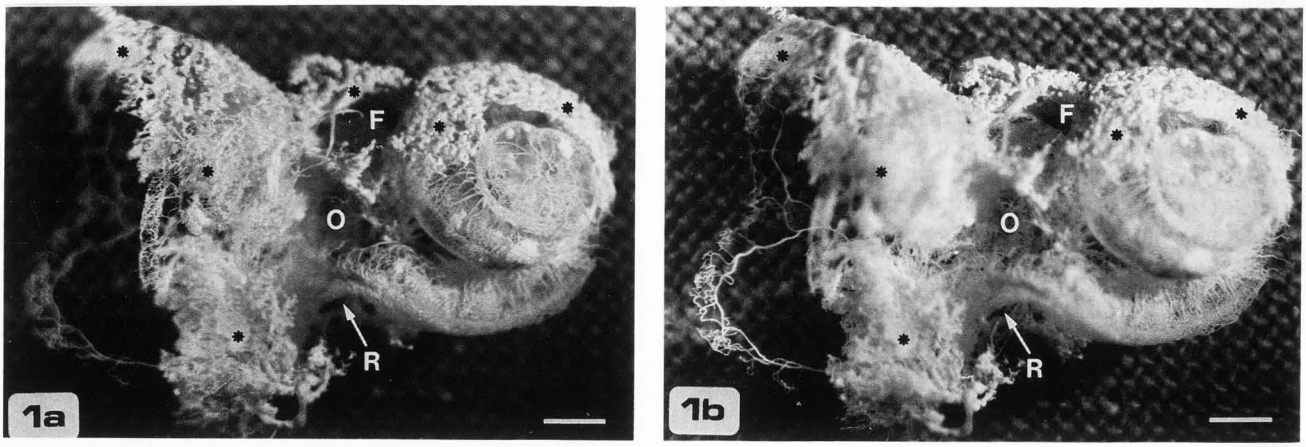
The vascular system of the inner ear plays a crucial role in the function of this sensory organ by providing metabolites to the surrounding tissue as well as ions and other constituents of cochlear fluids. Its disturbance can be involved in the pathogenesis of hearing loss, vertigo and balance disorders (Axelsson and Ryan, 1988). Although the location and minute size of the system make it poorly accessible and technically difficult to study, the vascularization of the inner ear was investigated as early as 1887 (Schwalbe, 1887). Until recently, contrast injection methods combined with light microscopy of thick sections were used to visualize the vascular network of the cochlea in several mammalian species including man (Axelsson, 1968; Axelsson and Ryan, 1988). These methods allow to study the spatial conception of cochlear vasculature, but do not provide a truly three-dimensional image. Furthermore, it is not possible to precisely visualize details due to the limited resolution of the light microscope. The use of vascular corrosion casts examined by scanning electron microscopy (SEM) allows to achieve both goals, and is a major breakthrough in this field. So far, the vascular system of the inner ear has been investigated using that method in rats (Miodoński *et al.*, 1978), mice (Hoshino and Ishioka, 1982), guinea pigs (Nakai *et al.*, 1986) and gerbils (Tange and Wijburg, 1986).

This is the first SEM study of human fetal material. We used the corrosion cast technique to examine the inner ear vasculature in 18-21 week fetuses.

Materials and Methods

Five human fetuses (2 male, 3 female) aged 18-21 gestational weeks, with crown-rump lengths ranging from 170 mm to 209 mm were obtained after spontaneous abortions from the Obstetric Clinic of the Medical Academy in Cracow.

After the abortion, the thorax of each fetus was opened to expose the heart and large vessels. The heart apex was cut off and a cannula was inserted via the left ventricle into the aorta and held in place by a ligation at the level of the ascending part. The vascular system of



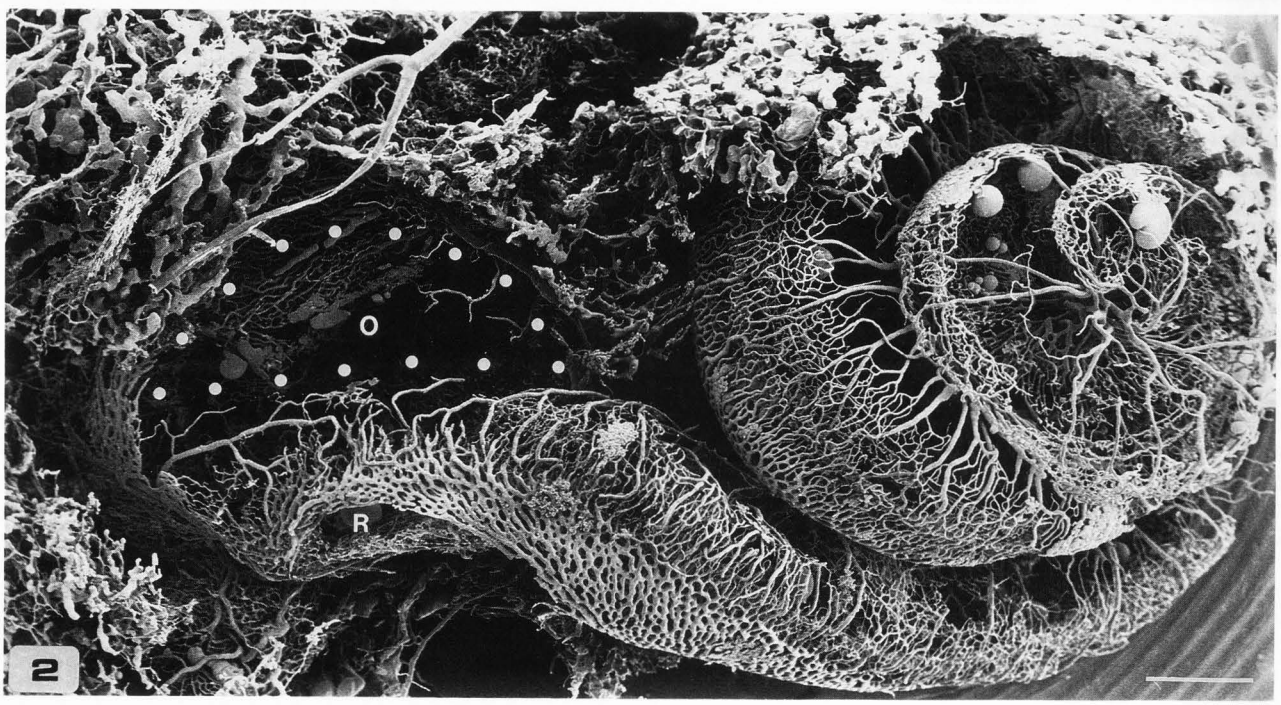


Figure 1. Low magnification light micrographs of the human fetal inner ear vascular cast preparation. The two photographs (**a, b**) were made at different focal depths in order to visualize the vessels of cochlea and semicircular ducts. R: round window; O: oval window; F: facial nerve canal; *: vessels of the partially removed ossified otic capsule. Bar = 1 cm

Figure 2. Low magnification scanning electron micrograph of the human fetal cochlea including its basal end. R: round window; O: oval window. Bar = 1000 μm.

