Scanning Microscopy

Volume 7 | Number 3 Article 13

6-17-1993

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Kunihiro Mizuta
Hanamatsu University

Osamu Nozawa
Hanamatsu University

Hirofumi Morita
Hanamatsu University

Tomoyuki Hoshino
Hanamatsu University

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Mizuta, Kunihiro; Nozawa, Osamu; Morita, Hirofumi; and Hoshino, Tomoyuki (1993) "Scanning Electron Microscopy of Age-Related Changes in the C57BL/6J Mouse Cochlea," *Scanning Microscopy*: Vol. 7 : No. 3 , Article 13.
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Scanning Microscopy, Vol. 7, No. 3, 1993 (Pages 889-896)
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

0891-7035/93$5.00+.00

SCANNING ELECTRON MICROSCOPY OF AGE-RELATED CHANGES IN THE C57BL/6J MOUSE COCHLEA

Kunihiro Mizuta, Osamu Nozawa, Hirofumi Morita, and Tomoyuki Hoshino*

Department of Otolaryngology, Hamamatsu University School of Medicine, 431-31, Hamamatsu, Japan

(Received for publication March 4, 1993, and in revised form June 17, 1993)

Abstract

Hair cells and nerve fibers inside the organ of Corti of the C57BL/6J mouse, which is known as the precocious presbycusis model, were studied using the scanning electron microscope. For this study, we used thick serial sections cut from celloidin blocks.

In the 5-week-old mice, hair cell loss was not seen. The upper tunnel radial fibers crossed the upper part of the tunnel of Corti and entered the Nuel's space between the outer pillar cells. The fibers varied in diameter and showed many varicosities. The basilar fibers emerged into the tunnel of Corti beneath the upper tunnel radial fibers and crossed the floor of tunnel slightly curving basalward. The outer spiral fibers ran along the lateral wall of the Nuel's spaces, sometimes buried in the cytoplasm of Deiters' cells. The nerve endings were clearly seen on the modiolar sides of the outer hair cell bases in the basal turn.

In the 30-, 42- and 60-week-old mice, hair cell degeneration was seen both at the basal and apical portions, more pronounced in the former. The outer hair cells were affected more than the inner hair cells. In the basal turn where most of outer hair cells had degenerated, the upper tunnel radial fibers disappeared while the basilar fibers remained. These results suggest that degeneration of the efferent fibers occur earlier than those of the afferent to the outer hair cells.

Key words: Presbycusis, C57BL/6J mouse, inner ear, cochlea, organ of Corti, nerve fiber, efferent nerve, afferent nerve, nerve ending, scanning electron microscope.

*Address for Correspondence:
Tomoyuki Hoshino
Department of Otolaryngology
Hamamatsu University School of Medicine
3600 Handa-cho
Hamamatsu, 431-31 Japan
Telephone number: 053-435-2252
FAX number: 053-435-2253

Introduction

Our knowledge of the innervation pattern of the organ of Corti has been greatly advanced by using the electron microscope. It is now known that some efferent fibers cross the tunnel of Corti radially (upper tunnel radial fibers) and make large granulated nerve endings on the outer hair cells (Kimura and Wersäll, 1962; Smith and Rasmussen, 1963; Spoendlin and Gacek, 1963). Other efferent fibers enter the inner spiral or the tunnel spiral bundle innervating the inner hair cell area (Liberman, 1980; Ginzberg and Morest, 1983). The afferent fibers divide into two types: most of them are associated with the inner hair cells and only about 5% (in the cat) cross the tunnel of Corti toward the outer hair cells (basilar fibers) (Spoendlin, 1969, 1972, 1984). These studies were done mainly using the transmission electron microscope (TEM).

Only a few studies have been performed using the scanning electron microscope (SEM); photomicrographs of the nerve fiber arrangement inside the organ of Corti in the guinea pig and rabbit (Bredberg, 1977a, b), cat (Mizuta *et al.*, 1990), dog (Morita *et al.*, 1992), and human fetus (Hoshino and Nakamura, 1985; Hoshino, 1990) have been published. SEM studies on the mouse are rare probably because of the difficulties in preparing tiny specimens for viewing in side of the organ of Corti.

The mouse can serve as a good model for the study of pathological degeneration of the auditory system, because of several mutants with hereditary inner ear abnormalities (Deol, 1968; Steel and Bock, 1983) and early progressive hearing loss (Mikaelian *et al.*, 1974). The C57BL/6J strain of mice was selected for the present study because it is predisposed to early hearing loss and is often cited as a model for human presbycusis (Park *et al.*, 1990). The purpose of this study is to demonstrate age-related changes of the hair cells and the nerve fibers inside the organ of Corti in the C57BL/6J mouse using the SEM.

