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MICROVASCULATURE OF NORMAL AND HYDROPIC LABYRINTH

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Abstract

The microvasculature of the inner ear in guinea pigs and humans was observed with a scanning electron microscope using corrosion casting method. Alterations in the inner ear vasculature which occurred in association with experimental endolymphatic hydrops were also investigated. The results thus obtained are summarized as follows:

1. In the cochlea and vestibule, the arteries, coiled arterioles, and the veins are endowed with their respective characteristic morphologic features and play a role in the regulatory mechanisms of circulation.

2. The point in humans which is most different from guinea pigs was that coiled arterioles in the cochlea and the coil-like traveling of the anterior vestibular artery is not outstanding.

3. Arteriovenous anastomoses were demonstrated to exist in lateral wall of cochlea and utricular macula, a finding suggesting the existence of a regulatory mechanism for local blood flow.

4. Endolymphatic hydrops was noted to be preferentially associated with vascular abnormalities in the lateral wall of the cochlear duct and in the saccular macula, among other vestibular structures.

Key Words: Blood vessels, microcorrosion cast, cochlea, vestibule, semicircular canal, endolymphatic hydrops, human, guinea pig, scanning electron microscopy

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Introduction

The microvascular system of the inner ear has been studied for a long time because of its morphological as well as functional properties (Axelsson, 1986; Hodde *et al.*, 1977; Nakai *et al.*, 1986; Tange and Hodde, 1985). In some cases of hearing impairment, dizziness and vertigo due to inner ear disorders, circulatory disturbance of the inner ear has been implicated as the underlying cause and, in fact, circulatory agents are in common use in the treatment of these diseases. Hence, the blood flow regulation in the inner ear, both from morphological and clinical points of views, was a challenge for us to investigate.

The present study investigates blood-flow regulation in the inner ear of guinea pig and human from the morphological viewpoint by using the corrosion casting method. In a further attempt to study local blood-flow impairment or local vascular disturbance in the inner ear occurring in association with an inner ear disorder, experimental endolymphatic hydrops was produced in animals, and the microvasculature of the inner ear was observed using the casting method.

Materials and Methods

One hundred and eighty-five mature Hartley guinea pigs weighing 300-400 g and temporal bones from two human males 45 and 48 years old were used. Prior to the commencement of the study of guinea pigs, the appearance of the external acoustic meatus and the tympanic membrane as well as Preyer auricle reflex were confirmed to be normal. Microvascular casts were made using intravascular injection of the Mercox CL-2B-5 resin (Dainippon Inc.). The resin was mixed with hardener at 20 ml : 0.6 g.

Animals anesthetized with pentobarbital (30 mg/kg; intraperitoneally) were fixed in a supine posture. A medial incision was made from the abdomen to the neck. Below the diaphragm, the abdominal aorta and the inferior vena cava were ligated. A midline cut was made through the sternum so that the heart was visible. The pericardium was carefully separated, and above it, the ascending aorta was exposed. A needle was inserted

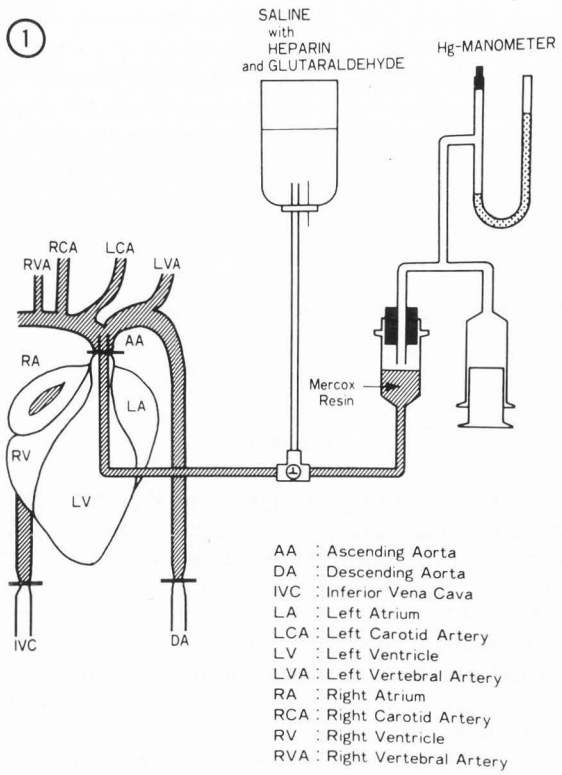

Figure 1. Schema for Mercox resin injection.

