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SCANNING ELECTRON MICROSCOPY OF THE MICROVASCULAR SYSTEM IN THE INNER EAR

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Abstract

In the present work, vessels casts in the inner ear of the rat and guinea pig, prepared by casting method using Mercox resin, were subjected to scanning electron microscopic examination and following results were obtained: 1) In adult guinea pig, numerous capillary nets were found in the following parts: stria vascularis, spiral ligament, spiral prominence, Corti's organ, spiral ganglion, plexus cochlearis, semicircular ampulla, saccule, utricle, and endolymphatic sac. These were consistent with functionally and morophologically important areas in the inner ear. 2) In the central side of the area with capillary nets, arterioles were found to run throughout, like a complex coil, and peripheral capillary diameter was found to be unchanged in an experiment in which the injection pressure was altered, thus autoregulation of blood flow into these important areas is assumed. 3) Vessels in the planum semilunatum were found to form a specific loop-shaped route, where secretion and reabsorption of endolymph is thought to occur. 4) After kanamycin injection into the tympanic cavity, stenosis was observed in capillary nets in the cochlear lateral wall. 5) In guinea pigs on the 30th day of fetal life, the main stem of the inner ear vessel had already formed; however, the peripheral capillary nets were as yet immature in form and vessel density was low.

KEY WORDS: Blood Vessels, Polyester Resin, Micro Corrosion Cast, Cochlea, Vestibule, Semicircular Canal, Endolymphatic Sac, Fetus Inner Ear, Kanamycin Ototoxicity, Scanning Electron Microscopy.

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Introduction

Because of its unique morphology as delicate sensory organs, the vascular system of the cochlea, vestibule and semicircular canal have been studied extensively. Schwalbe[6] as early as in 1887 and Nabeya et al[4] in 1923 have investigated the vasculae system in various kinds of animals and human beings. More detailed investigation have been made since microcirculer disturbance of the inner ear was considered one of the causes of inner ear deafness, vertigo or other balance disorders. To better understand the control of peripheral blood flow in the inner ear, it is important to study morphological characteristics of the micro-vessels of the inner ear. To this end, a number of investigators investigated capillaries of the cochlea (Symour[7], Nomura[5], Axelsson[1], Hodde[3] and Tange[8]) and that of the vestibule (Hawkins[2]).

To understand the distribution of controlling vessels, the structure of micro-vessels and the features of blood vessel running is required for researches into the causation and disease state of the inner ear disease. In order to clarify the metabolic state and function of the sensory organ and the secretion and absorption, etc., of the inner ear fluid, we should make physiological and biochemical investigation and in addition, investigate in detail the distribution of capillaries existing in the subepithelial part. In this study, the authors morphologically investigated the normal picture of structure of micro-vessels over the inner ear, ear damaged by an ototoxic drug and development in a three-dimensional way in guinea pigs and rats.

Materials and Methods

Materials

A hundred and twenty-five mature Hartley guinea pigs weighing 300-400 g were used for the present study. In addition, 35 white rats of Wistar strain and 20 fetuses of guinea pigs each on the 30th, 40th and 60th gestational days. Prior to the commencement of the study, findings on the external acoustic meatus and the tympanic membrane as well as Preyer auricle reflex were confirmed to be normal. The animals were

anethetized with pentobarbital (30mg/kg) injected intraperitoneally.

Method

Microvascular casts were made using intravascular injection of the Mercox CL-2B-5 resin (Dainippon Inc & Chemicals Inc.). The resin was mixed with a hardener at 20 ml : 0.6 g.

Animal anesthetized with pentobarbital (30 mg/kg) injected intraperitoneally were fixed in a lying posture. A median incision was made starting at the abdomen to the neck. Below the diaphragm, the aorta abdominalis and the vena cava inferior were ligated. After that, the Jugular vein at the neck was exposed on the both sides for ligature. Then, a median cut was given to the sternum so that the heart was visible. The epicardium was carefully separated and above it, the aorta ascendens was exposed. A needle was inserted into the left ventricle through the aorta ascendens and fixed. Heparin (37°C) added saline of 500 ml was injected into the needle and afterwards, the jungular vein on the both sides was cut. After the injection of 500 ml of saline, it was confirmed that the blood flow from the jugular vein completely disappeared. Then, 100 ml of 2.5% glutaraldehyde was injected. With perfusion pressure made constant at 120-150 mmHg by mercury manometer, about 50 ml of the mixture of Mercox resin with the hardener was injected. About one hour later, it was confirmed that the resin was fully hardened. Then, the target region was removed, washed with water and kept in 8N HCl solution at 60°C for one hour. The bathing completely dissolved the bone tissue and soft tissue of the inner ear into the solution but never damaged Mercox resin.

