WASHINGTON UNIVERSITY SCHOOL OF MEDICINE

Department of Otolaryngology - Head & Neck Surgery

MICROSCOPIC ANATOMY OF THE INNER EAR

Barbara A. Bohne, Ph.D.
Professor of Otolaryngology (Neurobiology)

and

Gary W. Harding, M.S.E.
Research Associate Professor of Otolaryngology

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PREFACE

This Atlas was developed as a teaching aid for individuals interested in cochlear anatomy and pathology. The goal is to provide multiple illustrations of the cellular arrangements within the inner ear. The same structures are shown in flat preparations and radial, horizontal or tangential sections. Comparison of the different views should help the interested person to understand the complex 3-dimensional structure of the organ of Corti.

The sense of hearing depends upon the central auditory pathways as well as the peripheral auditory structures (i.e., external, middle and inner ears). This Atlas is focused on the hearing portion of the inner ear only. For a discussion of the anatomy and physiology of the external and middle ears and details about the central auditory system, one should refer to a textbook on clinical otology and neuroanatomy, respectively.

COMMON ABBREVIATIONS

B - Boettcher cell
BM - basilar membrane
C - Claudius cell
CP - cuticular plate
D - Deiters' cell
D1 - first row Deiters' cell
D2 - second row Deiters' cell
D3 - third row Deiters' cell
DP - Deiters' process
E - endolymph
ES - endolymphatic space
H - helicotrema
HC - Hensen cell
HS - Hensen's stripe
L - limbus
IAM - internal auditory meatus
IB - inner border cell
IHC(s) - inner hair cell(s)
IP - inner pillar
IS - inner sulcus cell
ISB - inner spiral bundle of nerve fibers
M - mesothelial cell
MNF(s) - myelinated nerve fibers(s)
N - Nuel space
O1 (or OHC1) - first row outer hair cell
O2 (or OHC2) - second row outer hair cell
O3 (or OHC3) - third row outer hair cell
OC - organ of Corti
OHC(s) - outer hair cell(s)
OP - outer pillar
OSB - outer spiral bundle of nerve fibers
OSB1 - first outer spiral bundle
OSB2 - second outer spiral bundle
OSB3 - third outer spiral bundle
OSL - osseous spiral lamina
P - perilymph
RA - radial afferent
RM - Reissner's membrane
SG - spiral ganglion
SGC - spiral ganglion cell
SM - scala media
SPL - spiral ligament
st - stereocilia
ST - scala tympani
StV - stria vascularis
SV - scala vestibuli
T - tunnel
TEM - transmission electron microscopy
TM - tectorial membrane
TSB - tunnel spiral bundle of nerve fibers
VB - blood vessel of the basilar membrane
VTL - blood vessel of the tympanic lip of the osseous spiral lamina

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INTRODUCTION

The cochlea is the hearing portion of the inner ear that is housed in the petrous portion of the temporal bone. It consists of a triangular-shaped, fluid-filled channel, the membranous labyrinth, that is housed within the bony labyrinth (otic capsule). The membranous labyrinth spirals around a central bony canal, the modiolus, that contains the auditory division of the eighth cranial nerve and blood vessels to the cochlea. The membranous labyrinth is anchored to the bony labyrinth at the spiral ligament, the lateral edge of the triangle, and at the lip of the osseous spiral lamina medially. By anatomical convention, structures toward the modiolus are medial while those toward the spiral ligament are lateral. Except for its attachments laterally and medially, the membranous labyrinth is separated from the bony labyrinth by fluid-filled channels.

Several different techniques have been used to prepare the inner ear for microscopic examination. The entire temporal bone can be decalcified and embedded in a support medium such as paraffin, plastic or celloidin. Sections can then be cut parallel to the long axis of the cochlea (e.g., p. 5). Several hundred sections are made from each cochlea and generally every fifth to tenth section is stained and examined microscopically (e.g., Schuknecht, 1993). This technique allows one to study the otic capsule and the relation of damage in the membranous labyrinth to pathological changes in the overlying bone and middle ears. However, detailed quantitative studies of the sensory epithelium are either difficult or impossible.

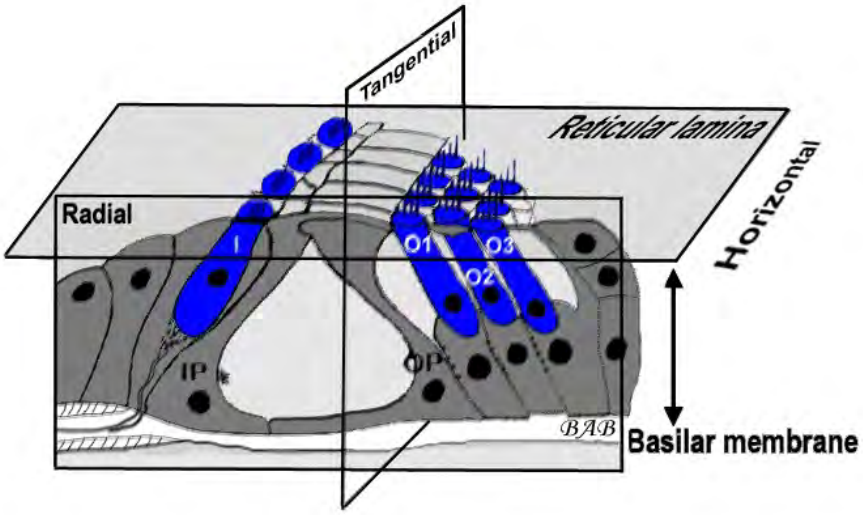
The membranous labyrinth (cochlear duct) is unique in that it need not be sectioned in order to be examined microscopically. Portions of the cochlear duct can be removed from the otic capsule, placed flat on a microscope slide in a liquid medium and cover-slipped (e.g., Engström et al., 1966). The various cells in the organ of Corti can then be examined by "optically sectioning" or using a microscope to focus at successively deeper layers within the epithelium. Tissues prepared this way are called "surface" or "flat" preparations. With this technique, long stretches of the sensory epithelium can be examined and the numbers of damaged or missing sensory (hair) cells can be readily determined.

The standard technique for making surface preparations of the inner ear involves dissecting the temporal bone when it is immersed in a buffered salt solution or alcohol. However, because of the delicate nature of the inner-ear epithelium, mechanical artifacts can occur while the tissue is being dissected. Some tissue may suffer distortion or actually be destroyed during the dissection. The technique used to prepare the specimens illustrated in this Atlas involved embedding the entire cochlea in epoxy resin [Durcupan (araldite)] prior to removal of the otic capsule. This procedure eliminates dissection artifacts and allows preparation and microscopic examination of the cochlear duct from apical to basal tip. Complete details of this technique have been published previously (Bohne, 1972; Bohne & Harding, 1993).

All illustrations in this Atlas were taken of the chinchilla cochlea. Although there are interspecies differences in certain cochlear parameters (e.g., number of cochlear turns, density of hair cells, innervation ratio, etc), the basic structure of the organ of Corti and the different cell types are the same across mammalian species.

The photomicrographs in this Atlas, except those on pp. 5, 6, 14 and 43, were made from cochleae that were fixed in a buffered solution of 1% osmium tetroxide. The dissected cochleae were examined and photographed using a Wild phase-contrast microscope. Selected regions within the cochleae were sectioned with an LKB or RMC ultramicrotome. One-micron-thick sections (pp. 7, 9, 12, 13, 41, 47-48, 56 and 58, were mounted on glass slides, stained with a methylene blue-azure II solution (Richardson et al., 1960), cover-slipped and examined by bright-field microscopy. Thinner sections were mounted on formvar-coated slot grids, stained with uranyl acetate and lead citrate and examined by transmission electron microscopy (pp. 10-11, 14-24, 29, 30, 33 and 62).

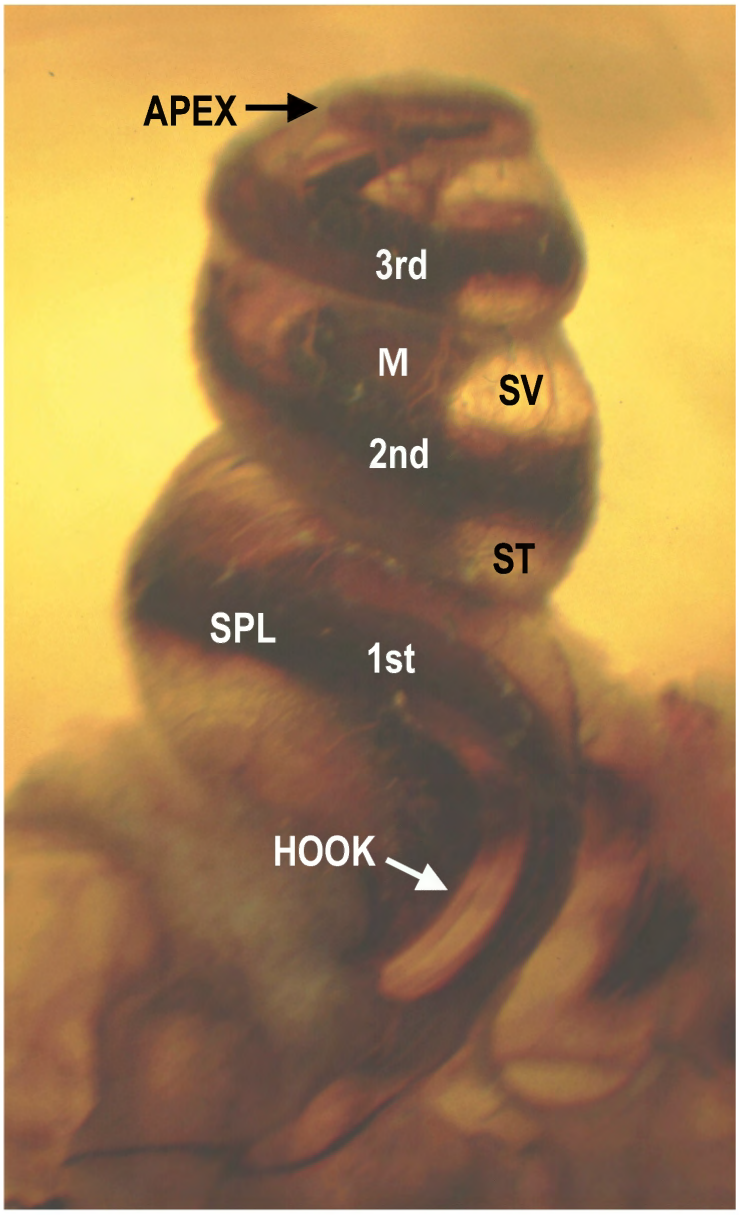
ANGLES for VIEWING the ORGAN of CORTI



This is a schematic view of the organ of Corti (adapted from Spoendlin, 1966) showing the three planes in which the organ can be observed: **Radial** - perpendicular to both the inner (IP) and outer pillar (OP) heads and the basilar membrane, passing through one cell in each sensory-cell row [i.e., inner hair cell (I) and three rows of outer hair cells (1, 2, 3)] (pp. 5-11); **Horizontal** - parallel to the endolymphatic surface of the organ of Corti (i.e., reticular lamina) and the basilar membrane, passing through a number of hair cells in each row simultaneously. This view can be either a sectioning plane (pp. 20, 23, 33) or a focal plane in a surface preparation (pp. 25-28; 30-32; 34-40; 42-46); **Tangential** - parallel to the pillar heads and perpendicular to the basilar membrane, passing through one row of sensory cells at a time (pp. 12-14, 66).

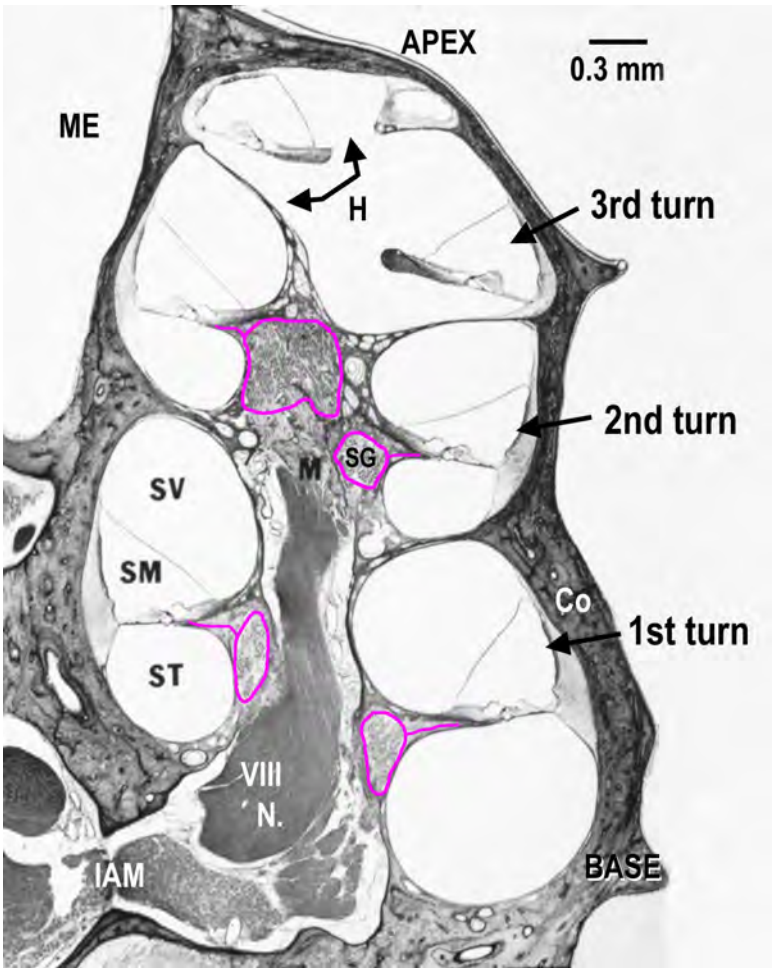
To understand anatomical relations, examine a flat preparation (e.g., pp. 26-28; 31-32; 34-40) of the undamaged organ of Corti under a phase contrast microscope and simultaneously look at a photomicrograph of a radial section of the organ of Corti. In your mind, draw straight lines across the radial section and observe how a section cut parallel to the basilar membrane (or a focal plane through the organ) would pass through the various cells. Compare what is visible in the radial section to the horizontal view seen in the flat preparation.

WHOLE COCHLEA with COCHLEAR BONE REMOVED



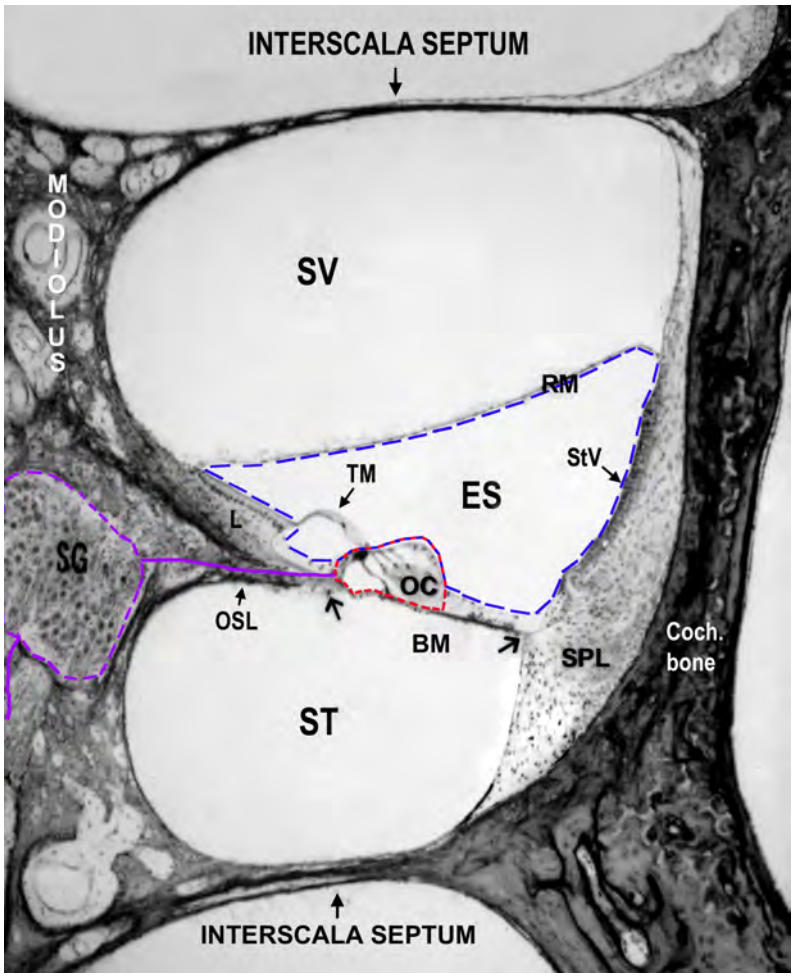
Plastic-embedded whole-mount of the cochlea following removal of cochlear bone. All fluid channels [i.e., scala tympani (ST); scala vestibuli (SV); scala media (under SPL label)] as well as the soft tissue are filled with polymerized plastic. The soft tissue and fluid spaces of the cochlea spiral around a central bony canal, the modiolus (M), that contains the axons of the spiral ganglion cells. The base of the cochlea (hook) is near the round window (not visible) and is located caudally in the middle ear space; the apex occupies a rostral location. Each turn or spiral of the cochlea is numbered (1, 2, 3) and is separated from the next turn by a bony shelf, the interscala septum. The chinchilla cochlea has 3.5 turns (i.e., 3 full turns plus the half-turn formed by the hook). SPL -spiral ligament and stria vascularis.

MID-MODIOLAR SECTION of the COCHLEA



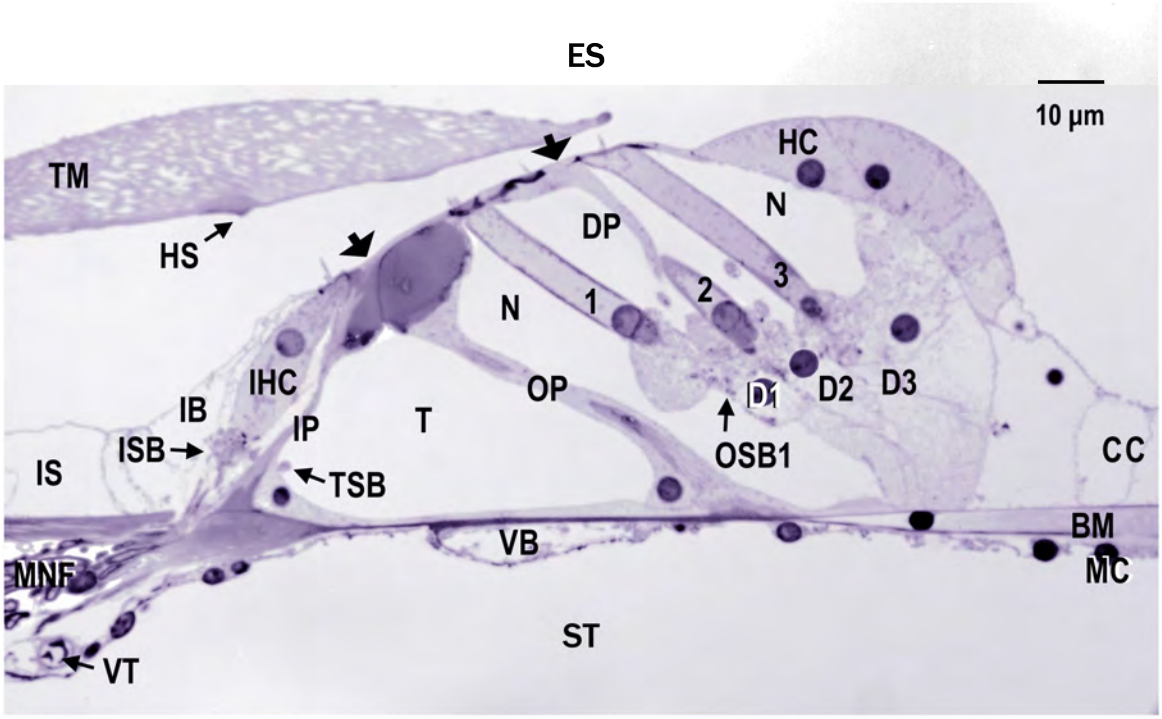
Cross-section of the chinchilla cochlea that was decalcified, embedded in celloidin and sectioned parallel to its long axis. The cochlear bone (Co) separates the soft tissue and fluid spaces of the cochlea from the middle ear space (ME), except at the round window. The cochlear duct spirals around a central bony canal, the modiolus (M). The spiral ganglion cells, are found in Rosenthal's canal (purple outline) which spirals at the periphery of the modiolus. The axons of the spiral ganglion cells form the eighth nerve (VIII N.) which nearly fills the modiolus. The eighth nerve exits the temporal bone through the internal auditory meatus (IAM) then enters the brainstem and synapses in the cochlear nuclei. The cochlear turns decrease in size from the base to the apex. The cochlear duct [also scala media (SM)] is a triangular-shaped fluid channel that is surrounded by two other fluid channels - scala vestibuli (SV) and scala tympani (ST). Scalae vestibuli and tympani are in communication at the apex of the cochlea through the helicotrema (H). These scalae are filled with a fluid called perilymph that is ionically similar to cerebrospinal fluid (i.e., high [Na+], low [K+]). The cochlear duct, excluding the organ of Corti, is filled with endolymph, a fluid that is similar to intracellular fluid in its ionic composition (i.e., high [K^+], low [Na^+]).