Materials and Methods

Twelve C57BL/6J mice were used in the study: 4 at 5 weeks, 4 at 30 weeks, 2 at 42 weeks and 2 at 60 weeks of age. Under general anesthesia by pentobarbital

intraperitoneal injection, 2% phosphate buffered glutaraldehyde (pH 7.4) was gently introduced into the inner ear within 5 minutes after decapitation. Temporal bones were kept in the same fixative *in toto* overnight. They were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) solution. After washing and dehydration, they were embedded in celloidin by the conventional celloidin method (Schuknecht, 1974), over a period of about 3 weeks. The hardened cochleas were serially cut in 80-100 μm thick sections using a sliding microtome. There were 13-15 sections per cochlea with 80 μm sections. The sections were dehydrated in a series of 70% to 100% ethanol. After removing celloidin in ether-ethanol (1:1) solution, selected sections were put in ether and then into t-butyl alcohol. About 1 ml of the t-butyl alcohol in a 10 ml glass container was frozen for 10 minutes in the refrigerator. We employed the t-butyl alcohol freeze-drying method (Inoue and Osatake, 1988). The dried sections were mounted on metal stubs with carbon paste and coated with gold, using a sputter coater (JEOL, JFC-1100). The specimens were observed using a SEM (HITACHI, S-800). After observation, some sections were detached and inversely remounted for observation of the reverse side of the same area.

Results

The present thick sectioning method was previously reported as being advantageous to observe innervation in the organ of Corti (Mizuta *et al.*, 1990). Using this method, not only nerve fibers in the fluid space of the organ of Corti but also sensory cell stereocilia may be clearly studied. Quantitative study of hair cell loss was not done, because it was difficult to peel off the tectorial membrane completely in the basal turn. A comparison was attempted between young adult group and the aged group.

The cochlear duct of the mouse is composed of 2 coils and its length from the apex to the round window is about 4.7 mm in an average of 5 cochleas. This length is almost the same as reported by Garfinkle and Saunders (1983).

Young adult mice (5-week-old mice)

At this stage, both the inner and outer hair cells showed no degenerative changes in either coil (Fig. 1A). Many fascicles of the upper tunnel radial fibers were seen in horizontal sections of the tunnel of Corti (Fig. 1B). The upper tunnel radial fibers emerge between the inner pillars, crossed the tunnel of Corti well above the floor in bundles of 2-5 fibers and entered the Nuel's space between the outer pillar cells. A few fibers, however, cross the tunnel at a lower levels (Fig. 2A arrow). The fibers varied in diameter from 0.15 μm to 0.8 μm and showed varicosities (Figs. 2A and 3A). The tunnel spiral bundle was not found in any specimen.

Most of the basilar fibers had diameters between 0.15 μm and 0.5 μm and ran on the floor (Fig. 2B), with only a few running above (Fig. 2A, arrowhead), curving slightly basalward. Sometimes, the fibers became

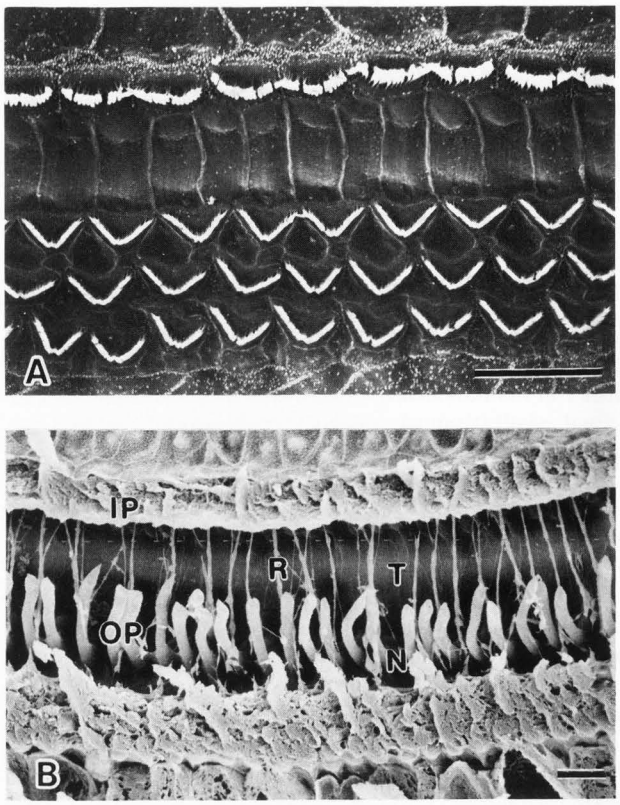

Figure 1. Surface view of the organ of Corti (**A**) and view of a horizontally cut organ of Corti (**B**) in a 5-week-old young adult mouse. **A**: Hair cell loss is not seen. Lower basal turn. **B**: Fascicles of the upper tunnel radial fibers (R) run from the modiolar side (top) to the strial side (bottom). The ratio of fascicles to outer pillar cells (OP) is roughly 1:1. IP: inner pillar cell; T: tunnel of Corti; N: Nuel's space. Upper basal turn, 3.1 mm from the apical end. Bar = 10 μm.