The specimens prepared according to the above procedures were air dried, coated with gold and examined with a scanning electron microscope (Hitachi S-405).

Results and Comments

Inner ear was fed by labyrinthine artery, derived from basilar artery. Labyrinthine artery becomes common cochlear artery after branching off of the anterior vestibular artery (AVA) and then, join the internal auditory canal. Then, after branching off of the posterior vestibular artery (PVA), common cochlear artery becomes the cochlear artery (Fig. 1). The spiral modiolar artery, after branching off from the common cochlear artery, ascends in a spiral manner around the cochlear nerve in the modiolus. This artery makes its diameter smaller as it ascends and at the apex, it consists of several radiating arteriolae which are distributed on the lateral wall of apex (Fig. 2). In the mid-way, some blood vessels branch off from the spiral modiolar artery in each turn and run in a complicated, coiled way. These vessels are called coiling arteriole (Fig. 3). Among them, the blood vessels existing at the upper end of scala vestibuli are called upper coiled arteriolae and those existing at the level of spiral lamina, lower coiled arteriole. The microvascular system of the inner ear in rats is not so complicated as that in guinea pigs and appears a winding, meandering shape rather than the coiled shape (Fig. 4).

Using a manometer with mercury, we increased infusion pressure of resin from 100 mmHg up to 400 mmHg and measured the diameter of blood vessel of cochlear lateral wall at the individual pressure levels. As a result, the diameter of blood vessel at each pressure level did not significantly differ from each other. Thus, the presence of coiled structure on the central side of such blood vessels is a favourable vascular structure for pressure absorption and maintenance of blood flow from the viewpoint of hemodynamics.

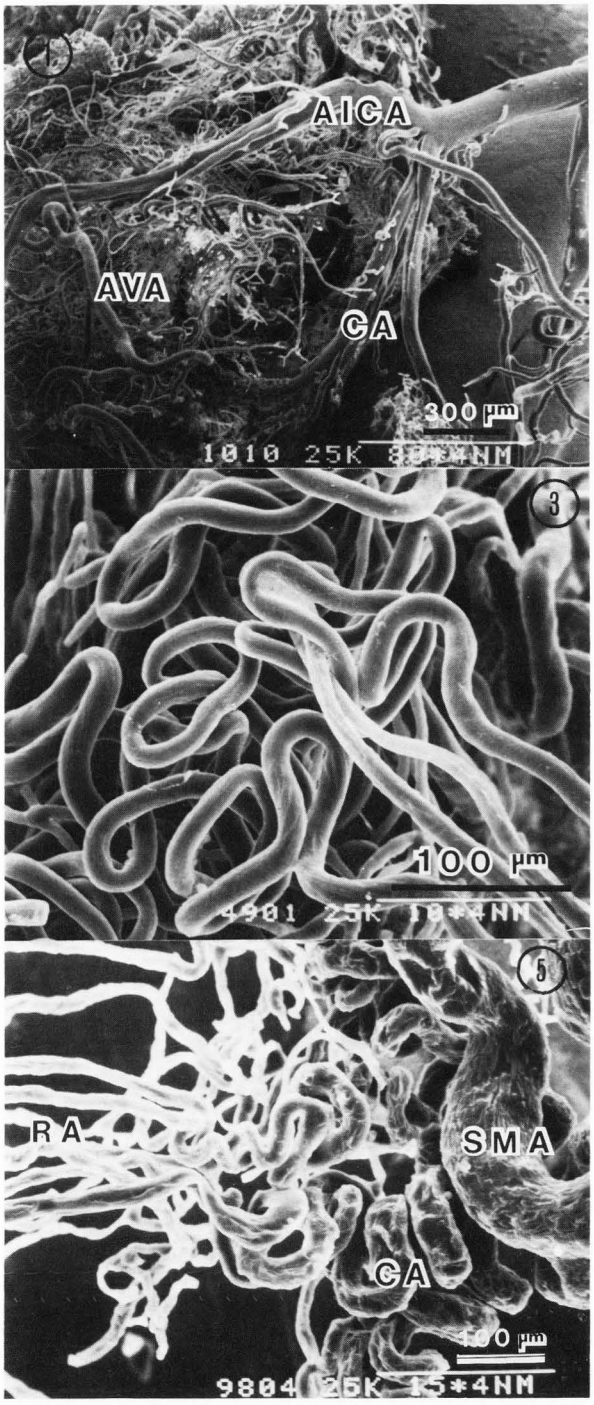
In guinea pigs on the 30th day of fetal life, the cochlear blood vessels consist of spiral modiolar artery, coiling arteriolae, radiating arteriolae, etc. Its structure is monotonous and the vascular wall is uneven (Fig. 5). In guinea pigs on the 60th day, vascular structure around the modiolus has been completed and is almost the same as that of mature guinea pigs. The spiral modiolar artery becomes thicker and its spiral arrangement becomes closer. Especially, a group of blood vessels distributed on the lateral wall are developed. The upper and lower coiled arteriolae have been almost completed, although unevenness is noted inside the blood vessels.

