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Kwang-Sun Lee
University of Ulsan

Robert S. Kimura
Harvard Medical School

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ULTRASTRUCTURAL CHANGES OF THE VESTIBULAR SENSORY ORGANS AFTER STREPTOMYCIN APPLICATION ON THE LATERAL CANAL

Kwang-Sun Lee[1] and Robert S. Kimura[2,*]

[1]Department of Otolaryngology, Asan Medical Center, College of Medicine, University of Ulsan, Seoul, Korea
[2]Dept. Otolaryngology, Harvard Medical School and Massachusetts Eye and Ear Infirmary, Boston, MA, U.S.A.

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Abstract

Early changes in the vestibular sense organs resulting from the application of a streptomycin sulfate soaked Gelfoam® pledget on the fenestra of the lateral semicircular canal were studied by transmission and scanning electron microscopy. Three days after the application, lesions were present in the central part of the lateral crista. The type I sensory cells were more affected than the type II cells. These sensory cells showed mitochondrial swelling, cytoplasm protrusion at the cell apex, inclusion of multiple vacuoles, fusion or loss of stereocilia, and pyknotic nuclei. Seven days after the drug application, the sensory cell damage extended to all three cristae and macula utriculi. The lesions were very extensive after ten days and the sensory cells had almost equally disappeared in all three cristae; the lesion in the macula utriculi was smaller and the macula sacculi was unaffected. At fourteen days, the lesions appeared less severe. Thus, a single application of a small amount of streptomycin on the lateral canal fenestra affected all vestibular sense organs, except the saccule, in a short time. The strong affinity of aminoglycosides for the cristae suggests possible entrapment of the drug at the ampullae. This local drug application technique to the canal will be useful in studying vestibular function in animals, and it is applicable to controlling severe vestibular symptoms in human patients.

Key Words: Streptomycin, Lateral ampulla, Vestibular toxicity, Ultrastructural changes.

*Address for Correspondence:
Robert S. Kimura
Department of Otolaryngology
Massachusetts Eye and Ear Infirmary
243 Charles Street
Boston, MA 02114

Telephone number: 617 573 3592
FAX number: 617 573 3939

Introduction

One of the main goals for the treatment of Menière's disease is the prevention of recurrent attacks of vertiginous symptoms. Various medical and surgical treatments have attempted to ablate the vestibular function in order to control the vestibular symptoms. Streptomycin sulfate (SM), which is well known for its toxicity to vestibular sensory cells, has been used for about four decades for the treatment of intractable Menière's disease. The routes of the aminoglycoside administration have varied from the systemic injection [4, 15, 20] to the topical application to the tympanum [2, 8, 11, 13, 14, 15] and the lateral canal [1, 5, 6, 7, 12, 16].

Vestibular ablation by systemic administration usually requires a large amount of the drug and is expected to produce significant ototoxicity. The ototoxicity of the aminoglycosides is usually dose dependent and its effectiveness can be achieved by careful dose control. The tympanic application has merit because a smaller dose of aminoglycoside is used, thus lessening the ototoxicity. However, the outcome of the vestibular ablation has been variable and risk to hearing cannot be excluded.

A modified modality of topical treatment has recently been introduced with application of the ototoxic drug closer to the peripheral vestibular apparatus [5, 6, 7, 12]. In animal studies, gentamicin (GM) and SM were applied to an artificially made fenestra on the lateral canal and were found to affect primarily the vestibular sensory cells with very little consequence to cochlear sensory cells. Recently, SM application to the lateral canal has been performed clinically in Menière's patients [1, 16].

The present study was conducted to investigate early morphological changes in vestibular sensory cells and differential lesions among the vestibular sense organs resulting from the application of a small amount of SM to the lateral canal.

Materials and Methods

A total of seven guinea pigs, Charles River Duncan

Hartley strain, weighing between 250 to 300 gm were used for this study. The animals were anesthetized with sodium pentobarbital (35 mg/kg) and operated on bilaterally under sterile conditions. A coronal incision was made in the retroauricular region, and the temporalis muscle and soft tissue over the dorsal bulla were elevated. After opening the dorsal bulla, the lateral semicircular canal close to the ampulla was drilled and the perilymphatic space was exposed by removing the bony cap. The fenestra was covered with a small piece of Gelfoam® (about 2 mm in diameter) soaked with streptomycin sulfate (1 gm in 3.5 ml distilled water). For the control study, a fenestra was made and a saline soaked Gelfoam® pledget was applied on the fenestra. The size of the fenestra was about 0.14 mm^2.

The survival times of the animals after the drug application were 3, 7, 10 and 14 days. All fourteen ears were examined. Both ears of one animal were used for the fixative control. One ear each from two animals was used for the saline soaked Gelfoam® control and the opposite ears were used for the 3 and 14 day drug treatments. Both ears from four animals were used for drug treatment for 3, 7, 10, and 14 days.

At the time of sacrifice, under deep anesthesia, the posterior wall of the external ear canal was partly removed to expose the oval and round windows. The stapes was subluxated and a small hole was made in the round window membrane and the cochlear apex. One percent osmium tetroxide was perfused directly into the oval and round windows by micropipette until the cochlea was dark. Our prior test of fixatives showed that initial fixation with osmium instead of Karnovsky's fixative yielded better structural preservation for this study. The animal was decapitated and the temporal bone was quickly dissected out. The temporal bone was immersed in one percent osmium for 1 hour and post-fixed with modified Karnovsky fixative (2% glutaraldehyde and 2.5% paraformaldehyde) for 2 hours. In 70% ethanol, the three cristae, utricle and saccule were dissected out under the surgical microscope. The specimens were embedded in Spurr® resin and sectioned for transmission electron microscopy (TEM) or coated with gold for scanning electron microscopy (SEM). These specimens were examined with a JEOL 100CX transmission electron microscope and a JSM 35CF scanning electron microscope.

Results

Control for fixation and fenestration of the lateral canal

When the specimens were fixed primarily with one percent osmium, *in vivo*, and post fixed with modified Karnovsky fixative, globular masses which were small cytoplasmic protrusions in the apical surface of the sensory cells were rarely seen in the cristae and maculae (Figs. 1a and 1b).

Figure 1 (on the facing page 109). Scanning electron micrographs of control ear for fixation. **1a.** Superior crista. Surface is round and covered with sensory stereocilia. **1b.** Higher magnification of Fig. 1a. No globular masses are visible in the central part of the crista. **1c.** Transmission electron micrographs of control ear for the fenestration and Gelfoam® application on the lateral canal. Lateral crista. The sensory cells in the peripheral part show normal appearance. A globular mass is partly shown in the endolymph surface of the sensory cell (open arrow). (Bars = 10 μm).

In the control specimens with the saline soaked Gelfoam®, the type I and II sensory cells were intact. The stereocilia at the central part of the lateral crista remained intact (Fig. 1c), however, globular masses were present next to the stereocilia, as was the case in the superior crista of three day specimens (Figs. 2c and 2d).

Three days after SM application

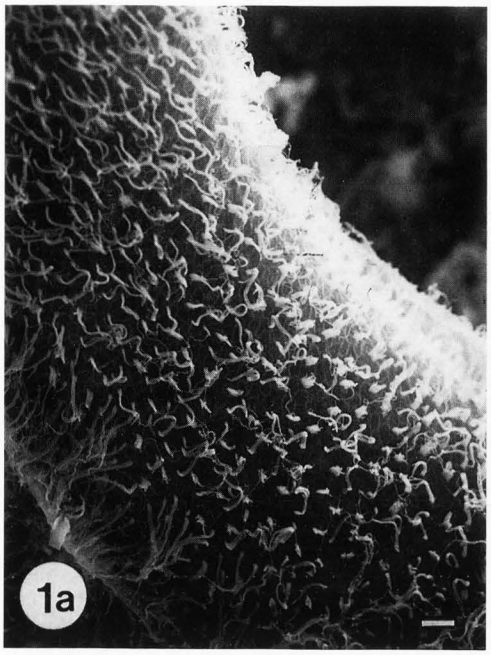
SEM showed obvious lesions in the SM applied central part of the lateral crista. The sensory cell stereocilia were missing, and the cytoplasm was protruded toward the endolymph surface, forming globular shaped masses (Figs. 2a and 2b). TEM revealed that cytoplasmic changes were most common in type I cells; mitochondria were swollen, cytoplasmic vacuoles were numerous, stereocilia were missing or pushed aside by the cytoplasmic protrusion, and the nuclei were pyknotic. The nerve chalices became small and irregular in shape, and thus, differential characteristics of some type I and II cells became obscure in pathological cells (Fig. 3a).

The lesions of the superior crista were less apparent than in the lateral crista. The sensory cell stereocilia were intact, though several globular masses were shown next to the stereocilia in the central part of the crista (Figs. 2c and 2d). In the posterior crista, few globular masses were found (Figs. 2e and 2f).

The stereocilia on the sensory cells of the macula utriculi were normal in appearance, although pyknotic nuclei and mitochondrial swelling in the type I sensory cells near the striola were found by TEM (Fig. 3b). The sensory cells of the saccule showed no abnormalities.

