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SCANNING ELECTRON MICROSCOPY OF THE CELLOIDIN-EMBEDDED INNER EAR SECTIONS

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Abstract

The nerve fibers running inside the organ of Corti were studied in cats by scanning electron microscopy (SEM). The fixed temporal bones were decalcified and embedded in celloidin according to the conventional method. Thick serial sections (100-150 μm) were cut parallel to the basilar membrane. After removing the celloidin, the sections were freeze-dried in t-butyl alcohol. Though some outer hair cells had been deformed, the nerve fibers were well preserved for analysis.

Some new findings were observed in the present SEM study. The tunnel spiral bundle in a kitten took an atypical course on the floor of Corti's tunnel apart from the previously reported site. In the barely-patent tunnels of Corti in kittens, nerve fibers were stuck together into thick bundles and took different courses compared to those in the mature cochleas. Filiform projections of nerve fibers were climbing around the first row of the outer hair cells in the immature cochlea. These were thought to be growing ends of efferent nerves which would later grow into nerve endings.

Key Words: scanning electron microscope, cat, inner ear, nerve fiber, organ of Corti, Corti's tunnel, Nuel's space, tunnel spiral bundle, afferent nerve, efferent nerve.

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Introduction

The surface preparation technique has usually been used to observe Corti's tunnel or Nuel's space with the scanning electron microscope (SEM). Several photomicrographs obtained with this technique in the guinea pig (Wright and Preston , 1975. Bredberg , 1977a, b), rabbit (Bredberg, 1977a, b) and human fetus (Hoshino and Nakamura, 1985) have been published. The reticular membrane of the organ of Corti is lifted up with a fine needle, sharpened forceps or a knife blade to show the inside of the organ of Corti. It is rather difficult, however, to constantly obtain good results with this technique. In addition, it is very difficult to observe bilateral sides of a cutting plane and to consistently obtain views of desired structures. To overcome these disadvantages, we made thick macrosections of the decalcified temporal bone. Decalcification and embedding in celloidin produced artefacts in some elements, such as shrinkage of the outer hair cell bodies. The procedure, however, did not distort the nerve fibers inside the organ of Corti. This was confirmed by comparing specimens prepared by the present procedure and ones prepared by the surface preparation technique. The main purpose of this paper is to introduce this method and demonstrate the interior view of the organ of Corti obtained with this thick section method.

Materials and Methods

Cochleas from 3 adult cats and 6 kittens were examined. The exact birth dates of the kittens were not ascertained. They weighed 80, 90, 93, 200, 300 and 300 grams. The animals were anesthetized with an intraperitoneal pentobarbital injection. They were decapitated and the middle ear bullas were opened. The stapes were removed and 2% phosphate-buffered glutaraldehyde (pH 7.4) was gently introduced into the inner ear through the round and oval windows within 5 minutes of decapitation. The dissected temporal bones were further fixed in glutaraldehyde. They were decalcified in 5% trichloroacetic acid and neutralized in 5% sodium sulfate solution. Some specimens were decalcified in 10% EDTA solution. They were then dehydrated in graded

ethanol and transferred into ether-alcohol (1:1). They were infiltrated and embedded in celloidin by the conventional celloidin method (Schuknecht, 1974), over a period of about 12 weeks. The hardened temporal bones were serially cut at 100-150 μm using a sliding microtome. We selected horizontal sectioning so that the cutting plane would be parallel to the basilar membrane. The sections were put between pieces of paper and stored in 70% ethanol. After removing celloidin in ether-alcohol (1:1) solution, selected sections were put in absolute ethanol and then into t-butyl alcohol for freeze-drying using the procedure of Inoue and Osatake (1988).

In the process of drying, the sections were put between two small pieces of wire mesh to prevent them from curling. The sections were gently washed 3 times in t-butyl alcohol. A minimal volume was used for the 3rd wash so that the surface of the mesh was lightly covered (about 1.5 ml). The container was placed in the refrigerator. T-butyl alcohol freezes in 5-10 minutes at temperatures below 25.6° C when the volume is as small as 1.5 ml. The sections frozen in the container were then allowed to dry in a vacuum evaporator evacuated by a rotary pump for about 1 hour. The dried sections were mounted on metal stubs with carbon paste, coated with gold using a sputter coater (JEOL JFC-1100), and observed with a scanning electron microscope (Hitachi S-800).