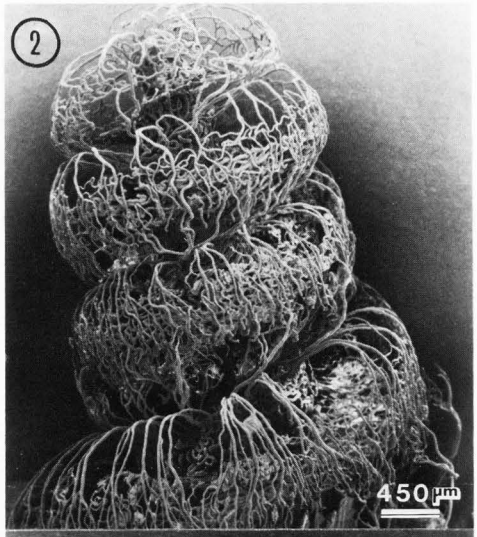
In adult cochlea, after formation of a coil, the radiating arteriole descends the lateral wall of scala vestibuli in almost a straight manner, branches off into several vessels slightly above the stria vascularis and then, runs towards the spiral ligament and the stria vascularis. The way of vascular arrangement in this region varies depending on animal species (Fig. 6).

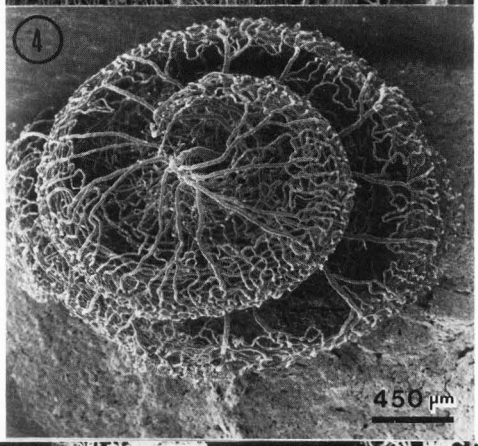
In guinea pigs, the number of radiating arteriolae is very high and for this reason, distribution of blood vessels at the stria vascularis and the spiral ligament is complicated. Vascular arrangement at the two sites is less regular. Anastomosis is not so frequently observed between the stria vascularis and the spiral ligament.

The capillary net of stria vascularis, then, descends the lateral wall of cochlea, forms collecting venule and then, returns to the common modiolar vein. On the other hand, the blood vessels of spiral ligament, although some of them are anastomosed to the capillary net of stria vascularis, branches off in most cases into a couple of vessels inside the ligament. Then, a part of the branched vessels goes to spiral prominence and forms a vascular net at that site. The remainder descends as it has descended and enters the collecting venule (arterio-venous anastomosis). As it goes to a lower turn, the density of radiating arteriolae is higher and thus, the vascular density of stria vascularis and spiral ligament is higher.