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the fetus was subsequently perfused manually by a sequence of solutions, with the efflux drained via the umbilical veins and incised posterior tibial vessels.

The perfusion started with 800 ml of prewarmed (37°C) heparinized saline (30 I.U./ml) containing 3% Dextran, molecular weight (M.W.) 70,000 (Gannon, 1978) and 0.025% Lidocaine. Fixation was carried out with 200 ml of 0.08% glutaraldehyde in 0.15 M cacodylate buffer, pH 7.4, at 37°C (Paine and Low, 1975). Finally, 60 ml of casting medium consisting of 8 ml Mercox CL-2B (Vilene Comp., Tokyo) and 2 ml methylmethacrylate (Fluka) containing 0.2 g initiator, was injected. Following the injection, the fetuses were kept overnight in water at 60°C in order to accelerate and complete resin polymerization (Miodoński *et al.*, 1981; Lametschwandtner *et al.*, 1990).

After polymerization of the resin, the temporal bones were removed and decalcified in several passages

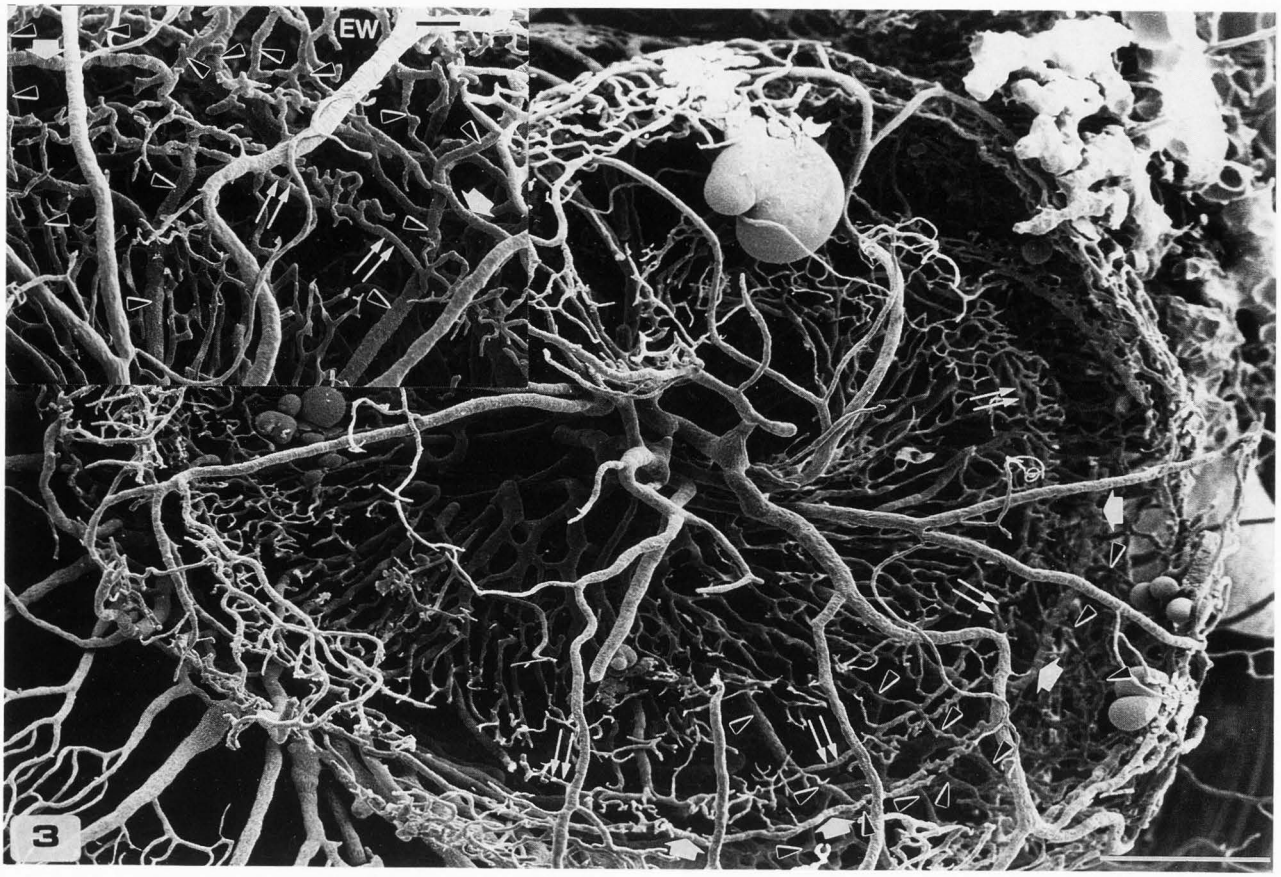


Figure 3. The apical turn of the cochlea, showing the vasculature of the spiral lamina (arrows: the vessel of the basilar membrane; double arrows: the vessel of the tympanic lip). Note connections between the vessel of the basilar membrane, as well as radiating arterioles of the spiral lamina supplying it, with the vessels of scala tympani (arrowheads). Large ball-like structures are artifactual extravasations of resin. Bar = 500 μm. Inset: peripheral region of spiral lamina showing the presence of connections between its vessels and the vessels of the external wall (arrowheads). EW: external wall. Bar = 100 μm.

of 3%-5% trichloroacetic acid followed by rinsing in distilled water. Then the specimens were macerated in 8-10% NaOH at 38°C. The resulting vascular casts were carefully and thoroughly cleaned in hot tap water followed by several rinses in distilled water. The final cleaning was accomplished by immersing the casts for 3-5 minutes in 2% formic acid. The casts were again carefully washed in distilled water, frozen in the latter, freeze-dried and examined under Olympus SMZ stereomicroscope. Afterwards, they were mounted onto specimen stubs using colloidal silver and "conductive bridges" (Lametschwandtner *et al.*, 1980), coated with gold and examined in a JEOL JSM 35-CF scanning electron microscope operated at 20-25 kV.

Results

Light microscopic examination of the vascular casts revealed that the vasculature of the cochlea and semicircular canals had been well preserved and the vessels of the ossified otic capsule had been partially retained (Figs. 1a,b). As shown at a low magnification of SEM, cochlear development reached two and three-fourth turns and its general vascular pattern corresponded to that described in adult humans. The radiating arterioles ran apicobasally to the extensively developed vascular network of the external wall, showing a typical cascade-like arrangement (Fig. 2).

In the spiral lamina, radiating arterioles supply a relatively dense capillary network of the limbus as well as well developed vessels of the tympanic lip and of the basilar membrane, commonly termed marginal vessels (Fig. 3). The branches of the radiating arterioles and marginal vessels, especially of the basilar membrane (VSBM), have several connections with the collecting venules of the external wall (Fig. 3, inset).