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into the left ventricle through the ascending aorta and fixed. Heparinized saline (37 °C) in volume of 500 ml was injected and the right atrium was cut. After the saline injection, it was confirmed that no more blood escaped from the right atrium (Fig. 1). A volume of 100 ml of 2.5% glutaraldehyde was injected. With perfusion pressure held at 120-150 mm Hg by mercury manometer, about 50 ml of the mixture of Mercox resin with the hardener was injected (Fig. 1). About one hour later, it was confirmed that the resin was fully hardened. The temporal bones were then removed, washed with water and kept in 8 N HCl solution at 60 °C for one hour. This latter solution completely dissolved the bone and soft tissue of the inner ear into the solution but never damaged the Mercox resin.

In the two humans, the dura mater was incised after a craniotomy from anterior cranial fossa and the brain base was exposed. Cannulation of the anterior inferior cerebellar artery was performed and the vessel was perfused with a heparinized physiological saline, and injected with Mercox resin. After the temporal bone was removed, the excess tissue was dissolved in 8 N HCl solution for 24 hours.

The vascular casts, prepared according to the above procedures, were air dried, coated with gold, and examined with a Hitachi S-405 scanning electron microscope (SEM). Some of the temporal bones were embedded in styrene resin and subsequently cut into sections.

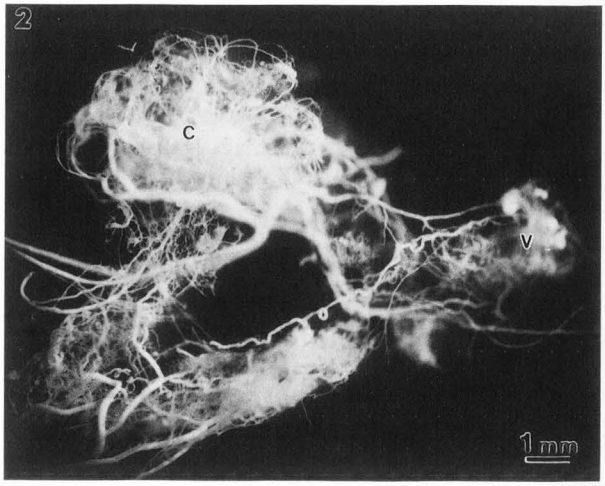


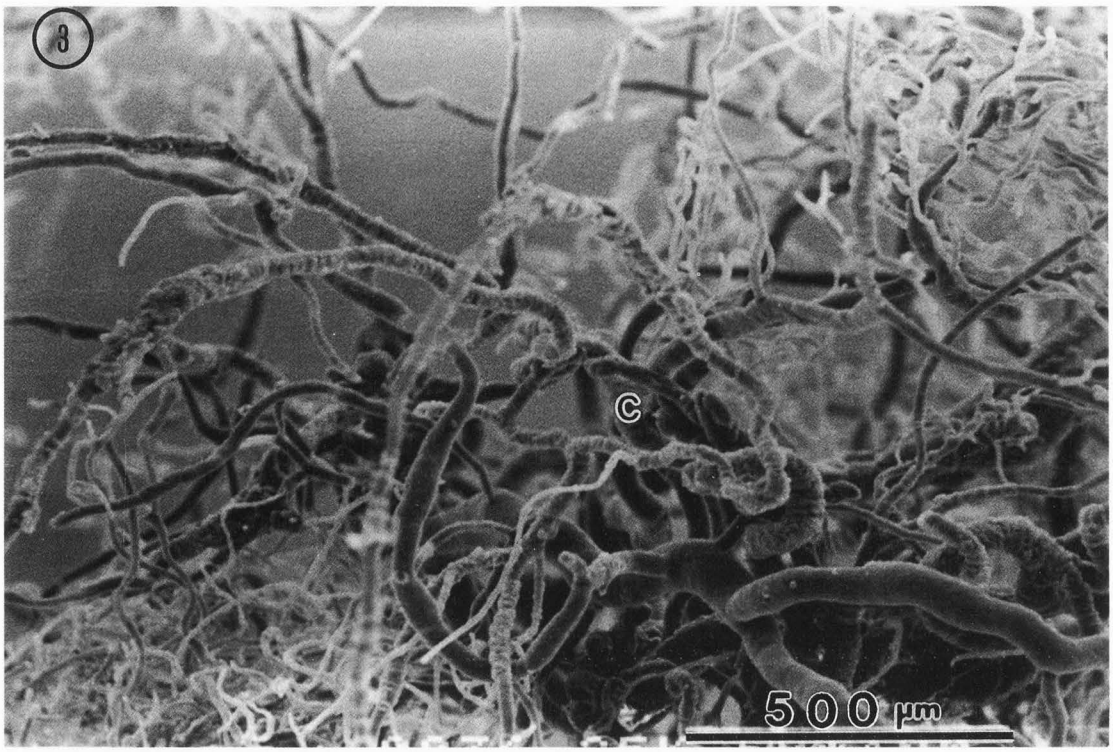
Figure 2 (above). Blood vessel of human inner ear. C: cochlea V: vestibule.