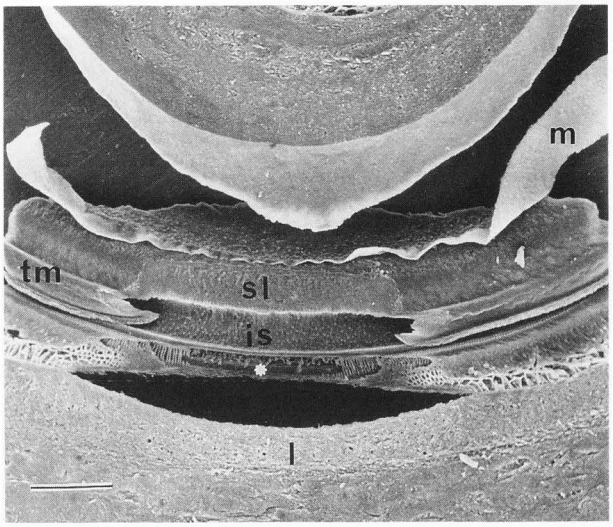

Fig. 1. Low magnification view of a thick section. At the center of the horizontally cut organ of Corti, the floor (*) of Corti's tunnel is exposed over a 350 μm span. is: inner sulcus, sl: spiral limbus, tm: tectorial membrane, m: Reissner's membrane, l: spiral ligament. Bar equals 200 μm (kitten 300g, lower middle turn)

Results

We could not tell the difference in quality of specimen preservation in two groups which were prepared by the different decalcifying agents, 5% trichloroacetic acid and 10% EDTA.

A low magnification photomicrograph of a thick section is shown in Fig. 1. The floor of Corti's tunnel of the middle turn was exposed over a 350 μm span in the center of the cut surface of Corti's organ. Figure 2 shows two facing surfaces of a cut plane from the organ of Corti, the upper figure (A) is the under side of one section and the lower figure (B) is the upper surface of the adjacent section. The tunnel base is seen in Fig. 2-A (t), and the ceiling of the tunnel (c) and the outer spiral bundles (o) in the Nuel's space are seen in Fig. 2-B.

Nuel's space of another cat is shown in Fig. 3. The cell bodies of the outer hair cells (Fig. 3 : oh) are not smooth and round, seemingly due to fixation and/or preparation artefacts. The outer spiral bundles (Fig. 3 : o), however, does not undergo much change. The caplike coverings of the cell membrane of Deiters' cells which cover the lower parts of the outer hair cells are also clearly seen (Fig. 3 : arrow).

The nerve fibers on the base of Corti's tunnel are shown in Fig. 4. The basilar fibers, probably afferent in nature, take basalward spiral courses on the bottom of the tunnel. It is hard to trace individual single nerve fibers because they were partially covered and sealed by cytoplasm of the pillar cells. In most instances, the tunnel spiral bundle runs just medial to the stalks of the inner pillar cells (Fig. 4-insertion). In one kitten, the tunnel spiral bundle ran longitudinally apart from the stalk at the bottom of the tunnel (Fig. 4). Though not confirmed in the basal turn, this atypical running of the tunnel spiral bundle was seen all through the middle and the apical turns.

Tunnel medial fibers which appear through or under the tunnel spiral bundle run through the upper portion of the tunnel space and enter Nuel's space between the outer pillar cells (Fig. 2-B, 4-insertion). There was some variation in the course of these tunnel medial fibers in mature cochleas. Though many fibers run high in the tunnel space, some fibers descended on the floor in the middle of the tunnel or ascended again, running high after touching the tunnel floor.

In the growing tunnel of Corti in kittens, numerous nerve fibers are packed together into thick bundles (Fig. 5, b). Though they run in longitudinal and radial directions, the pattern is different compared to the mature nerve network (Fig. 5). In Figure 5, the tunnel space still retained amorphous substance. Tunnel medial fibers run in this substance at a little higher level than the tunnel base, but not as high as in the mature tunnel. Some spiral fibers on the tunnel base could be traced as far as 35 μm in this photomicrograph.