Higher magnification revealed the main constituents of the external wall microvasculature: capillary network of scala vestibuli, partially drained by its own collecting venules, vessels of stria vascularis and the draining system of collecting venules at scala tympani. The

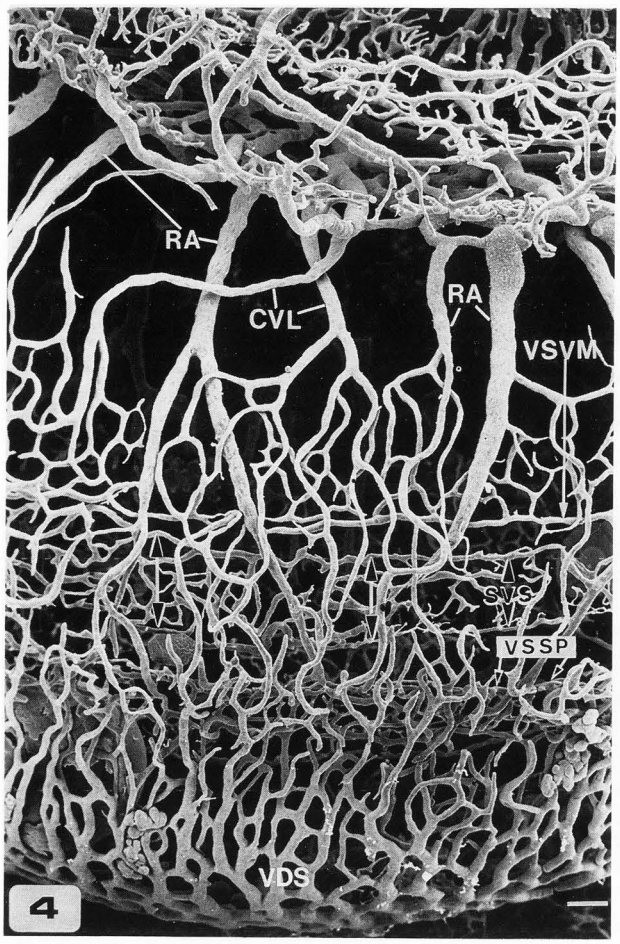


Figure 4. The external wall of the cochlear middle turn with its cascade-like arrangement of blood vessels. RA: radiating arterioles; CVL: collecting venules of scala vestibuli; SVS: vessels of stria vascularis; VDS: venous draining system of scala tympani; VSSP: vessel of spiral prominence (black arrows); VSVM: vessel of vestibular membrane. Bar = 100 μm.

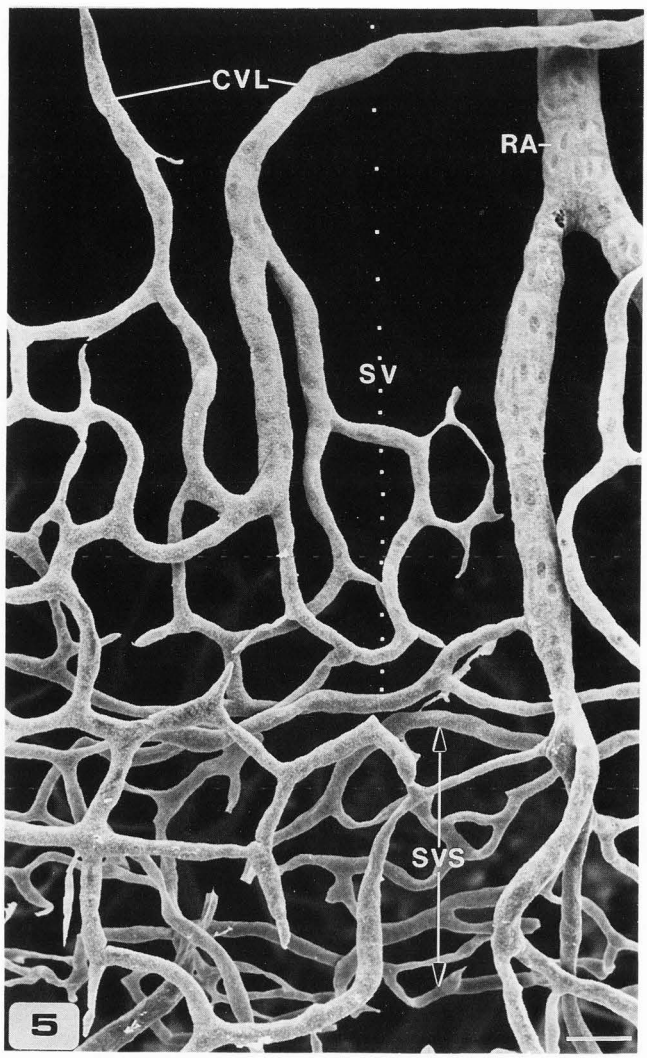


Figure 5. The vascular network of scala vestibuli (SV) and stria vascularis (SVS). RA: radiating arteriole; CVL: collecting venules. Dots mark the zone of scala vestibuli. Bar = 50 μm.

vessels of the vestibular membrane and spiral prominence could also be distinguished (Figs. 4, 5). The radiating arterioles were sometimes seen to communicate directly with the venous draining system of scala tympani via arterio-venous anastomoses (Figs. 4, 6). The latter draining system showed some unusual features: the vessels had a sinusoidal character and formed a dense network with circular or elongated meshes (Fig. 7). The venules at the basilar membrane which directly drained that sinusoidal network were moderately developed (Fig. 8).

At the basal end of cochlea (Fig. 9), in the region between the oval window and round window the radiating arterioles from the vestibular branch of the vestibulo-cochlear artery met their counterparts running from the opposite direction and together produced a small capillary network at the level of scala vestibuli (Fig. 10). The capillaries of stria vascularis and the venous draining system became gradually narrower but retained their network character. The latter emptied into several larger veins that had not yet formed the vein of the round window, as well as with venular branches which joined the posterior vestibular vein. The vascular network of utricle could be seen at the bottom of the oval window (Fig. 9).

The vasculature of all semicircular ducts showed a similar pattern: a long arterial branch was observed to run along the concave side of the endosteal wall, sending off several short twigs that supplied a relatively dense capillary network of the membranous ducts (Figs. 1, 11, 12). Two such branches entered each duct from both ends, meeting and anastomosing near its summit. At the site of entry, the arteries supplied the ampullar part of the duct, however, the vascular network of this area was