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embedded in the pillar cells' cytoplasm and ran separately as single fibers (Fig. 2B).

The outer spiral fibers ran along the Nuel's space at the modiolar side of the Deiters' cells, partly buried in their cytoplasm but easily traced by the presence of many microvilli (Fig. 3B, *). The nerve endings could be clearly seen on the modiolar side of the outer hair cells' bases (Figs. 3A and 3B).

Aged mice (30-, 42- and 60-week-old mice)

In aged mice, degeneration of the outer hair cells was noted with considerable individual variations (compare Figs. 4C and 5C with Fig. 7C). The degenerative changes of the hair cells in the four representative locations in the basal and apical turns are shown in Figures 4 and 5. These areas are indicated on the diagram in Figure 6. Since the disarrangement of stereocilia might have been an artifact, this evaluation of degenerative changes was based only on sensory cell loss. The outer hair cells were affected more severely than inner hair

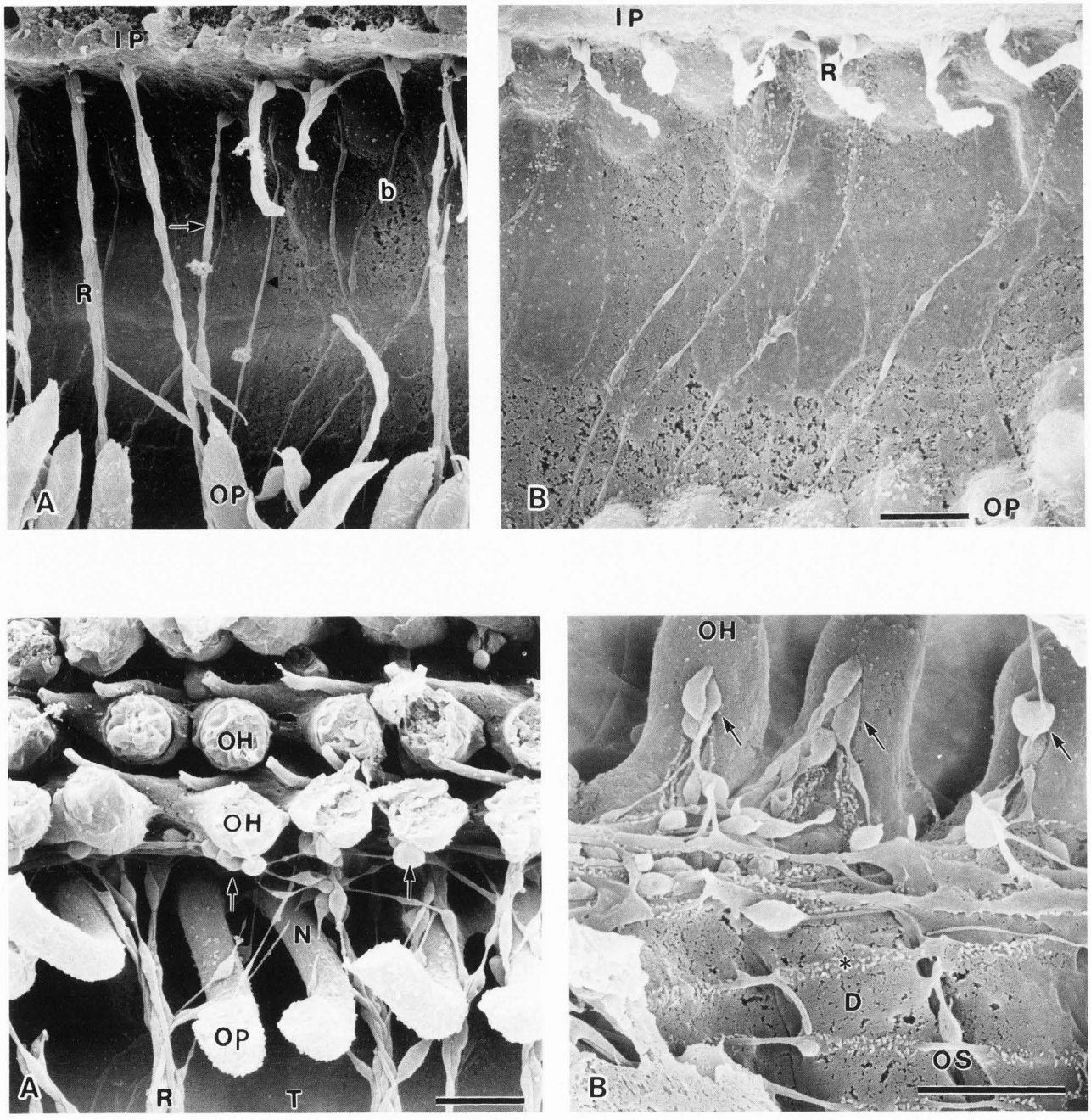

Figure 2. The upper tunnel radial fibers and the basilar fibers. **A**: The tunnel of Corti is seen from above. Upper tunnel radial fibers emerge from between inner pillars (IP) and run radially through the