Seven days after SM application

The lesions extended from the lateral ampulla to all three cristae and macula utriculi. In the lateral crista, the number of stereocilia in the central part was smaller than that of the three day survival group (Fig. 4a) and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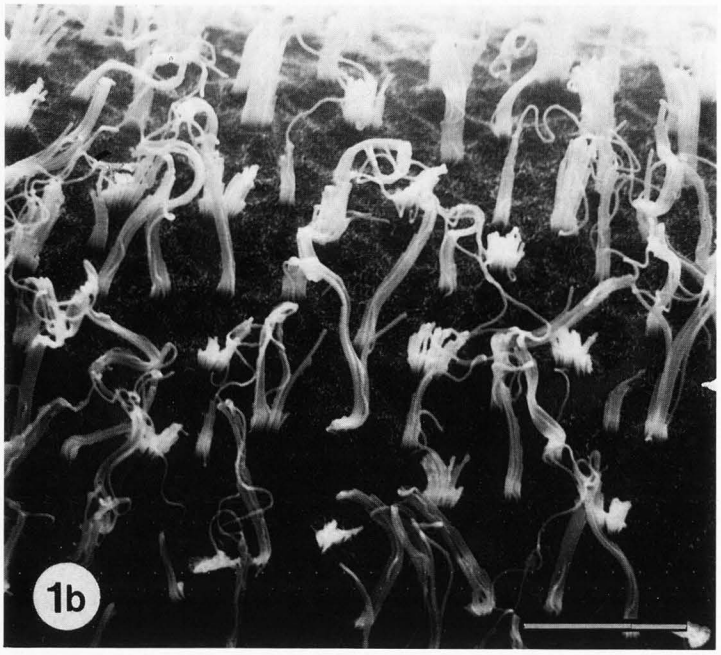
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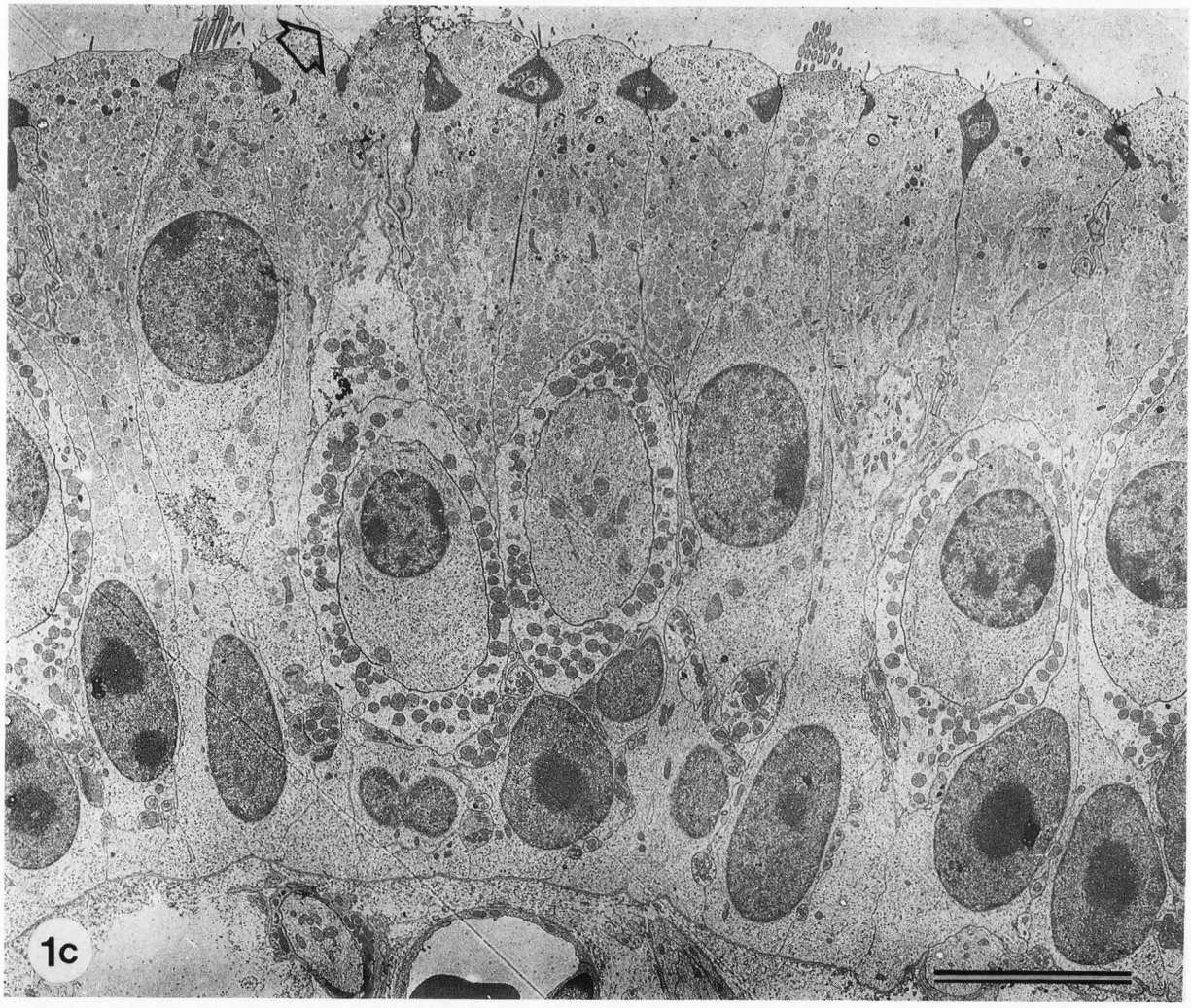
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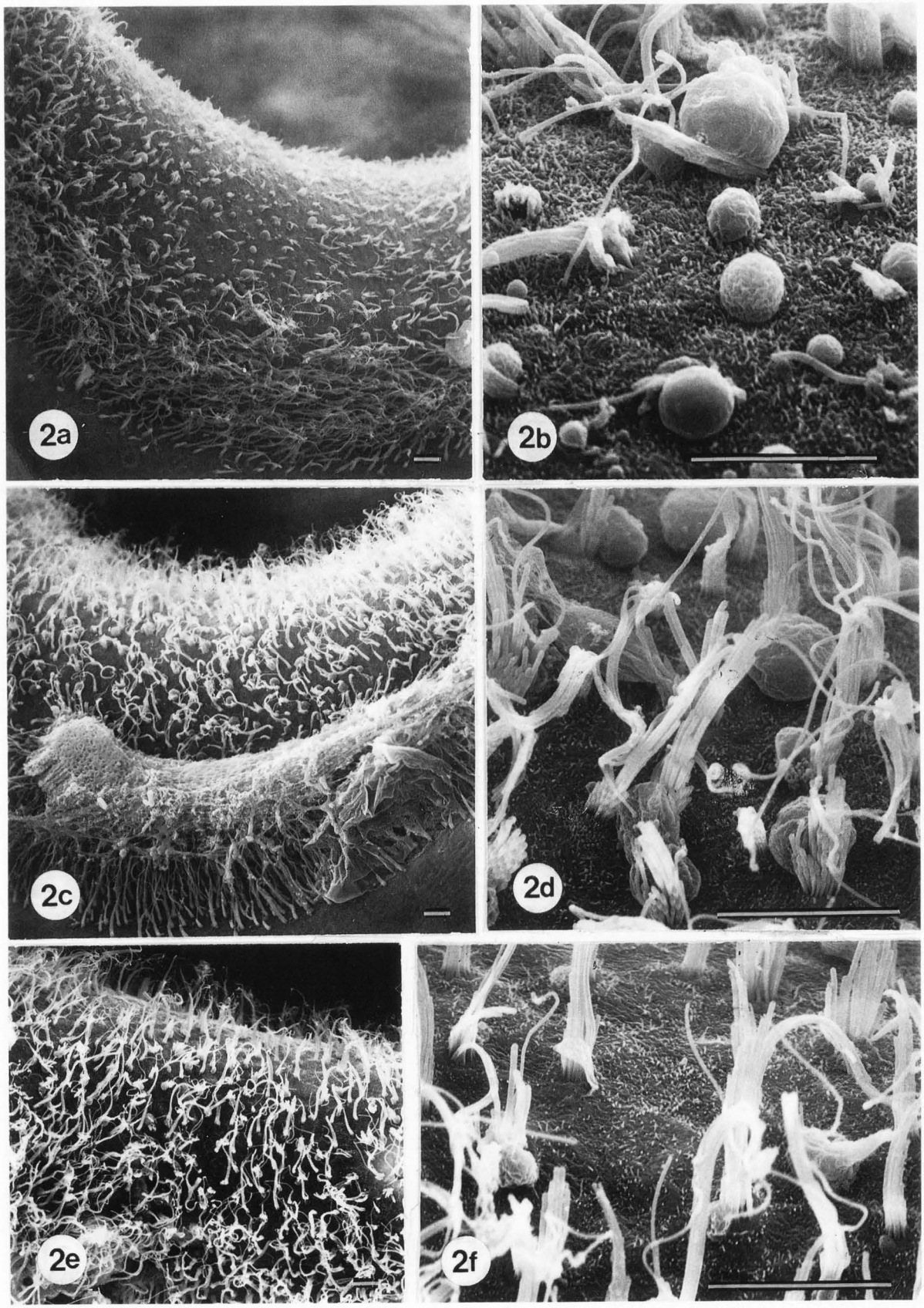
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Figure 2 (on the facing page 110). Scanning electron micrographs of three day specimens. **2a.** Lateral crista. Central part shows loss of sensory stereocilia, whereas the peripheral part has normal sensory stereocilia. **2b.** Higher magnification of Fig. 2a. Sensory stereocilia are decreased in number and various sizes of globular masses are scattered on the crista. **2c.** Superior crista. The sensory stereocilia are intact in the central and peripheral parts. **2d.** Higher magnification of Fig. 2c. Several globular masses are shown next to stereocilia. **2e.** The posterior crista shows normal sensory stereocilia. **2f.** Higher magnification of Fig. 2e. The globular masses are fewer compared to the lateral and superior cristae. (Bars = 10 μm).

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the remaining stereocilia were fused or enclosed by cytoplasmic extrusions to form giant stereocilia with various shapes (Fig. 4b). In the TEM examination, the type I and II sensory cells were missing in the central part of the lateral crista (Fig. 5a). The nerve chalices were degenerated at the same time. The type II cells in the peripheral part of the crista contained some vacuoles, dense inclusion bodies, and pyknotic nuclei (Fig. 5b).

In the superior and posterior cristae, the central part showed damaged sensory cell stereocilia though these lesions were milder than those of the lateral crista (Fig. 4c). The stereocilia in the central part were fused, whereas the stereocilia in the peripheral part were normal (Fig. 4d). In the TEM examination, type II cells showed cytoplasmic changes but a small number of these cells were normal in the peripheral parts of the superior and posterior cristae.