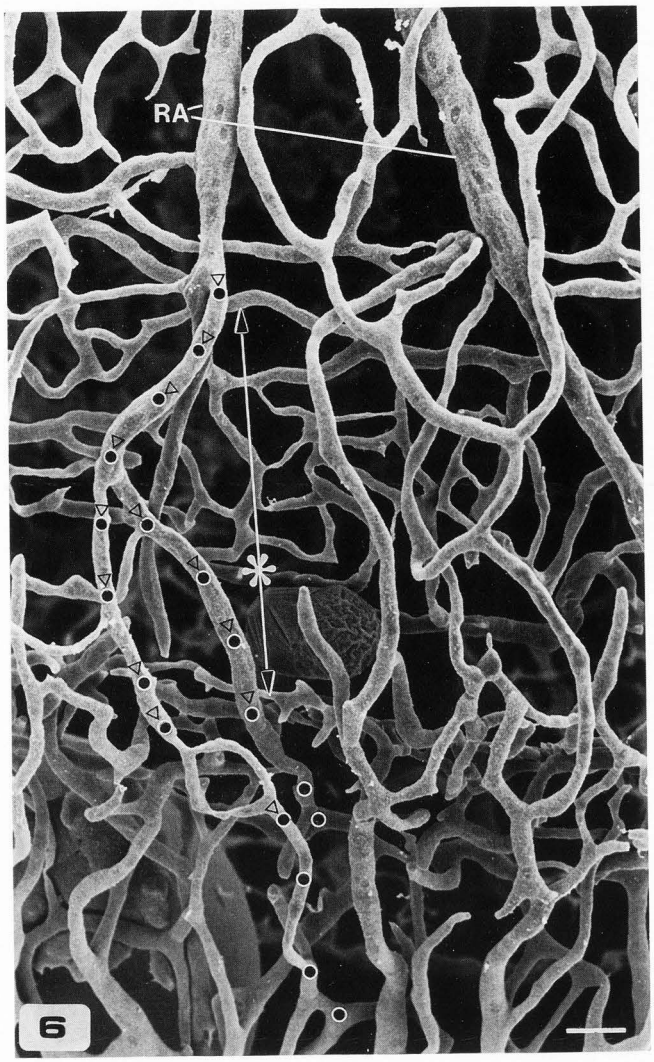
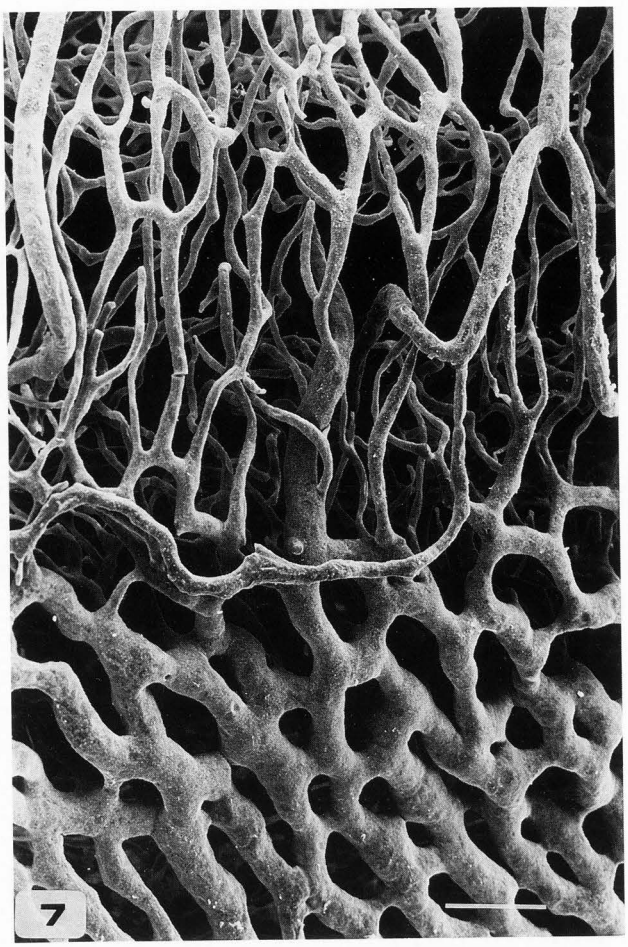


Figure 6. A fragment of the external wall (middle turn) showing the vascular network of stria vascularis. RA: radiating arterioles; dotted: arteriovenous anastomosis; rosette: stria vascularis. Note typical arterial nuclear imprints on the surfaces of RA casts. Bar = 50 μm.

covered by the vessels of the otic capsule and hence poorly visible. In spite of a close vicinity of both systems, there were no connections between the ampullar and capsular vessels. The vascularization of the concave side of the endosteal wall was rather poor. The capillary network of semicircular canal was drained by a long vein accompanying the respective arterial branch at that wall (Fig. 13). In such pair of vessels (as well as in other large vessels observed in the entire cochlear cast), the vessel type could easily be recognized according to the pattern of nuclear imprints (Fig. 13, inset).

Discussion

The microvascular system of the adult human cochlea was described in detail in the classic studies of



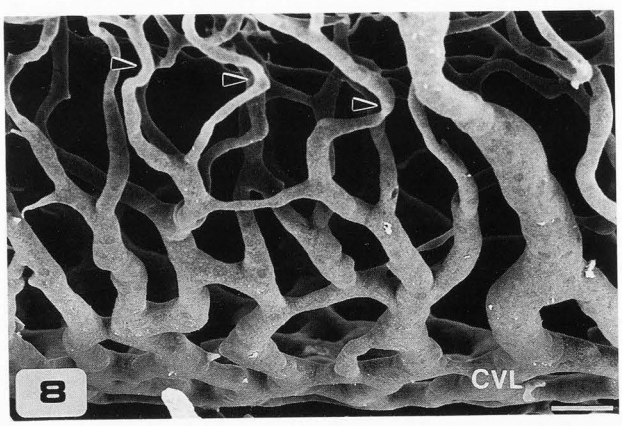
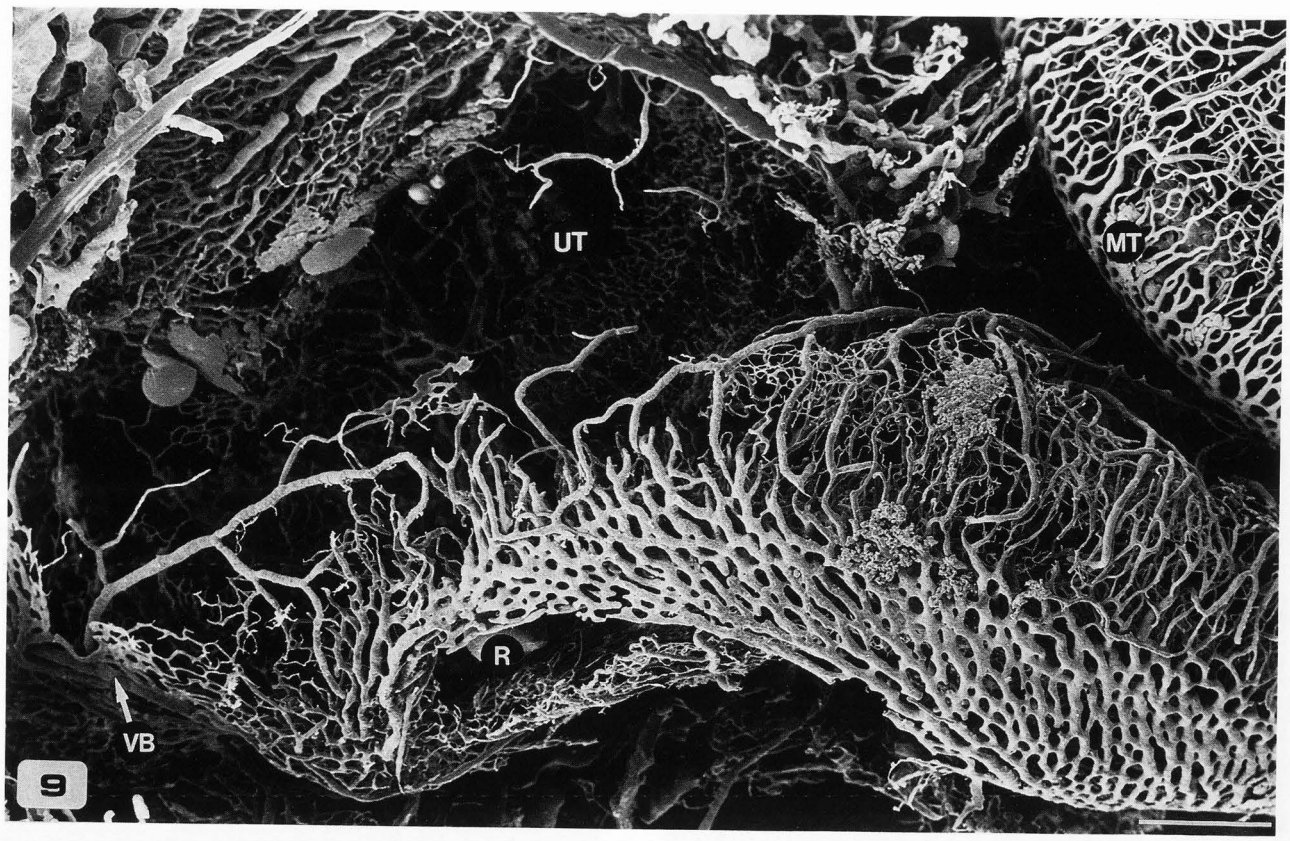