The regulation of blood flow into the capillary area of cochlear lateral wall is made by the autonomic nervous system distributed in the modiolar blood vessel, which is the main trunk of these capillaries, or made by the above-stated complicated coil-like structure of the basal part of radiating arteriole. In addition, chemical regulation by prostaglandin and other substances distributed in the cochlea is also







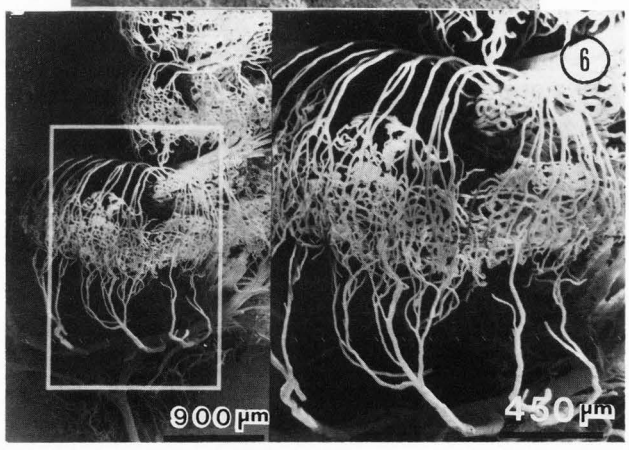


Fig. 1: Blood vessels located on the skull base of temporal bone in a guinea pig. AICA: Anterior Inferior Cerebellar Artery. CA: Common Cochlear Artery. AVA: Anterior Vestibular Artery.
Fig. 2: Vascular structure of the guinea pig cochlea.
Fig. 3: Coiled arterioles of the guinea pig cochlea.
Fig. 4: Vascular structure of the rat cochlea. Observation at the apical turn clearly reveals that the common cochlear artery connects with radiating arterioles at the apex.
Fig. 5: Cochlear blood vessels in guinea pig on the 30th gestational day. The common cochlear artery, coiled arterioles and radiating arterioles are seen, although simple in structure. There are imprints on the walls of the blood vessels. SMA: Spiral Modiolar Artery. CA: Coiled Arterioles. RA: Radiating Arterioles.
Fig. 6: Blood vessels of lateral wall of the guinea pig cochlea.

considered. Intra-venous administration of prostaglandin E_1 resulted in a significant dilatation of radiating arteriole. Accordingly, there is a possibility that chemical substances including prostaglandin may be involved in the regulation of blood flow into the cochlear lateral wall (Fig. 7).

Injection of kanamycin into the tympanic cavity produced cord-like changes in the blood vessels of the lateral wall, especially in the capillary area of stria vascularis. These changes were more prominent in a basal turn (Fig. 8). Observation on the normal capillary of the modiolus reveals that arteriolae branching off from the spiral modiolar artery enter the spiral ganglion where the arteriolae form a dense capillary net. On many occasions, a route of venous return starts from the spiral ganglion and goes in a medial direction back to the posterior spiral vein.

On the modiolar wall, a cotton-like capillary net is also observed in the circumference of the spiral modiolar artery. These capillaries seem to correspond to so-called plexus cochleaaris. Transmission electron microscopy reveals fenestra in this capillary.

Below the Corti tunnel, usually, one capillary ascends the basilar membrane in a spiral manner. This is seen in human beings and guinea pigs, but not in adult rats or mice. The main vein of cochlea is the posterior spiral vein which spirally descends with the cochlear modiolus as the center. The posterior spiral vein joins in the collecting venule on the lateral side, which is formed by the capillary net composing the lateral wall of cochlea, and on the medial side, joins in such blood vessels as collect venous blood from the spiral lamina, the spiral ganglion and cochlear modiolus. Then, at the basal site, the resulting vein further joins in the vein of round window and the posterior vestibular vein and then, reaches the inferior cochlear vein.

The inferior cochlear vein starts around at the round window, runs along with the cochlear aquaeduct and flows into the inferior petrosal sinus. At the vestibule, there are two arteries, that is, the anterior and posterior vestibular arteries.