In the macula utriculi, the sensory cell stereocilia were mostly absent at the striola, and other stereocilia were adhered or adjacent to globular masses (Figs. 4e and 4f). In the TEM examination, type I sensory cells and their nerve chalices were absent and type II sensory cells containing numerous vacuoles remained in the peripheral part of the striola (Fig. 5c).

Ten days after the SM application

The lesions were similar in extent and severity in all three cristae. The sensory cell stereocilia were missing in the central and peripheral parts of the cristae and were replaced by numerous large globular masses (Figs. 6a and 6b). In the TEM examination, all the sensory cells were missing in the central and peripheral parts of the cristae. The sensory cell areas were occupied by the expanded cytoplasm of the supporting cells. The density of the secretory granules in the enlarged supporting cells was relatively less, although some cells showed dense cytoplasm (Figs. 7a and 7b).

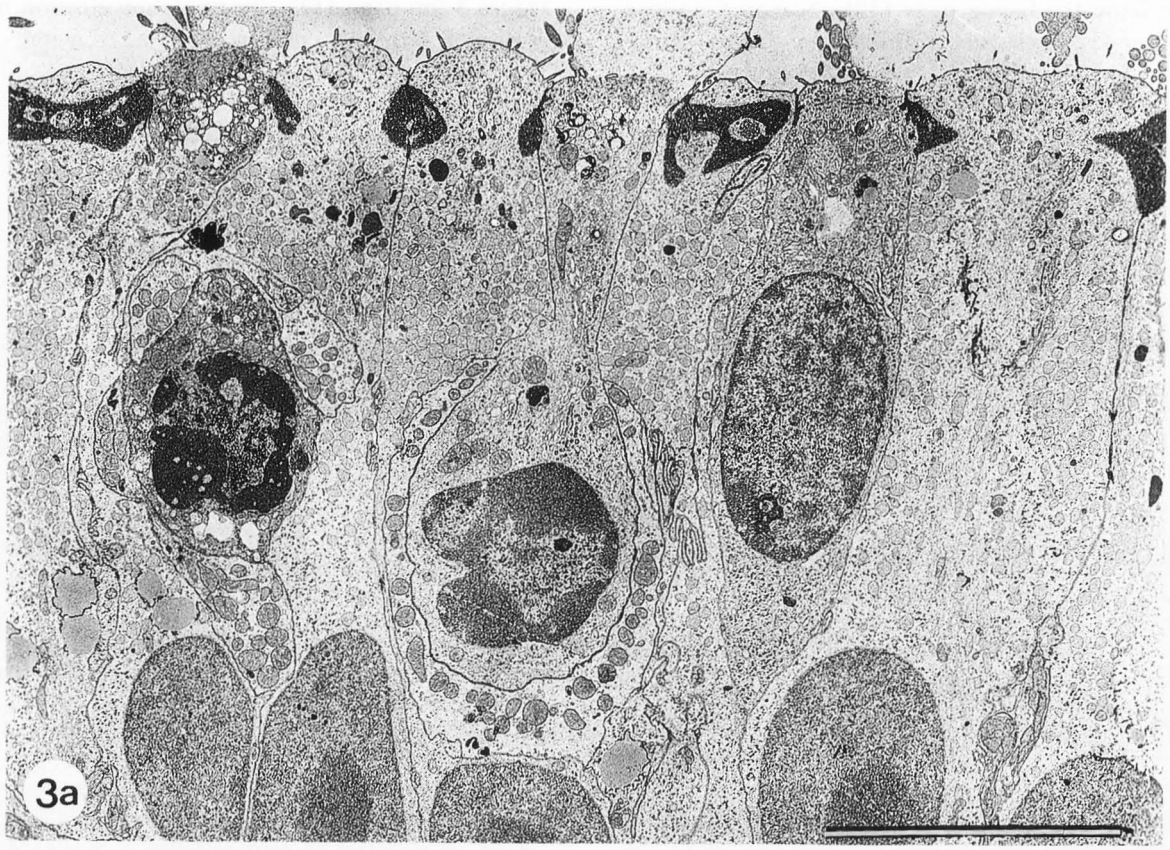
Figure 3 (on page 112). Transmission electron micrographs of three day specimens. **3a.** Lateral crista. Type I sensory cells in the central part show cytoplasmic protrusion. There are multiple small vacuoles in the subcuticular area, and the nucleus is pyknotic. (Bar = 10 μm). **3b.** Macula utriculi. Two type I sensory cells show pyknosis and the mitochondria show vacuolar changes. (Bar = 1 μm).

Figure 4 (on page 113). Scanning electron micrographs of seven day specimens. **4a.** Lateral crista. Many sensory stereocilia are missing in the central part. (Bar = 100 μm). **4b.** Higher magnification of Fig. 4a. The remaining stereocilia are fused, forming giant stereocilia of various sizes. (Bar = 10 μm). **4c.** Superior crista. The sensory stereocilia remain in the central part. (Bar = 100 μm). **4d.** Higher magnification of Fig. 4c. The stereocilia are partly fused in the sensory cell surface, whereas the distal parts are not yet fused. (Bar = 1 μm). **4e.** Macula utriculi. The number of the sensory stereocilia is decreased around the striola. (Bar = 10 μm). **4f.** Higher magnification of Fig. 4e shows globular masses next to the sensory stereocilia. (Bar = 10 μm).

Figure 5 (on page 114). Transmission electron micrographs of seven day specimens. **5a.** Central part of the lateral crista. The sensory cells have disappeared and the spaces that the sensory cells occupied are filled by supporting cells. The density of the secretory granules in the supporting cells is decreased because of expansion of the cytoplasm. **5b.** The peripheral part of Fig. 5a. The type I sensory cells are absent in the peripheral part. The type II sensory cells show dense cytoplasmic inclusions and pyknotic nuclei. **5c.** Macula utriculi. The type I sensory cells are absent and the type II sensory cells show multiple vacuoles in the cytoplasm. (Bars = 10 μm).

Figure 6 (on page 115). Scanning electron micrographs of ten day specimens. **6a.** Lateral crista. Almost all sensory stereocilia are replaced by globular masses. **6b.** Higher magnification of Fig. 6a shows globular masses. **6c.** Macula utriculi. The sensory stereocilia remain and many globular masses are seen next to the stereocilia. **6d.** The macula sacculi shows normal sensory stereocilia, and globular masses are absent. (Bars = 10 μm).