Figure 7. Cochlear external wall. A transition between the capillaries of the stria vascularis and the venules of the draining system, forming a dense network, can be seen. Bar = 100 μm.

Figure 8. A close-up view of the basal turn, bottom region of the external wall. Arrowheads: venules at the basilar membrane; CVL: collecting venules. Bar = 50 μm.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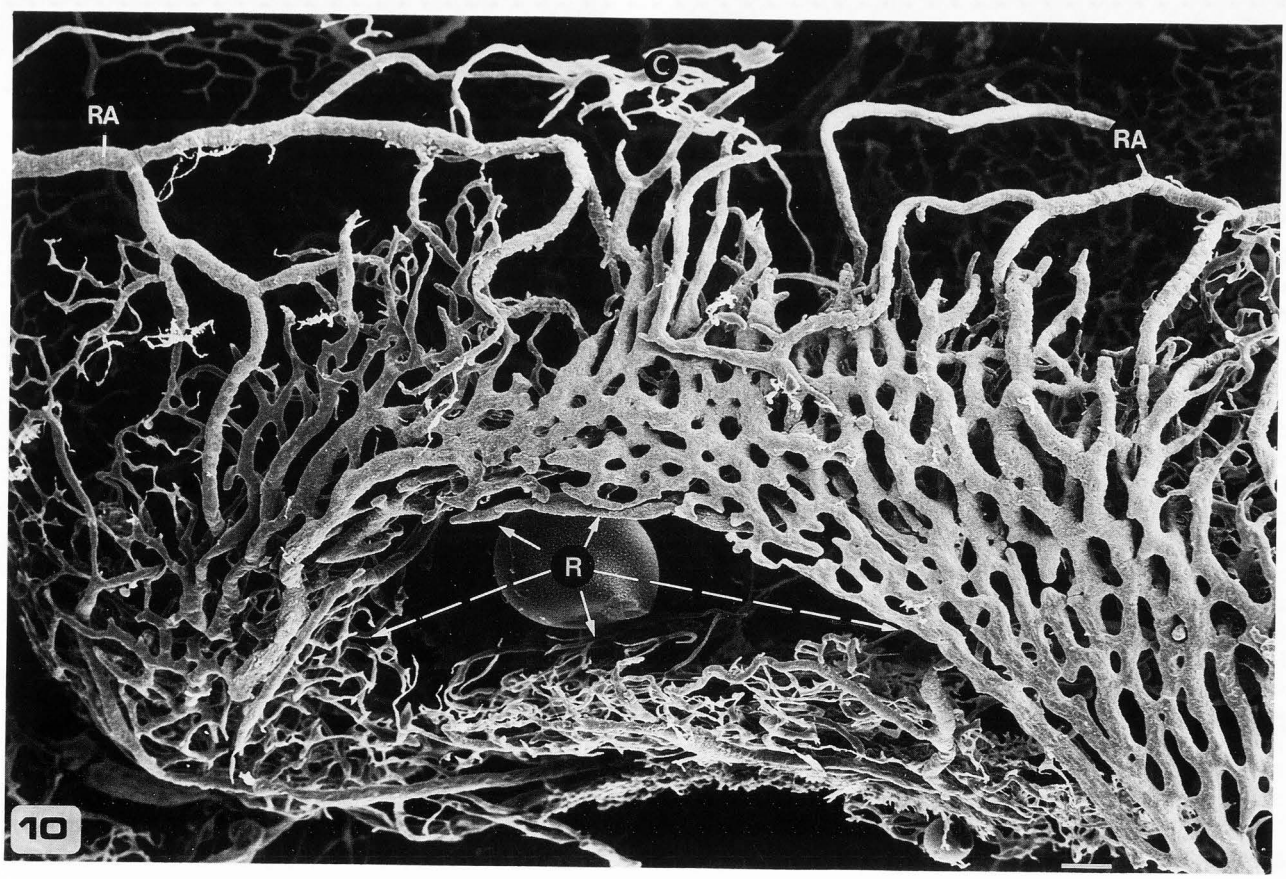
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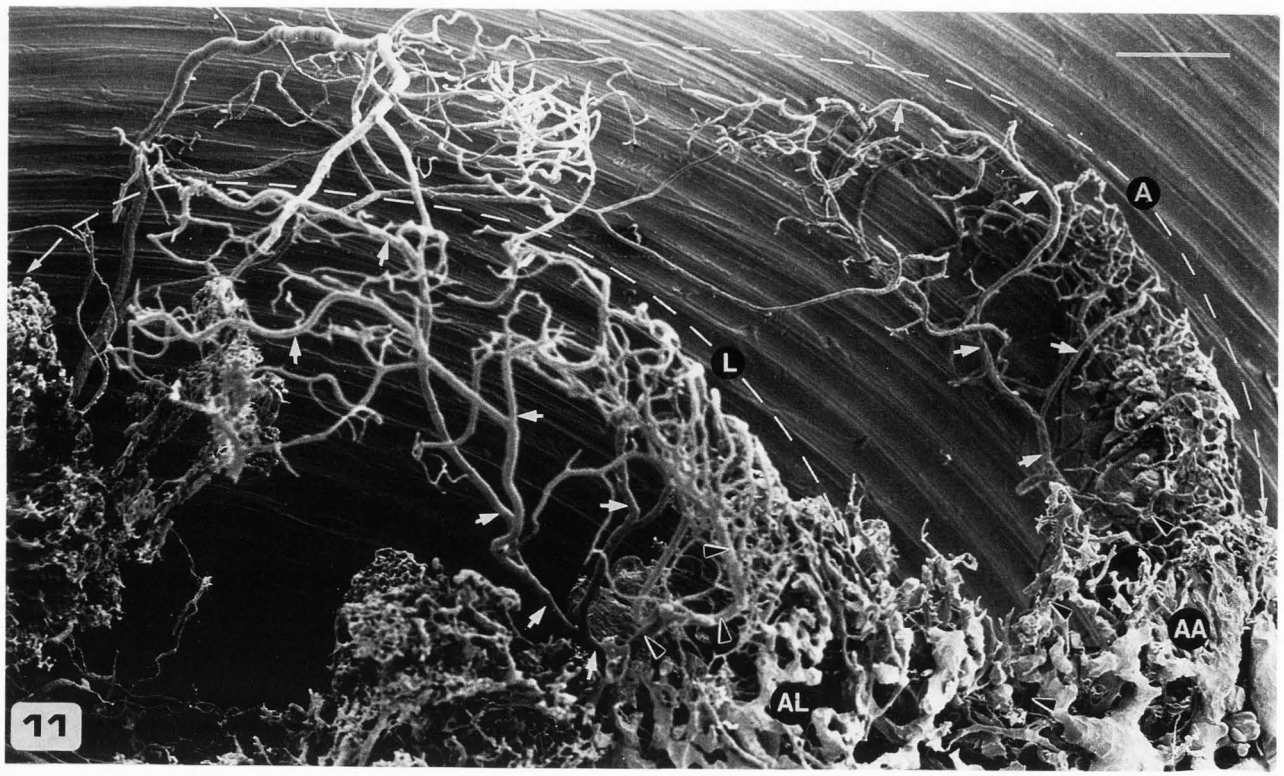