upper part of the tunnel of Corti. A radial fiber which crosses the tunnel at a lower level can also be seen (arrow). Most of the basilar fibers run on the tunnel floor (b), but occasionally a fiber runs slightly above the tunnel floor (arrowhead) (5-week-old mouse, upper basal turn, 3.1 mm from the apical end). **B**: Floor of the tunnel of Corti. Basilar fibers run on the floor curving toward the base (to the left of the picture). Note the absence of the tunnel spiral bundle in Figures 2A and 2B. Five-week-old mouse, upper basal turn, 3.4 mm from the apical end. OP: outer pillar cell; R: stumps of the upper tunnel radial fibers cut during preparation. Bar = 5 μm

Figure 3. Nuel's space viewed from above (A) and from the modiolar side (B). **A**: Upper tunnel radial fibers (R) show large variation in diameter and many varicosities. **B**: Outer spiral fibers in the first outer spiral bundle on the lateral wall of the Nuel's space. Most of them are concealed by Deiters' cell cytoplasm (*). Nerve endings (arrow) are seen on the lower medial part of outer hair cells. T: tunnel of Corti; N: Nuel's space; OP: outer pillar cell; OH: outer hair cell; D: Deiters' cell; OS: outer spiral fiber. Five-week-old mouse, upper basal turn, 3.4 mm from the apical end. Bar = 5 μm.