The degenerative changes of the macula utriculi were generally less severe than those of the three cristae. The sensory cell stereocilia near the striola were absent, however, some stereocilia in the peripheral part still remained intact (Fig. 6c). In the TEM examination, type I and II sensory cells were absent near the striola.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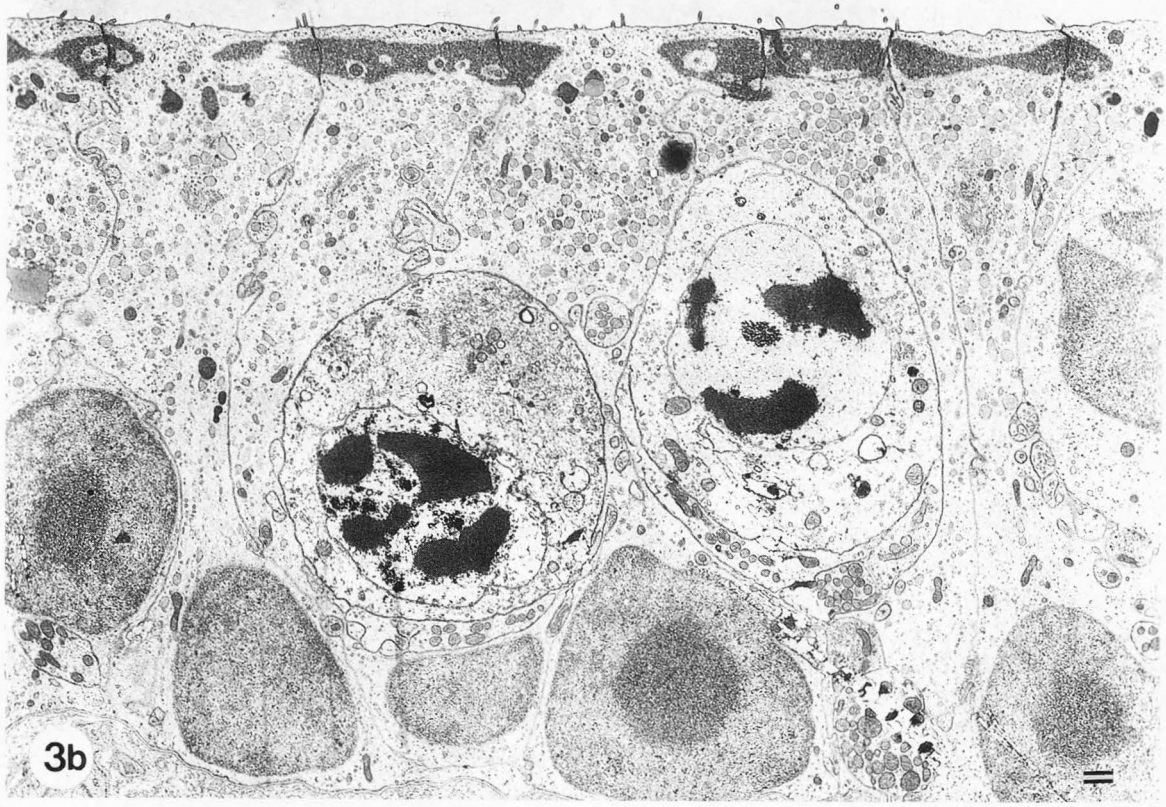
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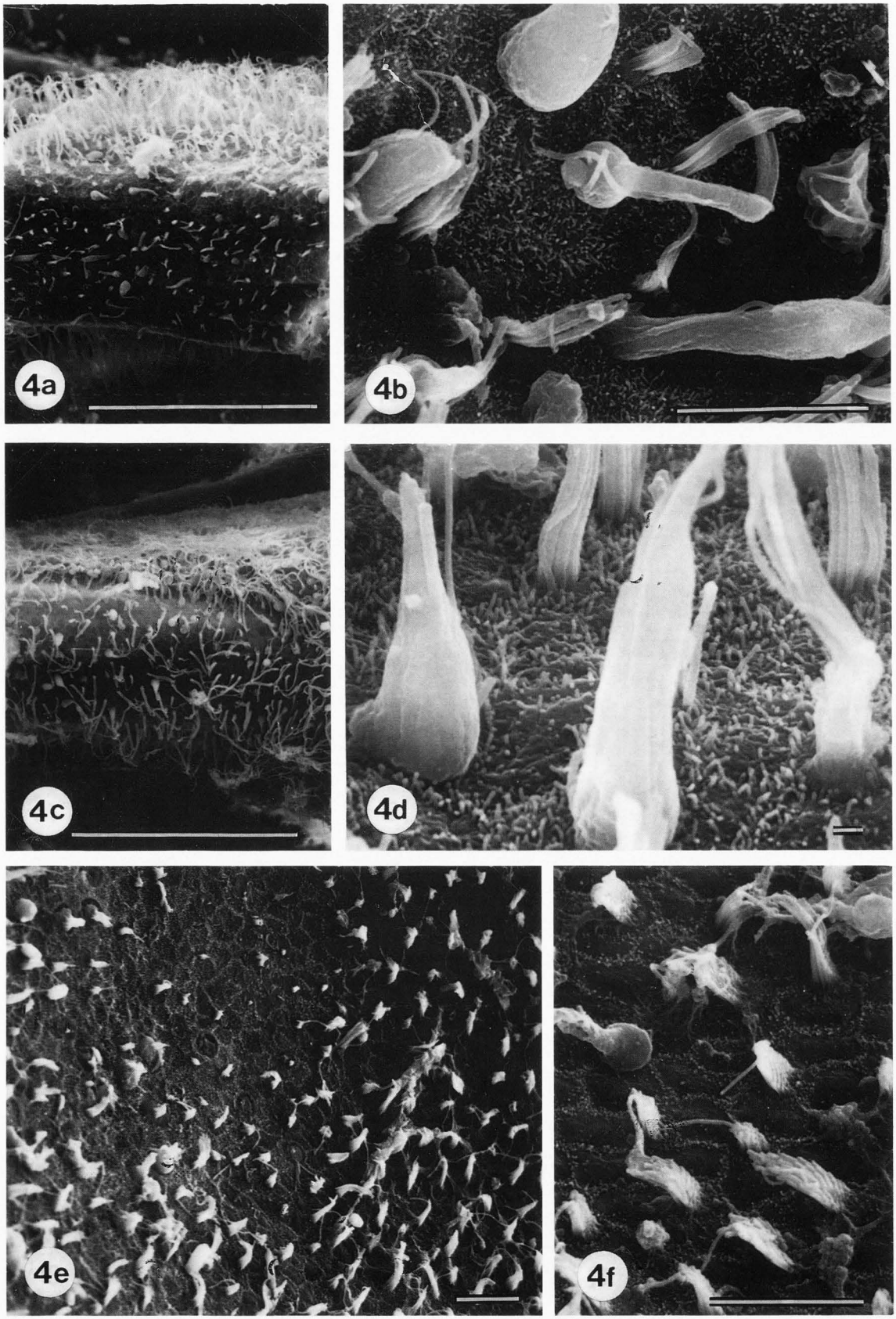
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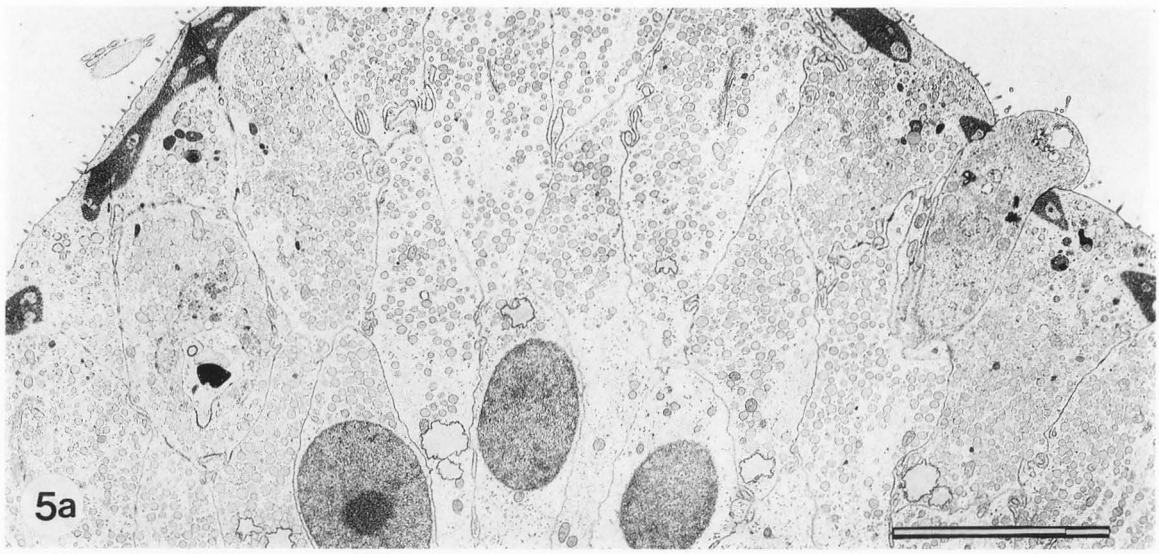
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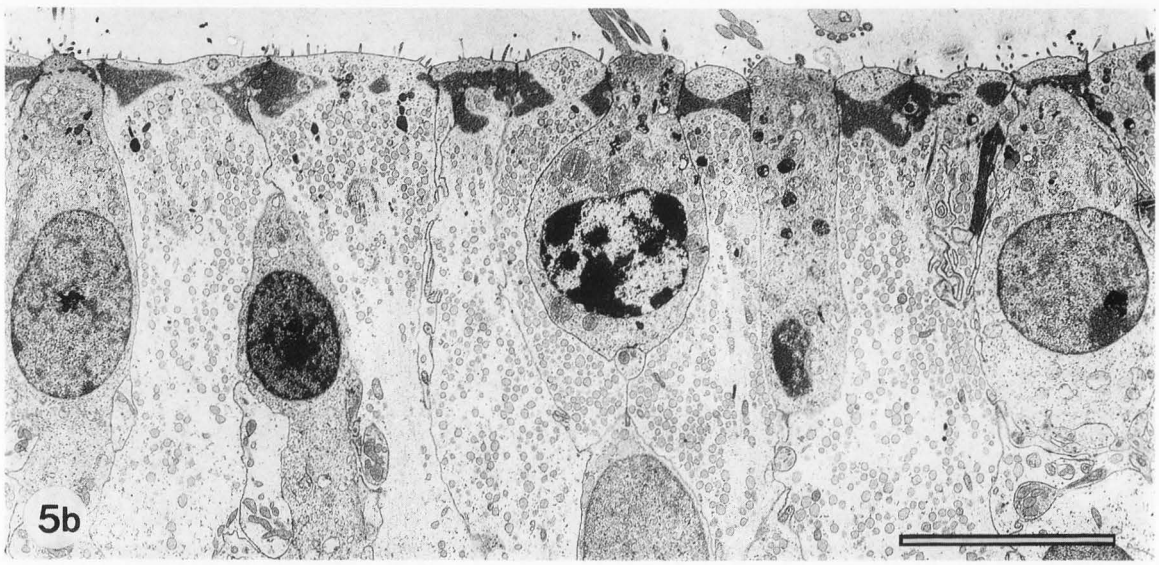
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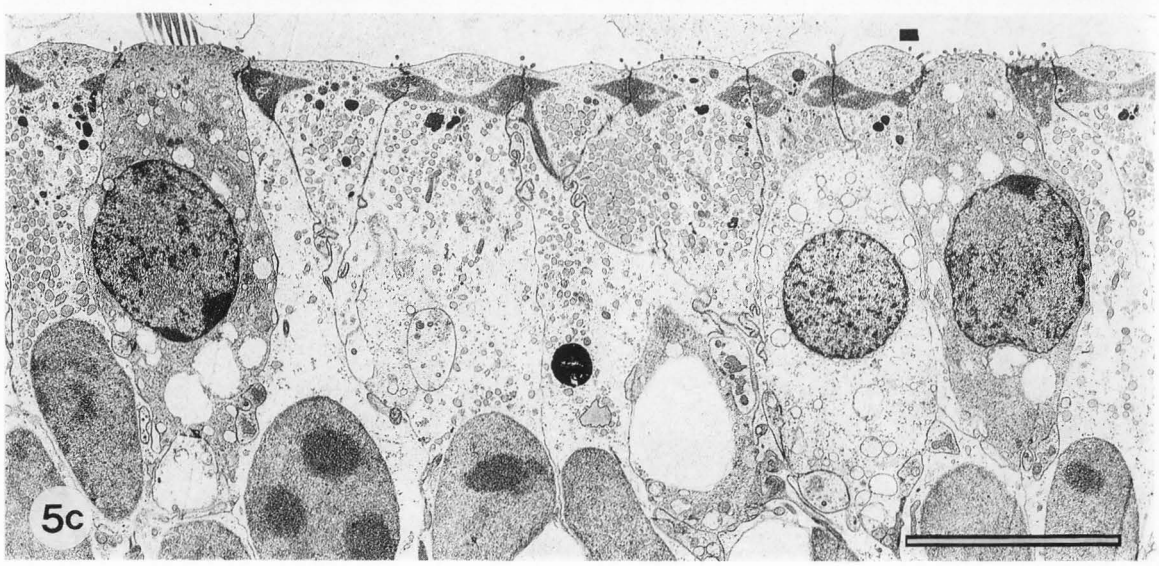