Figure 11 (above). The vasculature of the anterior (A) and lateral (L) semicircular ducts as well as endosteal canals. The capillary bed is supplied by branches of the vestibulo-cochlear artery (arrows) and drained by veins (arrowheads) that escape into the vein of vestibular aqueduct. The vasculature of the ampullar parts (AA, AL) is masked by the vessels of bony otic capsule. Bar = 500 μm.

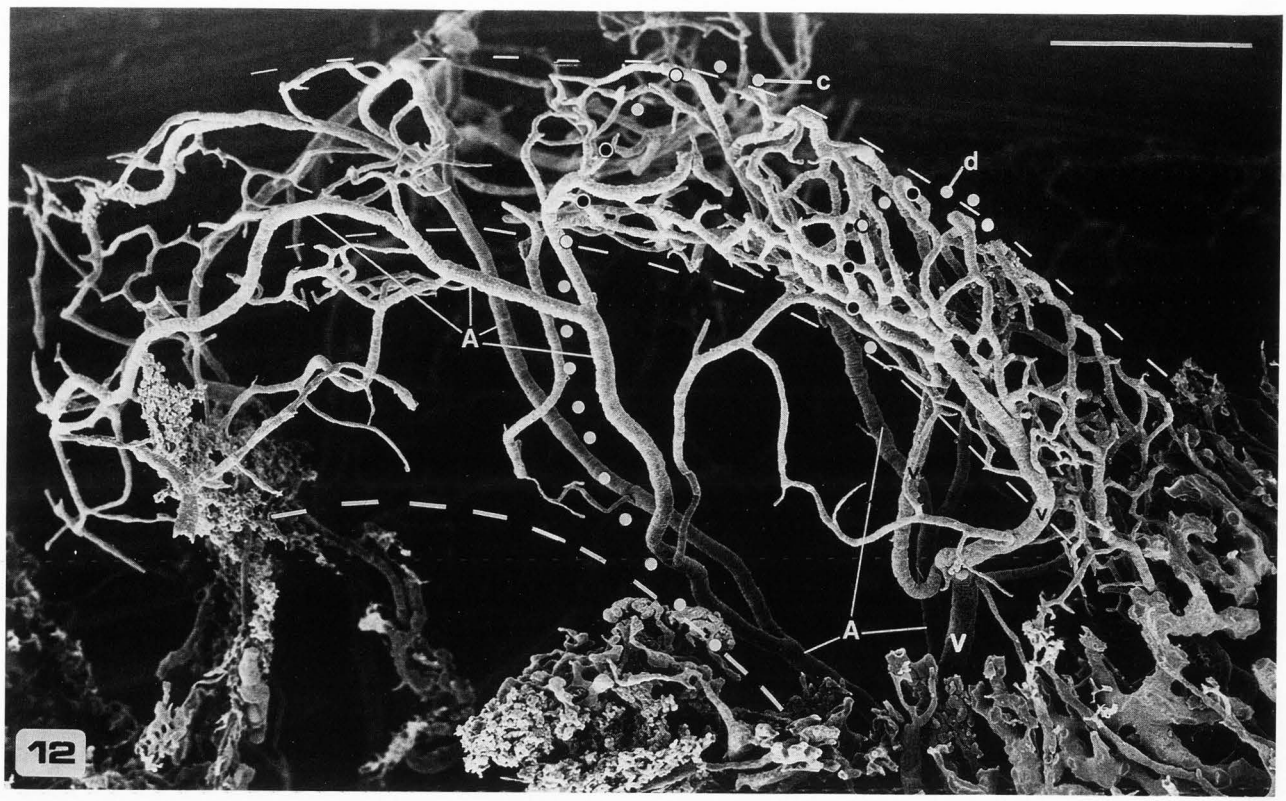
Figure 9 (facing page top). A panoramic view of the cochlear basal end. R: round window; MT: middle turn; UT: utriculus; VB: vestibular branch of the vestibulo-cochlear artery. Bar = 500 μm.

Figure 10 (facing page bottom). A region of the cochlear basal end located between the two windows, with radiating arterioles (RA) running from opposite directions and producing a capillary network (C) at the site of their encounter. R: round window. Bar = 100 μm.

Nabeya (1923) and Axelsson (1968), based on Berlin blue injection method. A developmental study on fetal and postnatal vascularization of the cochlea was published by Johnsson (1972) who used osmium-stained sections and phase contrast microscopy. Recently, a corrosion cast study by Nakai *et al.* (1992) included some observations on human inner ear. To our knowledge, the vascular system has not been yet examined in human fetuses by using the corrosion cast technique and scanning electron microscopy, that offers three-dimensional images with high resolution.

The general vascular pattern of the fetal inner ear is identical with that described for the adults by Axelsson (1968). There are, however, some differences that are due to prenatal development. In the spiral lamina, the limbus vessels form a dense network, whereas they are sparse in adults. Instead of typical arcade-like pattern of the peripheral vasculature that includes vessels of tympanic lip and basilar membrane, we observed a more irregular arrangement of the vessels, predominantly of the venous type. The initial branches of the radiating arterioles that supply the spiral lamina also showed a less serpentine course than described in the adult human cochlea.