The rest of the blocks, after having been cleared of styrene resin with propylene oxide, were prepared in the usual manner, and examined by SEM.

In addition, we induced experimental hydrops (right side) in 5 guinea pigs by cauterizing the endolymphatic sac with 10% silver nitrate (Yazawa *et al.*, 1985). Their inner ear vessels were observed by SEM using the casting method. In the present study, the animals were used at 2 months after the experimental production of the hydrops, their left inner ears serving as controls.

Results

The inner ear is supplied by the labyrinthine artery which is derived from the basilar artery. The labyrinthine artery becomes the common cochlear artery after branching off the anterior vestibular artery and then, enters the internal auditory canal. Then, after branching off from the posterior vestibular artery, common cochlear artery becomes the cochlear artery (Fig. 2). After branching off the common cochlear artery, the spiral modiolar artery ascends in a spiral manner around the cochlear nerve in the modiolus. The diameter of this artery becomes smaller as it ascends. At the apex, it consists of several radiating arterioles which are distributed to the lateral wall of apex (Fig. 3). In the center of the modiolus, some blood vessels branch off from the spiral modiolar artery in each turn and run in a complicated, coiled way in guinea pigs. These vessels are called coiling arterioles (Fig. 4). Among them, the blood vessels existing at the upper end of scala vestibuli are called upper coiled arterioles and those existing at the level of spiral lamina, lower coiled arterioles. The microvascular system of the inner ear of humans is not as complicated as that in guinea pigs and has a winding, meandering shape rather than the coiled shape (Fig. 5).