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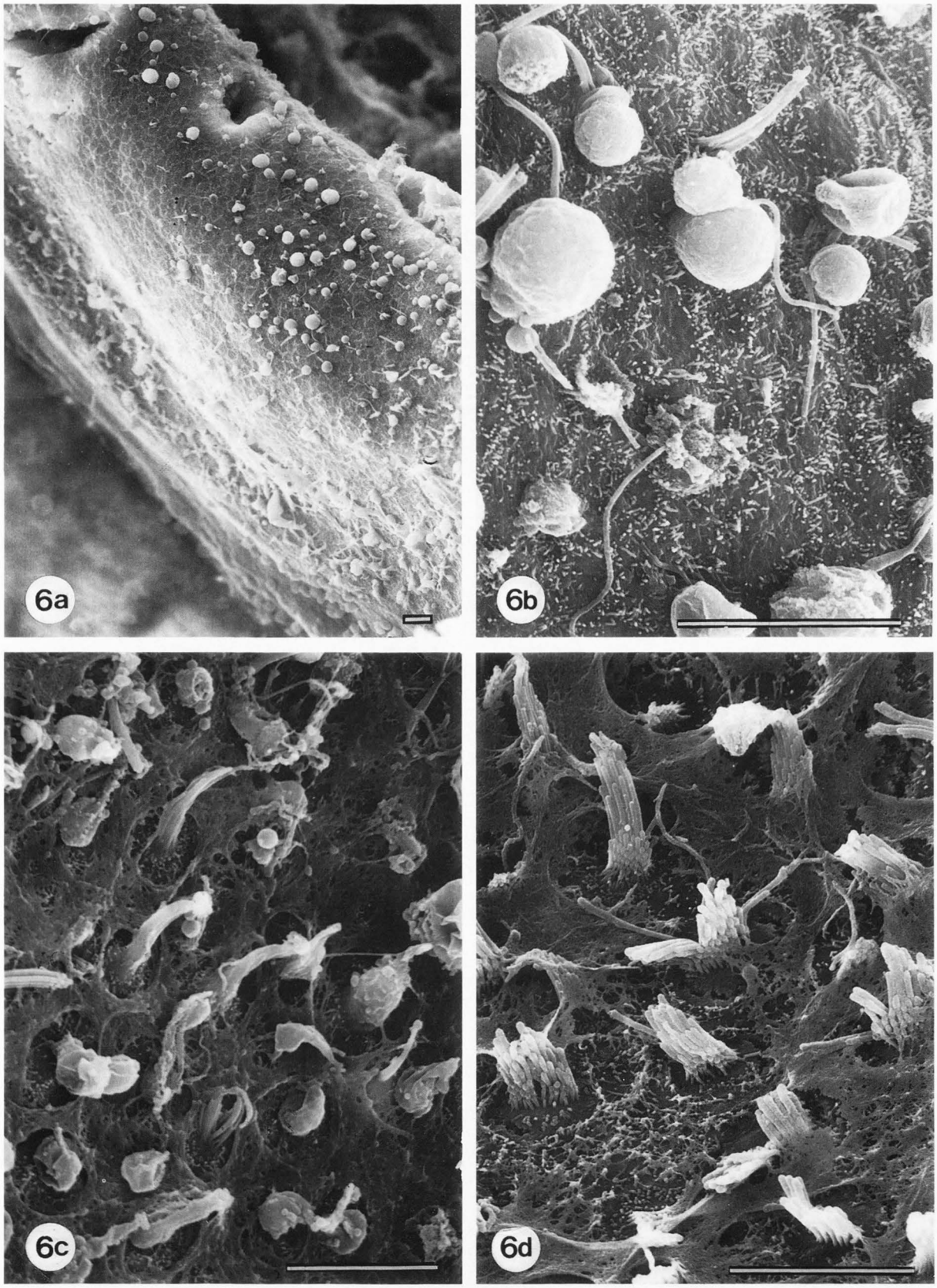
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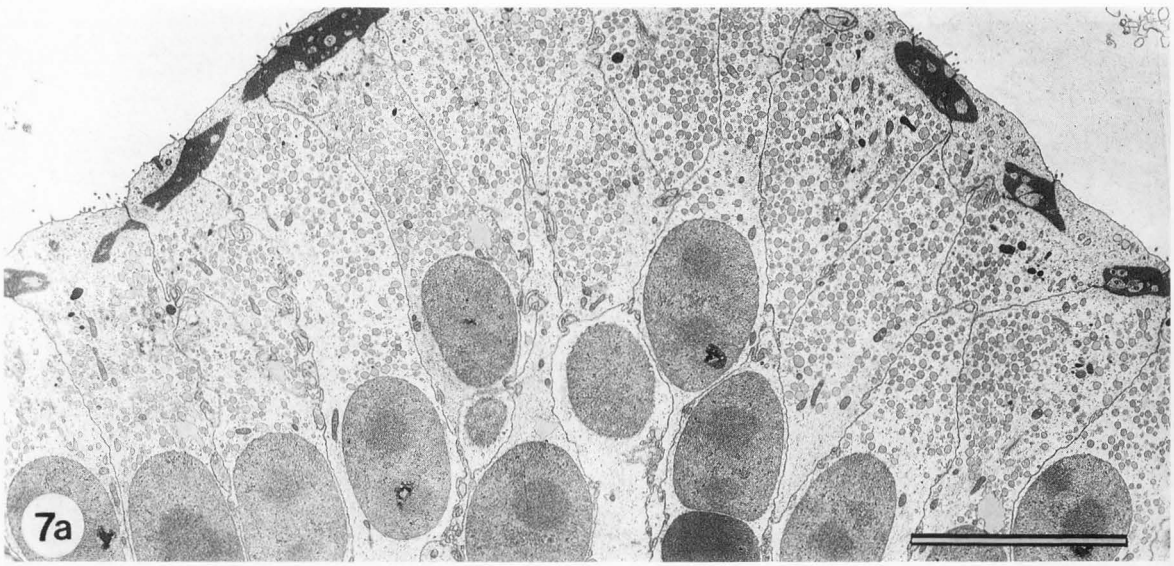
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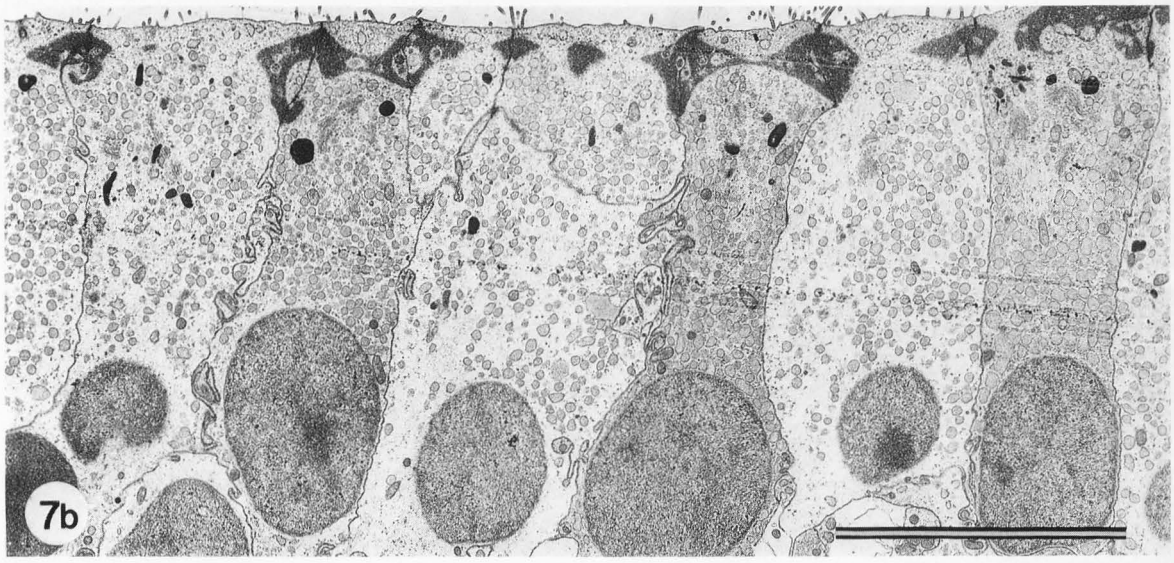
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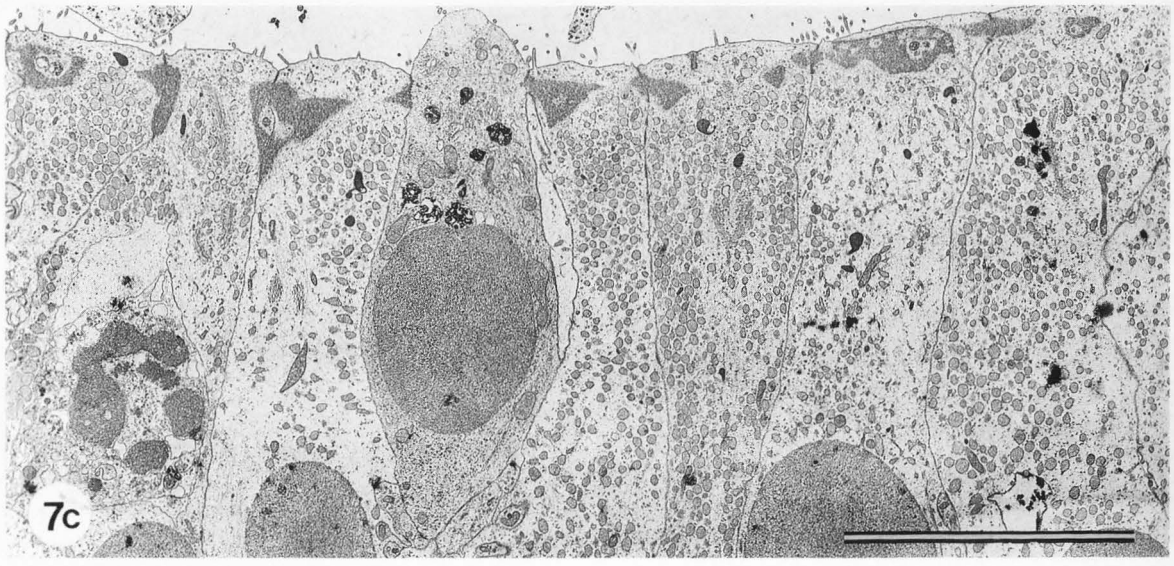
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Figure 7 (on the facing page 116). Transmission electron micrographs of ten day specimens. **7a.** Central part of the superior crista. The sensory cells are absent and only supporting cells remain. **7b.** The peripheral part of the superior crista shows supporting cells with dense cytoplasm. **7c.** A degenerating type II sensory cell in the macula utriculi. (Bars = 10 μm).