In the external wall, the most conspicuous feature differing from that system in adults is the character of draining venules at scala tympani. They have a sinusoidal form of a dense network with irregular meshes. We consider such vascular arrangement as a fetal feature, and during further pre- and postnatal development this network has to be transformed into a system of apicobasally directed collecting venules with their typical omega-shaped loops at the level of the spiral ligament. Our observations correspond well with those reported by Johnsson (1972) who described in 11-24 week fetuses at the level of scala tympani "an undifferentiated, primitive



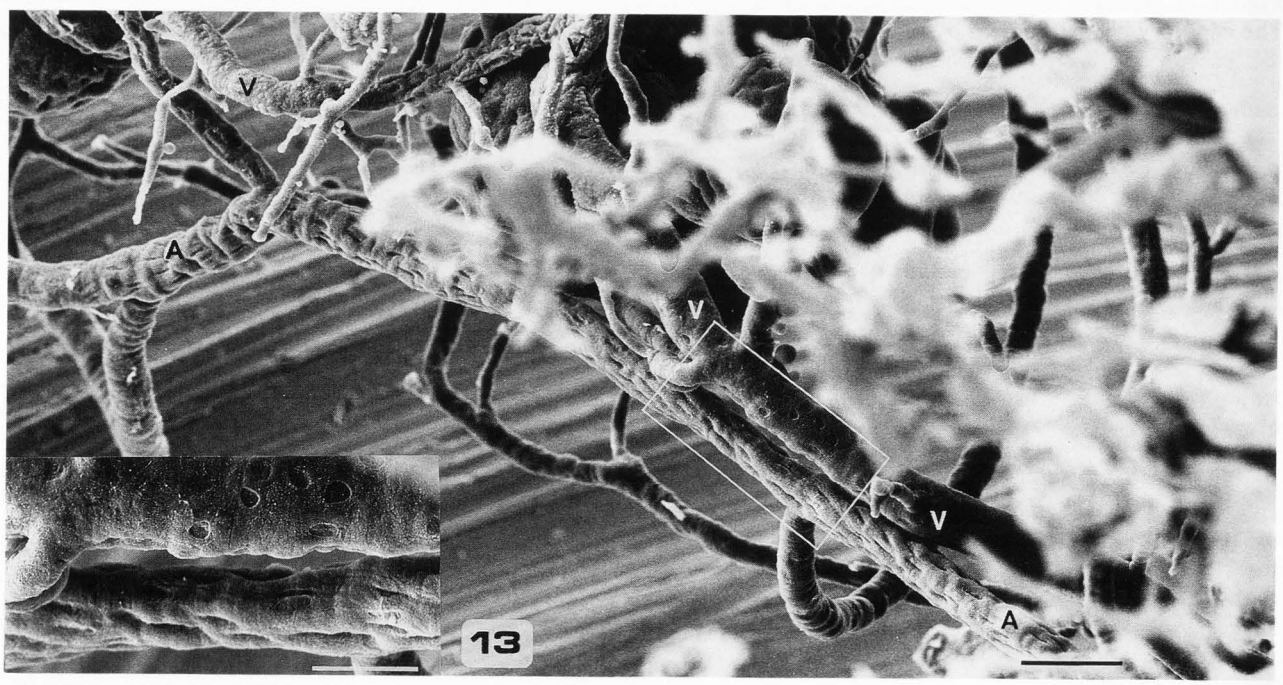


Figure 12. The lateral semicircular duct (d) and its endosteal canal (c) with their respective perimeters and borders marked with dotted lines. The main arterial vessel (A) gives off short twigs to the capillary network. V: the main venous vessel. Bar = 500 μm.

Figure 13. A pair of large vessels, artery (A) and vein (V), running along the endosteal wall of the semicircular canal. Rectangled area is shown at higher magnification in the inset. Bar = 100 μm. **Inset**: typical nuclear imprints on the surface of vascular casts. Bar = 50 μm.

vascular plexus" which could no longer be seen in the postnatal material.

We observed the connections between the radiating vessels of the spiral lamina as well as marginal vessels with the vasculature of the external wall. Such connections have been reported to occur only occasionally in the adult human cochlea (Axelsson, 1968), but they are absent in guinea pig and gerbil (Axelsson *et al.*, 1986; Nakai *et al.*, 1986; Tange and Wijburg, 1986). These connections have been suggested to be of functional importance in the fetal period but disappear afterwards (Johnsson, 1972; Axelsson and Ryan, 1988). The vessel of the basilar membrane also seems to play a significant role in the cochlear development, since it is located directly beneath the organ of Corti, and in gerbils, it has been shown to undergo regression after the maturation of the organ is completed (Axelsson *et al.*, 1986). In our material, this vessel was quite prominent and the studies of human fetuses of a similar gestational age demonstrated that the organ of Corti was still developing and the auditory stereocilia were at the very beginning of their formation (Fujimoto *et al.*, 1981). A strikingly large vessel of the basilar membrane was observed in younger (11 and 14 week) fetuses by Johnsson (1972) and its size decreased with the pre- and postnatal age.

The stria vascularis network was extensively developed in human fetuses. This system may change in the third trimester, during the acquisition of high potassium content in the cochlear endolymph. In the gerbil cochlea, showing at birth the developmental level roughly equivalent to that of human fetus at 5 months, the stria vascularis expands in the postnatal period parallel with increasing potassium level in endolymph (Axelsson *et al.*, 1986).

Arterio-venous anastomoses between radiating arterioles of scala vestibuli and collecting veins of scala tympani are quite numerous in the basal turn of fetal cochlea. They allow a direct shunt of blood between the two compartments, bypassing the spiral vessels, and may play a role in the regulation of blood flow through spiral vessels, especially stria vascularis. At the level of scala vestibuli, the capillary network was relatively well formed and draining was provided by separate collecting venules running apically and joining the vein of scala vestibuli. Such venules are present in adult human cochlea, but absent in other species (Axelsson and Ryan, 1988). The region of the Reissner's membrane was found to be avascular, as also observed by Johnsson (1972), although single vessels were described there in 75% of the fetal cochleas examined by Okano *et al.* (1978).

In this study, the vasculature of the semicircular ducts has been visualized for the first time using the corrosion cast technique. Our results confirm the description of Nabeya (1923). The capillary network surrounding the semicircular canals is supplied by long arterioles running along the concave side of the duct from its both ends and anastomosing at its summit. This was also reported (but not illustrated) in the inner ear of guinea pig (Nakai *et al.*, 1986).

At higher magnifications in SEM, we could clearly distinguish arterial and venous vessels, according to their typical nuclear imprints produced on the cast surfaces by the endothelial lining. In arteries, the imprints are elongated, with their long axis parallel to that of the vessel, whilst in veins they are roundish, shallower and more randomly distributed (Miodoński *et al.*, 1976). Except for the collecting venules of scala tympani that still showed some primitive features, the arterial and venous vessels were fully differentiated at this stage of prenatal development.