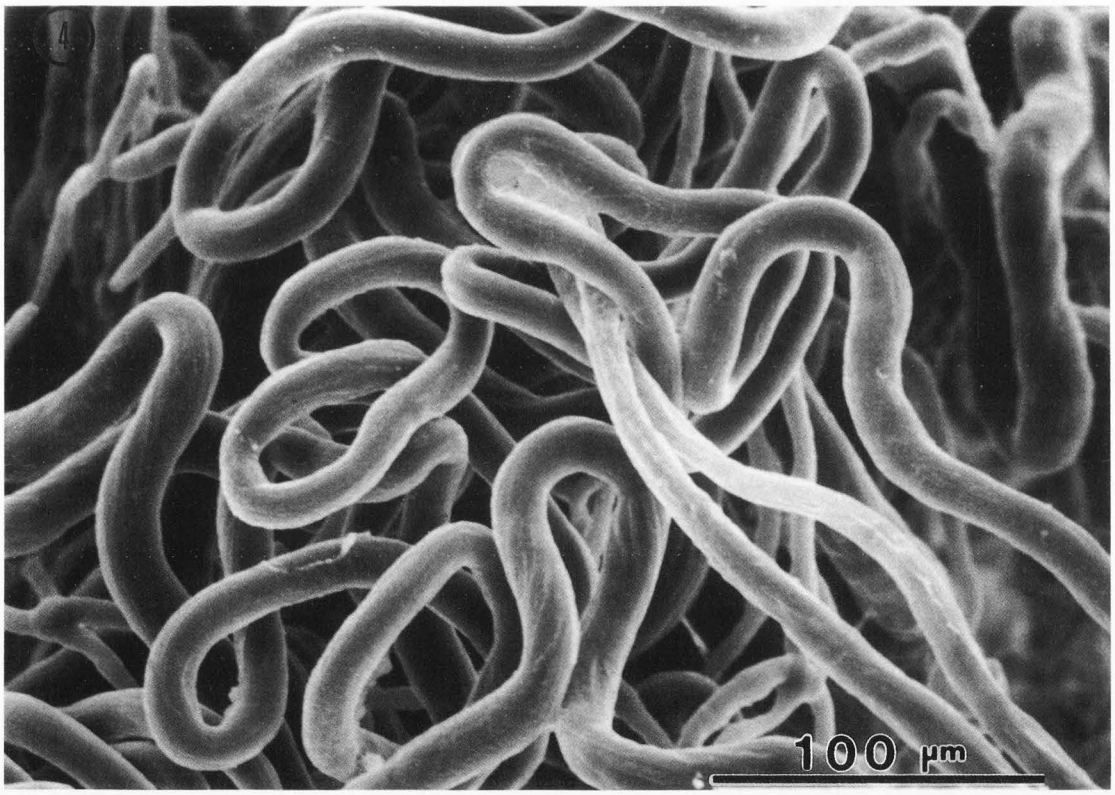
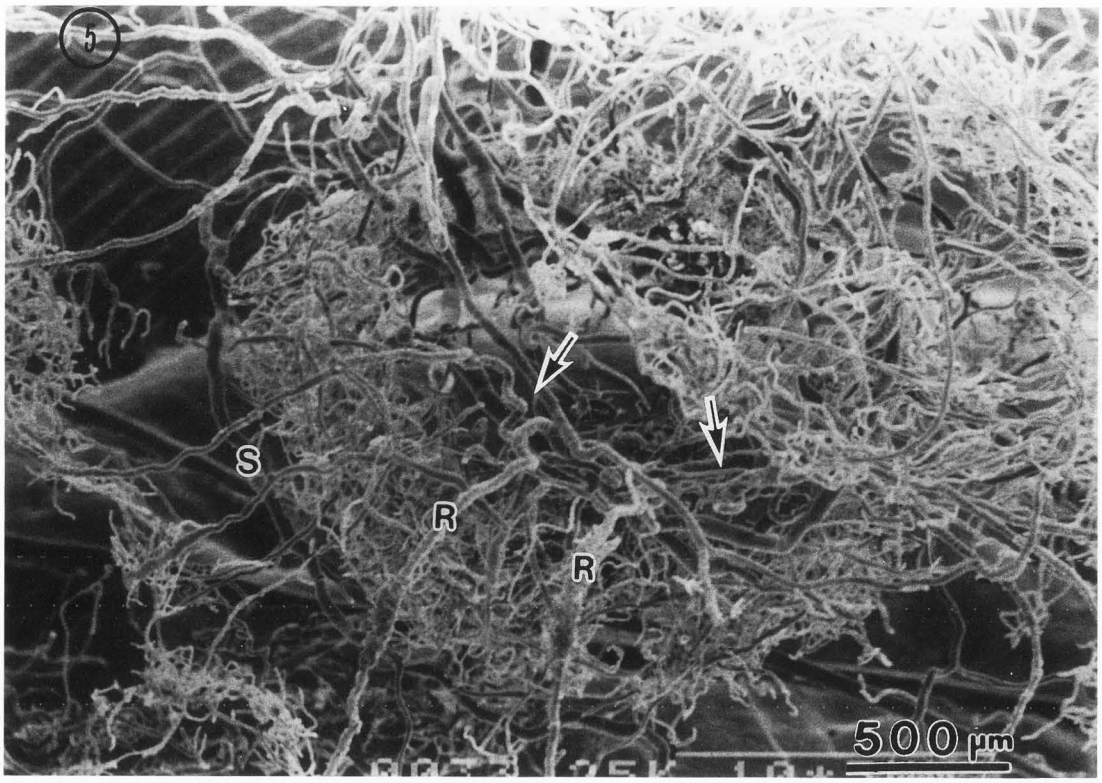


Figure 3. Microvasculature of apex in human cochlea. C: terminal of common cochlea artery.

Figure 4. Coiled arteriole of guinea pig cochlea is running in a complicated coiled way.