The anterior vestibular artery parallels the utriculo-ampullar nerve in the superior vestibular canal, runs spirally in almost the center of bony canal and then, forms a coil several times. Afterwards, it distributes blood vessels in the utricule, the superior-lateral ampulla and the semicircular canal (Fig. 9). The posterior vestibular artery branches off from the common cochlear artery and immediately after that, forms a coil. It distributes blood vessels in the saccule, the posterior ampulla and the posterior semicircular canal. Vessels in the planum semilunatum were found to form a specific loop-shaped route, where secretion and reabsorption of endolymph is thought to occur.

These vestibular arteries necessarily form coils in a complicated manner just before the sensory epithelium, like the coiling arteriolae in the cochlea.

A roughly two-layered capillary network was observed around the utricle. The utricle received the blood supply from just anterior and posterior to the coil of anterior vestibular artery. In the central part of the utricle a dense vascular network was formed (Fig. 10). Some arterioles reach the venous system directly without branching in this region. On the opposite side, arterioles met venule from the ampulla, and the blood flows returned to vestibular veins. Figure 11 shows the endolymphatic sac. Crossing the sigmoid sinus medial to the anterior semicircular canal, a capillary network was widened to fan shape. The intermediate portion was more abundant than other portions. The blood vessels in this area continued from the PVA or the posterior meningeal artery. The sigmoid sinus was recognized as a return route to the venous system. On detailed examination at the intermediate portion, blood vessels looked like sinuses and were flat and relatively large in diameter in upper portion. There were fine vessels in the lower portion as in the other part of the inner ear. All the routes of venous system do not present complicated running patterns as the arterial system does. At the vestibule, blood flow from each sensorial epithelium returns to the vestibular vein and that from endolymphatic sac, to the sigmoid sinus (Fig. 12).

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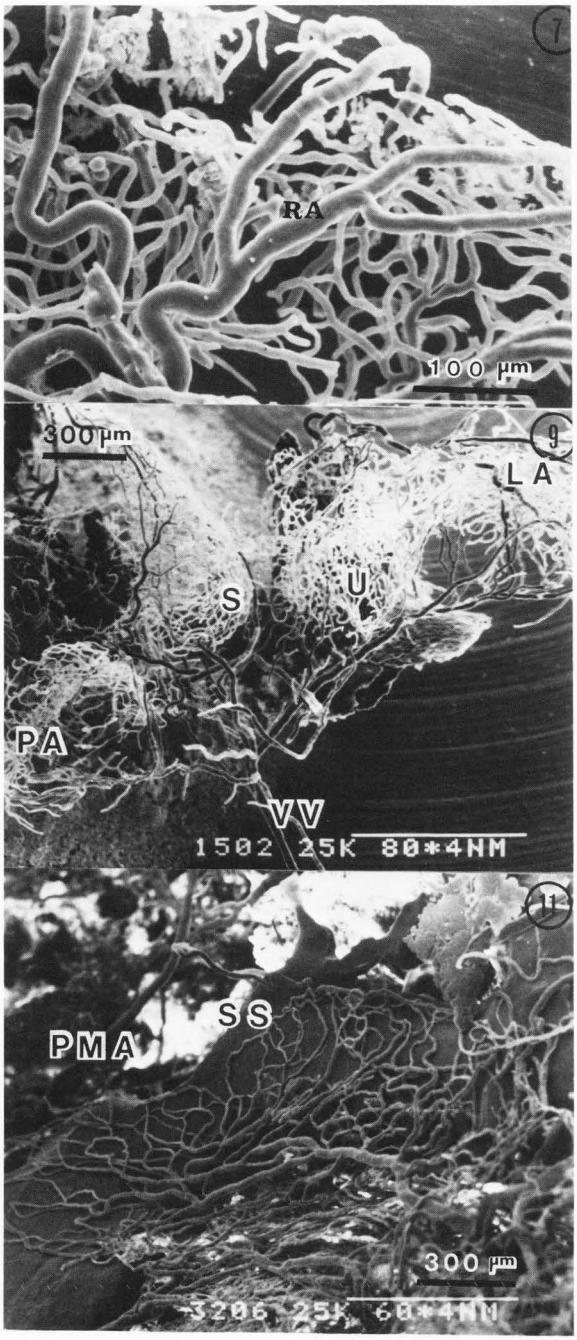
Discussion with Reviewers

M. Anniko: How much kanamycin was injected into the typanic cavity?

Authors: 50 mg KM in 0.3 ml saline per day was injected for 5 days in a total dose of 250 mg, and for 10 days in a total dose of 500 mg.