RADIAL SECTION of a COCHLEAR TURN



Higher power radial view of the 2nd turn of the cochlea (see p. 5). Scala media is triangular in shape. Its boundaries are: Reissner's membrane (RM) which separates scala vestibuli (SV) from scala media; the basilar membrane (BM) (from middle to right arrow in scala tympani) which separates scala tympani (ST) from scala media; and the stria vascularis (StV) which is located just medial to the spiral ligament (SPL; a periosteal thickening of the cochlear bone). The endolymphatic space (ES) is outlined in blue. The limbus (L) is a periosteal thickening of the osseous spiral lamina (OSL) to which is attached an extracellular structure, the tectorial membrane (TM). The organ of Corti (OC) (red outline) contains the sensory cells that are responsible for transducing mechanical waves in the cochlear fluids into electrical impulses. The spiral ganglion cells (SG) are found in Rosenthal's canal (purple outline), which is located at the lateral edge of the modiolus, just medial to the OSL (from left to middle arrow in scala tympani). The peripheral processes (purple line) of the SG neurons extend from the cell bodies to hair cells in the OC. A spiral bony partition, termed the interscala septum, runs from the cochlear base to the helicotrema and separates scala vestibuli in one turn from scala tympani in the adjacent turn.

RADIAL SECTION of the ORGAN of CORTI



This is a 1-micrometer-thick, radial section of the 2nd turn of the chinchilla organ of Corti. The organ of Corti consists of sensory cells, supporting cells and fluid spaces. The **sensory cells** consist of the inner hair cell (IHC; p. 35) and three rows of outer hair cells (1, 2, 3; p. 35). The **supporting cells** are divided into two types - those containing bundles of microtubules and those without microtubules. The inner pillar (IP) and outer pillar cells (OP) contain microtubules and have similar structures. Their bases, in contact with the basilar membrane (BM), are large and contain the nuclei and the termination of the microtubular bundles (p. 32). Their heads are also large and contain darkly stained material. Their slender bodies, comprised of straight parallel bundles of microtubules surrounded by a minimal amount of cytoplasm, form the sides of the triangularly shaped fluid space called the tunnel (T; p. 44). Deiters' cells (D1, D2, D3; pp. 37-39), one located beneath each outer hair cell, contain smaller bundles of microtubules. Deiters' cells have octagonal bases in contact with the BM. These cells extend toward the surface of the organ of Corti and expand slightly in the area below the hair-cell bases to form the Deiters cup region. At this point, a slender Deiters process (DP; p. 37) projects from each cell up to the surface of the organ of Corti, where it enlarges to form a phalangeal process. The reticular lamina (arrows; p. 31) is formed by the heads of the inner and outer hair cells, headplates of inner pillar cells and phalangeal processes from the outer pillar head and the three rows of Deiters' cells. Supporting cells without microtubules are inner border (IB) and inner phalangeal cells (not visible) around the inner hair cells and Hensen's cells (HC; 40) forming the lateral margin of the organ of Corti. (Cont. next page).

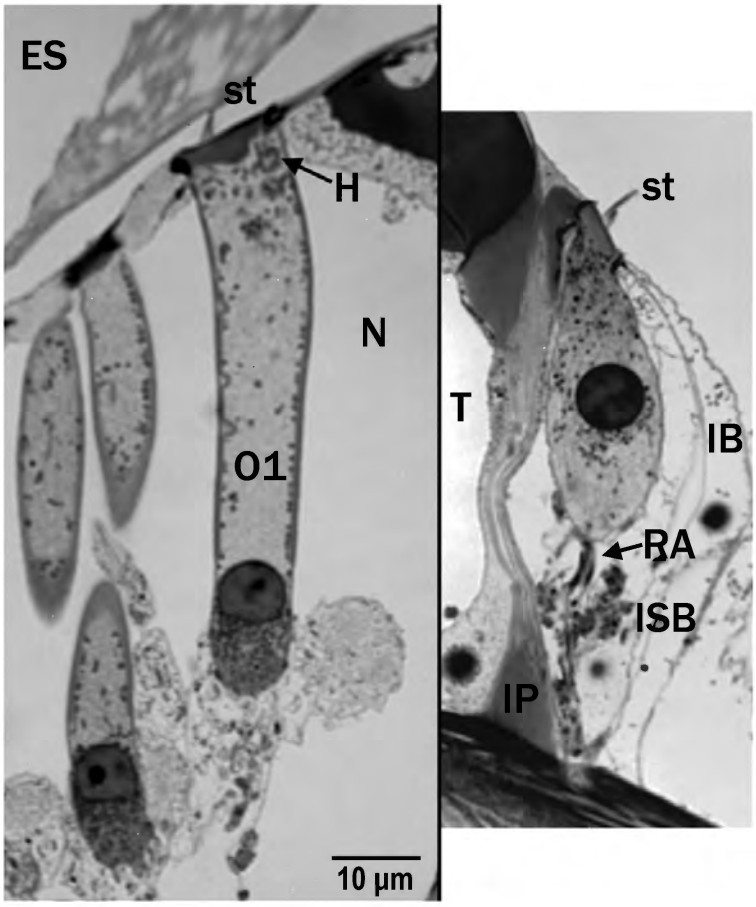
In addition to the fluid-filled tunnel space (T), Nuel spaces (N) surround each of the outer hair cells. All of these spaces are filled with a fluid similar or identical to that which fills scala tympani. The fluid spaces within the organ of Corti are sometimes called Cortilymph spaces with the fluid being termed "Cortilymph".

The peripheral processes (MNF; or dendritic processes; p. 42) of the spiral ganglion neurons (p. 41) are myelinated until they penetrate the BM and enter into the organ of Corti. Within the organ of Corti, the nerve fibers form several distinct bundles: inner spiral bundle (ISB) beneath the inner hair cell, tunnel spiral bundle (TSB) within the tunnel space near the inner pillar base, and outer spiral bundles (e.g., OSB1) between the rows of Deiters' cells, one beneath each row of outer hair cells (pp. 43, 44).

Inner sulcus cells (IS) cover the area from the medial edge of the organ of Corti to the lip of the limbus. Claudius' cells (C) cover the BM lateral to the organ of Corti. On the scala tympani side of the BM, several mesothelial cell nuclei (M) can be seen. Also visible here is the vessel of the tympanic lip (VT; p. 46) of the osseous spiral lamina and the vessel of the basilar membrane (VB) which is non-functional in this region of the adult chinchilla cochlea.

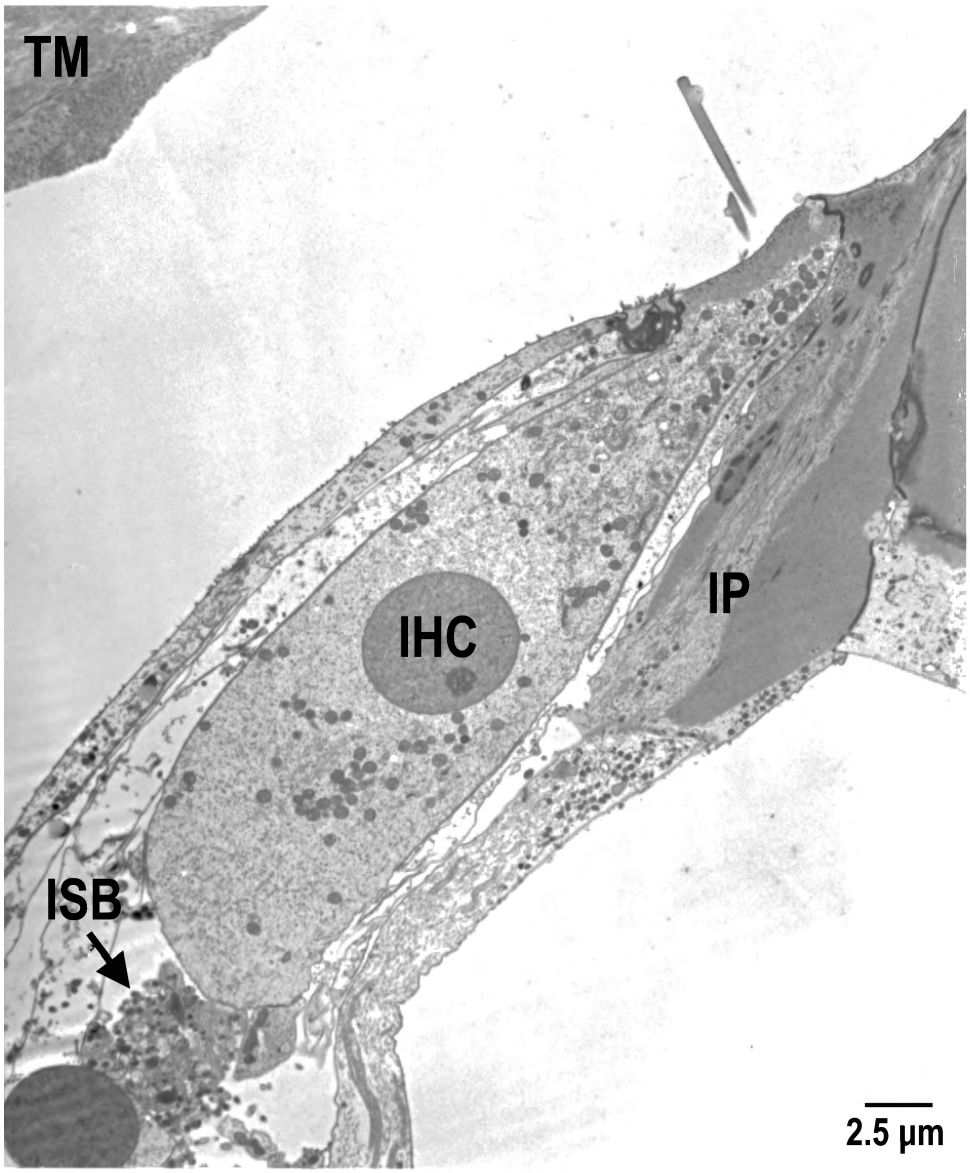
In vivo, the tectorial membrane (TM; p. 26) is found in close proximity to the reticular lamina. The tallest stereocilia (p. 30) on the outer hair cells project into the tectorial membrane. On the other hand, IHC stereocilia abut on the lateral side of Hensen’s stripe (HS). In most histological preparations of the inner ear, the tectorial membrane is shrunken and displaced from its *in-vivo* position due to the processes of fixation and dehydration. ES - endolymphatic space; ST - scala tympani.

RADIAL SECTION of an OHC & an IHC



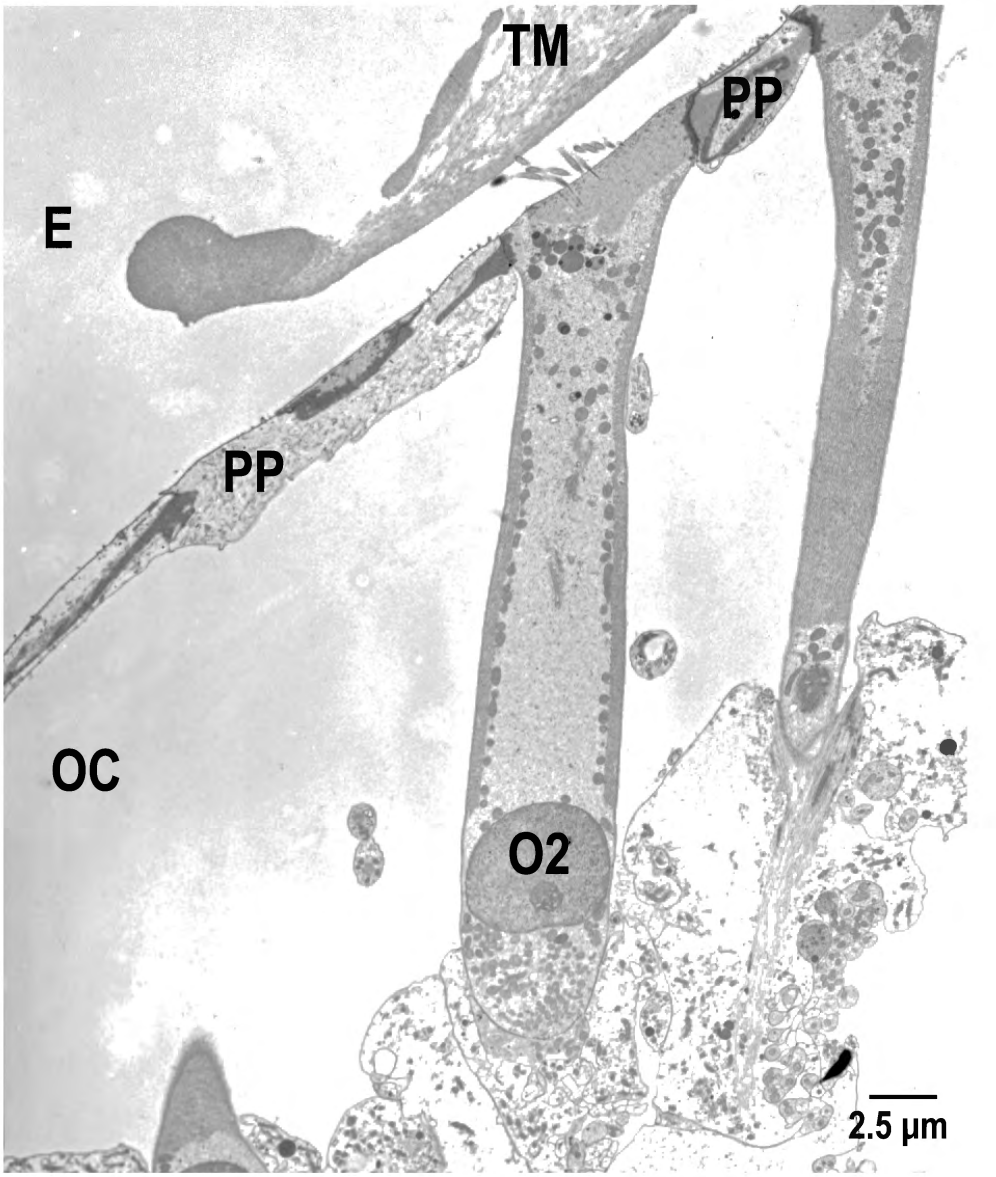
One-micrometer-thick radial sections of an outer hair cell (O1) and an inner hair cell. Stereo-cilia (st) project from the apical surface of each cell into the endolymphatic space (ES). At the reticular lamina, the supporting cell processes surrounding the hair cells and the dark-staining tight junctions are visible. Medium-staining cuticular plate substance is seen just beneath the apical membrane of each hair cell. The basolateral surfaces of OHCs are freely exposed to the fluid (i.e., Cortilymph in older literature) within the Nuel (N) spaces and tunnel (T). Organelles have a specific distribution in OHCs. Lysosomes, mitochondria and occasionally Hensen's bodies (i.e., H - whorl of smooth endoplasmic reticulum) are found in a subcuticular location. A single row of mitochondria (dark dots) is found in the mid-portion of the cells, just inside the plasma membrane. The nucleus is located near the cell's base along with an infranuclear group of mitochondria. Efferent and afferent nerve endings (not visible here) synapse on the OHC base near the Deiters cup region. The IHC is completely surrounded by inner border (IB) and inner pillar (IP) cells. Inner phalangeal cells, located between adjacent IHCs are not visible here. Mitochondria, lysosomes and rough endoplasmic reticulum appear as dark dots and strands scattered in the cell's cytoplasm. The inner spiral bundle (ISB) of nerve fibers is located between the IHC base and the spiral lamina. Radial afferents (RA) can be seen extending from the spiral lamina toward the IHC base.

RADIAL TEM of an IHC



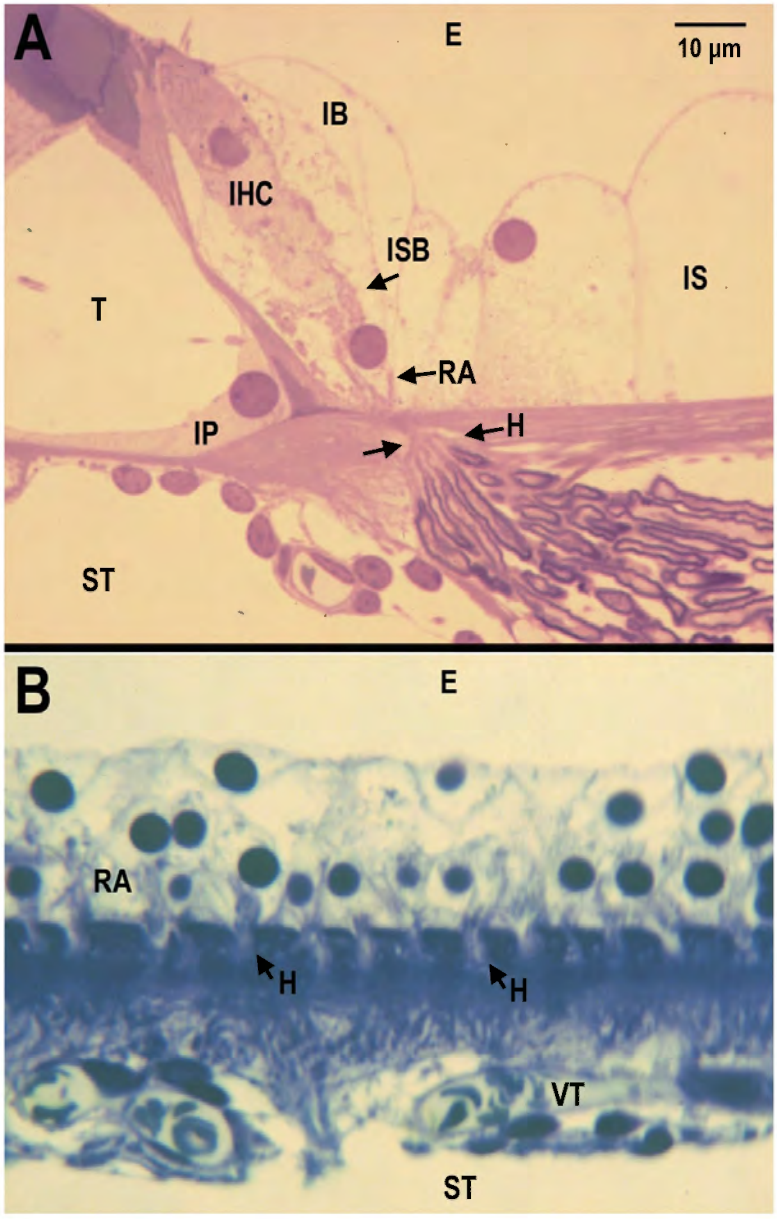
Low power TEM of a radial section through an IHC in the 3rd turn. The cell is flask-shaped with a centrally located nucleus. Stereocilia (i.e., elongated microvilli) project from its apical surface into the endolymphatic space. Dark-staining cuticular plate substance is found just beneath the surface membrane of the cell. The cell's cytoplasm contains scattered organelles including mitochondria, vesicles and strands of endoplasmic reticulum. Nerve endings from type I spiral ganglion cells are applied to the base and basolateral sides of the cell. The non-myelinated nerve fibers in the inner spiral bundle (ISB) appear in cross-section. IP -inner pillar; TM - tectorial membrane.