Nerve endings on the outer hair cells were clearly visible in the basal turn (Fig. 6-Insertion). In the immature cochlea, these nerve endings were not observed yet. Instead, filiform projections were seen on the sides of the hair cells and on Deiters' cells (Fig. 6). In this 93 grams kitten, Corti's tunnel above the upper

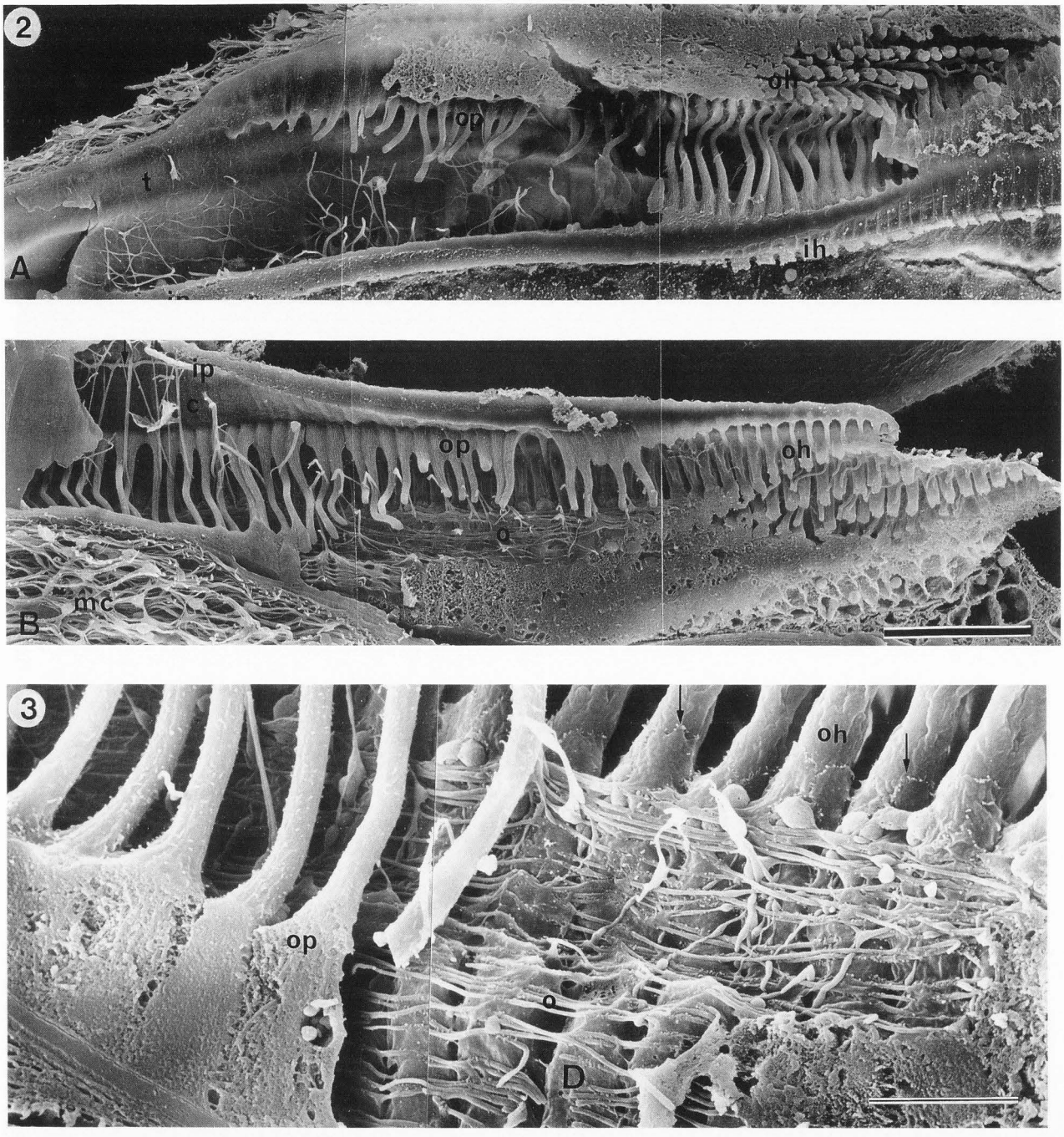

Fig. 2. Views of a horizontally cut organ of Corti. A: Under side of the Corti's tunnel. B: Upper surface of Corti's tunnel and Nuel's space. Left side of the figure is basalward. Nerve fibers running basalwards on the tunnel floor are seen in A. The tunnel spiral bundle (arrow) and the outer spiral bundle (o) in Nuel's space are seen in B. ip: inner pillar cell, op: outer pillar cell, ih: inner hair cell, oh: outer hair cell, t: tunnel base, mc: mesothelial cell. Bar equals 50 μm (adult cat, lower middle turn)