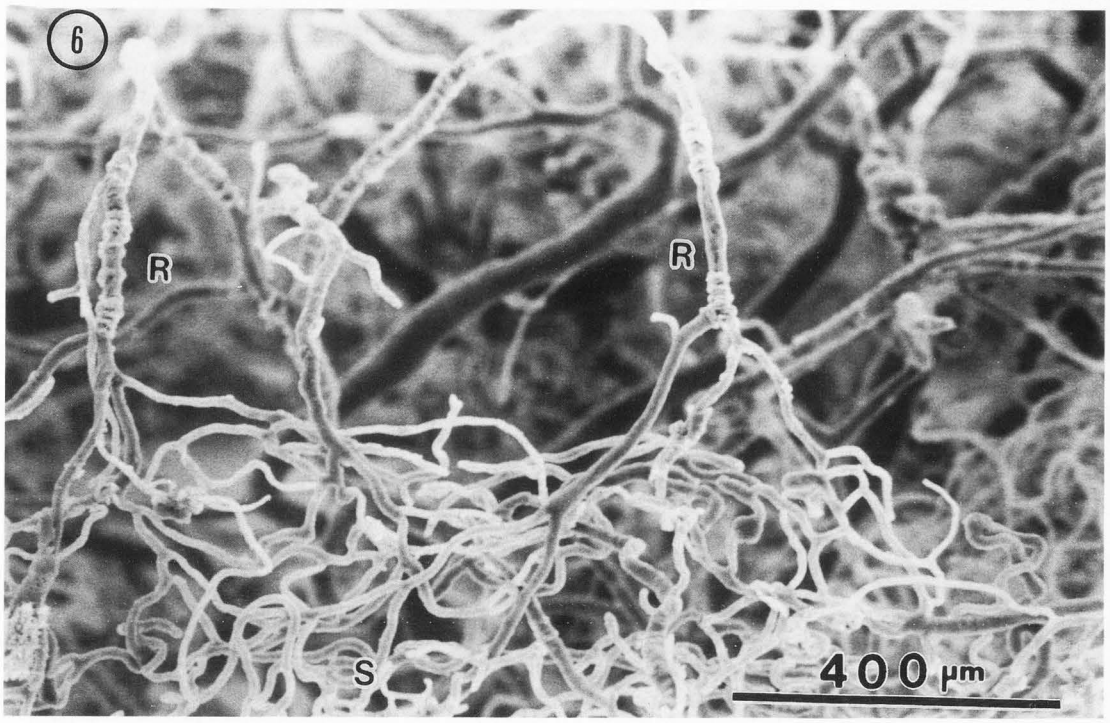
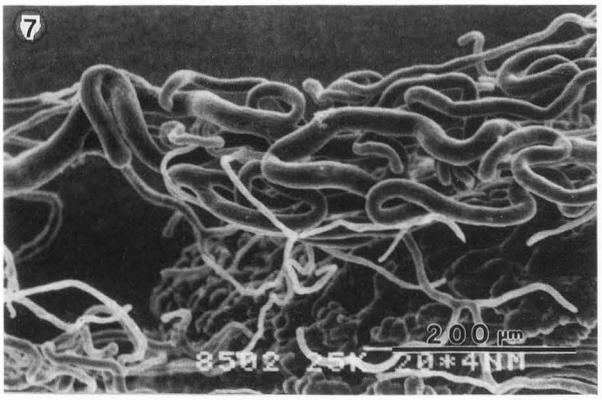
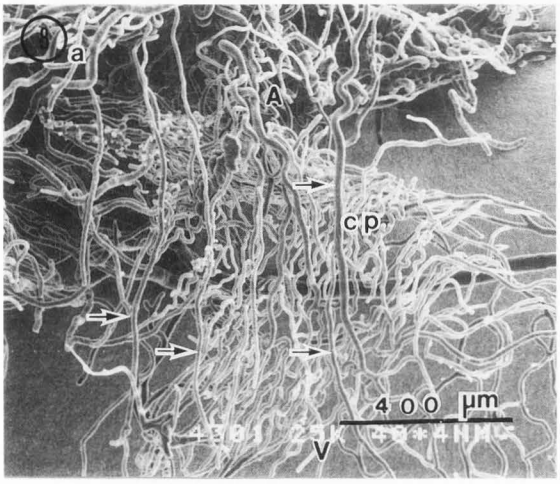


Figure 5. Coiled arteriole of human cochlea (arrow) is not so complicated as that in guinea pigs and appears as a winding, meandering shape rather than a coiled shape. R: radiating arterioles; S: spiral vessel.

Figure 6. Blood vessels of the lateral wall of the human cochlea. S: capillary net of stria vascularis. R: radiating arterioles.





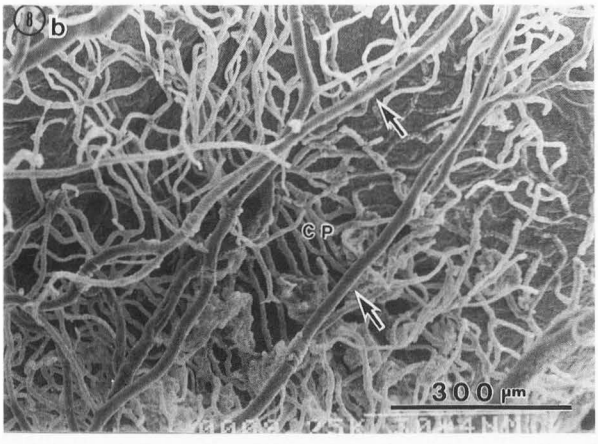


Figure 7. Coiled arterioles in the guinea pig vestibule. Immediately before the neuroepithelial area the arterioles form complex coils.