RADIAL TEM of an OHC



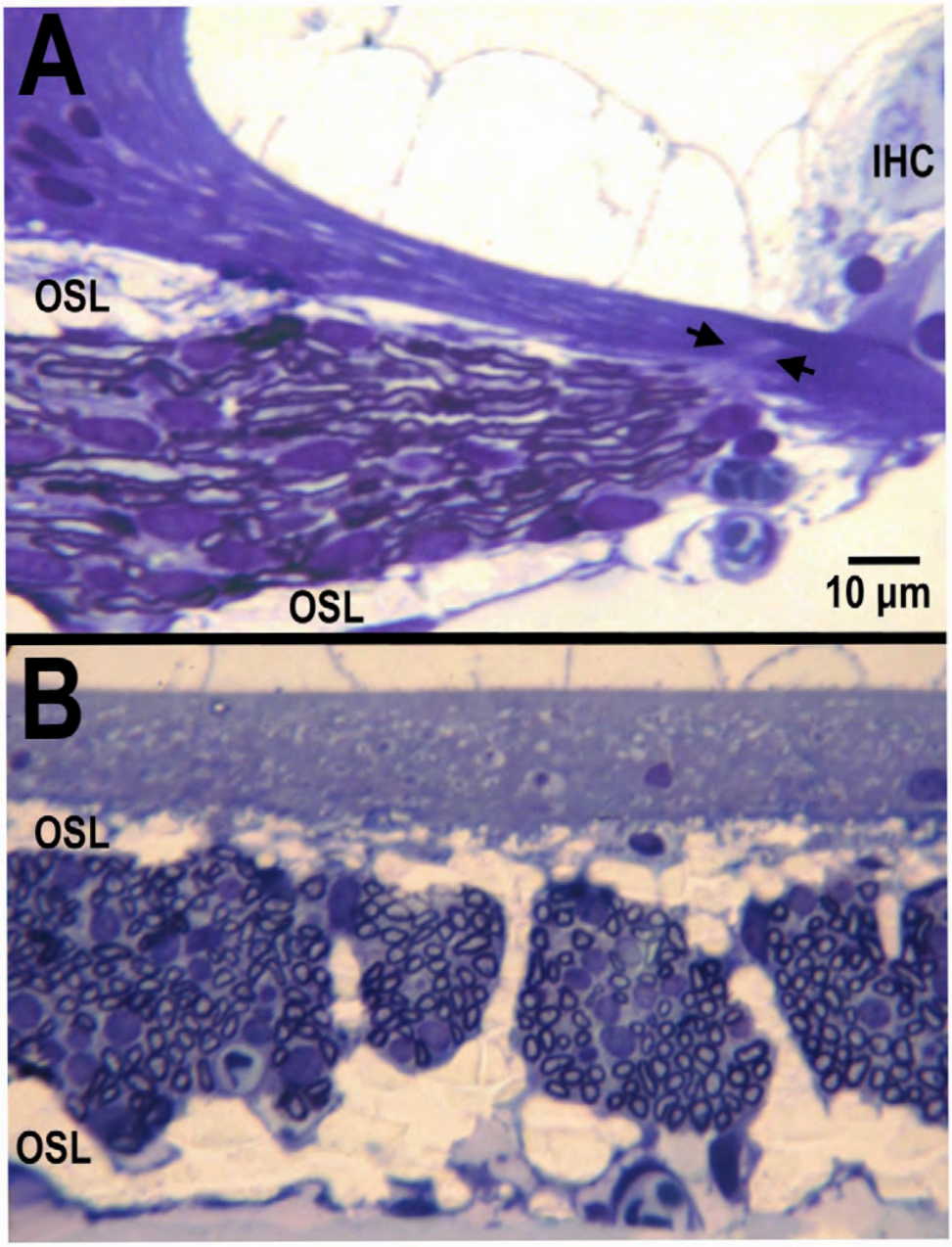
Low-power TEM of a radial section through a 2nd-row OHC (O2) in the first turn. The cell is long and cylindrical with its nucleus located near its base. Groups of organelles are found within the cell's cytoplasm: 1) mitochondria and lysosomes beneath the cuticular plate; 2) a single row of mitochondria just inside the peripheral membrane system of the mid-body region; 3) the infranuclear group of mitochondria beneath the nucleus near the nerve terminals. Stereocilia project from the apical surface of the cell, the tallest of which extend into the overlying tectorial membrane (TM). The hair cell apex is joined to the phalangeal processes (PP) of supporting cells by tight junctions. The tight junctions between sensory and supporting cells form a barrier between the endolymphatic space (E) and the fluid spaces within the organ of Corti (OC).

RADIAL & TANGENTIAL VIEWS of HABENULAE PERFORATA



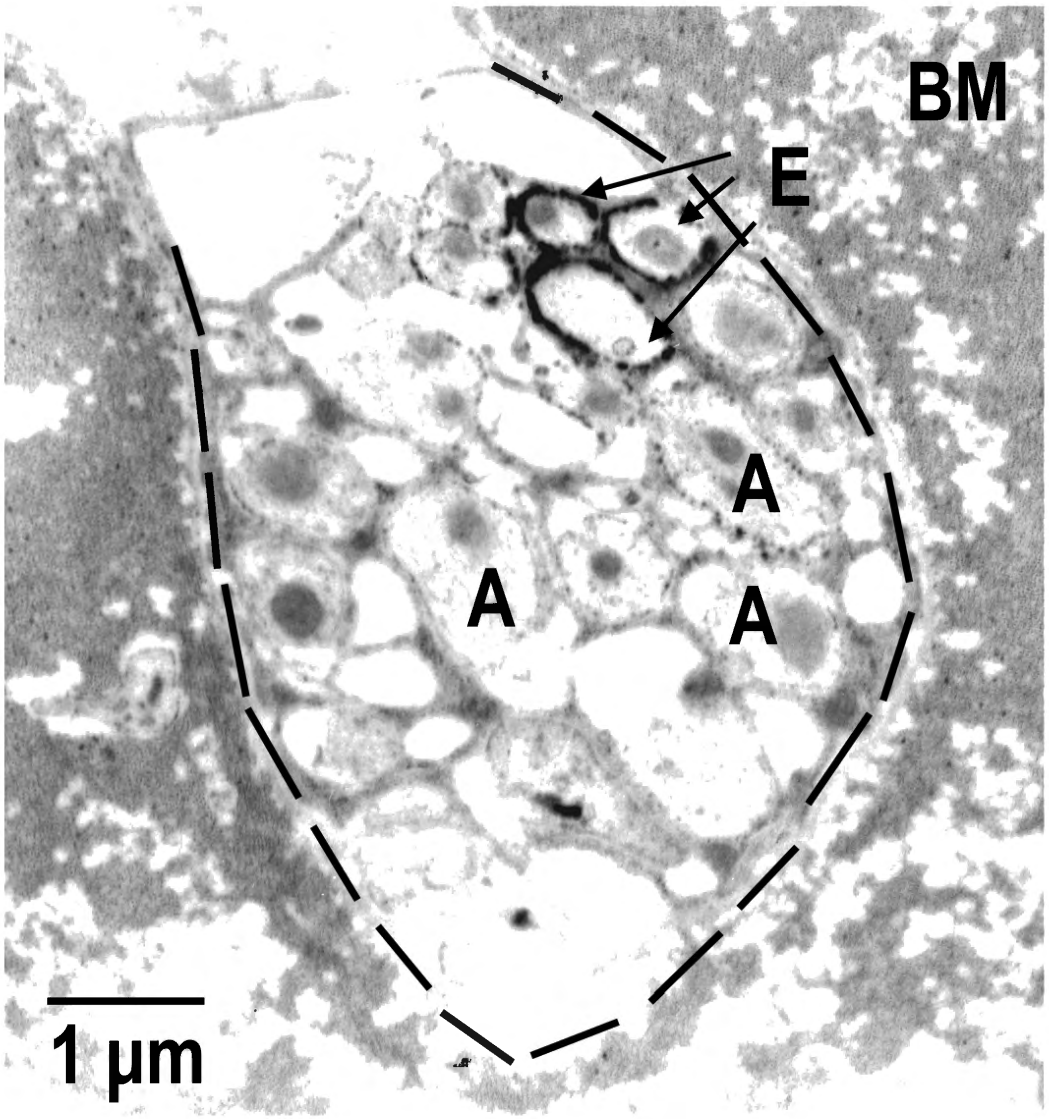
Stained sections of the organ of Corti in the inner hair cell area. A) Radial section showing the inner hair cell (IHC), inner pillar (IP), inner spiral bundle (ISB) of nerve fibers and habenula perforata (between arrows); B) Tangential section cut through about 13 habenulae perforata (arrows). Myelin sheaths on the nerve fibers are lost as they pass through the habenulae and enter the organ of Corti. Most fibers, termed radial afferents (RA), take a direct course to the nearest IHC. There is on average, one habenula per IHC. E - endolymphatic space; IB - inner border cell; IS - inner sulcus cell; ST - scala tympani; T - tunnel space; VT - vessel of the tympanic lip of the osseous spiral lamina.

MYELINATED NERVE FIBERS in the OSSEOUS SPIRAL LAMINA (OSL)



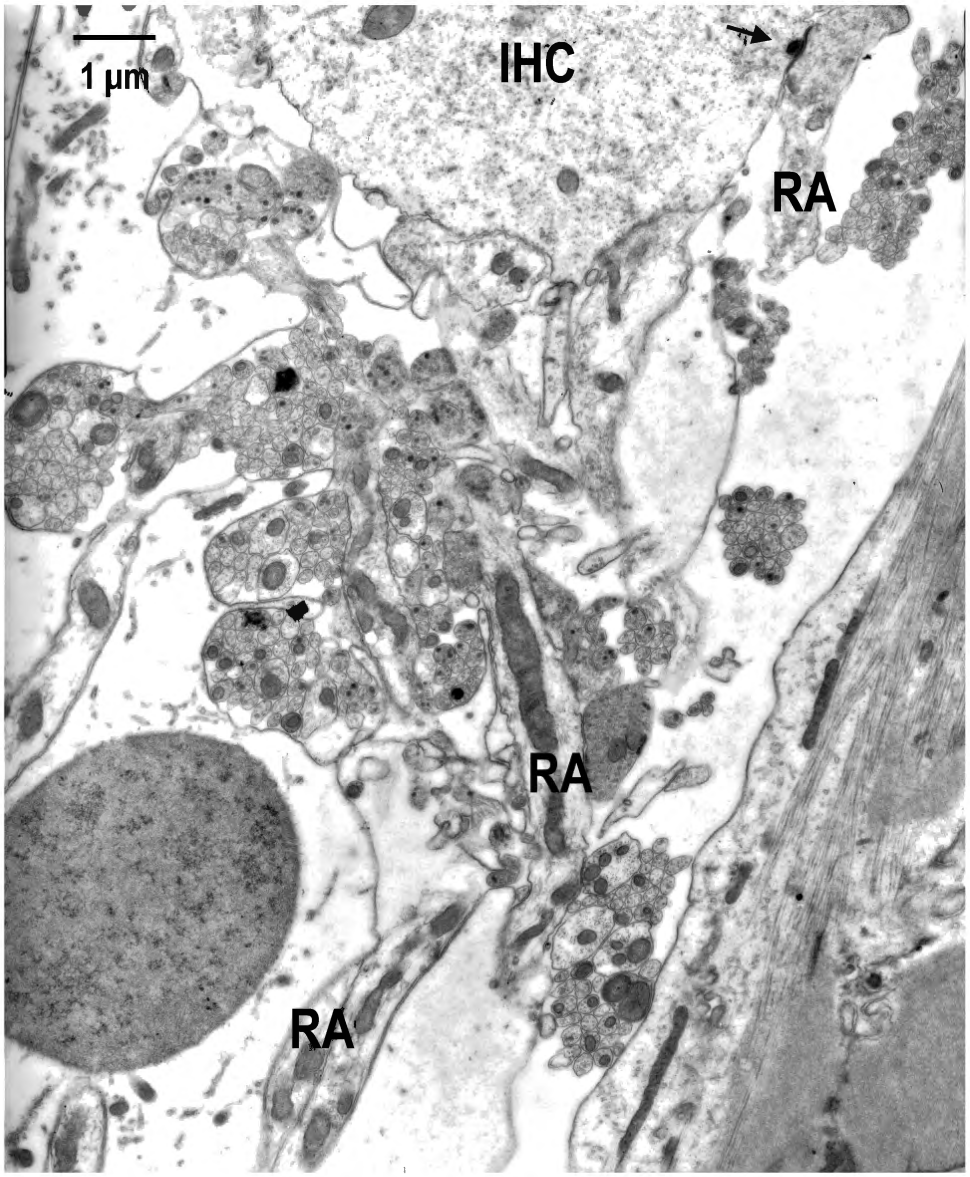
Stained, one-micrometer-thick radial (A) and tangential (B) sections of the peripheral processes of spiral ganglion neurons in the osseous spiral lamina (OSL). These processes are myelinated by Schwann cells (elongated, oval nuclei) until they pass through the basilar membrane by traversing a series of holes (i.e., habenulae perforata; between arrows in 'A') beneath the inner hair cells. The tangential section shows that the fibers are fairly uniform in diameter and are arranged in variable-sized bundles.

TANGENTIAL TEM of a HABENULA PERFORATA



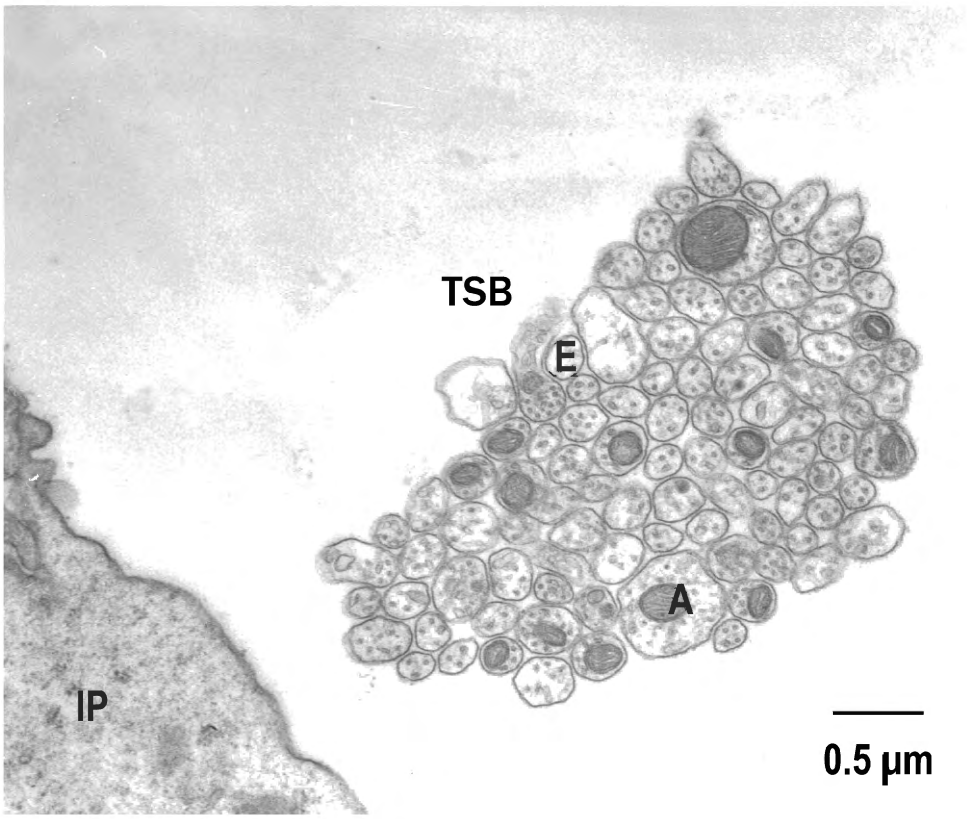
TEM of a tangential section through a habenula perforata (outlined with black dashed line). Habenulae are perforations in the basilar membrane (BM) that allow the peripheral processes of the spiral ganglion neurons to pass from the osseous spiral lamina into the organ of Corti. This is a control specimen that was fixed with paraformaldehyde and then stained for acetylcholinesterase (AChE) activity (i.e., marker for efferent fibers). This habenula contains approximately 22 cross-sectioned nerve fibers, three of which are positive for AchE activity (E), as evidenced by the dark precipitate on their limiting membranes. The rest of the fibers that have little or no precipitate are afferent nerve fibers.

RADIAL TEM of INNER SPIRAL BUNDLE of NERVE FIBERS



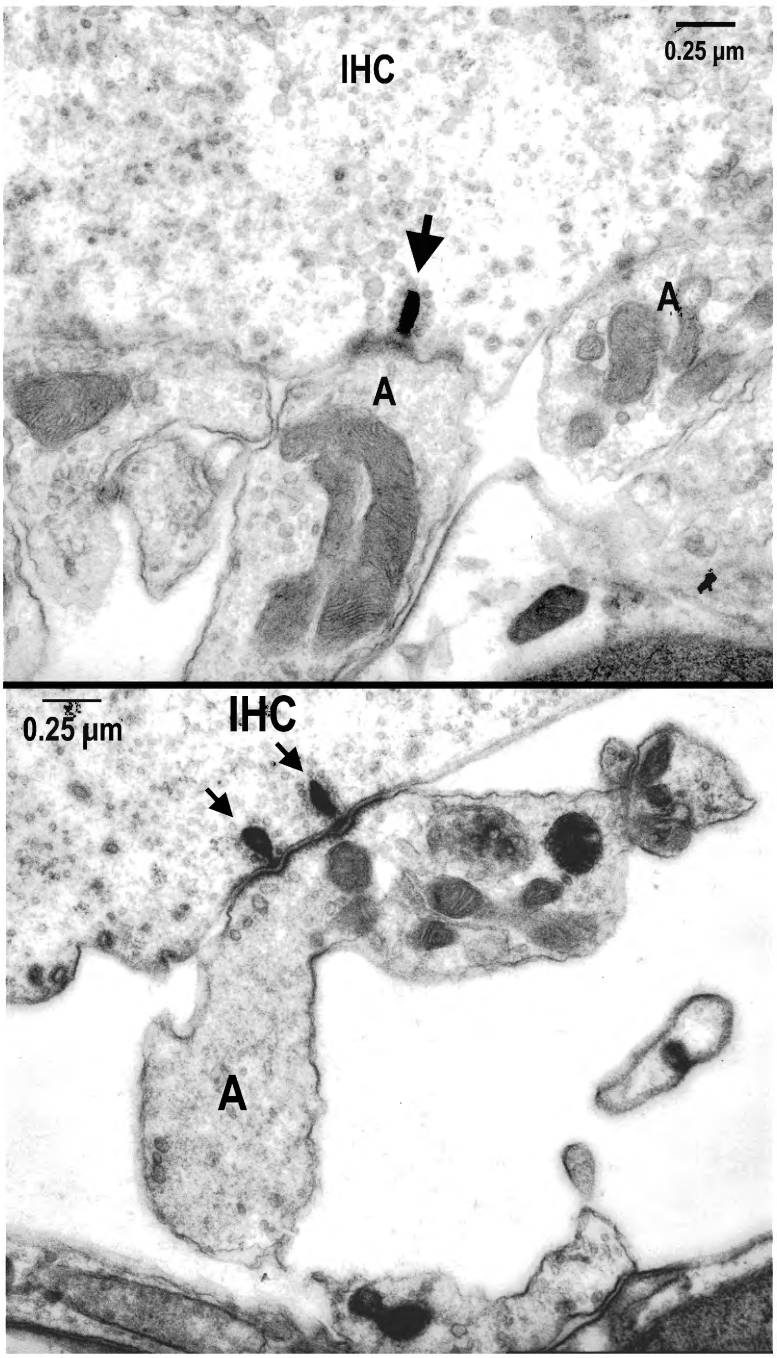
TEM of a radial section through the cross-sectioned inner spiral bundle of nerve fibers beneath the inner hair cell (IHC). The peripheral processes of the type I spiral ganglion cells, termed radial afferents (RA) take a nearly direct course from a habenula to the nearest IHC. The arrow points to a synaptic bar in the hair cell where a radial afferent is synapsing. Most of the fibers in the ISB have small diameters and belong to the efferent system.

RADIAL TEM of TUNNEL SPIRAL BUNDLE of NERVE FIBERS



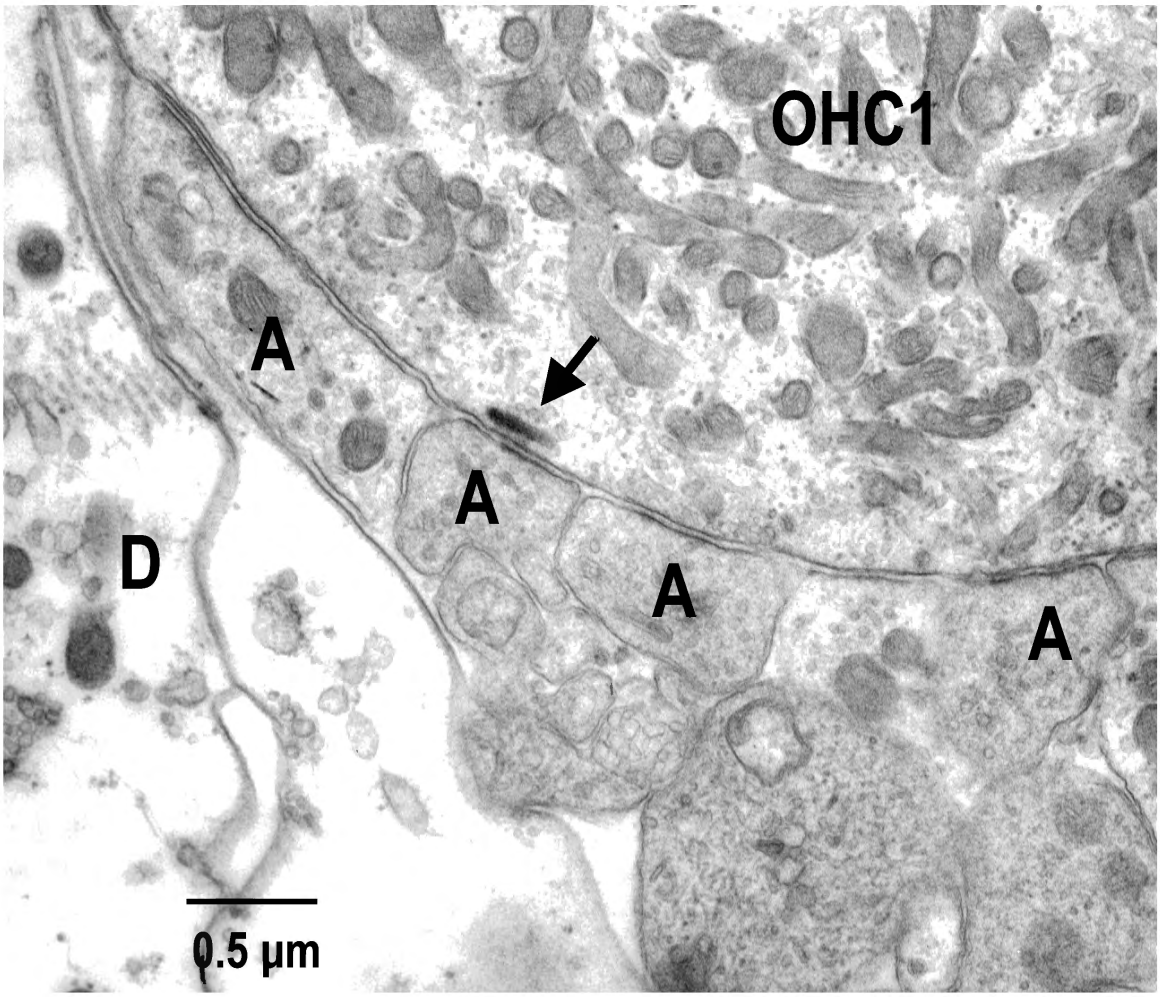
The tunnel spiral bundle (TSB) of nerve fibers is found within the tunnel space near the inner pillar foot (IP). Most of the fibers in the TSB are small in diameter and belong to the efferent system (E). There is an occasional larger diameter fiber that belongs to the afferent system (A).