Fig. 3. Outer wall of Nuel's space. Outer spiral fibers (o) gradually climb the surface of the first row of Deiters' cell bodies (D) up to the bottom of the first row of the outer hair cells (oh). op: outer pillar cell, arrows: Deiters' cell covering. Bar equals 10 μm (kitten 200g, upper middle turn)

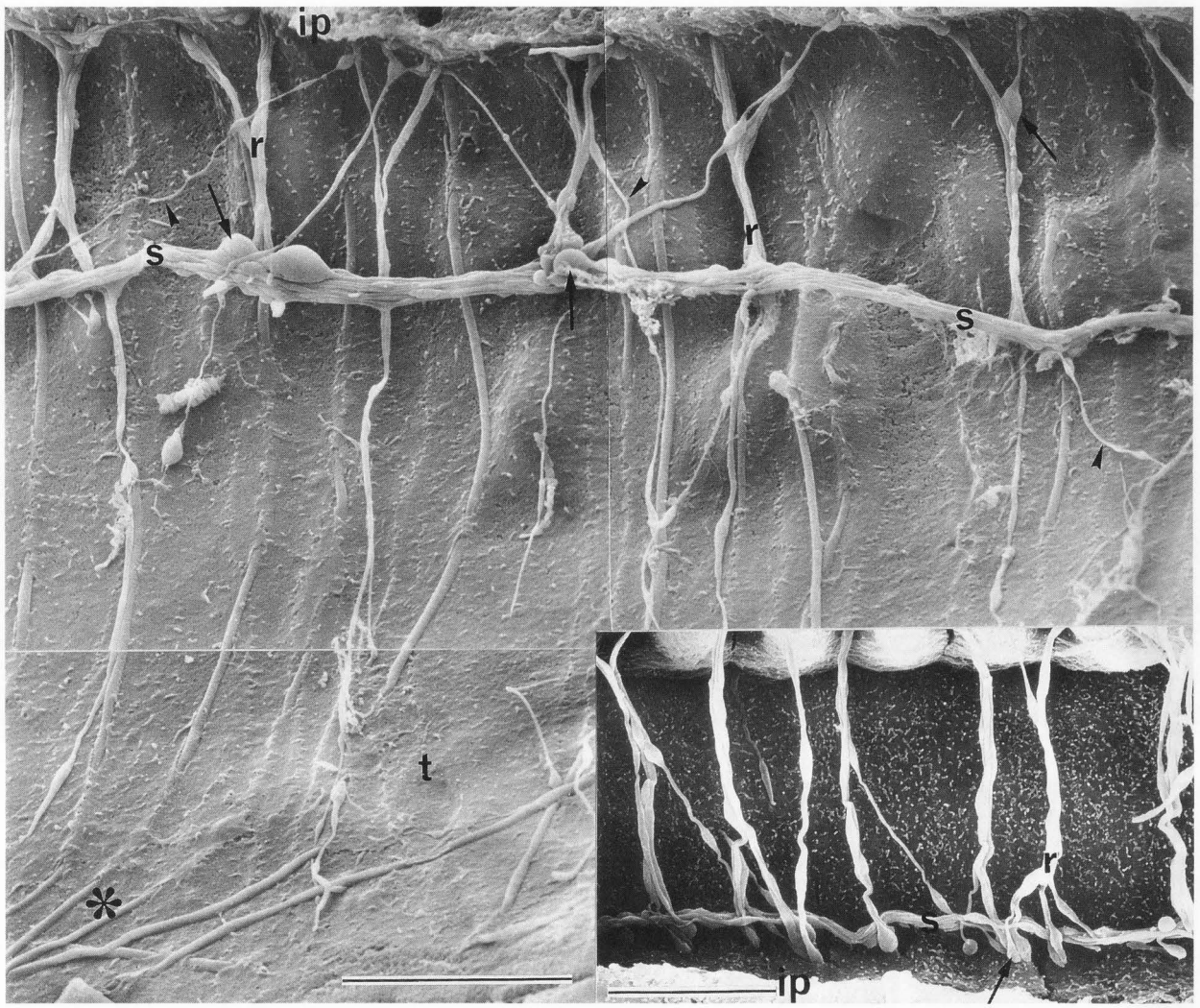

Fig. 4. Nerve fibers in the Corti's tunnel. The tunnel spiral bundle runs some distance apart from the emerging points of radial fibers. This bundle is usually located at the stalk of the inner pillar cells (insertion). Tunnel spiral fibers (s) and radial fibers (r) have varicosities (arrows) but tunnel basilar fibers (*) do not have them. The basilar fibers (*) emerge into the tunnel from between inner pillar cells (ip) at the medial corner of the tunnel and run spirally basalwards. Diameters of nerve fibers are variable. There are some very thin fibers (arrowheads).
s: tunnel spiral bundle, t: tunnel base. Bar equals 10 μm (kitten 200g, upper middle turn. Insertion: kitten 300g, lower basal turn)