Figure 8. Microvasculature in the guinea pig (**a**) and human (**b**) utricles. In both, arteriovenous anastomosis (arrows) is observed below the capillary plexus (CP). A: Arterial side, B: venous side.

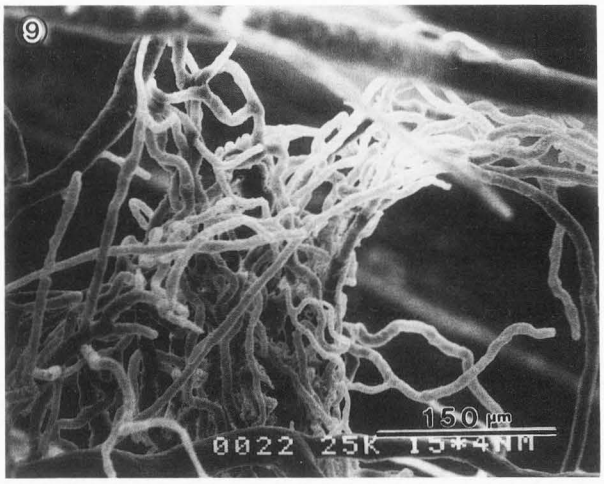


Figure 9. Microvasculature of the human superior and lateral semicircular canal.

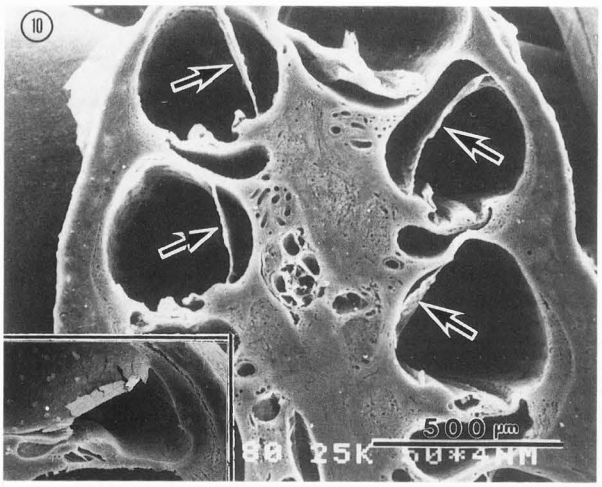
In guinea pigs, the number of radiating arterioles is very large and for this reason, the distribution of blood vessels to the stria vascularis and the spiral ligament is complicated. The vascular arrangement at these two sites is less regular. Anastomoses are infrequently observed between the stria vascularis and the spiral ligament. The capillary net of stria vascularis, descends the lateral wall of cochlea, forms the collecting venule and then, joins the common modiolar vein (Fig. 6).

In the vestibule, there are two arteries, namely, the anterior and posterior vestibular arteries.

The anterior vestibular artery parallels the utriculoampullar nerve in the superior vestibular canal, runs spirally near the center of the bony canal and then, coils several times in guinea pigs. Afterwards, it distributes blood vessels in the utricule, the ampullae of the superior and lateral semicircular canal (Fig. 7). In humans the coiled anterior vestibular artery which is characteristic for guinea pigs is not clear and the artery meanders.

The posterior vestibular artery branches off from the common cochlear artery. It distributes blood vessels in the saccule, the posterior ampulla and the posterior semicircular canal.

On the lower side of the capillary plexus immediately subjacent to the epithelium of the utricular macula, arterioles stemming from the anterior vestibular artery were noted to run almost straight and to communicate with the venous system without branching. Because of their morphological characteristics, these vessels were considered to be arteriovenous (AV) anastomoses (Fig. 8). In the saccular macula, on the other hand, no distinct AV anastomoses existed on the lower side while two-layered capillary networks were found on the endolymphatic space side. In the semicircular ampullae, especially at the junction of the lateral and anterior ampullae, several arterioles that represent a terminal portion of the anterior vestibular artery were seen running in almost straight lines without forming connections with