AFFERENT NERVE FIBER SYANPSES on an IHC BASE



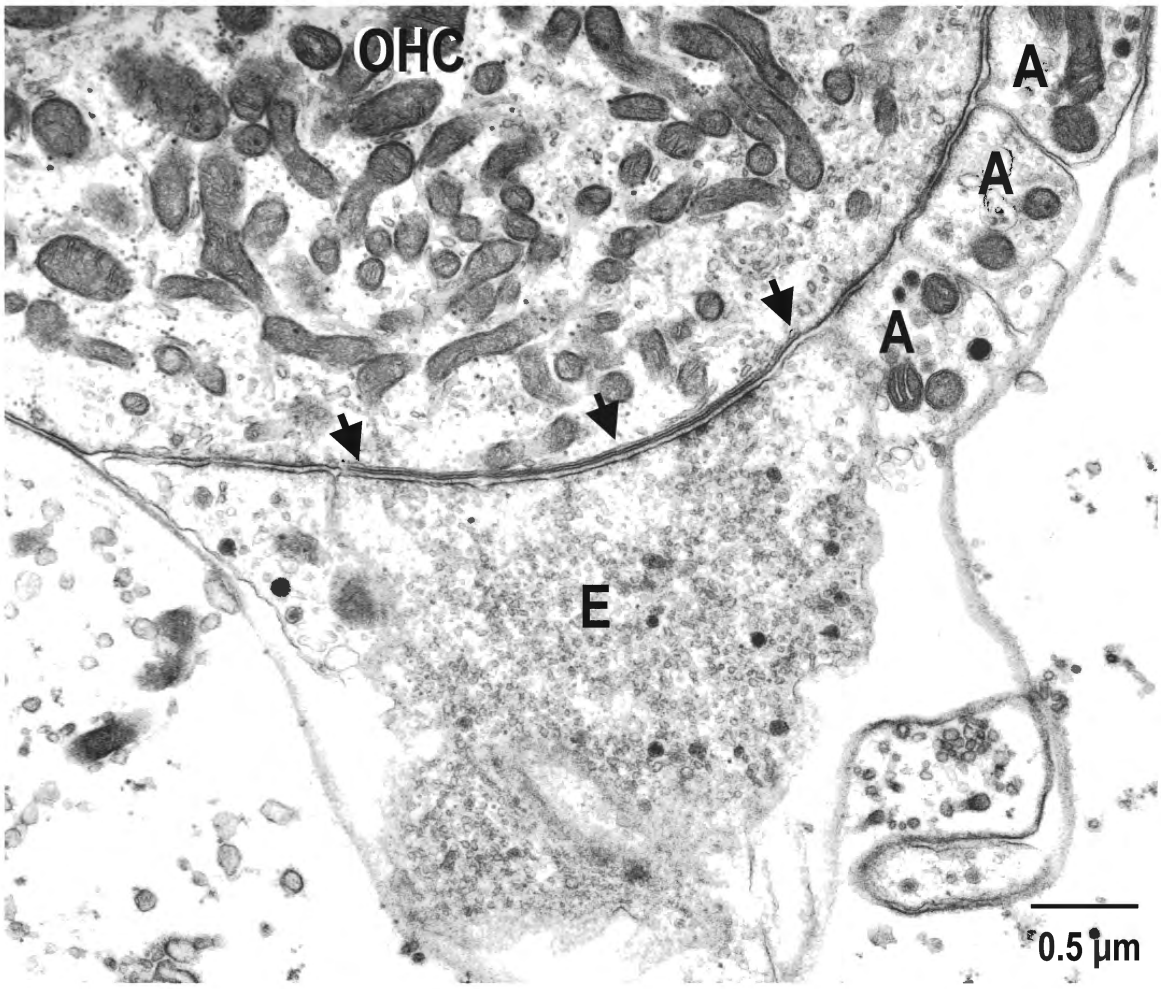
Synapses between radial afferents (A) and the base of the inner hair cell (IHC) often have synaptic bars (arrows) associated with them. Synaptic bars are dark-staining, oriented perpendicular to the plasma membrane of the hair cell and are surrounded by a single row of vesicles. The plasma membranes on either side of the synapse are thickened and darker-staining.

AFFERENT NERVE FIBER SYNAPSES on an OHC BASE



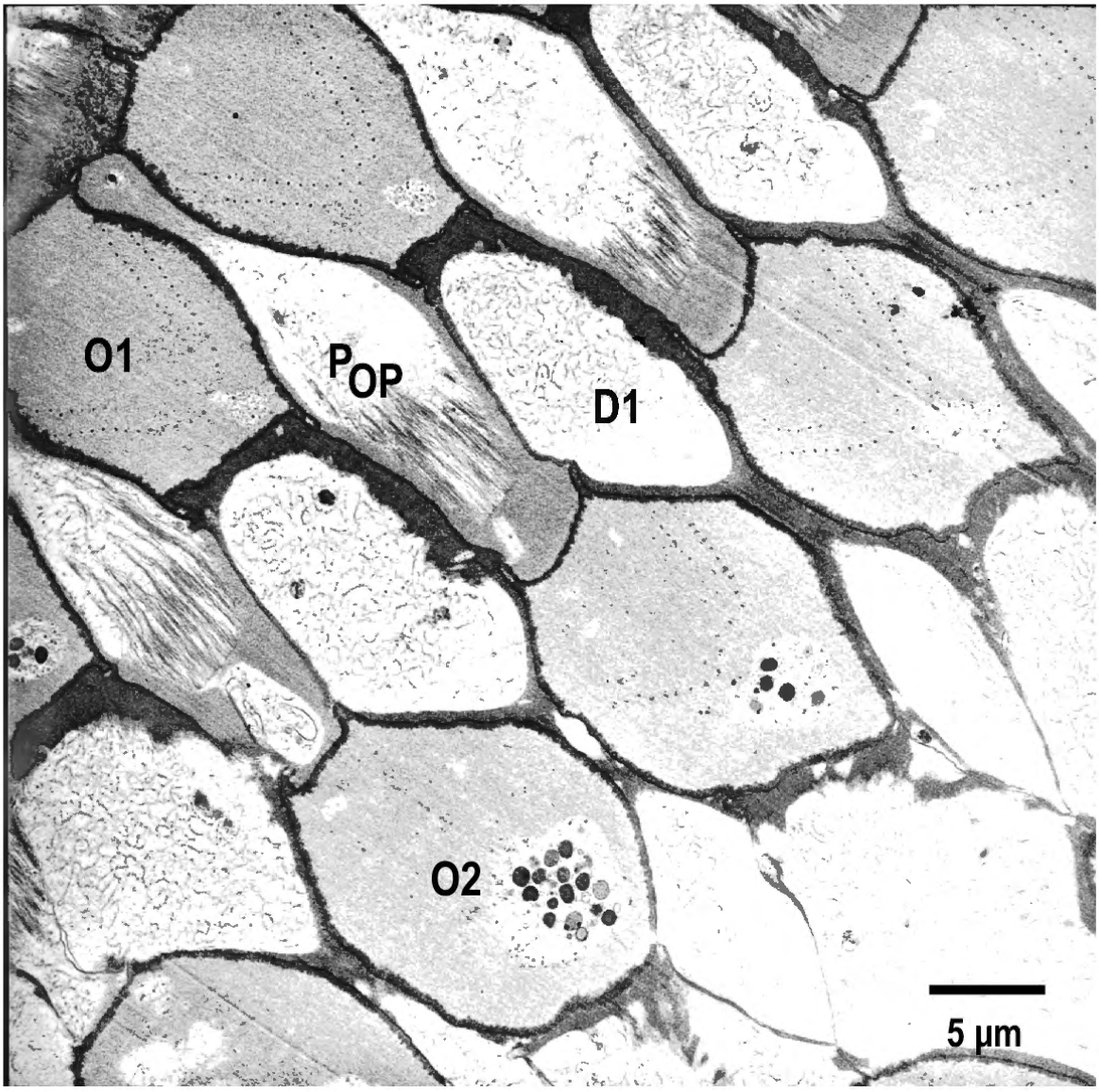
TEM of a radial section through a 1st row outer hair cell (OHC1). Afferent nerve fibers form small bouton endings (A) on the base below the nucleus. A synaptic bar (arrow) surrounded by synaptic vesicles is occasionally seen in the hair-cell cytoplasm adjacent to afferent synapses. The pre- and post-synaptic membranes are darker and thicker than the rest of the plasma membrane. D - Deiters' cell.

EFFERENT NERVE ENDING on an OHC BASE



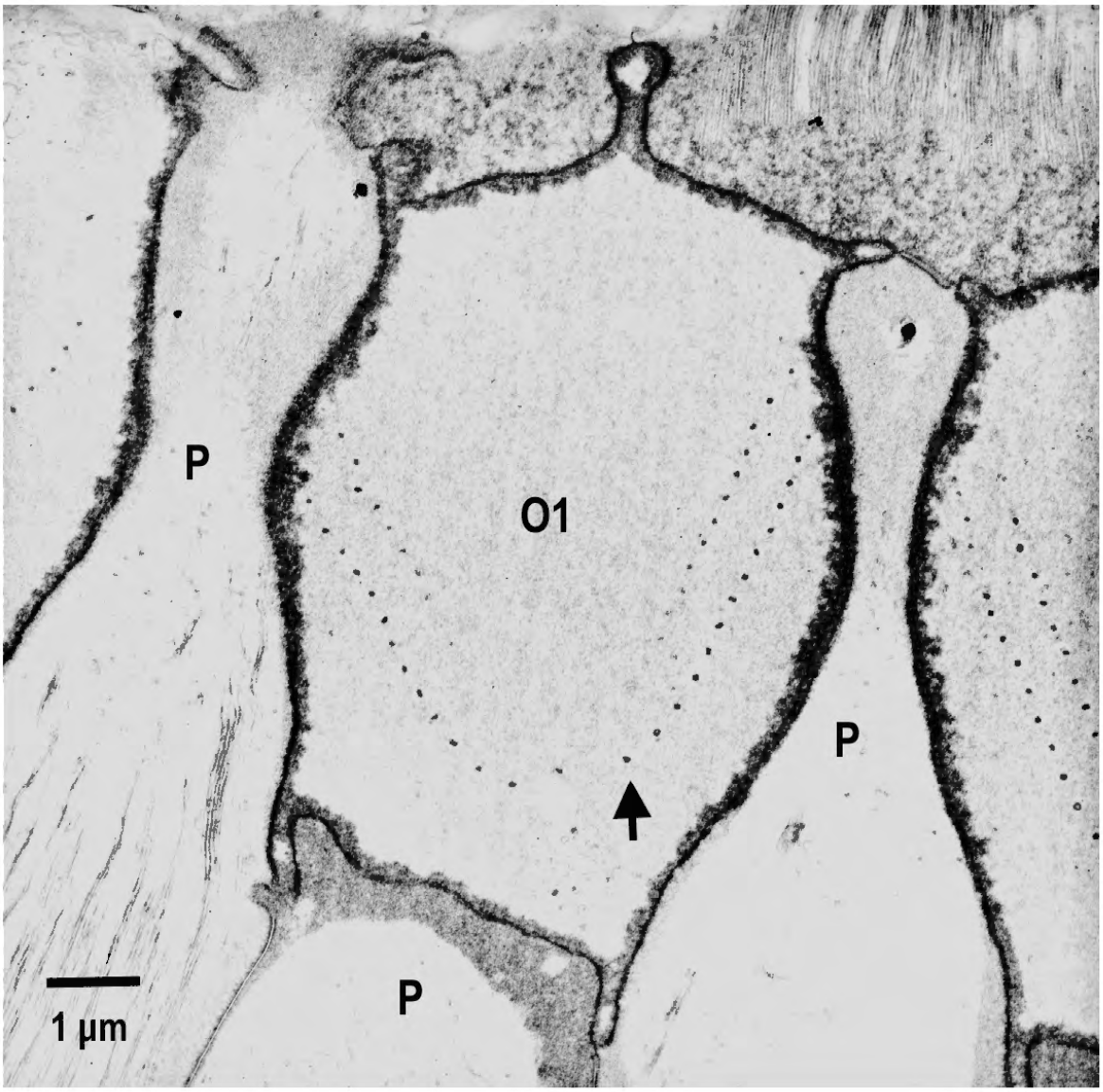
Efferent endings (E) are large and contain a number of synaptic vesicles adjacent to the synapse. There is generally a large number of mitochondria in the nerve ending opposite the synapse but none are visible here. In the hair cell at the synapse is a flattened cisterna of smooth endoplasmic reticulum (arrows) that is called a subsynaptic cisterna. A - afferent endings.

HORIZONTAL TEM THROUGH the RETICULAR LAMINA



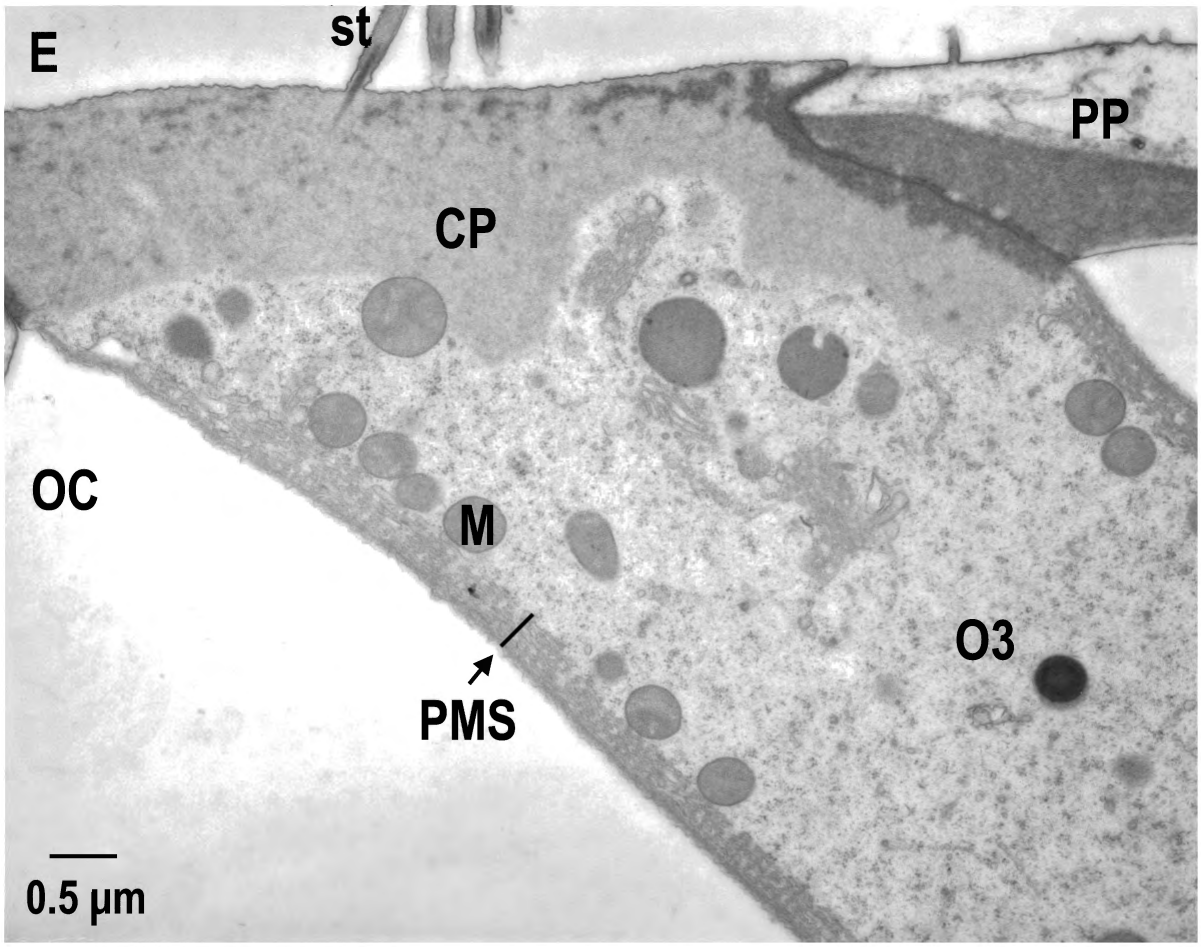
TEM of a horizontal section through the reticular lamina of the organ of Corti. The section passed through the cuticular plates of the 1st (O1) and 2nd (O2) rows of outer hair cells. Stereocilia rootlets appear as dark dots in many of the hair cells. Each hair cell is separated from its neighbors by phalangeal processes from supporting cells. D1 - phalangeal process from a 1st row Deiters' cell; Pop - phalangeal process from the outer pillar head.

HORIZONTAL TEM through an OHC APEX & PHALANGEAL PROCESSES



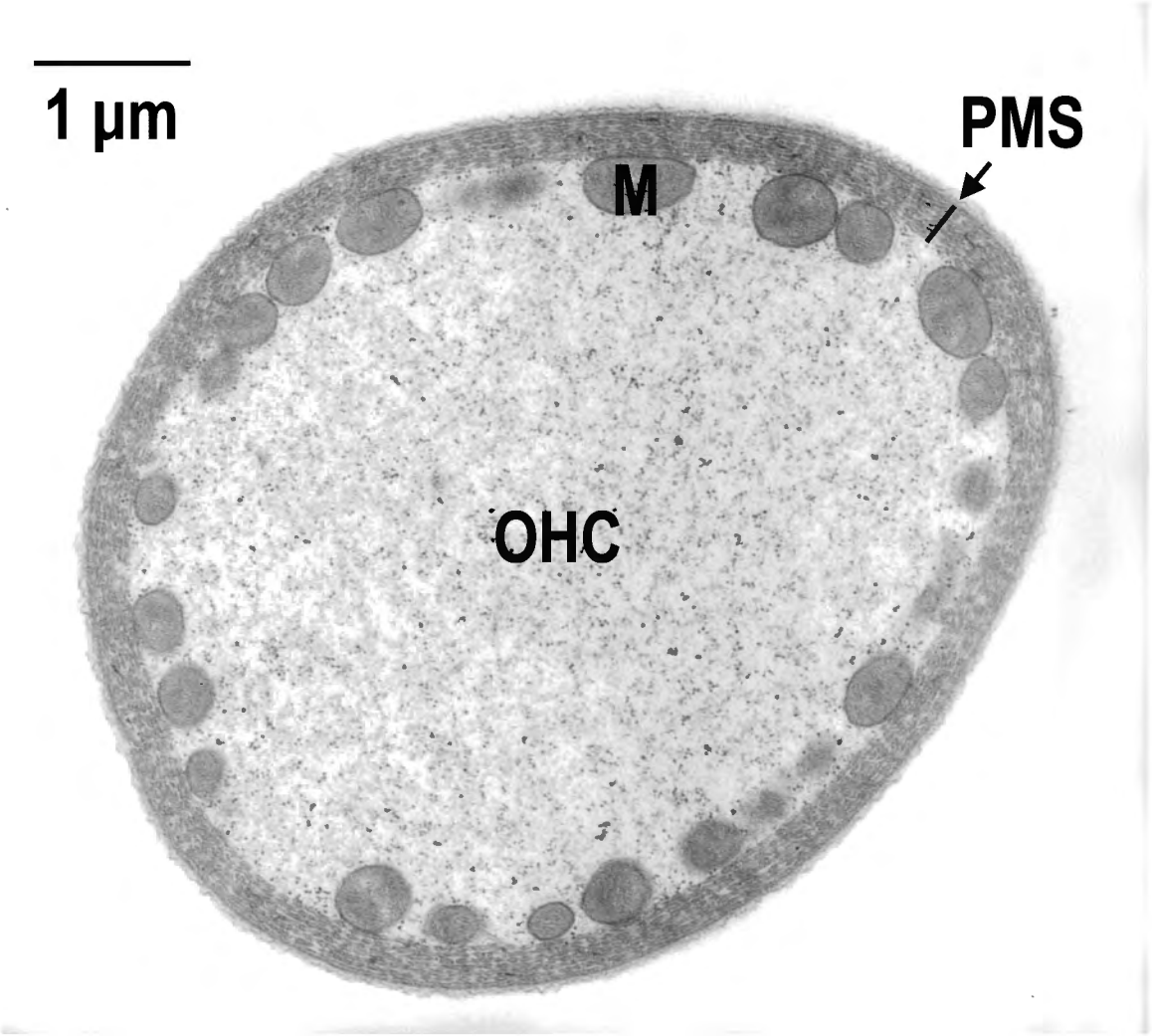
TEM of a horizontal section through the apex of a 1st row outer hair cell (O1). The tight junctions between hair cell and phalangeal processes (P) of supporting cells are darkly stained and encircle the hair-cell apex like a belt. The arrow points to a cross-sectioned rootlet of a stereocilium.