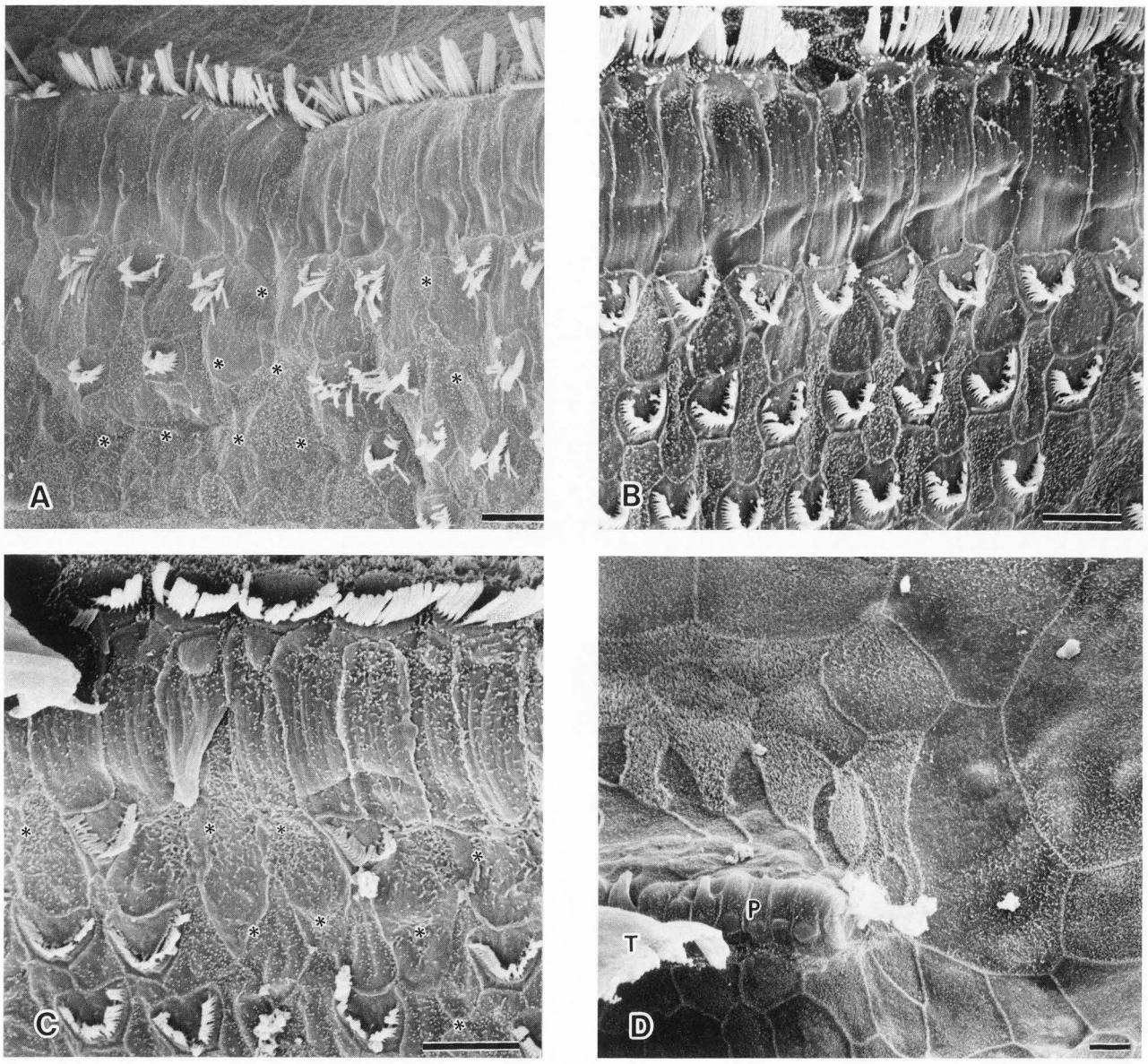

Figure 4. Surface views of the organ of Corti in 42-week-old mouse. Hair cells are better preserved in the upper (**A**) and lower (**B**) apical turns. In the lower basal turn (D) only a few pillar cells (P) remained. Some scars, left by missing hair cells, are exemplified by asterisks. T: tectorial membrane. Bar = 5 μm.

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cells (Figs. 4A, 4C, 5A, 5B, 5C and 7C). Degenerative changes advanced gradually from the basal to the upper turns, but moderate hair cell loss was also seen in the apical portion of the cochlea (Figs. 4A and 5A). In 30-week-old mice, the loss of a few pillars occurred in the basal turn, but in 42- and 60-week-old mice, this degeneration was more severe; sometimes the entire organ of Corti collapsed and became a flat scar (Figs. 4D and 5D).

In the basal turns of aged mice, where all of the outer hair cells and most of the inner hair cells were gone, the upper tunnel radial fibers were either greatly reduced or were absent, while the basilar fibers and the outer spiral fibers remained (Fig. 7). The number of the basilar fibers in these mice did not appear to be reduced when compared with those of the 5-week-old mice.

Discussion

The SEM study of the nerve fibers is efficient for visualizing a three dimensional view of their arrangement to study their continuity and interrelations.

The arrangement of nerve fibers inside the organ of Corti in the mouse was similar to that of guinea pig and rabbit, which were described by Bredberg (1977a, b). For example, the efferent fibers that ran radially in