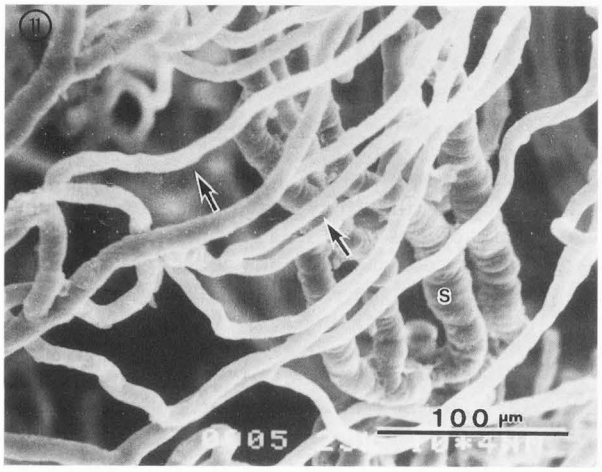


Figure 10. Guinea pig cochlea with endolymphatic hydrops. Reissner's membrane (arrows) is extended. Inset shows the normal cochlear duct.

Figure 11. Microvasculature of lateral cochlea wall in experimental endolymphatic hydrops. Dilatation of capillaries in the stria vascularis (S) and narrowing of vessels (arrows) at the AV anastomosis of the spiral ligament are observed.

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the microvasculature of the ampullae. Capillary networks formed a saddle like structure which corresponded to crista and were observed to be most dense at the top (Fig. 9).

Twelve months after the production of experimental endolymphatic hydrops (Fig. 10), the vasculature of the lateral cochlear walls was noted to have undergone changes that varied from place to place even in the same turn of a particular animal. Thus, within the same turn, the dilatation of capillaries of the stria vascularis and the narrowing of vessels at AV anastomoses of the spiral ligament were prominent when compared to the control side in some places, while, conversely, the narrowing of capillaries of the vascular stria and the dilation of vessels at AV anastomoses of the spiral ligament were evident in others (Fig. 11). Moreover, there were areas where no marked changes in the vascular system were discernible. In the saccular macula, notably on its epithelial side, vessels of the capillary plexus were found to be of reduced caliber. These changes were particularly pronounced at the center of the macula. No vascular abnormalities in the capillary plexus and AV anastomoses were observed in the utricular macula, nor were noteworthy abnormalities seen in capillaries of the semicircular canal ampullae or in vessels of the semicircular canals.

Discussion

A correct understanding of the anatomic basis for blood-flow regulation not only in the cochlea but also in neuroepithelial areas of the vestibule is of great importance from both the fundamental and the clinical viewpoint. In the vestibule, like in the cochlea, there is a characteristic blood vessel pattern over the entire length of the structure. The coiling of arterioles in the vestibule, as in the upper and lower coiled arterioles of the cochlea of guinea pigs, provides a blood pressure buffering function and, by virtue of its elongated blood vessels, favors the retention of blood, thereby ensuring constant blood supply to the neuroepithelial areas situated peripheral to the arterioles (Nakai *et al.*, 1986; Nakai *et al.*, 1990).

The point most different between guinea pigs and humans was that the coil-like appearance of the coil arteriole and anterior vestibular artery in humans is not outstanding, and the artery runs almost tortuously.

Of particular note among the present observations, is the existence of AV anastomoses immediately subjacent to the capillary plexus of the utricular macula. These communicating vessels are considered to subserve blood-flow regulation to functional vessels, i.e., the capillary plexus. It thus became obvious that a regulatory mechanism for local blood flow exists in this tissue. A similar vascular structure that is affiliated with such functional vessels and serves as a collateral pathway was also noted at the junction of the ampullae of the superior and lateral semicircular canals.

In inner ears affected by endolymphatic hydrops, capillaries of the vascular stria and vessels of AV anastomoses of the spiral ligament were found to be of irregular caliber, and vessels of the capillary plexus of the saccular macula were narrowed. These findings point to the possibility that endolymphatic hydrops gives rise to circulatory disturbances of the lateral walls of the cochlea and of the saccular macula. Implicated as the cause of this circulatory impairment of the cochlea is damage to the vascular system on the lateral wall of the cochlear duct at the attachment of Reissner's membrane. The damage arises from elevated endolymphatic volume and stretching of the membrane.

The results of our present experimental study of endolymphatic hydrops provide ample presumptive, although not definitive, evidence of circulatory disturbance occurring in the cochlea vascular system. It also seems possible, however, to interpret the vascular changes in the lateral wall of the cochlear duct as a consequence of a local defense mechanism against a load of endolymphatic hydrops. The importance of AV anastomoses at the spiral ligament in the maintenance of blood supply to the stria vascularis is thus suggested.