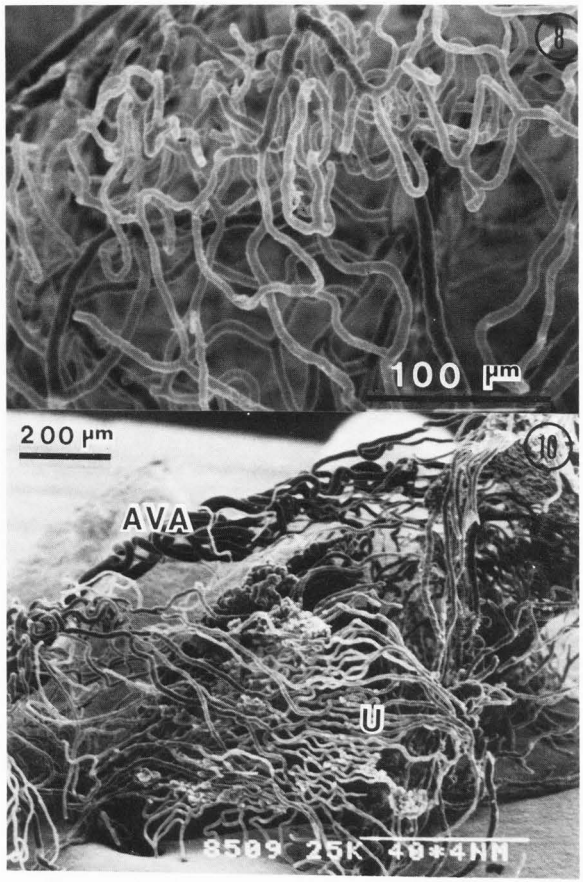


Fig. 7: Radiating arterioles (RA) in the rat given Prostaglandin E_1. The arterioles are dilated.
Fig. 8: Blood vessels of lateral wall in the guinea pig after KM injection into the tympanic cavity. Abnormal blood vessels appear.
Fig. 9: Blood vessels of the rat vestibule. LA: Lateral Ampulla. U: Utricle. S: Saccule. PA: Posterior Ampulla. VV: Vestibular Vein.
Fig. 10: Blood vascular system in the region of anterior vestibular artery of the guinea pig. AVA: Anterior Vestibular Artery. U: Utricle.
Fig. 11: Blood vascular system of the guinea pig endolymphatic sac. SS: Sigmoid Sinus. PMA: Posterior Meningeal Artery. The capillary network of the endolymphatic sac is seen across the sigmoid sinus.

M. Anniko: What do you consider to be the mechanism for kanamycin effects on the micro-vasculature in the lateral wall?
Authors: It was considered that KM administration caused stria vascularis atrophy, with which a change occurred in capillaries. It was also considered that KM had directly affected endothelial cells of capillaries.

M. Anniko: What was the dose for intravenous administration of prostaglandin E_1? Was there a uniform dilatation of radiating arterioles or did differences occur between the apical and basal parts of the cochlea?

Authors: 50 μg prostaglandin E_1 was intravenously injected. There were not substantial differences according to the types of turns, but the lower turn showed a greater effect of prostaglandin E_1.

D. J. Lim: You have stated that the specimens were kept in 8N HCl solution at 60°C for one hour, which presumably completely dissolved the bony and soft tissue of the inner ear. Is there any variation from one specimen to another, because some specimens are from young animals and the extent of calcification would differ? If the tissue was not complete dissolved, would you leave it in the solution longer? If kept in the solution longer, would it damage the Mercox?

Authors: All specimens can be completely dissolved for almost one hour. Even if they are put in 8N HCl solution for more then one hour, so much damage was not observed.

D. J. Lim: One of the concerns about the use of the microvascular technique for determining capillary diameter under experimental conditions is that the capillary diameter may be flexible and changed by the pumping pressure of the fixative or resin. How do you determine the safe pressure that you used that presumably represents physiologic condition.
Authors: The lowest injection pressure allowing vessel casts of complete shape to be obtained was considered as safe pressure.