RADIAL TEM of an OHC APEX & PHALANGEAL PROCESS



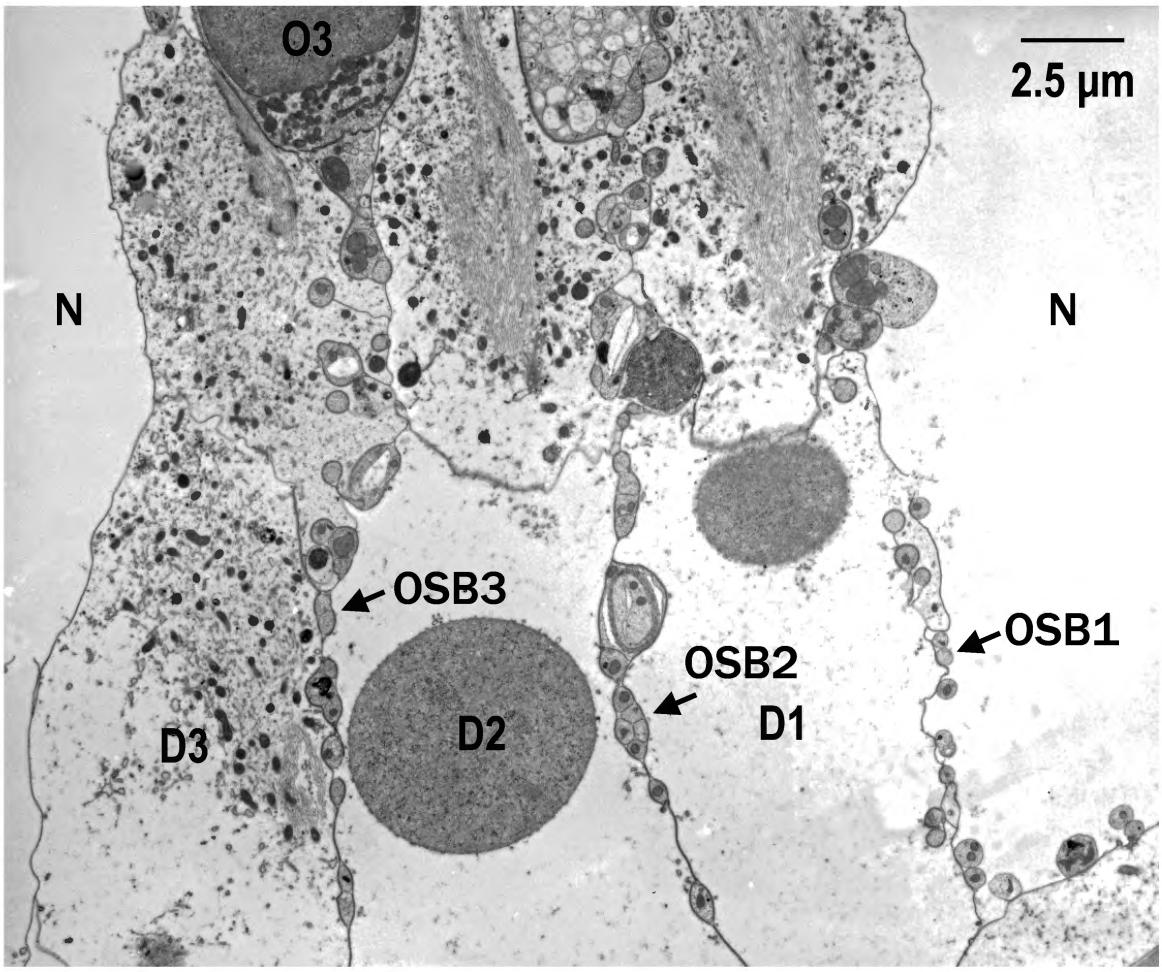
TEM of a radial section through the apex of a 3rd row outer hair cell (O3) showing three stereocilia (st), the cuticular plate substance (CP) and a group of subcuticular organelles. The tight junction between hair cell and phalangeal process (PP) of the supporting cell is darkly stained and forms a band around the cell apex separating the endolymphatic space (E) from the fluid (OC) within the organ of Corti. The peripheral membrane system (PMS) of the cell is just inside the plasma membrane. A row of mitochondria (M) is located just medial to the PMS.

HORIZONTAL TEM THROUGH MID-BODY REGION of an OHC



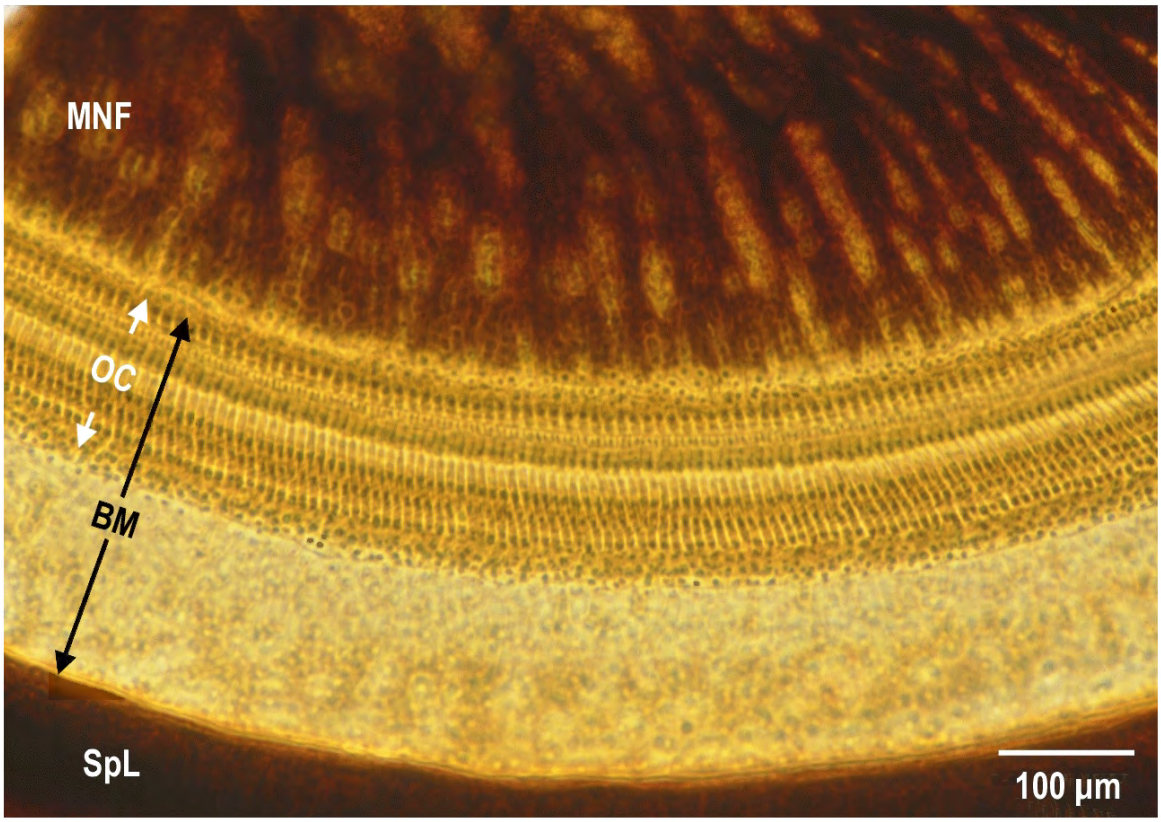
TEM of a horizontal section through the mid-body region of an outer hair cell (OHC). Adjacent to the plasma membrane are several rows of flattened cisternae of smooth endoplasmic reticulum. These cisternae form the cell's peripheral membrane system (PMS). A single row of mitochondria (M) is found just inside the peripheral membrane system. The central cytoplasm of the mid-body region of OHCs is usually devoid of organelles.

RADIAL TEM of OUTER SPIRAL BUNDLES of NERVE FIBERS



TEM of a radial section through the outer spiral bundles (OSB1, 2, 3) of nerve fibers between the Deiters cells' bases (D1, D2, D3) and beneath the corresponding row of outer hair cells (e.g., O3). Note that the nerve fibers are often contained within folds of the Deiters cells' membranes, especially OSB1. N - Nuel spaces.

COCHLEAR DUCT VIEWED at HORIZONTAL ANGLE

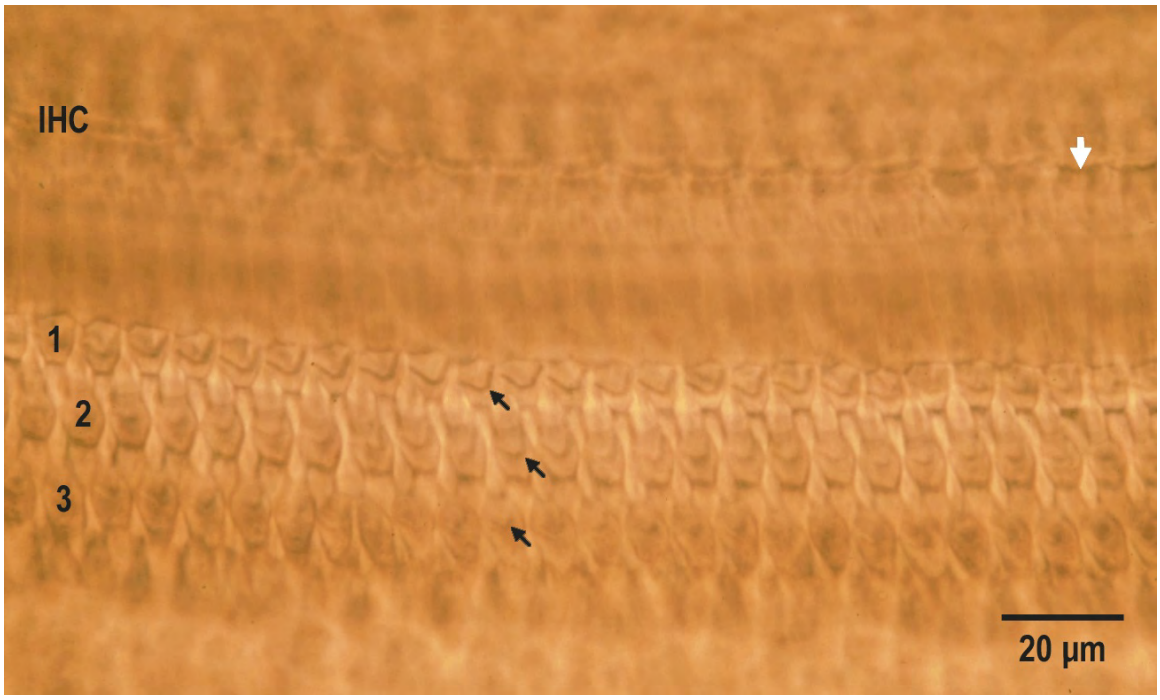


Low power view of the cochlear duct in the 1st turn as seen from Reissner's membrane. The basilar membrane (BM) extends from the lateral edge of the osseous spiral lamina that contains myelinated nerve fibers (MNF) to the spiral ligament (SpL). The organ of Corti (OC) covers that part of the basilar membrane nearest the osseous spiral lamina.

TECTORIAL MEMBRANE VIEWED at HORIZONTAL ANGLE

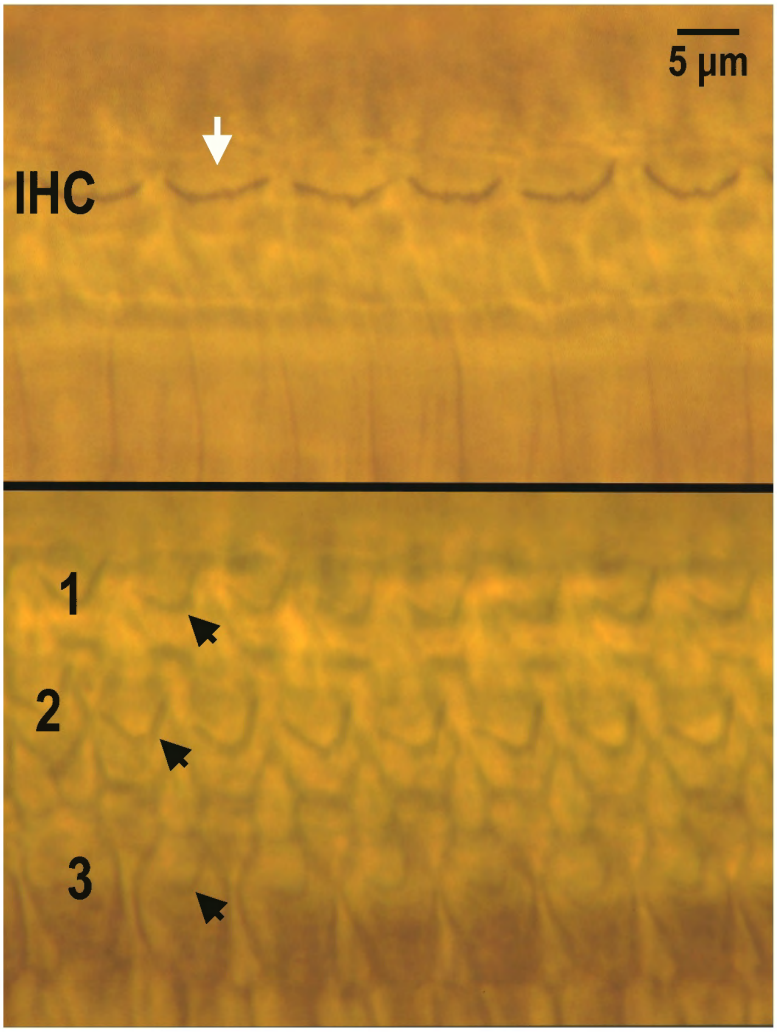
The tectorial membrane appears as faint striations running at a slight angle across the upper 4/5 of the photomicrograph. The arrows point to the free edge of the tectorial membrane which has rolled back upon itself. This artifact occurred as a result of the fixation, dehydration and/or embedding processes.

HAIR-CELL STEREOCILIA VIEWED at HORIZONTAL ANGLE



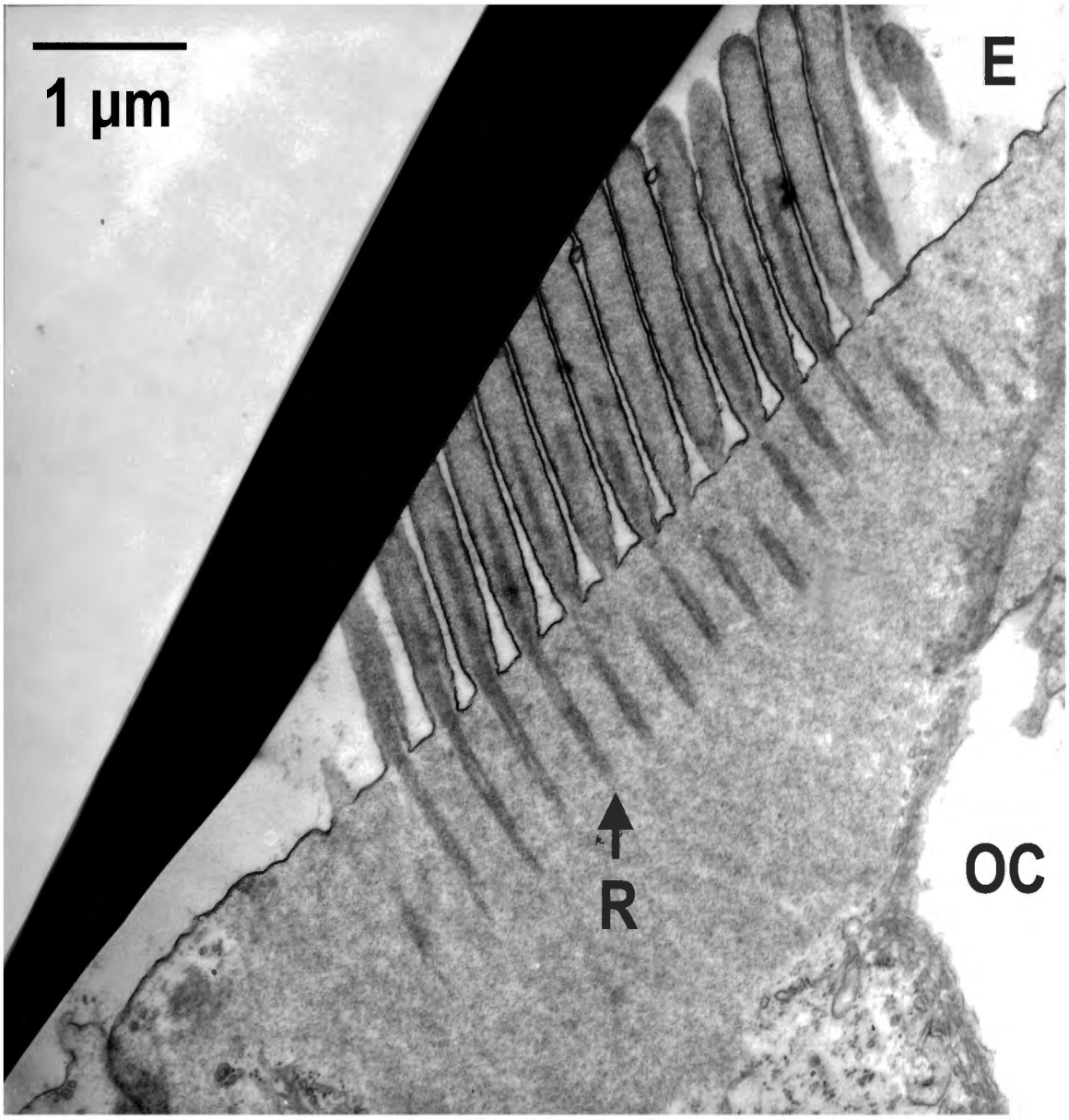
View of the endolymphatic surface of the organ of Corti where the stereocilia of the sensory cells are visible. The stereocilia (white arrow) form a nearly straight line across surface of inner hair cells (IHC) while they are arranged in a "W"- or "V"-shaped pattern (black arrows) on outer hair cells (1, 2, 3).

IHC & OHC STEREOCILIA VIEWED at HORIZONTAL ANGLE



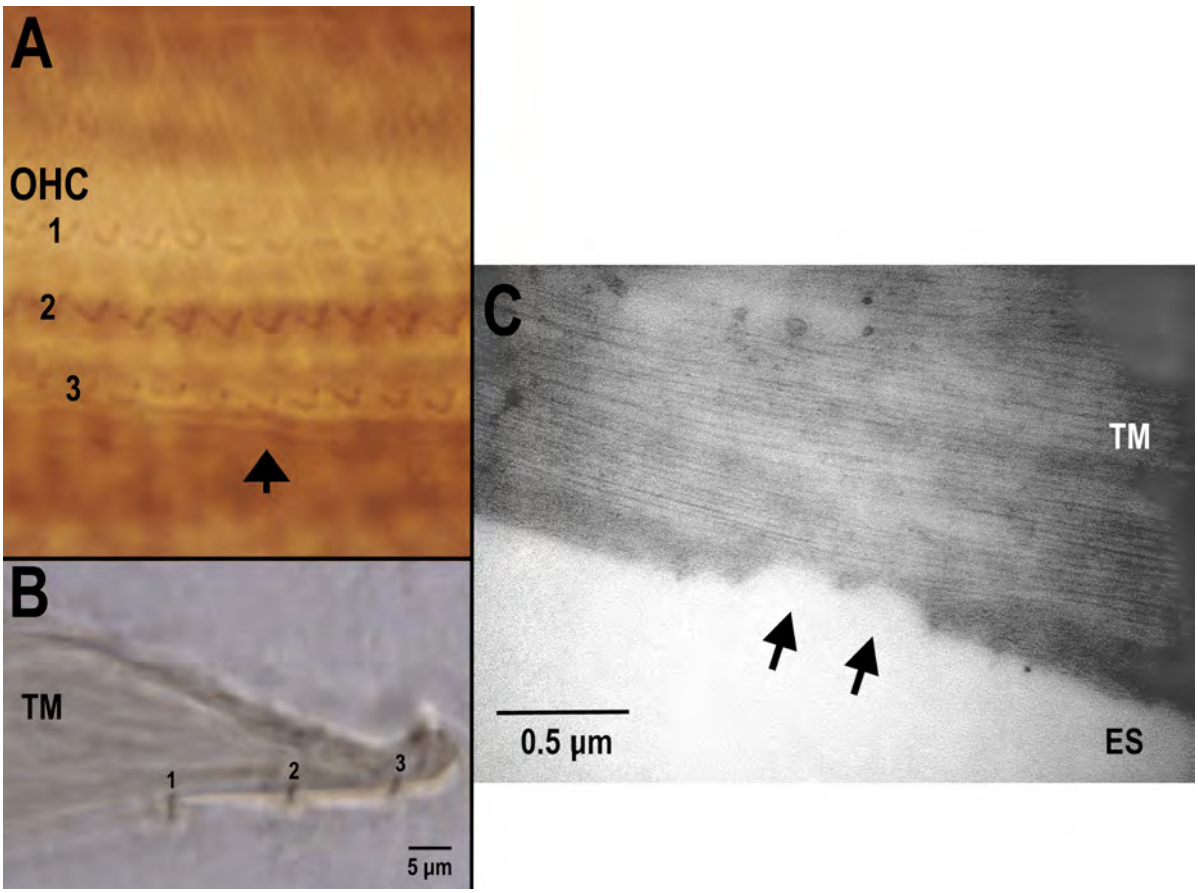
High power view of the endolymphatic surface of the organ of Corti. Stereocilia (white arrow) are arranged in several nearly straight rows across each inner hair cell (IHC). Black arrows point to "W"-shaped pattern of stereocilia on outer hair cells in the 1st (1), 2nd (2) and 3rd (3) rows.