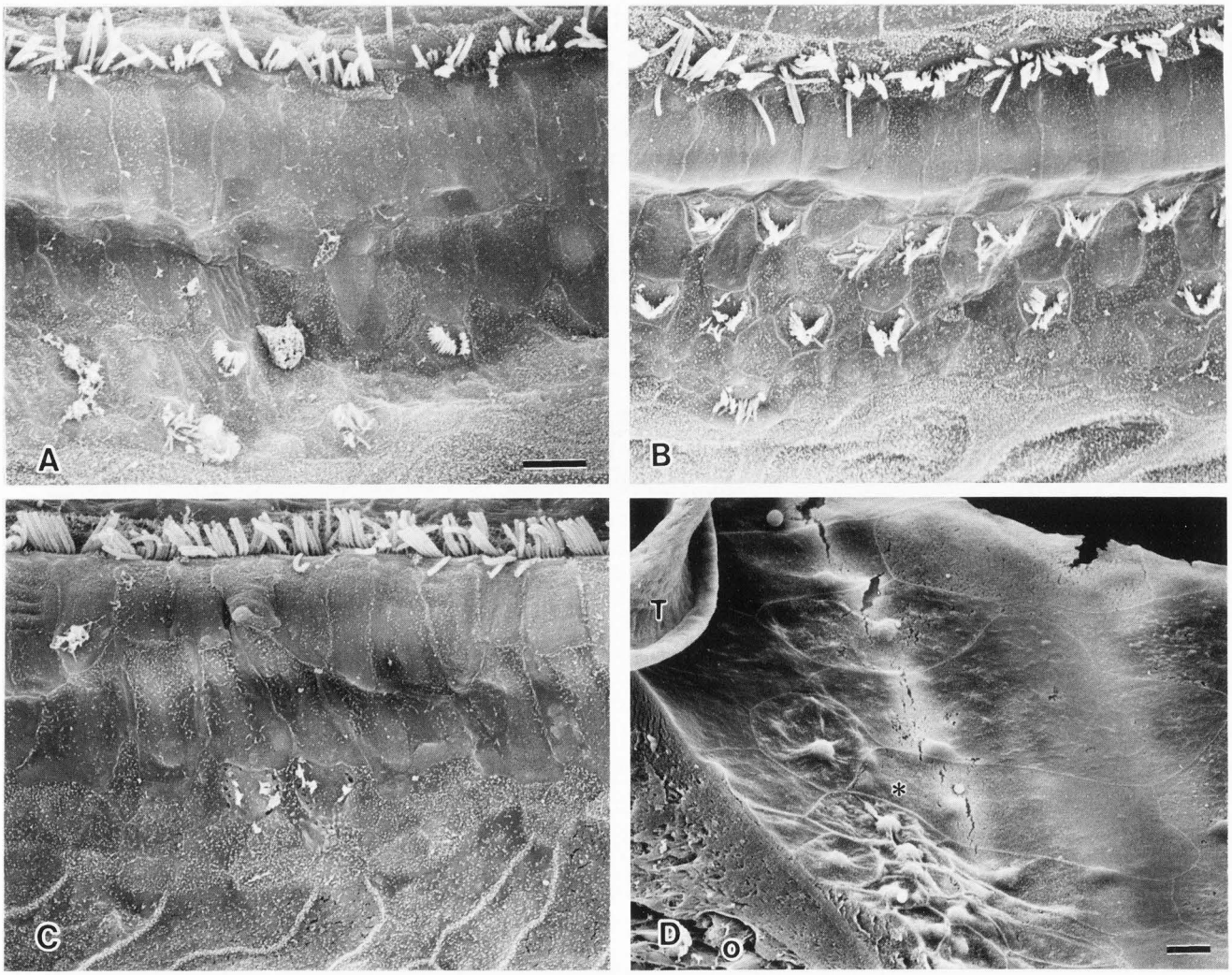

Figure 5. Surface views of the organ of Corti in a 60-week-old mouse. Degeneration of the organ of Corti is more severe than that of 42-week-old mice. The loss of outer hair cells can be seen in the upper (**A**) and lower apical turn (**B**), and in the upper basal turn (**C**). In the lower basal turn (**D**), the organ of Corti becomes atrophied. The organ of Corti disappeared completely (*). T: tectorial membrane; O: osseous spiral lamina. Bars = 5 μm (in A, B, and C) and 10 μm (in D).

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the upper part of the tunnel of Corti varied in diameter and showed varicosities, and the afferent fibers that ran on the tunnel floor were sometimes wrapped by the pillar cells. Low level tunnel radial fibers reported in the guinea pig (Brown, 1987) were also found in the present study (Fig. 2A arrow).

Since the tunnel spiral bundle was not found at all in our specimens, it appears that the tunnel spiral bundle is absent in the C57BL/6J mouse (Figs. 2A and 2B). This bundle was reportedly present in the house mouse

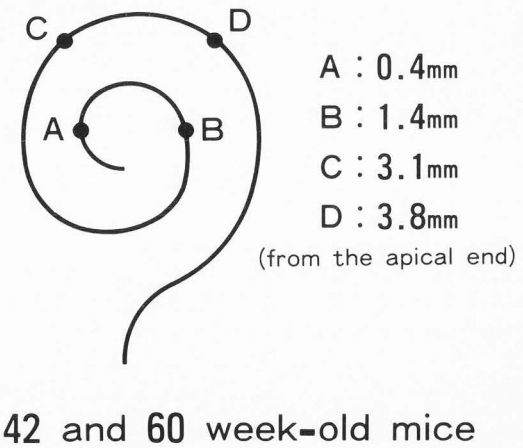

Figure 6. Location of four representative areas in the basal and apical turns which were shown in Figure 4 and 5. **A**: 0.4 mm, **B**: 1.4 mm, **C**: 3.1 mm, and **D**: 3.8 mm from the apical end respectively.