According to our results, and those obtained by other authors (Nabeya, 1923; Axelsson, 1968; Johnsson, 1972), it seems that the fetal features of inner ear vascularization observed in the second trimester of prenatal development disappear during the remodelling of the vascular system that occurs in the third trimester and in the early postnatal period.

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Discussion with Reviewers

S. Aharinejad: How long after death were the fetuses perfused?

Authors: After about 90-120 minutes.

S. Aharinejad: Does Lignocaine still influence vascular wall in a material perfused several hours post mortem? Same question arises when using heparin.

Authors: We have since long used heparin and Lidocaine (Lignocaine) as routine components of the perfusion fluid and have never observed any artifactual effects of these compounds on the vascular wall. Considering the low concentrations of the drugs used, one could only expect some slight vasodilatatory effect of lidocaine, if the smooth muscle cells are still able to respond.

S. Aharinejad: We could not find arterio-venous anastomoses between scala tympani and scala vestibuli in cast guinea pig cochlea, but they occur in human fetal inner ear. Accepting that function follows the form, I can hardly understand this. Can you please explain?

Authors: Arterio-venous anastomoses between scala vestibuli and scala tympani are difficult to find, however, they were described in human cochlea by Axelsson (1968, cf. Figs 60 and 64) and they were also reported to occur in guinea pig (Axelsson, 1968, cf. Fig. 51; Axelsson and Ryan, 1988, cf. Fig. 5; Sugar *et al.* 1973, Acta Otolaryng. Suppl. 301, cf. Figs. 1 and 2; Hayran and Karatay, 1992, Acta Anat **145**: 55-60).

S. Aharinejad: In guinea pigs, branches of the modiolar artery form the so-called "coiled arterioles", also known as glomeruli of Schwalbe. Although the morphology of the cochlea might have been altered in your study because you used post mortem material, the excellent micrographs suggest that structures are well preserved. Nevertheless, I cannot recognize glomeruli at the scala vestibuli. On one side, arterio-venous anastomoses are present and on the other side glomeruli are absent. How are these two characteristics interrelated when the blood flow regulation is considered?

Authors: Arterial glomeruli or "spring-coil arterioles" are typical of guinea pig ear but they have not been observed in man (Axelsson, 1968; Axelsson and Ryan, 1988).

The arteriovenous anastomoses between scala vestibuli and scala tympani allow the shunting of blood past the anatomically distinguishable capillary networks located within the external wall of the cochlear duct and thus can be regarded as a device responsible for the regulation of both blood flow and pressure, especially in the region of the stria vascularis. It is known that strial vessels are characterized by much slower blood flow than that in the other vessels of spiral ligament, and they are also especially densely packed with blood cells (Hawkins, 1976, Arch. Oto-Rhino-Laryngol. **212**: 241-251). Since vasomotor innervation has not been demonstrated in the lateral wall, the flow regulation must depend on a (probably local) hormonal control.

S. Aharinejad: Did you observe a vein similar to the Hook vein described in rats?

Authors: It seems likely that several large collecting venules seen in Fig. 10 can join to form an equivalent to the Hook vein observed in rats. In our images, however, such a vein cannot be seen, since it is masked by other vessels.

B.A. Bohne: What were the causes of the spontaneous abortions of the five fetuses? Were autopsies done to determine if the fetuses had any congenital anomalies, especially of the vascular system?

Authors: In all cases, abortion was due to maternal disorders (hormonal disturbance, uterine malformations and tumors, renal insufficiency). Although autopsies were not performed, the fetuses did not show any anomalies as far as macroscopic inspection (including that of the intrathoracic large vessels) was concerned.

B.A. Bohne: What pressure was used to perfuse the resin through the vascular system? Was the perfusion pressure similar to the normal blood pressure of fetuses at 18-21 weeks?
Authors: We used manual perfusion (with a syringe), hence we cannot specify the pressure. Although the method does seem to be precise enough and outdated, we have had a long experience with it and have been able to achieve good casts. Moreover, it has been demonstrated that during the perfusion the input pressure is different from that measured inside the vascular bed (Lametschwandtner *et al.* 1990; Motti *et al.* 1987, Scanning Microsc. 1: 207-222). Last, but not the least, manual perfusion allows one to use much smaller quantities of the casting medium, thus being cost-efficient.

B.A. Bohne: What accounts for the fact that portions of the otic capsule remained on certain parts of the specimen but not on others? How can the remainder of the otic capsule be removed so that the ampullae can be examined?
Authors: This is due to the fact that at the studied developmental stage, certain parts of the otic capsule are already ossified, while the others are not. The employed decalcification/maceration procedure can completely remove the cartilaginous portions which are devoid of blood vessels. The ossified portions contain a dense vasculature which obscures the underlying vessels of ampullae. Although technically difficult, the only way to unmask the ampullar vessels would be to remove the vessels of the otic capsule using a micromanipulator.

B.A. Bohne: Do you have any plans for studying the vasculature of younger fetuses? It would be interesting to examine specimens in which the membranous labyrinth is just beginning to differentiate into its various parts (i.e., vestibule, cochlear duct).
Authors: If only we succeed to collect the appropriate number of younger fetuses (which is extremely difficult), we shall continue our studies in that direction.

T. Hoshino: How did you determine the location of certain vessels such as the ones in the tympanic lip, the basilar membrane and the vestibular membrane, after washing out all the surrounding soft tissue?
Authors: The identification of these vessels was based on their location in the entire vascular system which has a very precise architecture, allowing such identification even in the absence of the surrounding soft tissue.

T. Hoshino: You pointed out a poorly developed meshwork of capillaries in the hook portion. Was this finding not caused by an imperfect filling of the casting material?
Authors: Although some loss of strial capillaries during cast preparation cannot be excluded, this particular region of the stria vascularis of the basal end has been described to be extremely poorly developed in humans (Axelsson, 1968) and our results confirm that observation.

T. Hoshino: You showed a collecting venule indicated by the letters "CVL" in Fig. 4. The vessel seems to be a radiating arteriole from the status of branching and connection to the other radiating arteriole. Nuclear imprints are also seen in the same vessel in Fig. 5. What is your opinion?
Authors: CVL in Fig. 4 shows roundish nuclear imprints typical for venules, its course also deviates from that of a normal radiating arteriole. We observed further course and connection of this venule with a larger vein (not shown in Fig. 4).

Y. Nakai: You show the capillary network of scala vestibuli draining its own collecting venule (CVL) in Figs. 4 and 5. How did you judge this blood vessel is a venule? Does this venule exist in the upper turn, too? We did not observe this kind of venule in the adult cochlea.
Authors: The character of this vessel is indicated by its course and by the form of endothelial nuclear imprints (roundish). We have not observed such venules in the upper turn, which generally contains much less complex vasculature than the other turns.

Y. Nakai: How is the microvasculature in the crista ampullares, utricle and saccule in the inner ear?
Authors: This microvasculature was obscured by the vessels of the partially ossified otic capsule and could not be examined in detail.