Some type II cells in the peripheral area lost stereocilia but showed normal nuclei and nerve endings (Fig. 7c). The sensory cells of the macula sacculi did not show any degenerative changes (Fig. 6d).

Fourteen Days after SM Application

When the cupula remained on the crista, the globular masses appeared to be embedded in the thin cupula. The surface of the cristae uncovered by the cupula showed no globular masses (Fig. 8a). Loss of the sensory cells and possibly supporting cells changed the smooth curvature of the crista to an irregular and asymmetric surface (Figs. 8b and 8c). There were more cristae sensory cells in the fourteen day specimens compared to the ten day specimens, and the shape of the stereocilia was slender and twisted (Fig. 8d). Under the TEM, type II cells at the peripheral zone showed sensory cell stereocilia and nuclei, but they contained numerous vacuoles (Fig. 9a). The myelin sheaths of the vestibular nerve fibers under the basement membrane showed degenerative changes (Fig. 9b).

In the macula utriculi, undamaged sensory cell stereocilia remained on the periphery of the striola (Fig 8e). The type II cells at this location showed vacuoles in the supranuclear cytoplasm (Fig. 9c). Even in severe destruction of the macula utriculi, the sensory cells of the macula sacculi remained intact (Fig. 8f).

Discussion

Early changes of the vestibular sensory cells are limited to the lateral canal crista as expected because during the first few days SM may be more concentrated around the application site. The degenerative changes are shown foremost in the type I sensory cells in the central part of the crista, followed by the type II sensory cells, similar to the results reported by others [9, 19]. The high sensitivity of the type I cells is not clearly understood; however, it has been suggested that these cells are phylogenetically younger than the type II cells, have a higher metabolic activity, and are more readily affected by drugs which inhibit protein synthesis [17, 18].

The sequence of degeneration of vestibular sensory cells are: extrusion of apical cytoplasm, swelling of

Figure 8 (on page 118). Scanning electron micrographs of fourteen day specimens. **8a.** Lateral crista. Degenerated thin cupula covered on the surface of the lateral crista. Uncovered area (arrow) shows smooth surface with few sensory stereocilia. **8b.** Superior crista. Globular masses have disappeared and the slender and curly sensory stereocilia are shown in the central and peripheral parts of the superior crista. Note the indented surface of the crista. **8c.** Posterior crista shows changes similar to the superior crista. **8d.** Higher magnification of Fig. 8c shows curly and flaccid appearance of the sensory stereocilia. **8e.** Macula utriculi. Sensory stereocilia remain in the peripheral area. **8f.** Macula sacculi. The sensory cells show normal stereocilia. (Bars = 10 μm).

Figure 9 (on page 119). Transmission electron micrographs of fourteen day specimens. **9a.** Superior crista. Several type II sensory cells are shown in the peripheral part of the crista. (Bar = 10 μm). **9b.** Vestibular nerve fibers under the basement membrane of the lateral crista. Myelin sheath shows degenerative changes. (Bar = 1 μm). **9c.** Macula utriculi. The intracellular organelles in the supporting cells are dispersed. In the type II sensory cell, the supranuclear part shows vacuoles. (Bar = 1 μm).

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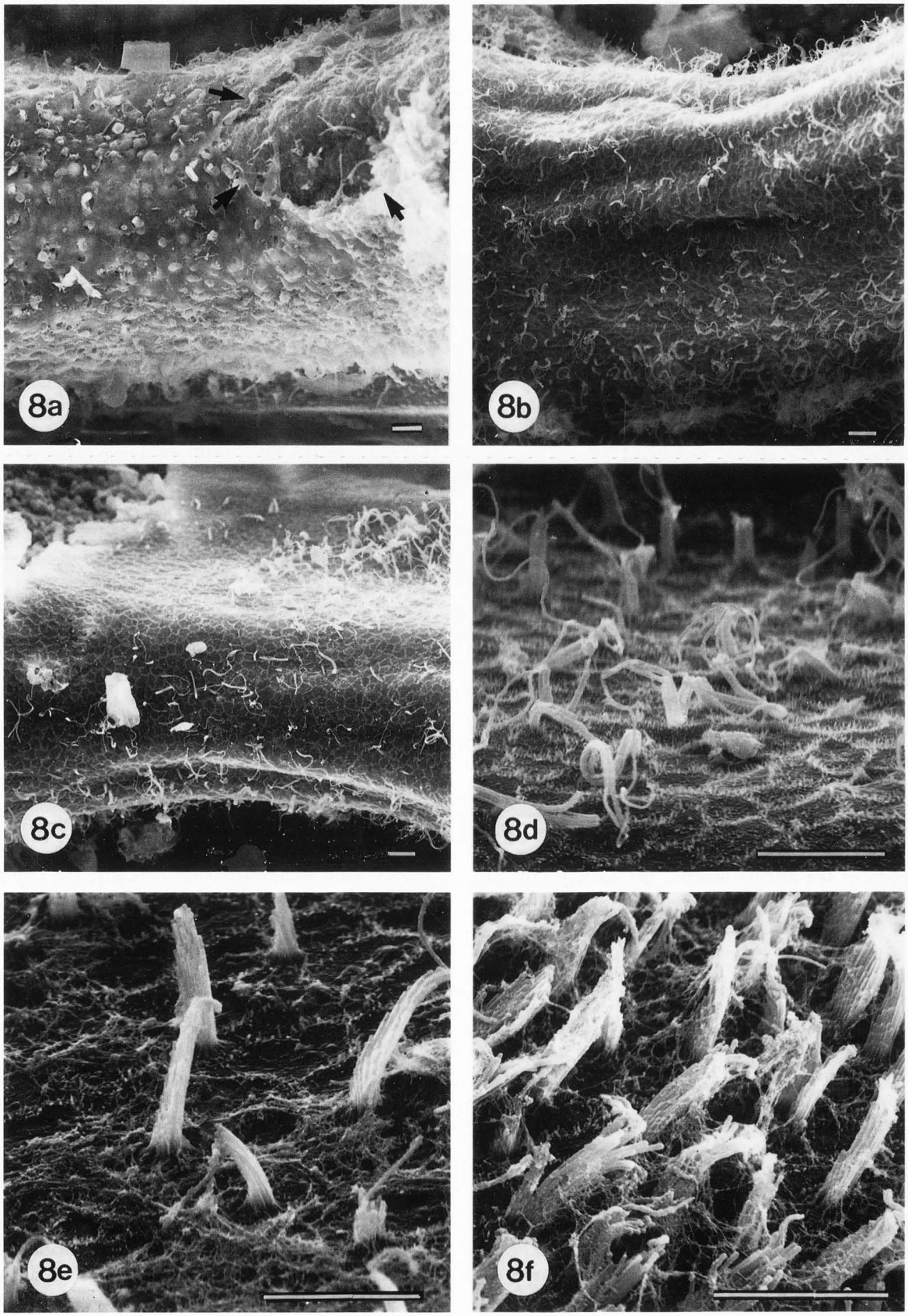
mitochondria, vacuolation of cytoplasm, formation of dense bodies, pyknosis of nuclei, and loss of stereocilia similar to those described earlier [18, 19]. Our findings suggest that degeneration of the stereocilia comes later than the cytoplasmic changes. These results are different from previous studies; when the aminoglycoside antibiotics were administered systemically, the changes in the vestibular organs were shown in the sensory stereocilia prior to the cytoplasmic changes [18]. These events are further followed by detachment of degenerating cells from the reticular lamina, fragmentation of the nuclear substance, gradual shrinkage of cells and nerve chalices, and enlargement or increased electron density of the supporting cells. The cell fragments appear to dissolve without phagocytic activity among the supporting cells. As these sensory cells disappear, the shape of the cristae becomes distorted and appears as irregular ridges. Degeneration of myelinated nerve fibers under the basement membrane may be a retrograde phenomenon due to the sensory cell degeneration. The pattern of degeneration may differ somewhat according to the type of the drug, dosage, and route of its application [18].