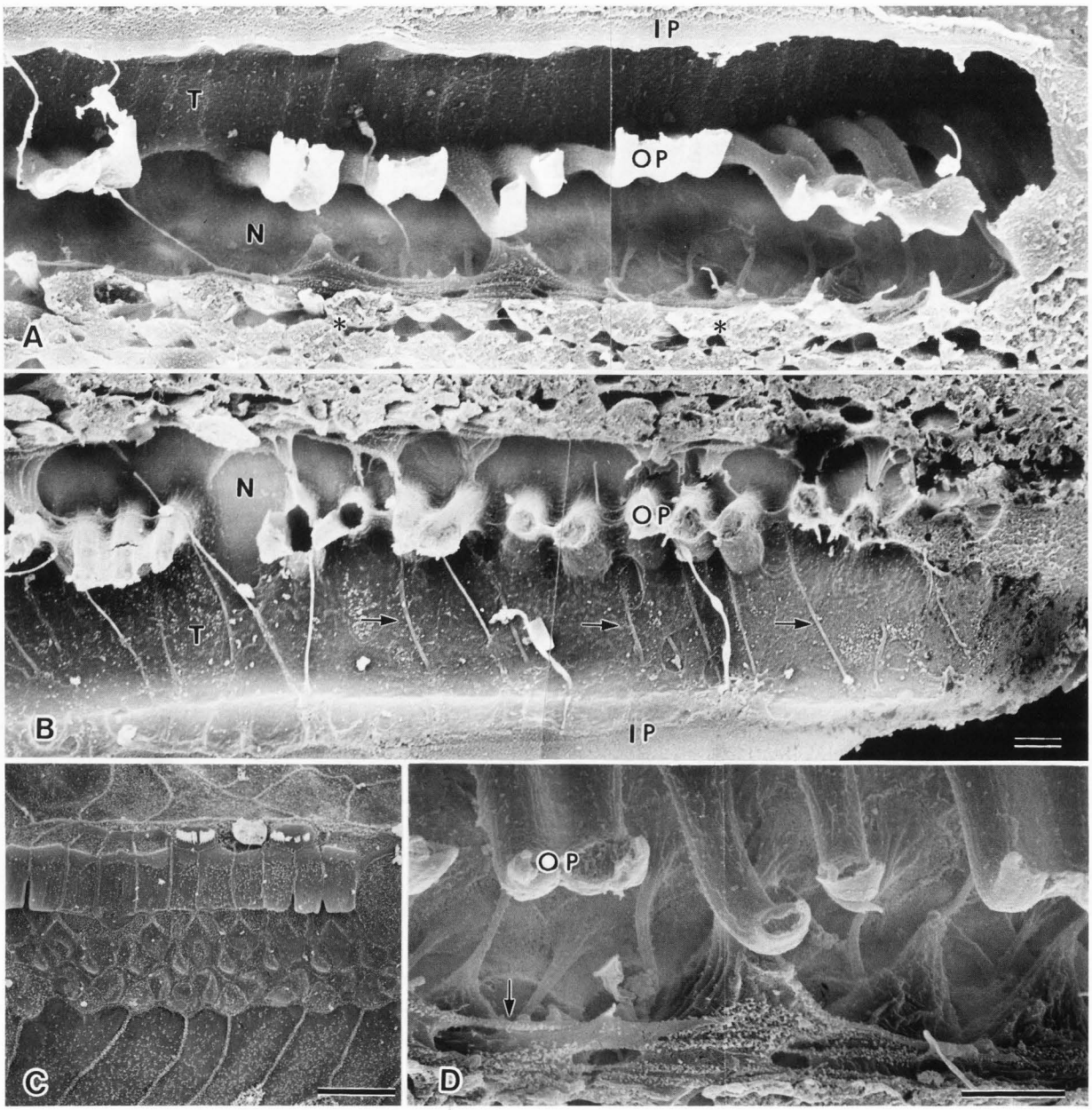

Figure 7. Loss of outer hair cells and corresponding nerve fiber degeneration. In the upper basal turn of a 30-week-old mouse, the outer hair cells and most of the inner hair cells have degenerated (**C**). In the same place, the upper tunnel radial fibers are greatly reduced or absent (**A**), while the basilar fibers present (Figure **B** arrows). Despite the loss of outer hair cells, outer spiral fibers persisted (arrow in **D**). The area interposed between 2 asterisks in A is shown in D under higher magnification. A, B and C were photographed within same area of the organ of Corti; A is viewed from below and B is viewed above. IP: inner pillar cell; OP: outer pillar cell; T: Corti's tunnel; N: Nuel's space. Upper basal turn, 3.1 mm from the apical end. Bars = 5 μm (in A, B and D) and 10 μm (in C).

(Ehret, 1979). This discrepancy between the C57BL/6J mouse and the house mouse may be caused either by their genetic difference or by the technique used in the study. The silver staining method which was used by Ehret may not be suitable to distinguish the exact location of a thin nerve fiber bundle running near the inner pillar cells. The tunnel spiral bundle is present in the guinea pig and the rabbit (Bredberg, 1977a, b), cat (Mizuta *et al.*, 1990), dog (Morita *et al.*, 1992), and human (Hoshino and Nakamura, 1985; Hoshino, 1990), but absent in the rat (Smith and Haglan, 1973) and the hamster (our unpublished data). Using a histochemical

technique, Sobkowicz and Emmerling (1989) have reported that there were 2 efferent spiral bundles near the inner pillar in the HA/ICR mouse: one was the inner spiral bundle, and the other, that they named, the inner pillar bundle. As the tunnel spiral bundle is simply thought of as an outlying portion of the inner spiral bundle (Wright and Preston, 1975), absence of the tunnel spiral bundle in the mouse may not be significant functionally.