middle turn was not patent. The filiform projections were seen all through the lower turns. These projections were thought to be growing ends of nerve fibers, probably efferent in nature.

Discussion

A section through the organ of Corti parallel to the basilar membrane should provide a view of its entire inside. In our study the specimens were sectioned obliquely in most cases. Therefore, views of only restricted spans of the inside of the organ of Corti were obtained. Though fragmented, various structures were constantly and easily observed. Using serial sections, we could exactly determine the distance of the observed object from the basal end.

The decalcification and embedding procedure resulted in some distortion of the cell surface of the organ of Corti, for example, the outer hair cell bodies. Thin structures such as nerve fibers, however, were minimally distorted. Another advantage of this method is that it can be applied to immature cochleas. It is difficult to lift up the reticular membrane in the immature or barely patent tunnel of Corti to obtain a good view. So, we believe that the present method is quite suitable for studying nerve fibers in the organ of Corti. If we make cutting planes at different angles, other structures in this organ

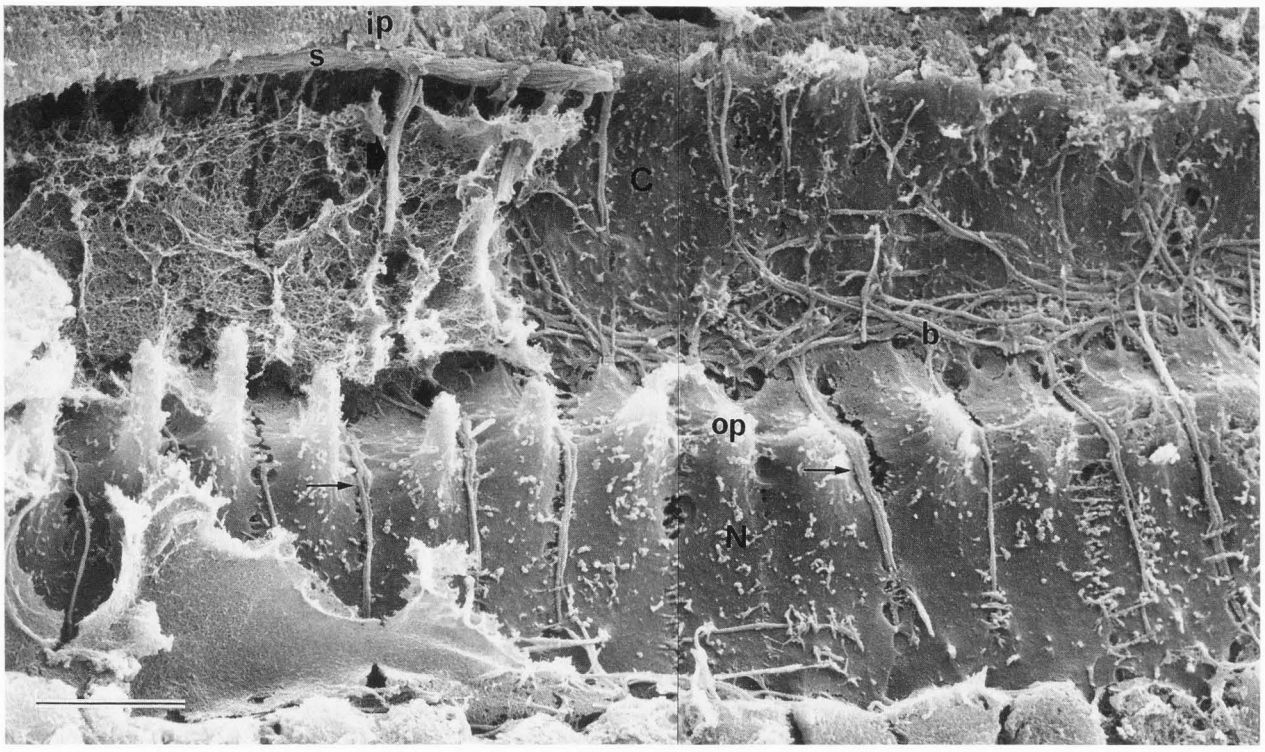

Fig. 5. Bottom of the immature tunnel of Corti and of Nuel's space. The bases of Corti's tunnel (C) and of Nuel's space (N) run from right to left in this picture separated by a row of outer pillar cell bases (op). The modiolus is in the upper part of the picture. Tunnel spiral bundle (s) runs at the base of the inner pillars (ip). Tunnel medial fibers (thick arrow) run radially in some amorphous substance. Tunnel basilar fibers (b) run basalwards stuck to the bottom of the tunnel space. Tunnel basilar fibers exit the tunnel between outer pillars and enter Nuel's space (small arrows). Bar equals 10 μm (kitten 80g, lower basal turn)

can be analyzed.