D. J. Lim: In the same vein, if poor filling of the capillary occurs, as in the case of kanamycin-injected animals, how do you make a distinction between the poor filling caused by technical failure and the vasocontriction caused by the experiment? Are there any good criteria one can use?
Authors: Poor filling does not normally occur only in a part of the specimen.

K. C. Hodde: Did you see vessel casts along the whole length of the semicircular canals?
Authors: Yes, I saw one or two vessels along the whole length of the semicircular canal.

K. C. Hodde: You speak about an autoregulatory principle because you have seen no difference in vessel diameters at different injection pressures. Could that also be caused by the possibility that the pressure at that level is so much reduced that the pressure differences have become ineffective, especially after previous fixation?
Authors: Since the injection pressure ranged from 100 mmHg to 400 mmHg, a considerable effect may have also been given to the peripheral part though after previous fixation.

K. C. Hodde: What would you consider judging the overall result to be the most vulnerable part of the inner ear microcirculation?
Authors: From an aspect of only the vascular structure, the vestibule which is characteristically closer to an end-artery is weaker than the cochlea, and especially the anterior vestibular artery system which has a long vessel length is considered the weakest part.

P. Hinojosa: What is the diameter of radiating arterioles before and after prostaglandin E_1 administration?
Authors: The diameter of radiating arterioles was 16 μm on an average for the control and was 23 μm on an average after administration of prostaglandin E_1, which was about 1.5 times dilatation.

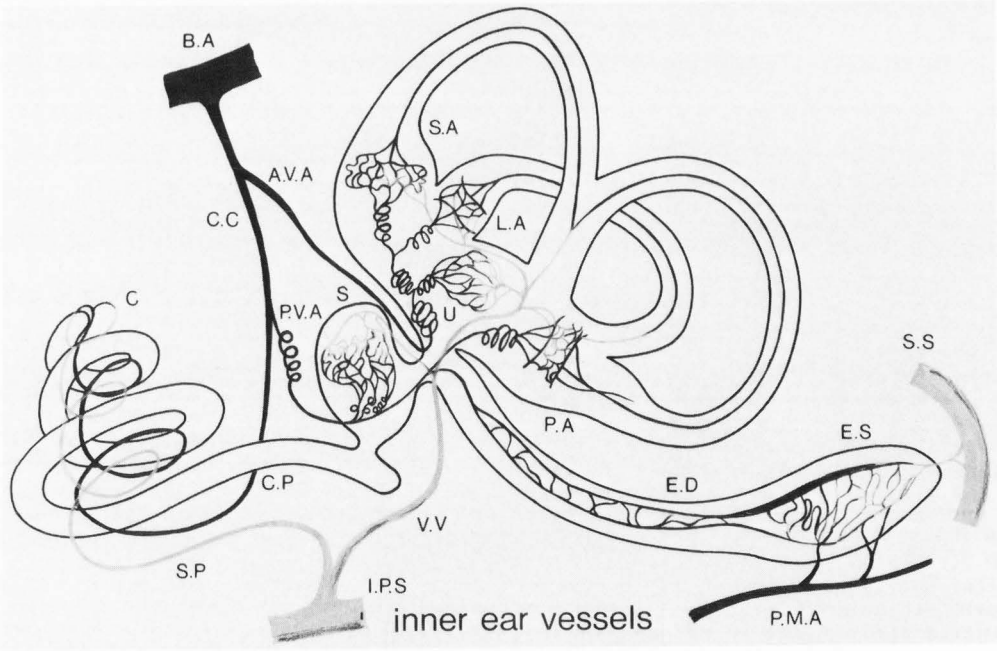


Fig. 12: Inner ear vascular system.

B.A : basilar artery
C.C : common cochlear artery
A.V.A : anterior vestibular artery
P.V.A : posterior vestibular artery
C.P : arteria cochlearis propria
P.M.A : posterio meningeal artery
C : cochlea
S : saccule
U : utricle
S.A : superior ampulla
L.A : lateral ampulla
P.A : posterior ampulla
E.D : endolymphatic duct
E.S : endolymphatic sac
I.P.S : inferior petrosal sinus
S.P : posterior spiral vein
V.V : vestibular vein
S.S : sigmoid sinus