The basilar fibers, afferent in nature, usually enter into the tunnel of Corti between inner pillars under the efferent upper tunnel radial fibers and run on the floor of the tunnel (Fig. 2B). In the CBA mouse, these afferent fibers (basal radial tunnel fibers) run free in the tunnel (Lim and Anniko, 1985). In our findings, most of basilar fibers ran attached to the floor and only a few of them took similar courses to the basal tunnel radial fibers (Fig. 2A, arrowhead). There may be some differences in the course of the basilar fibers in various species.

The nerve endings seen at the modiolar side of the first row of outer hair cells in the basal turn could not be defined as either afferent or efferent, because their connection to the upper tunnel radial fibers could not be traced. By cutting the efferent nerve supply at the 4th ventricle floor, Bredberg (1977a, b) showed degeneration of similar bud-like nerve endings and concluded that these endings were efferent in nature.

The inner ear changes of the C57BL/6J mouse have been observed with the light microscope (LM) and the TEM. Shnerson *et al.* (1982) studied 1-50 days old mice with the TEM and found that the degenerative process in the organ of Corti began 30-50 days after birth. Henry and Chole (1980), using the LM, reported that the C57BL/6J mouse had increased loss of outer hair cells in the basal and apical portions. In our findings, the most severe outer hair cell loss was seen at the basal portion, but degeneration also occurred in the apical region (Figs. 4 and 5). Such a degenerative pattern of hair cells has been observed in the cochlea of the aged human (Bredberg, 1968).

Sensorineural degeneration with aging is the most common form of pathology seen in the human cochlea (Johnsson *et al.*, 1990). The hair cells disappear first, and the nerve degeneration is presumably secondary. The change of the mouse cochlea also seem to be of the sensorineural type, because sensory and neural degeneration occur concomitantly (Fig. 7).

Willott (1991) reported that efferent terminals remained despite severe atrophy of the organ of Corti in old C57 mice and suggested that the efferent system may be rather robust despite sensorineural presbycusis. Our findings, however, suggest that the degeneration of the efferent fibers occur at a comparative early stage, at least earlier than the afferent fibers. We speculate that this difference is due to variability in different animals.

Some reports have been published concerning the relation between the loss of efferent and hearing function in the mutant mouse strains. Kikuchi and Hilding (1965) suggested that the absence of the efferent innervation could be correlated with the auditory dysfunction in the shaker-1 mutant mice. Contrary to this, Emmerling and Sobkowicz (1990) noted a the lack of correlation between the timing of efferent degeneration and the onset of deafness in the same strain of mice. So far, it is not clearly known what changes occur when efferent nerve fibers disappear while afferent fibers remain. Further studies are need to correlate auditory function with the changes in afferent and efferent nerves.

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Discussion with Reviewers

R.S. Kimura: Degeneration of outer hair cells is more obvious at the innermost row of basal turn in Figure 4C, while the loss appears to occur more at the outermost row of the apical turn in Figure 4A. Is this a consistent pattern of degeneration or is there any pattern among the different rows?
Authors: In degenerated outer hair cells in a 42-week-old mouse, there was no different degeneration of the outer hair cells in each of three outer hair cell rows.

R.S. Kimura: The cochlear length is mentioned in the **Results** section. How is the length determined from 80 μm thick serial sections?
Authors: We placed the specimens as horizontal as possible, and measured the length of sections using a string on their SEM images. The cochlea length was evaluated as a sum of these. Our values may be a little smaller than real length because specimens cannot be placed into a perfectly horizontal plane.

B.A. Bohne: How many ears developed flat scars in the basal turn? What was the size of these lesions?
Authors: Flat scars were seen in all ears of 42- and 60-week-old mice. We did not measure the size of scar because it is difficult for this method to measure the length of the hook portion.

B.A. Bohne: Do you think that some of the individual variation in the magnitude of damage has a genetic origin? Have you examined the ears of littermates? If so, do they have a similar amount of damage?
Authors: We have not examined littermates, but we speculate the damage has a genetic origin.

B.A. Bohne: Can you quantify the loss of nerve fibers in the organ of Corti in ears prepared for SEM?
Authors: No, we cannot. SEM is not a good tool for quantification. TEM should be used, but we have not tried.

B.A. Bohne: Why can't efferent and afferent fibers be distinguished on the basis of the fiber diameter?
Authors: Although efferent fibers have larger diameters than afferent fibers on the average, the diameter distributions overlap. It is more reliable to distinguish between efferent and afferent fibers based on their location and orientation rather than their diameters.