RADIAL TEM of OHC STEREOCILIA



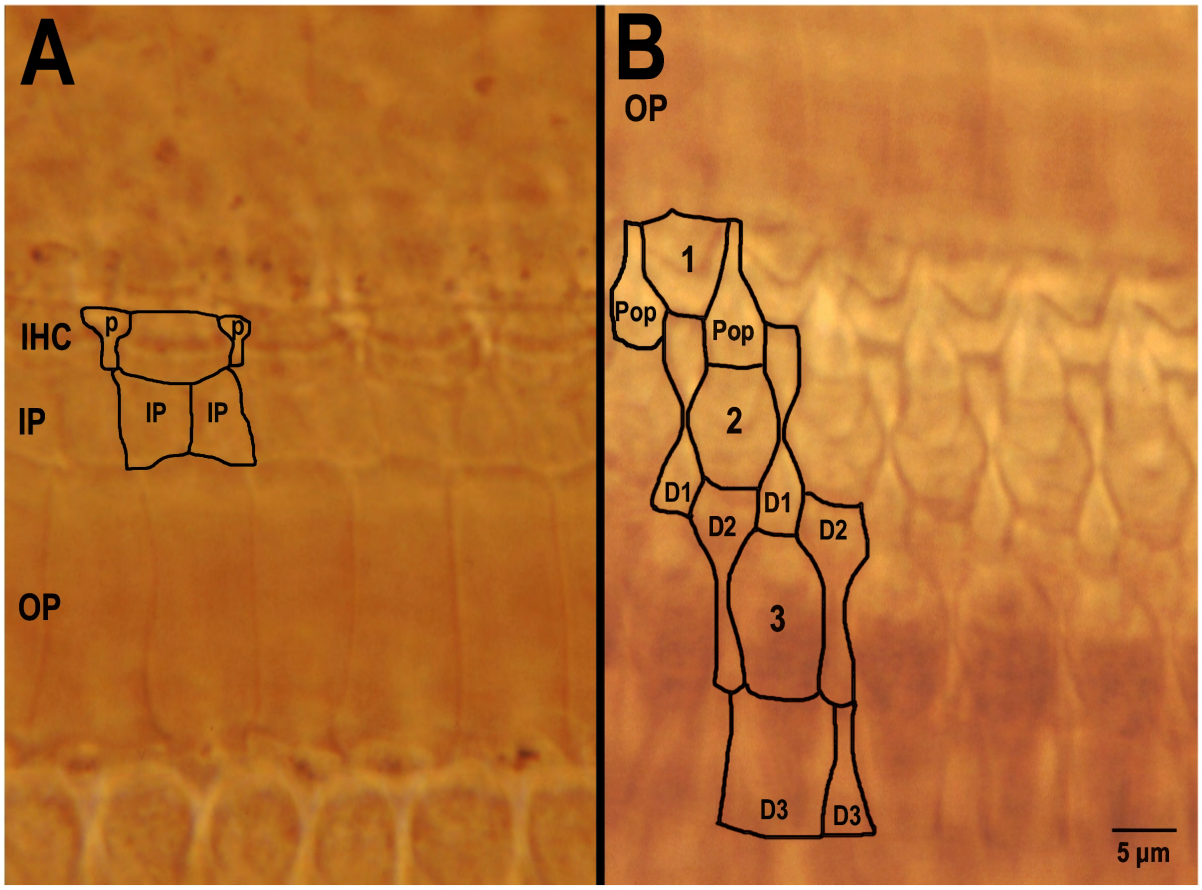
TEM of a radial section through the stereocilia on an outer hair cell. Each stereocilium has a dense core that continues down into the cuticular plate substance as a rootlet (R). The apical membrane and stereocilia are bathed in endolymph (E) while the basolateral surface of the cell is bathed in organ of Corti fluid (OC) (i.e., perilymph-like). The black band is a fold in the section.

RELATION of OHC STEREOCILIA to TECTORIAL MEMBRANE



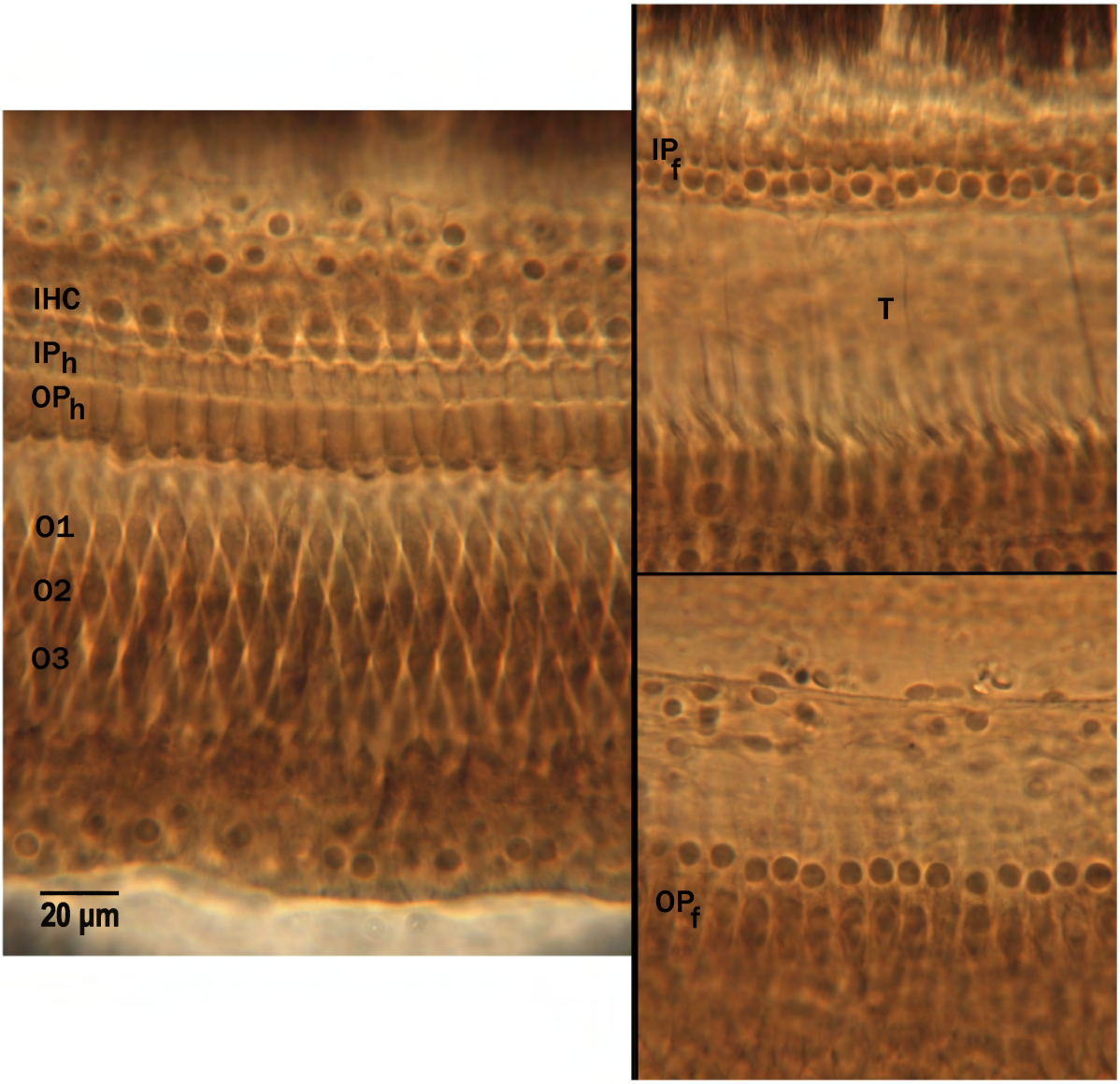
In animals with normal hearing, the tips of the tallest OHC stereocilia are inserted into the tectorial membrane (TM). Because the TM is 96% water, it shrinks and pulls away from the stereocilia when the cochlea is processed for histological examination using traditional techniques. On the other hand, the *in-vivo* relation between the OHC stereocilia and the TM can be ascertained if the cochlea is prepared using the 'survival-fixation' technique (Bohne et al., 1999; Nordmann et al., 2000). With survival-fixation, the stereocilia become tightly bound to the TM. When the cochlea is dehydrated, the TM shrinks as usual, but when it shrinks, it pulls the stereocilia off the OHC apices. The TM can then be examined for OHC stereocilia attachment. A) Phase-contrast view of the TM shows three rows (1, 2, 3) of 'W' patterns, corresponding to the rows of OHC stereocilia. Arrow points to lateral edge of TM. Note that the IHC stereocilia are not attached to the TM; B) Thick radial section of TM shows three stereocilia bundles projecting from its inferior surface; C) TEM of the inferior surface of the TM showing notches (arrows) for the OHC stereocilia. ES - endolymphatic space.

CELL RELATIONS in the RETICULAR LAMINA



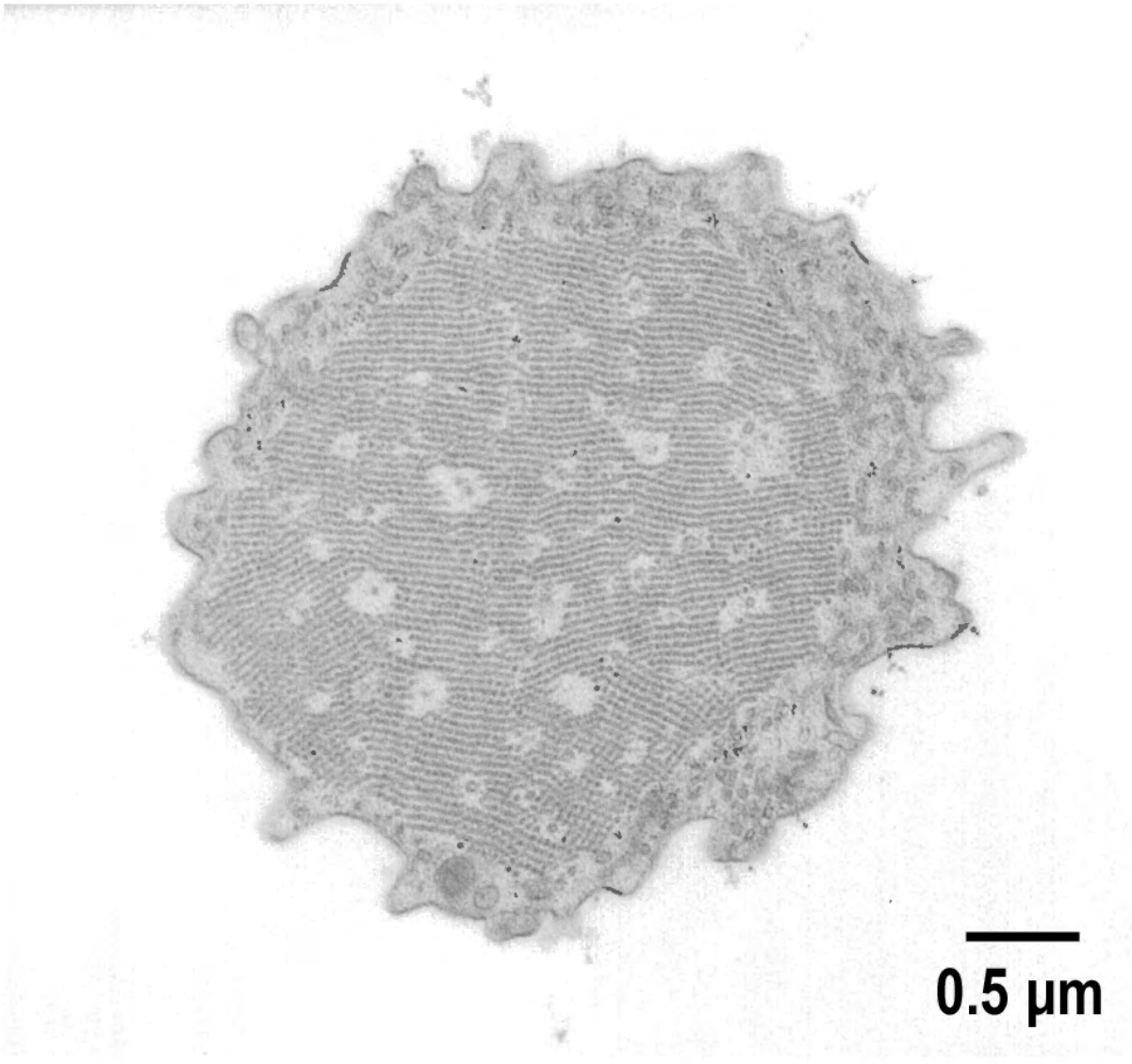
The reticular lamina forms the endolymphatic surface of the organ of Corti. Tight junctions (i.e., zonulae occludentes) are found between all cell membranes bordering on the endolymphatic space, including the reticular lamina. The reticular lamina is composed of the apices of the sensory cells separated by phalangeal processes of the supporting cells and the headplates of the inner pillar cells. A) Inner hair cells (IHC) are supported by the heads of one or two inner pillars (IP), phalangeal processes (p) from two inner phalangeal cells and one or two inner border cells, medial to the inner hair cells, which are out-of-focus here; B) Outer hair cells (OHC) in the 1st row (1) are held in the reticular lamina by the headplates of the inner pillars (not visible), phalangeal processes from two outer pillar heads (Pop) and one 1st row Deiters' cell (D1). Second row OHCs (2) are surrounded by phalangeal processes from one outer pillar head, two 1st row (D1) and one 2nd row (D2) Deiters' cells. OHCs in the 3rd row (3) are supported by phalangeal processes from one 1st row, two 2nd row and one or two 3rd row (D3) Deiters' cells. OP - outer pillar heads.

INNER & OUTER PILLAR HEADS & FEET



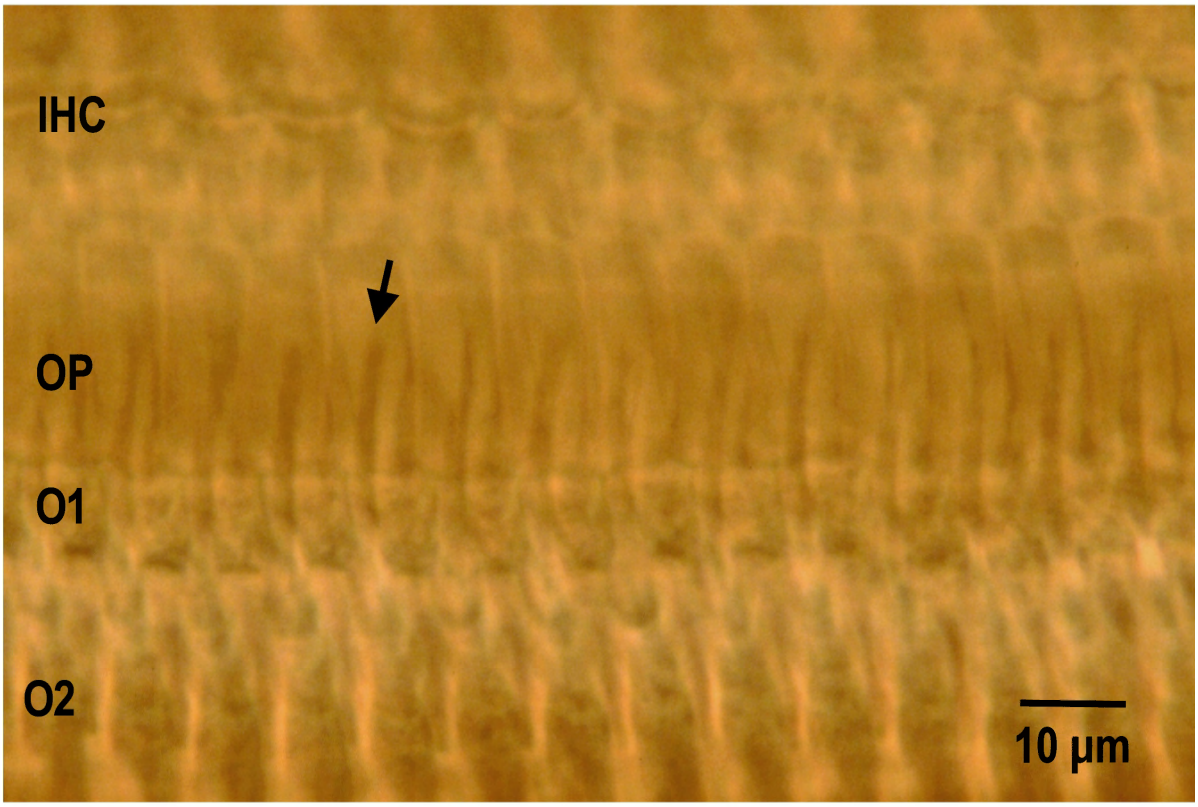
The inner pillar heads (IP_h) are smaller than the outer pillar heads (OP_h) and form part of the reticular lamina. A thin headplate (not visible) from the IP heads cover the OP heads. The bodies of the inner and outer pillars extend from their heads to their feet on the scala-media side of the basilar membrane. Because they contain intracellular bundles of microtubules, these cells are very important for maintaining the shape of the organ of Corti. The nucleus and termination of the microtubular bundles are visible in the inner (IP_f) and outer pillar feet (OP_f). IHC - inner hair cell; O1, O2, O3 - 1st, 2nd and 3rd rows of OHCs, respectively; T - tunnel.

HORIZONTAL TEM of OUTER PILLAR BODY



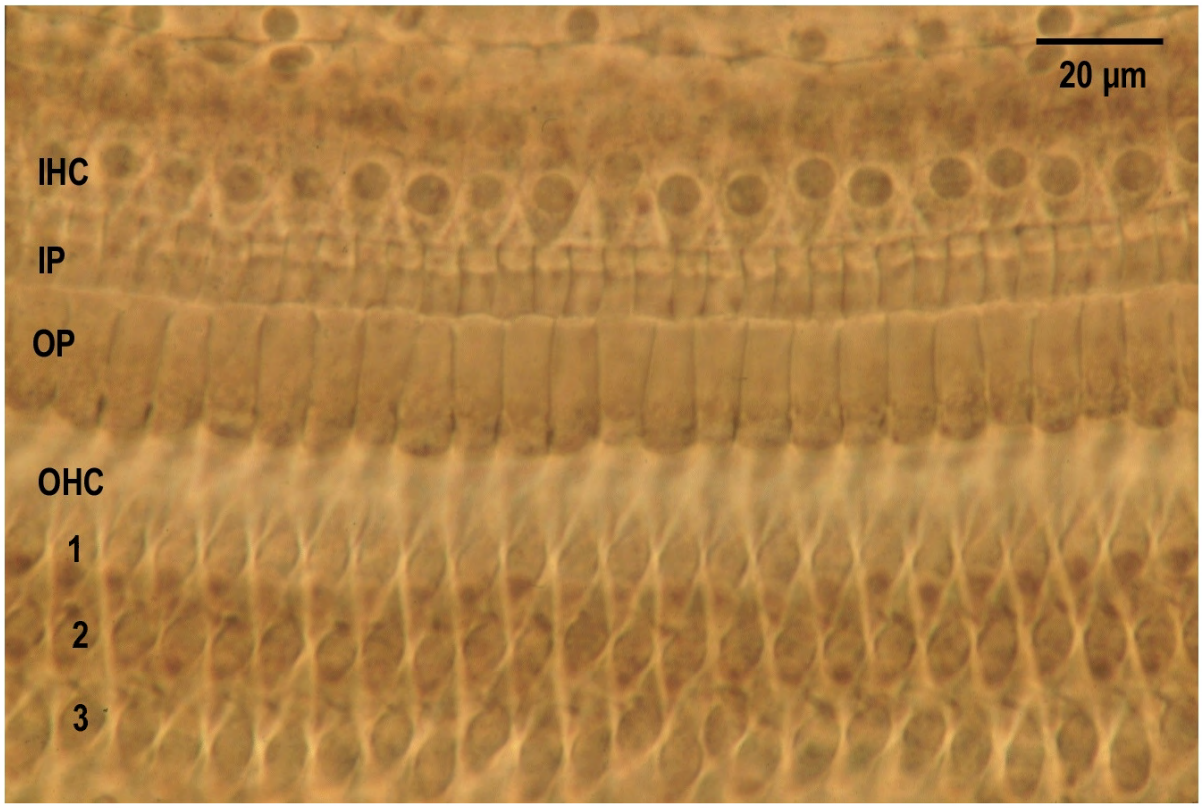
TEM of cross-section through the body of an outer pillar cell. The pillar body is composed of a parallel bundle of microtubules, with interspersed actin filaments (not visible here), surrounded by a thin rim of cytoplasm.