The freeze-drying method with t-butyl alcohol was reported to be suitable for biological specimens because it maintains the details of fine cell structures (Inoue and Osatake, 1988). The method was found to be applicable also to such large histological sections as used in the present study. We also tried to examine by this method some cats' cochlear sections which were fixed in formalin and mounted in the balsam for more than 20 years. Preservation of the outer hair cells and the outer spiral bundles was good enough to allow the evaluation at a higher magnification than a light microscope can achieve. Another advantage of this method is that the procedure does not require special equipment and can be easily accomplished.

The anatomy of the nerve fibers in the organ of Corti of the guinea pig, chinchilla, rabbit and human has been elaborately studied. In the cat, the nerve fiber network in the mature cochlea has been studied by transmission electron microscopy (Spoendlin, 1969. 1972. Ginzberg and Morest, 1984) and by light microscopy using horseradish peroxidase technique (Kiang et.al, 1982. Simmons and Liberman, 1988) or Golgi method (Perkins and Morest, 1975. Ginzberg and Morest, 1983). Developmental changes of nerve fibers during maturation has not yet been fully studied. Transiently appearing thready branchlets in the outer spiral bundles (Ginzberg and Morest, 1983) or filiform projections of probable efferent nerves have not been fully examined by TEM or SEM. Scanning electron microscopy seems to be suitable for observing these structures.

Relative locations of the tunnel spiral bundle, tunnel medial fibers, and tunnel basilar fibers in the cat were schematically shown by Liberman (1980). Our SEM observations showed a variation of the location of the tunnel spiral bundle. Liberman indicated that the tunnel spiral bundle usually runs 8-10 μm above the medial corner of the tunnel base against the stalks of the inner pillar cells. In our specimen, the tunnel spiral bundle runs at the bottom of Corti's tunnel throughout the middle and apical turns. Various running courses were also observed in the tunnel medial fibers in mature cochlea. As the counting of these tunnel medial fibers was used as the base of quantification of the afferent and efferent nerve supply (Spoendlin, 1969), visualization of nerve fibers by SEM will be useful for reconfirmation of the cochlea nerve supply.