Globular masses increase at the apical surface of sensory cells. The small cytoplasmic protrusions are known to occur in the normal vestibular sensory cells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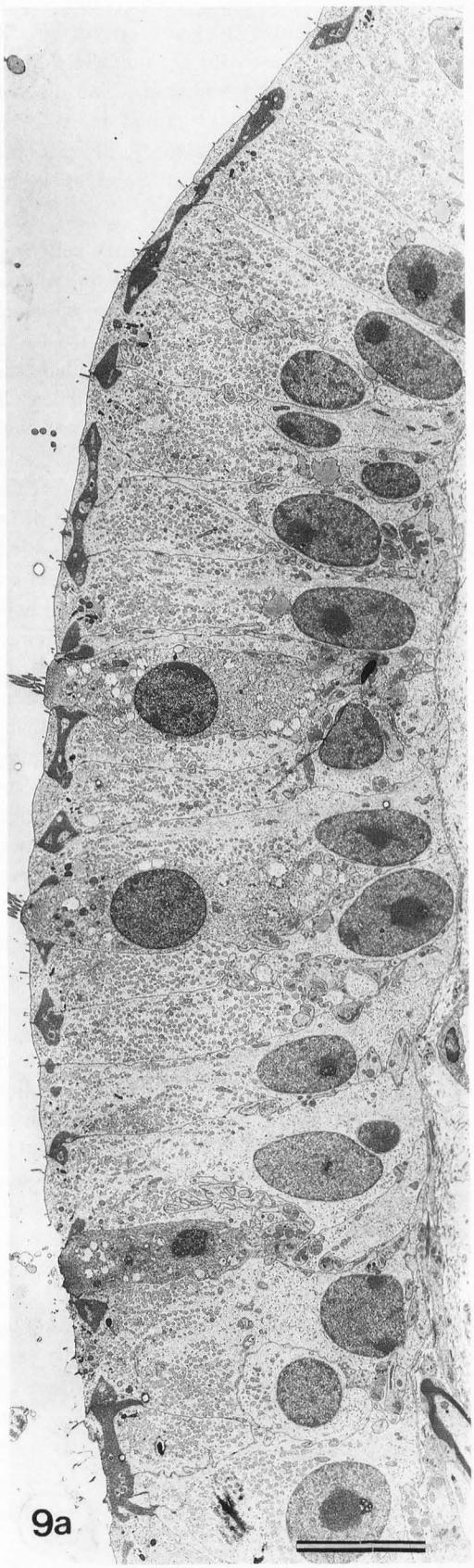
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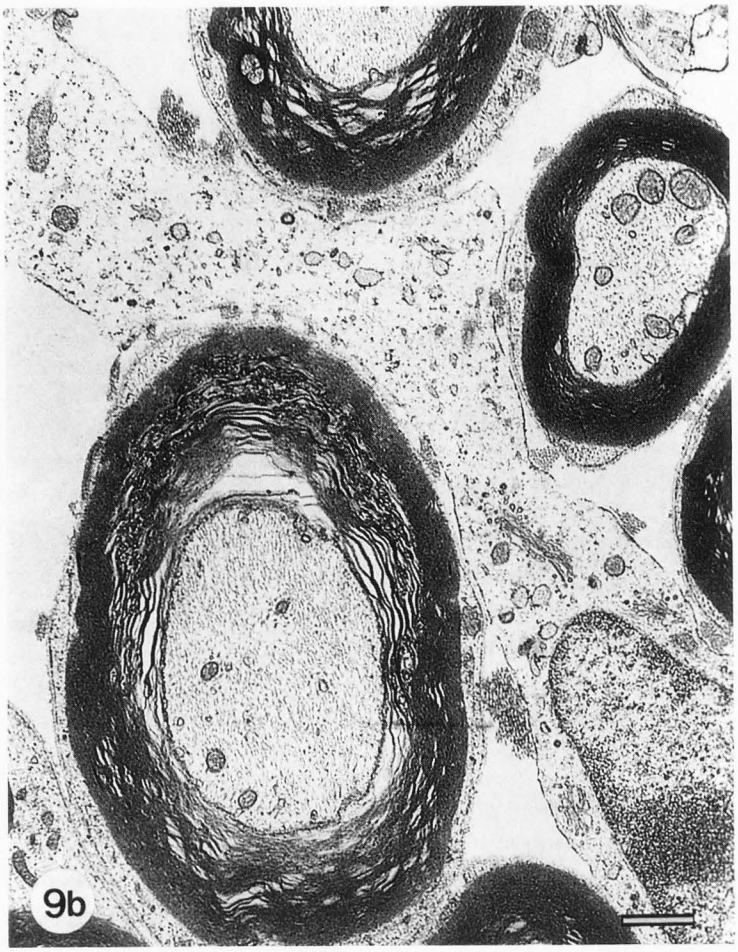
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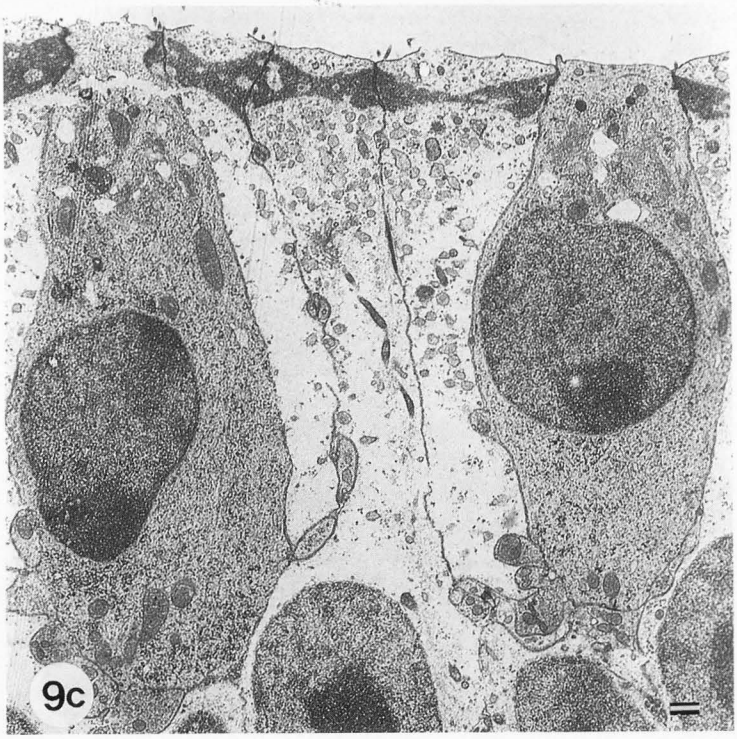
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[19]. It is not certain whether these protrusions are normal phenomena or induced by the fixatives or a delay in fixation which cause the postmortem changes. Our earlier study showed that the globular masses occurred in the specimens prefixed with the aldehyde type fixatives. In the present study of initial fixation of normal ears intravitally with osmium and subsequent fixation with glutaraldehyde and paraformaldehyde, globular masses were rarely observed. However, they appeared in the saline control specimens, indicating that all these masses shown in this study are not entirely due to the effect of the ototoxic drug. In the pathological specimens, the apical cytoplasm and membrane are apparently altered and the cytoplasm pushed out by the supporting cells. The large globular masses are often incorporated with stereocilia or even embedded in the cupular substance. The giant cilia are formed by the direct fusion of small stereocilia and/or by enclosure of the stereocilia by the cytoplasmic protrusion. Most of these giant cilia formations are of transitory nature because their number does not increase in the specimens with longer survival times.

At seven days, the lesions become obvious at the tops of the superior and posterior cristae and the striola of the macula utriculi. These findings suggest a gradual diffusion of the drug toward these sense organs from the lateral canal. The lesions occur also at the peripheral regions to a lesser extent. The reason for this difference in the distribution of the lesions is that the sensory cell population at the central part of cristae is far less in comparison to the peripheral region [10]. It is also interesting to note that the population of the type I cells is greater by 1.5 times than that of the type II cells throughout the cristae, including the central part in the normal specimens [10]. In the ten day specimen, the lesions are severe and are also seen in the sensory cells of the peripheral region in all three cristae. The extent of the lesions in the three cristae is almost identical. This finding suggests that the drug is evenly distributed to all three cristae and each of the canal organs is equally sensitive to the drug. The extent of the lesion is dependent on the amount of the drug entering the canal, its diffusion, and the sensitivity of the sensory cells. Thus, the smaller lesions observed in the fourteen day specimens in comparison to the ten day specimens may be due to variations in the amount of drug entering the fenestra. However, regeneration of sensory cells is another possibility because a more recent study [3] indicates that the utricular sensory cells of the guinea pig regenerate as early as four weeks after gentamicin intoxication.

While the severity of lesions in the three cristae is almost identical by visual estimates, the lesions in the macula utriculi appear less than those of the cristae. In our earlier light microscopic studies from the larger samples at a longer survival time, the utricular macula were affected slightly more than the cristae [6, 7]; however, evaluation of these data [7] indicate that the difference is not statistically significant. The discrepancy between this study and the previous one is likely due to differences in the methodology of specimen examination or the small number of samples in the current study.

The preservation of the saccular sensory cells suggests the cochlear sensory cells are very unlikely or rarely affected by use of the same lateral canal approach as in previous studies [6, 7]. The drug applied to the lateral canal probably takes both the endolymphatic and perilymphatic routes toward the saccule and cochlea. In both situations, the drug diffusion is interfered with either by a longitudinal flow of endolymph or a partial barrier imposed by the membrana limitans and trabecular meshwork, respectively. However, the absence of lesions in the macula sacculi may not entirely be due to a lack of SM reaching the macular area. The process not only involves a greater dilution of the drug in the huge cistern of perilymph, but also there are no cellular networks to retain the drug longer outside of the macula sacculi area. In the systemic injection of SM, the macula sacculi is also the least affected among the vestibular sense organs [7, 19]. However, the distribution patterns of the type I and II cells are almost identical with the ratio of the type I to type II, that is 2 to 1 at the striola and 1 to 1 at the periphery [10]. Another possibility is that the drug is quickly removed by rich vascular networks located at the subepithelial region of the saccule.

Our experiences indicate that the forceful injection of a known amount of aminoglycoside into the lateral canal affects cochlear sensory cells [5]. Damage to cochlear sensory cells is very small when SM paste is inserted into the lateral canal [12]. Drug delivery by a diffusion process appears to be superior to the injection method in this regard. Both of these procedures can be further improved. Even though it is more convenient to give the drug through the transtympanic and systemic routes, the organ of Corti is unavoidably affected. However, at the present time we do not know how extensive the lesion in the vestibular sense organs needs to be or which vestibular sense organs need to be affected in order to minimize or eliminate the disabling vestibular symptoms. The present study showed that only a very small amount of streptomycin is needed to affect all cristae and macula utriculi in less than ten days.

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Discussion with Reviewers

A. Forge: Please expand on the advantages of your fixation protocol? In what ways is it preferable to the use of osmium tetroxide alone or of primary fixation with aldehydes followed by osmium tetroxide post-fixation? One would have thought that the temperature of *in vivo* perfusion and the use of osmium tetroxide as primary fixative would not be conducive to the preservation of microfilaments.