PHALANGEAL PROCESSES between 1st ROW OHCs



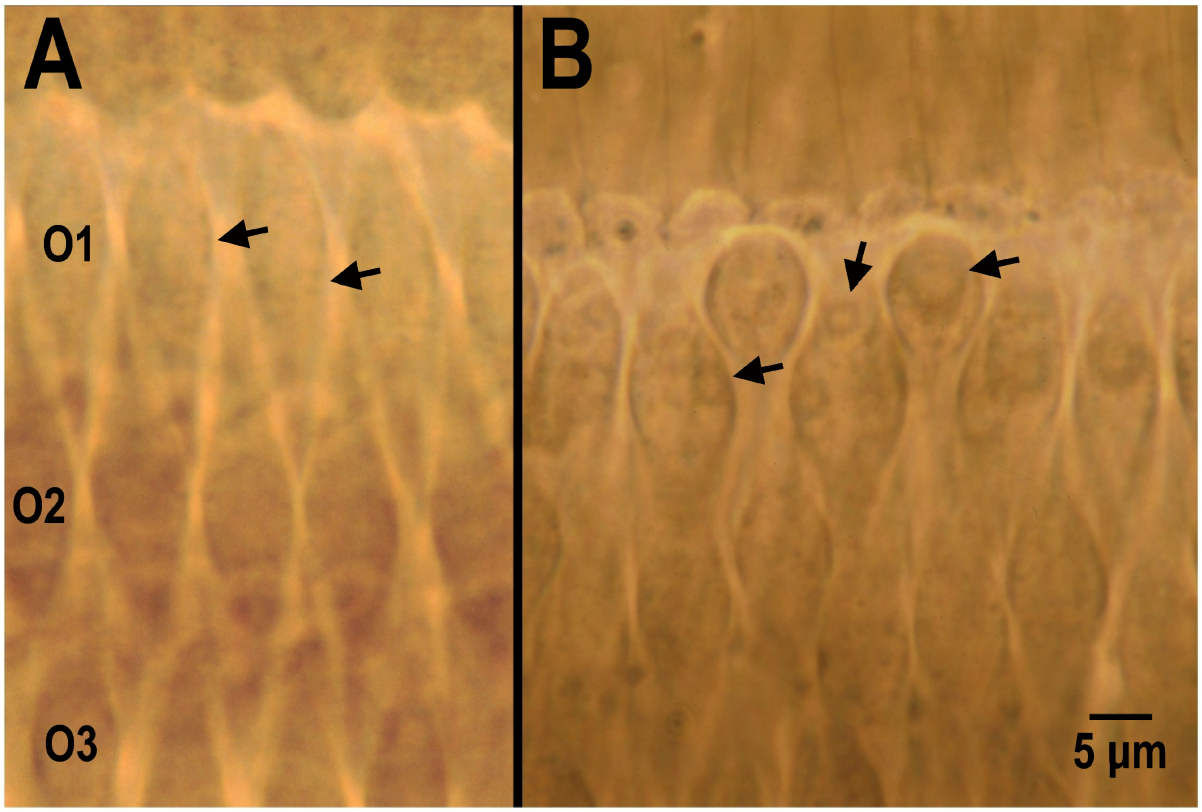
Microtubular bundle (arrow) in the phalangeal process from the outer pillar head (OP) is clearly visible. Stereocilia on the inner hair cells (IHC) are seen in reverse phase at left. Outer hair cells in the 1st (O1) and 2nd (O2) rows are slightly out-of-focus.

IHC & OHC BODIES VIEWED at HORIZONTAL ANGLE



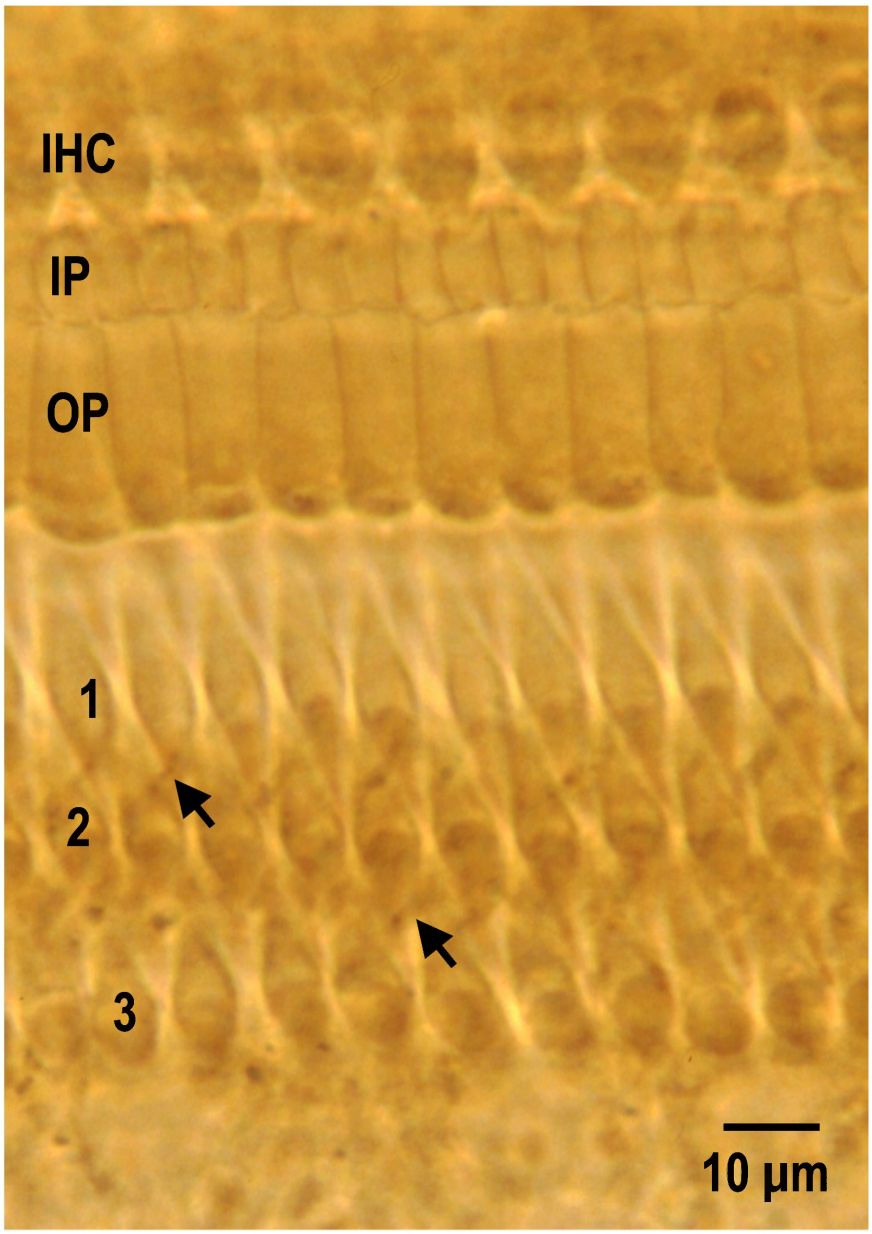
Focused below the reticular lamina on the bodies of the sensory cells. Inner hair cells (IHC) are flask shaped and contain large, centrally located nuclei. Outer hair cells (OHC 1, 2, 3) are long and cylindrical with their nuclei in a basal location. The heads of the inner pillar (IP) and the larger outer pillar cells (OP) separate the apices of the IHCs and OHCs.

INTRACELLULAR FEATURES of OHCs



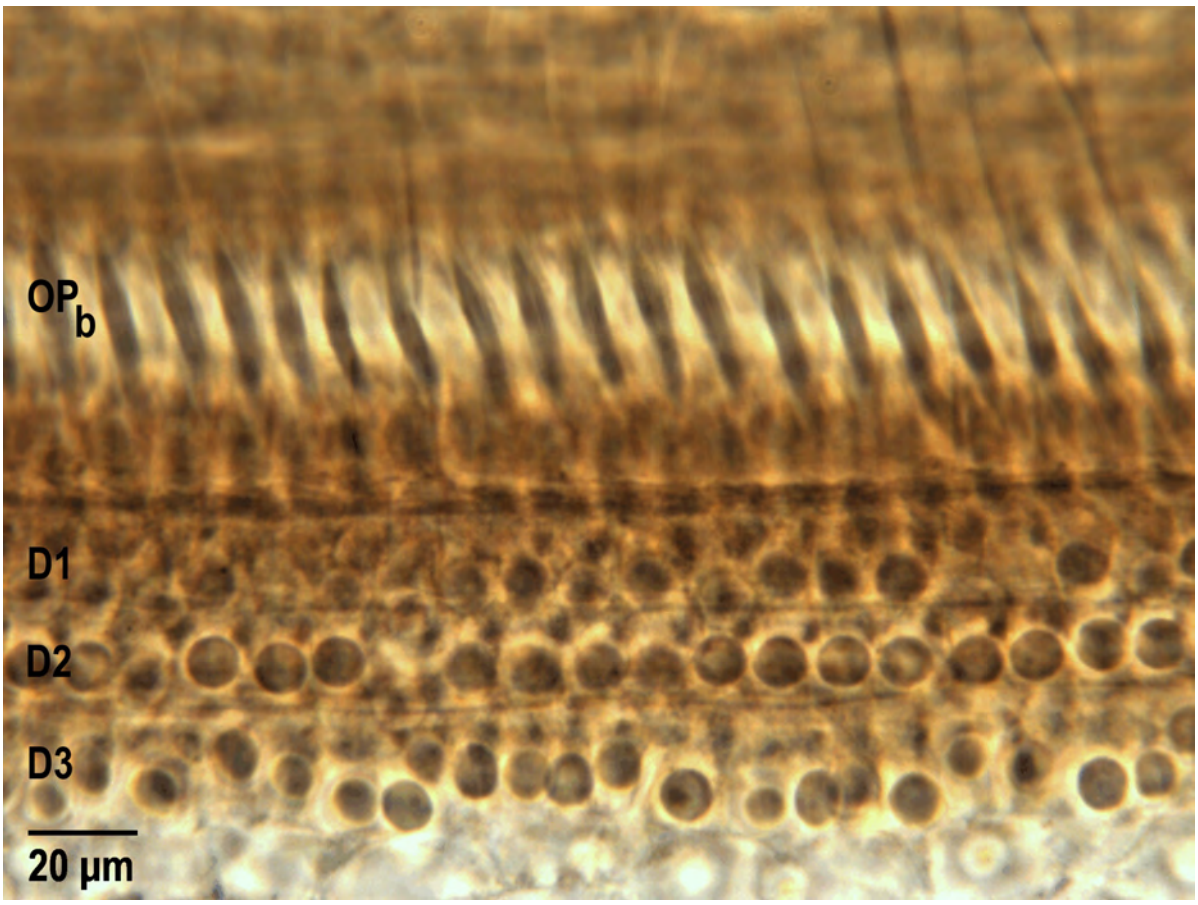
High power phase-contrast photomicrographs of outer hair cell bodies (O1, O2, O3). A) The mid-portion of the OHC bodies are nearly devoid of organelles except for the peripherally arranged mitochondria that are visible as small dark dots (arrows) adjacent to the plasma membrane. Note uniform spacing and orientation of OHC bodies in this control cochlea. B) Whorls of smooth endoplasmic reticulum, called Hensen's bodies (arrows), are found in some outer hair cells in a subcuticular location, especially in the upper turns of the cochlea.

SLENDER PROCESSES of DEITERS' CELLS



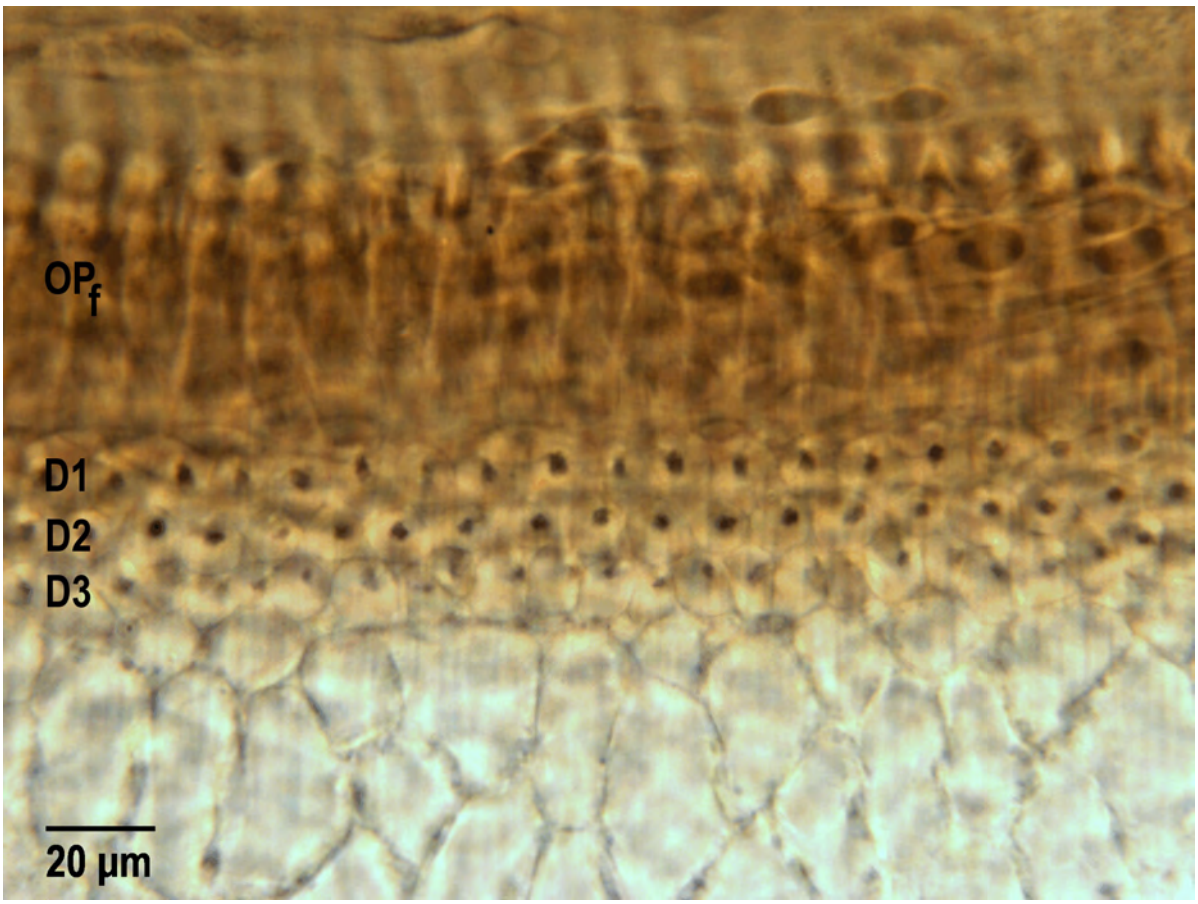
The Deiters cells have very complex shapes. These cells, along with the pillars (p. 32), are the only cells in the organ of Corti that contain parallel bundles of microtubules. Their phalangeal processes (p. 20 & 31), in contact with endolymph, surround the apex of each outer hair cell (1, 2, 3). A slender process (arrow) from each Deiters' cell base runs in an apical direction across three or four OHCs as it extends from the cell's base beneath an OHC body to its phalangeal process in the reticular lamina.

DEITERS' CELL NUCLEI



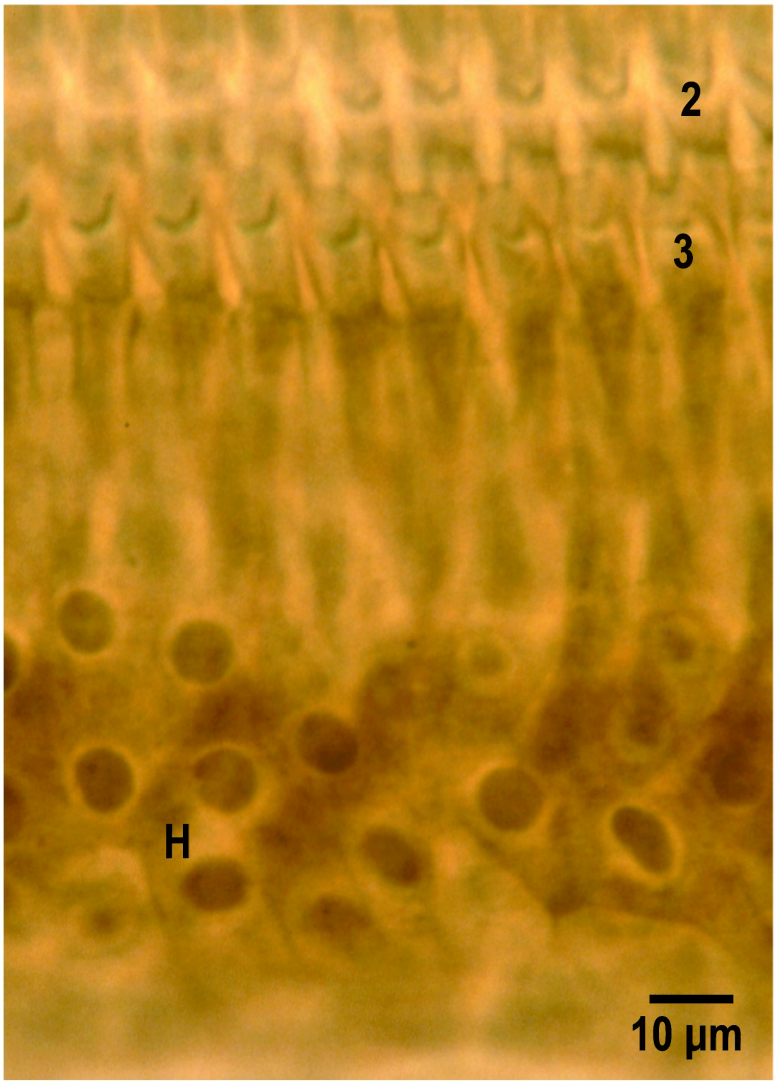
The nuclei of the Deiters cells (D1, D2, D3) are located about halfway between the basilar membrane and the OHC bases. The horizontal lines between the Deiters' cells represent some of the non-myelinated nerve fibers in the outer spiral bundles. In this focal plane, the bodies of the outer pillars (OP_b) are also visible.

DEITERS' CELL BASES on BASILAR MEMBRANE



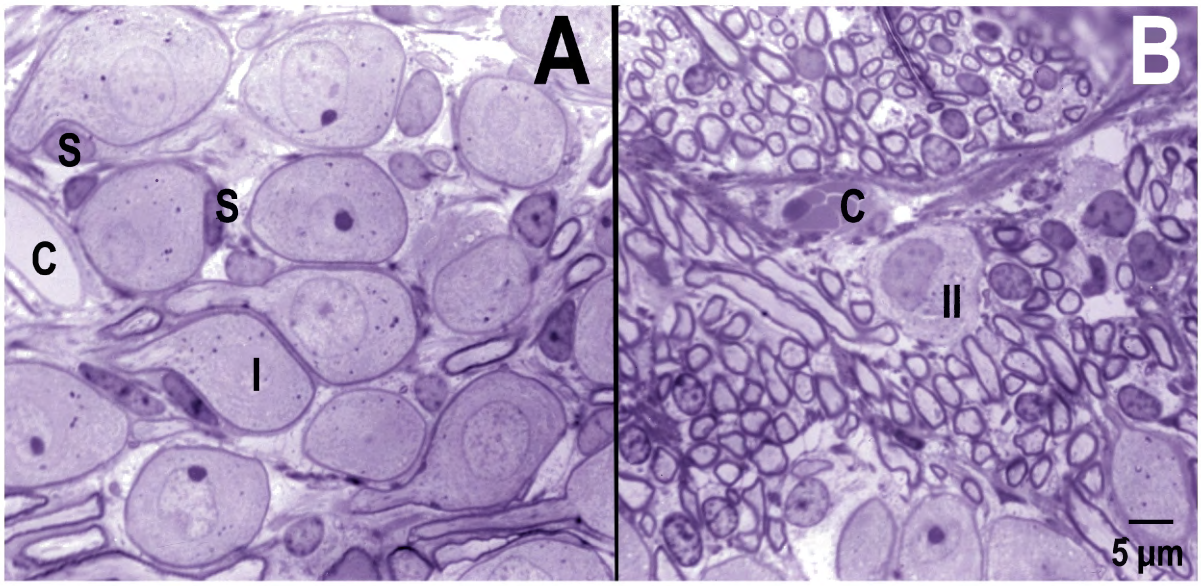
By focusing on the scala-media side of the basilar membrane, one can see the feet of the outer pillars (OP_f), slightly out-of-focus, and the bases of the three rows of Deiters'cells (D1, D2, D3). The dark dot in each Deiters' cell is its small, compact bundle of microtubules.