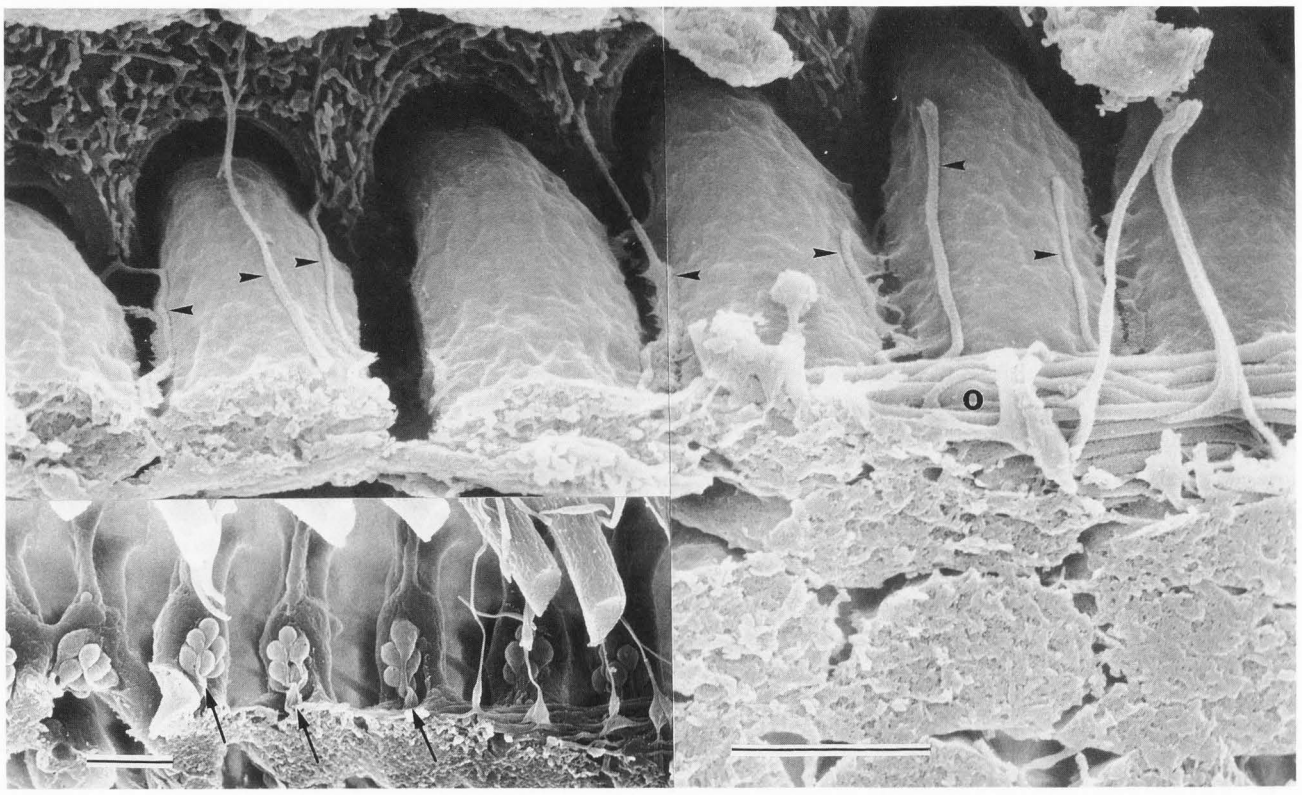

Fig. 6. Filiform nerve projections around the first row of the outer hair cells. In place of the mature nerve endings (arrows in the insertion), thin filiform fibers (arrow heads) were seen stuck on the side of the outer hair cells. Some of them reached the ceiling of Corti's tunnel. o: outer spiral bundle Bar equals 5 μm. (kitten 93g, upper basal turn. Insertion: kitten 300g, lower basal turn)

Acknowledgments

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Discussion with Reviewers

B.A. Bohne: Have you been able to determine how and where the basilar fibers enter the tunnel and the radial tunnel fibers exit the tunnel?
Authors: The basilar fibers enter tunnel space just below the radial fibers' entering points and run obliquely on the inner pillar. The radial tunnel fibers exit between the outer pillars about 2/3 height from the pillars' bottom.

R. Mount and R.V. Harrison: Was the animal illustrated in Figure 4, the only one where the tunnel spiral bundle was on the floor of the tunnel?
Authors: Yes, it was the only one cat which showed an atypical running of the tunnel spiral bundle.

R. Mount and R.V. Harrison: Within the cochlea illustrated in Figure 4, was the tunnel spiral bundle on the floor of the tunnel through out the cochlea or just in one area?
Authors: This atypical bundle was observed all through the middle and apical turns. We could not get good pictures in the basal turn of the kitten.

Y. Harada: Could the nerve fibers running between the foramina perforata and the tunnel be observed by this nethod? If so, please comment briefly.
Authors: We have not tried to observe this portion of the cochlea and have not yet been successful in getting good pictures.

Y. Harada: How were the conditions of the sectionized surfaces in the compositions of the Corti's organ, such as the outer hair cells for observing the intracellular architectures?
Authors: The cut surfaces of composing cells of the organ of Corti vary from cell to cell. Most cells including the outer hair cells and pillar cells showed solid cut surfaces but some cells as Hensen's and Claudius' showed empty spaces (Fig. 1). We do not think this method is suitable for observing the intracellular components.

Y. Harada: Is there another possible material for embedding the specimens?
Authors: Resin may be used for cutting small specimens but we haven't tried. Celloidin is good for cutting large sections.