In the vestibule, abnormalities in vascular structure were observed only in the saccular macula. A possible anatomical explanation for this could be that while the utricular macula is partly separated from the bony labyrinth and is proportionately less liable to be subject to endolymphatic volume, the saccular macula is in direct contact with the bony labyrinth and, accordingly, would be directly influenced by an increase in endolymphatic volume. Furthermore, as mentioned earlier, AV anastomoses in the utricular macula, by virtue of their structural specialty, are capable of regulating blood flow and thereby serving as a buffer-zone preventing circulatory disturbance.

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Discussion with Reviewers

M. Anniko: In animals with endolymphatic hydrops there occurred considerable irregularities in the caliber of capillaries of the stria vascularis, the AV anastomoses of the spiral ligament, and the capillary plexus of the saccular macula. Cannot these observations be the result of aging and not dependent on hydrops?

Authors: Irregularities in the caliber of these capillaries were most likely the result of endolymphatic hydrops because these changes could not be observed in the control side (experimental hydrops were produced just on the right side).

M. Anniko: How many human temporal bones were used? Did you find age-dependent variations of the microvasculature? Was their any difference between the two temporal bones from the same individual?
Authors: Two human temporal bones aged 45 and 48 years old were used in this study. We neither found any age-dependent variations nor any difference between the two temporal bones from the same individual. We need to study other temporal bones from younger and older ages to identify any age dependent variations.

B.A. Bohne: What criteria were used to determine vessel type and location within the labyrinth?
Authors: Observation of the whole microvasculature at low magnification enabled us to determine vessel type and location within the labyrinth.

B.A. Bohne: What is the justification for using 185 guinea pigs for this study?
Authors: We have examined vascular changes under many different conditions, over 200 normal inner ear vascular casts of guinea pig thus far. Therefor, we used the 185 normal vascular casts, as control, in this study.

B.A. Bohne: Were there problems with the vascular cast technique?
Authors: Resin injection and dissolution of tissue around the casts is comparatively easy. However, it is difficult to keep the whole structure of the cast without collapse when drying.

B.A. Bohne: Were vascular differences found across animals?
Authors: Vascular running and structure are fundamentally almost same across animals.

B.A. Bohne: How certain are you that the two human temporal bones, which were examined, are representative of the population? Are you certain that these individuals had normal labyrinthine function? Couldn't they have had aging changes in their vasculature?
Authors: Because of small number of human temporal bone, we do not have an exact idea as to whether the two bones examined in this study are representative of the population or not. These individuals had no hearing impairment and balance disorders before death. Aging changes were not observed in their vasculature.

S. Aharinejad: Recent work has shown that a longer perfusion with saline may be helpful for showing the lymphatics, based on the mechanism that a prolonged perfusion time may probably caused edema. Do you think that this could be true for your approach too?

Authors: A prolonged perfusion in this study was performed to remove blood cells completely. We have no idea in detail about the possibility that a prolonged perfusion time may cause edema.

S. Aharinejad: How long after death were the human temporal bones removed?
Authors: Human temporal bones were removed about 8 hours after death.

S. Aharinejad: The use of 8 N HCl may not damage the Mercox cast specimens when the maceration time is no longer than 4-6 hours. You used this solution for 24 hours for removing the soft tissue. Does not the risk of the cast damage exist in this case?
Authors: The use of 8 N HCl for 24 hours did not damage the cast at all.

S. Aharinejad: It is surprising that you dried your specimens by air. What about the shrinkage of cast specimens?
Authors: Although the shrinkage of the cast has not been examined so far, our previous study reveals that the diameter of the cast does not change with air drying.

S. Aharinejad: On the surface of almost all vessels shown in Figs. 3, 6, and 8, there are some circularly running structures, embracing the entire circumference of the cast vessels. Could these structures be plastic strips; if there are some, please comment upon the mechanism by which they might be caused.
Authors: These structures around the cast that you point out are not plastic strips but uncorroded perivascular tissue (not dissolved during preparation).

S. Aharinejad: On the surface of the "dilated capillaries" of the stria vascularis, there are numerous constrictions, defining the bulged areas. How could these constrictions be caused? Did you perform any histological examination for evaluating the morphological background of these constrictions? Are these capillaries equipped with pericytes?
Authors: We have no idea about the detailed mechanism of vasoconstriction in the stria vascularis. Blood sludging, which seemed to be caused by vasoconstriction, was observed under light and electron microscope. Capillaries of the stria vascularis are not equipped with pericytes.