Authors: We changed the fixatives and fixation methods to find the optimal fixation of the vestibular organs in the guinea pig. We preferred to use the decapitation and immersion method instead of cardiac perfusion because it was simple and time saving. The animals were decapitated and the temporal bones were quickly dissected out. Application of an aldehyde fixative was started within one minute and post-fixed with osmium tetroxide. However, aldehyde pre-fixed cristae and maculae showed globular masses. We changed to a single osmium tetroxide application (Table 1). The number of globular masses was markedly decreased in the osmium single fixed specimens (#2 left, #3 both). We thought the cause of the globular masses might be a delayed penetration of osmium and changed the fixation method from

Table 1. Different fixatives used in the decapitation and immersion fixation of the vestibular organs.

No.	Ear	Fixation Methods	Examination
1.	Left	**Pre-fixation:** 3% Glutaraldehyde; **Post-fixation:** 1.5% osmium	SEM & TEM
	Right	**Pre-fixation:** 3% Glutaraldehyde; **Post-fixation:** 1% osmium	SEM
2.	Left	**Fixation:** 1.5% osmium for 2 hours	TEM
	Right	**Pre-fixation:** Modified Karnovsky*; **Post-fixation:** 1.5% osmium	TEM
3.	Left	**Fixation:** 1% osmium for 2 hours	TEM
	Right	**Fixation:** 1% osmium for 2 hours; **Post-fixation:** Modified Karnovsky*	SEM

*Modified Karnovsky fixatives: 2% glutaraldehyde and 2.5% paraformaldehyde.

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decapitation-immersion to direct osmium tetroxide application, *in vivo*. We finally got good results as shown in Figures 1a and 1b. The problem with the single osmium fixed specimens was that the tissue was too soft to manipulate. When the specimens were post-fixed with the aldehyde fixatives, the tissue was consistently good enough for the microdissection.

A. Forge: Large cytoplasmic protrusions from the apical surface of hair cells that arise on the kinocilium side of the hair bundle and distort it, often occur in control tissues when fixation is sub-optimal. They also occur in cochlear hair cells for similar reasons. In these circumstances, how sure can the authors be that the surface protrusions are not artifacts? Globular masses are present on hair cells in ears exposed to a saline soaked Gelfoam® suggest that they occur in controls. It is also possible that the "globular masses" seen by SEM at the surface of cells without stereocilia (Fig. 2b) are different from the protrusions on cells with stereocilia. The former could be ejected remnants of degenerated hair cells. What do they look like in sections?
Authors: We agree that suboptimal fixation could be a factor in the production of the globular masses. As shown in Figures 1a and 1b, globular masses were not observed with optimal fixation. Another cause could be the aldehyde fixatives. Even with quick introduction of aldehyde fixatives into the vestibule, the globular masses were shown in the cristae and maculae. We do not deny the artifactual nature of these masses; however, in the pathological specimens, their number and size are increased. Small globular masses without stereocilia in Figure 2b might be from the pathological sensory cells or even supporting cells. The sizes of the masses are too small to be the ejected remnants of degenerated hair cells, but they may represent hair cell tops which lost stereocilia. Globular masses in the saline soaked Gelfoam® applied specimens might be a toxic effect of Gelfoam® or an effect of fenestra on the canal. However, their number and size were far less than those shown in the sense organs affected by the ototoxic drug.

B.A. Bohne: How did you ensure that the treated animals all received the same amount of SM?
Authors: We made a similar size fenestra on the lateral canal wall and applied the same size SM soaked Gelfoam® (2 mm in diameter). However, we do not know how much of the drug spread into the vestibular system. The control was limited to the size of the fenestra and Gelfoam®, so we could not rule out a variation in drug dosage.

B.A. Bohne: Why were the recovery times: 3, 7, 10 and 14 days, chosen for histological examination? Was any behavioral deficit noted in the SM treated animals at these intervals?
Authors: The time interval was arbitrary. We wanted to see the early changes of vestibular sensory organs after drug application. We did not examine the behavioral changes.

C.H. Norris: An important feature of this paper is that cytoplasmic changes occur prior to protrusion of cellular content and clumping of the stereocilia. Many other papers reporting on similar ototoxicities report stereocilia clumping as the early sign, and this has been confusing since the ototoxic agent is first found in the perilymph bathing the hair cells and not in the endolymph bathing the stereocilia.
Authors: The cytoplasmic changes occurred prior to

changes in the stereocilia. This is different from the earlier report that the fusion of the stereocilia is an early sign of aminoglycoside ototoxicity. The difference with earlier reports was the route of drug administration. In this study, the drug was applied directly on the fenestra of the lateral canal, and the drug could spread along the perilymphatic space by diffusion rather than the endolymphatic space. When the drug is administered parenterally, the drug might be secreted into the endolymphatic space by the secretory cells, such as dark cells.

B.A. Bohne: Do the globular masses eventually become detached into the endolymphatic space? Do they occur where the cuticular plate of the cell is deficient?
Authors: As shown in Figure 8a, the globular masses were attached or embedded into the cupula. We do not know whether the globular masses can be freely floating in the endolymph through the covered cupula or otolith membrane. In the SEM examination, the cytoplasmic protrusions usually occurred adjacent to the stereocilia. The cuticular plate of the vestibular sensory cells covers the whole surface, and the cytoplasmic protrusion usually showed the area where the cuticular plate was thin.

B.A. Bohne: Are sensory cells lost by extrusion from the endolymphatic surface of the vestibular organs or is there some other mechanism by which the dead cells are removed from the epithelium?
Authors: We think the degenerated sensory cells extruded into the endolymph side and embedded into the degenerated cupula or otolith membrane as showed in Figure 8a. However, dissolution of these cells without phagocytic activities may occur among the supporting cells.

B.A. Bohne: What was the condition of the cupula and otolithic membrane in the different ears?
Authors: We could not accumulate the data on the cupula and otolith membrane. The cupula and otolith membrane were usually flushed out from the surface of the crista or macula during the microdissection on purpose to examine the surface and/or to reduce knife marks from sectioning which were usually caused by the otoliths. The degenerated cupula in Figure 8a was strongly attached to the crista and observed on occasion.

C.H. Norris: Do the 14 day data really convince you of morphological regeneration?
B.A. Bohne: Do you think type II cells in the 14 day specimen are degenerating or recovering from their initial injury?
Authors: As we described in the **Discussion**, more sensory cells were present in the 14 day specimens. This could be a result of smaller drug effects or different susceptibilities to the drug due to individual variation. Be that as it may, we cannot rule out the possibility that they are in the process of degeneration. Regeneration could be another possibility because recent studies suggest the vestibular sensory cells can regenerate. We do not have evidence of the regeneration because we did not examine the stereocilia at a high magnification with the SEM, and no mitotic figures were found in the present TEM study. We tend to interpret the increase in sensory cells as regeneration; however, the number of samples is too small to make a definitive statement at this time.

A. Forge: Do the nerve fibers innervating the utricular macula degenerate as those of the cristae do, or do intact neural elements remain with the utricular sensory epithelium?
Authors: The sensory cell lesions in the macula utriculi were generally less severe than those in the cristae. In current study, the nerve fibers innervating the macula utriculi did not show degeneration. The swollen nerve endings were attached to the degenerating sensory cells, but, when the sensory cells were gone, the nerve endings were rarely observed within the sensory epithelial layer.

K.C. Horner: What behavioral dysfunctions were observed?
C.H. Norris: Does the possibility of long-term morphological regeneration imply functional restoration?
Authors: We did not conduct behavioral vestibular function tests. Functional restoration from regeneration poses an interesting question. This depends on the recovering cells, type I or type II, most likely type II cells which are of a primitive type. Our feeling is that function will be restored partially in long term survival animals.

C.H. Norris: Because some clinics are using gentamicin in place of streptomycin in this technique, it is important to note that all of antibiotic drugs of the aminoglycoside class can be ototoxic to all parts of the sensory epithelium of the inner ear. Differences between various members of the group are likely related to route of administration, dosage, and duration of treatment. However, you have previously shown [7] that gentamicin will produce increased lesions in all of the sensory cells, particularly in the organ of Corti, as compared to streptomycin when both are applied via the lateral semicircular canal. In addition, Hawkins (Trans. Am Acad Ophthalmol Otolaryngol. **63**: 206-218, 1959) has also shown that, given systemically in low doses, streptomycin has a predilection for vestibular sensory cells as compared to organ of Corti sensory cells. Do you have any data

on gentamicin that is analogous to this paper's streptomycin results?

Authors: When gentamicin is applied on the lateral canal of the hydropic cochlea, cochlear sensory cells are severely affected, but not in the normal ear. The effects of streptomycin and gentamicin administrations through the lateral canals of the normal ears are similar. We do not have the data on the changes in the cochlea and vestibular organs after the systemic administration of gentamicin.

C.H. Norris: As you know, this technique competes clinically with intratympanic aminoglycoside application for the treatment of vertigo. Do you think that transtympanic instillation would bias the ototoxic effect toward increased organ of Corti damage and hearing loss since the drugs would first pass through the round window membrane into scala tympani and have to traverse the whole organ of Corti prior to reaching the vestibule? Do you have any basic scientific data relating to the relative effectiveness of the two techniques?

Authors: There are reports on cochlear and vestibular sensory cell damage after drug application to the middle ear cavity. Our experience indicates that cochlear sensory cells are always affected by the middle ear approach even when the round window is covered with fat. We have histological serial sections, but the data is not yet collected from them.

A. Forge: In terms of treatment of Menière's disease, how useful would this procedure be if the saccular macula is unaffected and many hair cells remain in the utricular macula? Would a larger SM dose, sufficient to effectively ablate the entire vestibular sensory epithelia, also damage the cochlea?

Authors: The problem with Menière's disease is that we do not know which sense organ or organs are causing vertigo. If vertigo comes from the macular saccule, this procedure is not effective. A larger SM application could destroy the vestibular sensory organ more completely; however, cochlear sensory cells are likely to be affected.