HORIZONTAL VIEW of HENSEN'S CELLS



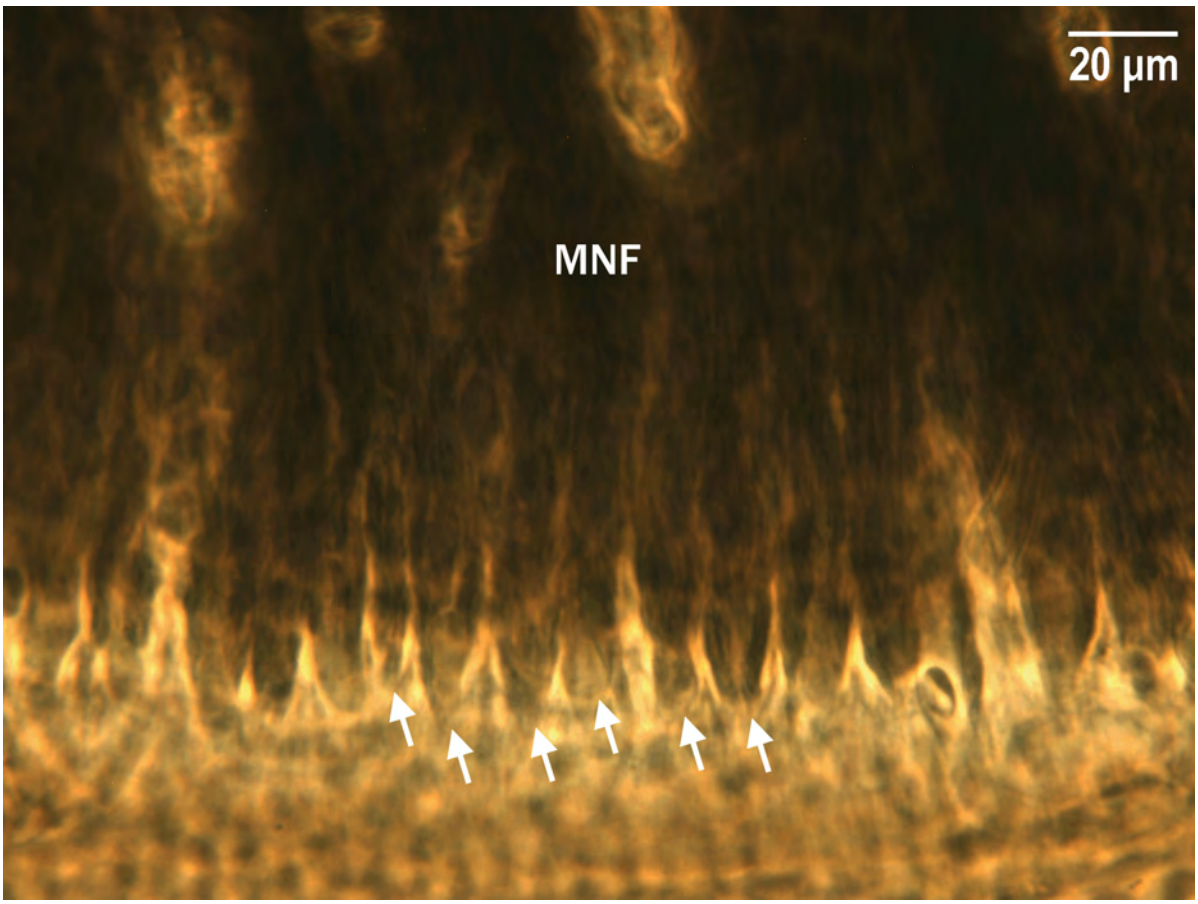
Hensen's cells (H) are lateral to the 3rd row of outer hair cells (3). In the upper turns of the cochlea (shown here), the Hensen cells can easily be distinguished from Claudius cells by their darker cytoplasm and slightly smaller size. However, in the cochlear base where the organ of Corti is much smaller, Hensen and Claudius cells have very similar appearances. 2 - 2nd row of OHCs.

RADIAL SECTIONS of TYPE I & II SPIRAL GANGLION NEURONS



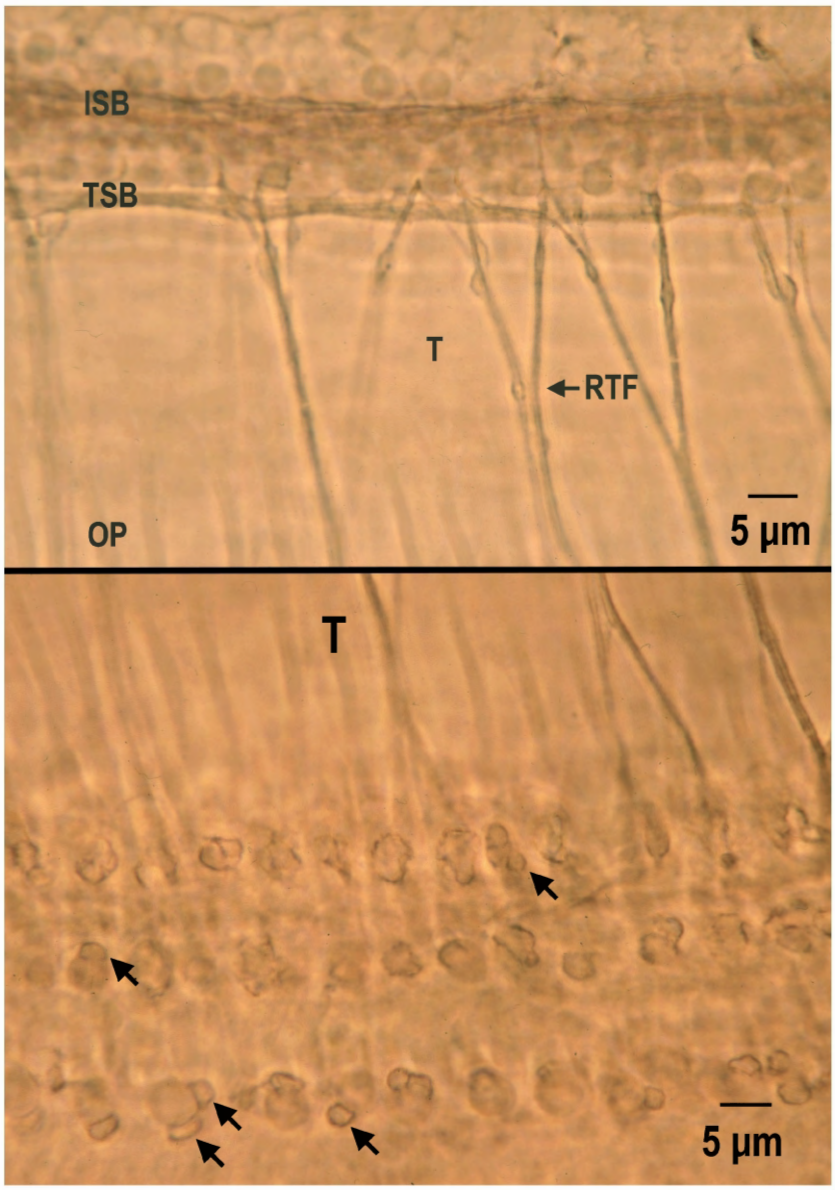
Radial sections of spiral ganglion neurons (i.e., SGNs) in Rosenthal's canal. A) Type I spiral ganglion neurons (I), which constitute the largest percentage of SGNs (i.e., 85-95%, depending on species), innervate the inner hair cells. These cells are large and have a round nucleus, a prominent nucleolus and a myelin sheath (i.e., dark line) surrounding the perikaryon, the central and peripheral processes. The myelin is formed by Schwann cells (S); B) Type II spiral ganglion neurons (II) innervate the outer hair cells. These cells are smaller than type I neurons, have a darker staining nucleus that usually is lobular in shape and eccentric in location. Type II neurons either have a 1-2-layer-thick myelin sheath or none at all. C - capillary.

HORIZONTAL VIEW of HABENULAE PERFORATA



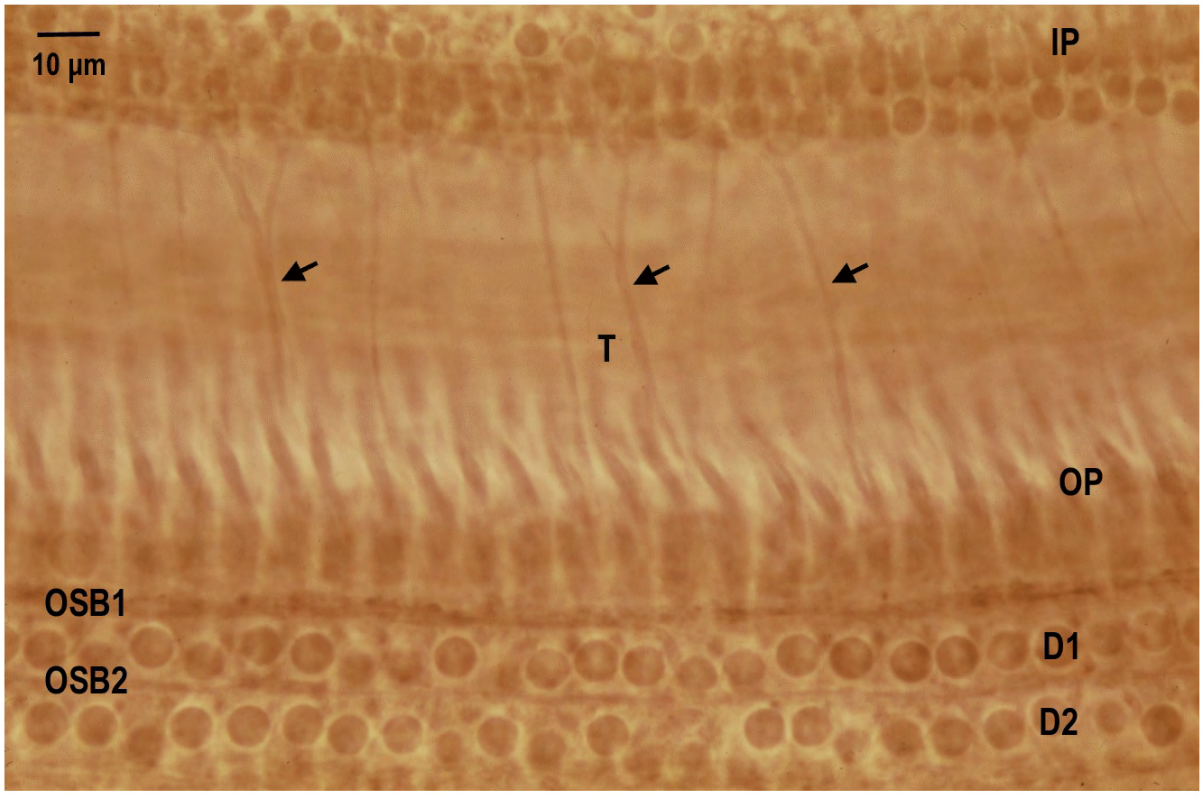
The peripheral processes (dendritic) of the spiral ganglion neurons are myelinated (MNF) as they traverse the osseous spiral lamina. The fibers form small bundles (arrows) as they approach the holes, called habenulae perforata, in the spiral lamina beneath each inner hair cell. The fibers lose their myelin sheaths just medial to the habenulae, before they enter the organ of Corti.

NERVE FIBER BUNDLES within the ORGAN of CORTI



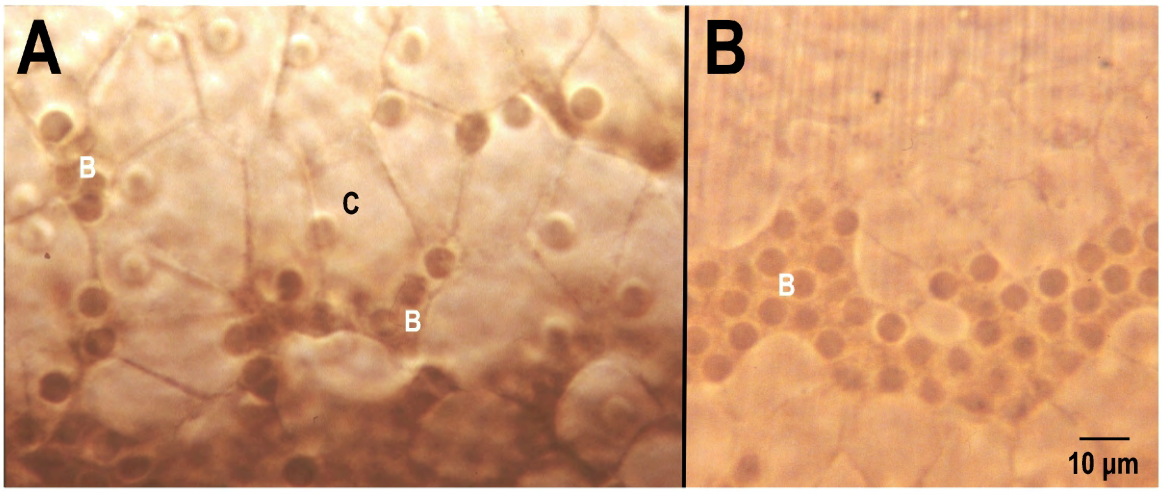
Within the organ of Corti, nerve fibers take different courses. Some fibers run spirally beneath the IHCs [i.e., inner spiral bundle (ISB)] or within the tunnel space (T) just lateral to the inner pillar feet [i.e., tunnel spiral bundle (TSB)]. Radial tunnel fibers (RTF) cross the tunnel, exit between adjacent outer pillar bodies (OP) and then enter the outer spiral bundles (one below each row of OHCs). This cochlea was stained for acetylcholinesterase (AChE) activity to demonstrate the efferent innervation of the organ of Corti. AChE is an enzyme that breaks down acetylcholine, the chemical transmitter at efferent synapses in the cochlea. Note that the ISB, TSB, many RTFs and large nerve terminals (arrows) on the OHC bases stain positively for AChE activity. The dilatations on the RTFs are efferent varicosities.

NERVE FIBER BUNDLES within the ORGAN of CORTI



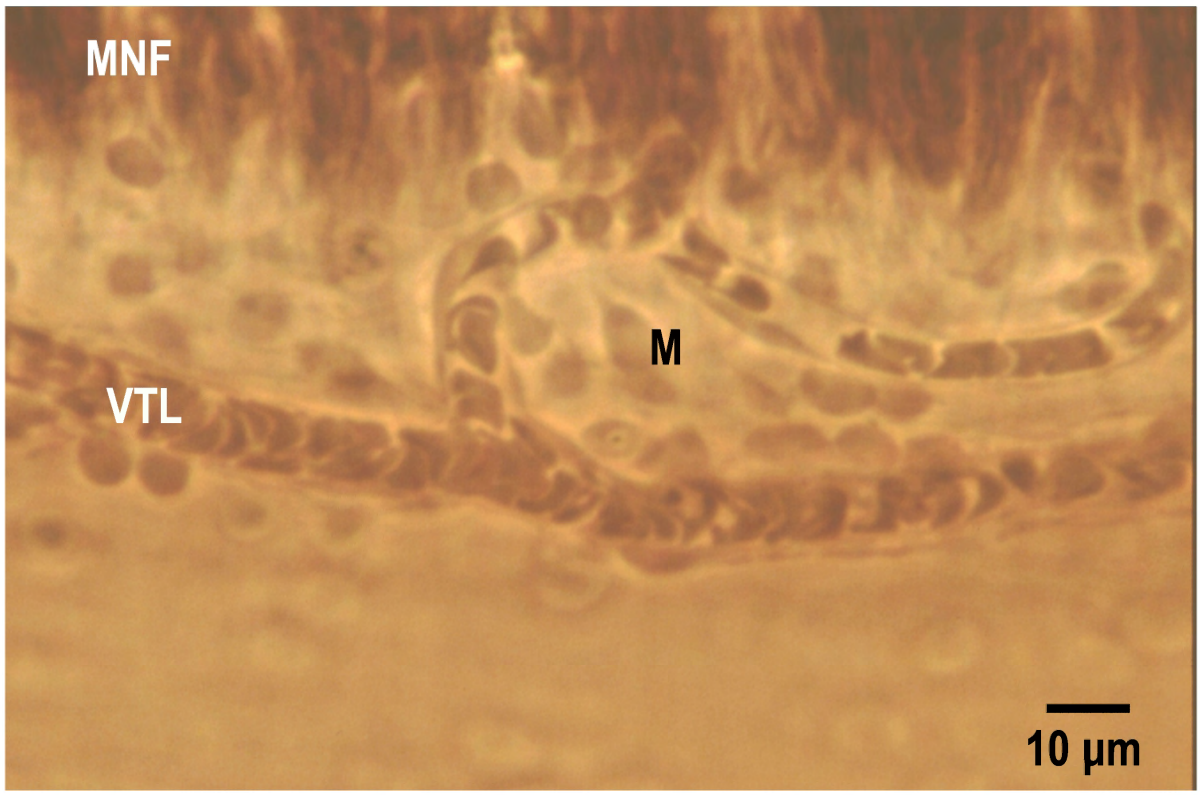
Radial tunnel fibers (arrows) are non-myelinated. They enter the tunnel space (T) by passing between the bases of adjacent inner pillar cells (IP), then take convergent and divergent courses before exiting the tunnel between the bodies of adjacent outer pillars (OP). The fibers turn toward the cochlear base and join either the 1st (OSB1), 2nd (OSB2) or 3rd (not visible) outer spiral bundles. Fibers in these bundles spiral toward the base for a distance of about 0.6 mm before synapsing on a number of outer hair cells in one, two, or all three rows. The nuclei of the Deiters cells (e.g., D1, D2) are visible between the nerve fiber bundles. In some species (e.g., cat, guinea pig), the radial tunnel fibers that cross the middle of the tunnel space are efferent (called upper tunnel crossing fibers) whereas afferent fibers to the outer hair cells cross on the floor of the tunnel (called basilar fibers). In the chinchilla, afferent and efferent fibers cross near the middle of the tunnel as small, mixed bundles. Only 5-15% of all afferent fibers to the cochlea synapse on the outer hair cells.

HORIZONTAL VIEW of BOETTCHER CELLS



Boettcher cells (B) are located on the basilar membrane near the spiral ligament in the basal half of the cochlea. They are separated from the endolymphatic space by a single layer of Claudius cells (C). Boettcher cells are smaller and more darkly stained than Claudius cells and have a variable distribution in the cochlea. A) Small, isolated clumps of Boettcher cells first appear in the second turn; B) In the middle of the first turn, the Boettcher cells form a lacy band. Near the round window (not shown), the band is continuous. The fibrils of the basilar membrane are visible in the upper half of 'B'.

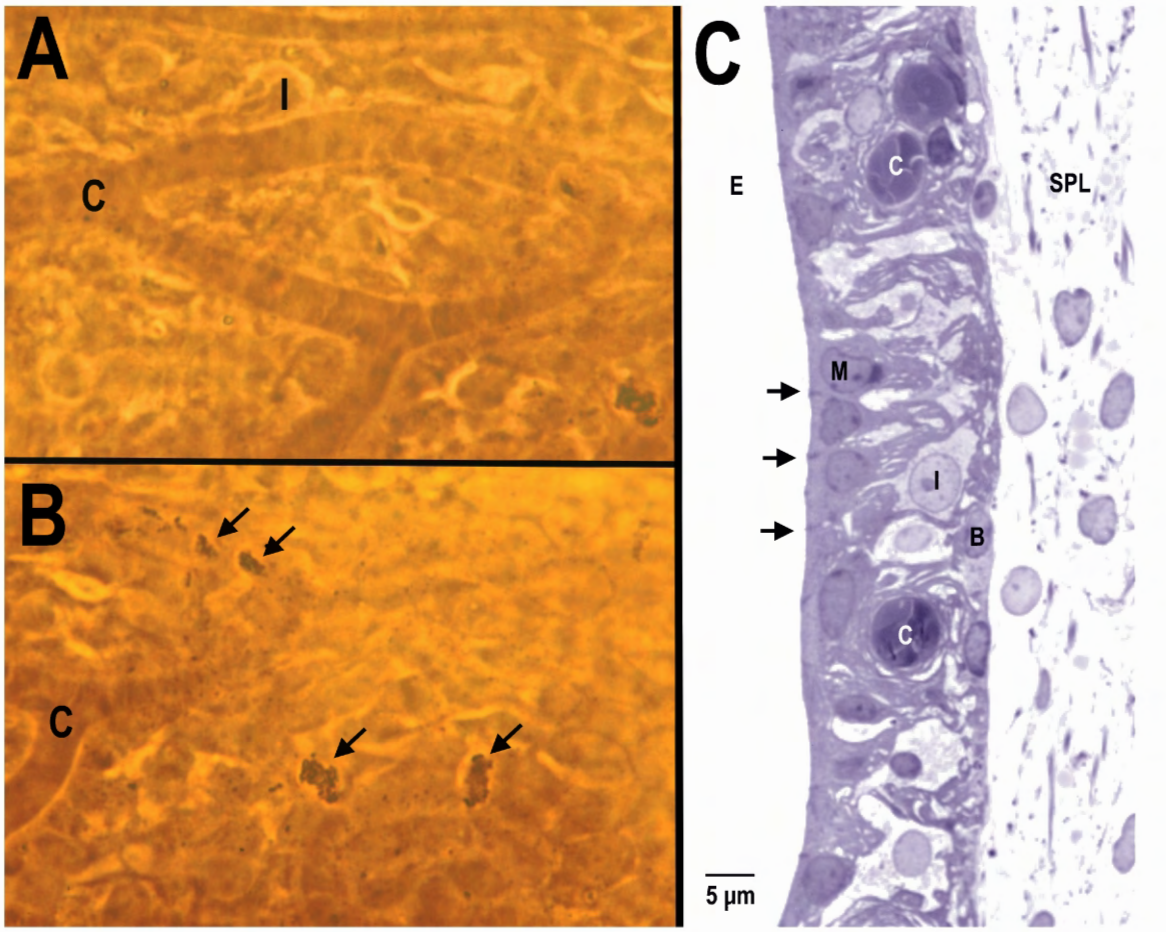
HORIZONTAL VIEW of BLOOD VESSELS below the BASILAR MEMBRANE



The blood vessels nearest the organ of Corti are located in scala tympani. The vessel that runs beneath the tunnel space is called the vessel of the basilar membrane (VBM; not visible here). The one that runs below the inner hair cell is termed the vessel of the tympanic lip (VTL) of the osseous spiral lamina. Because this cochlea was fixed by perfusing a buffered solution of osmium tetroxide through scala tympani, red blood cells were trapped inside all cochlear vessels that were functioning at the moment of fixation.

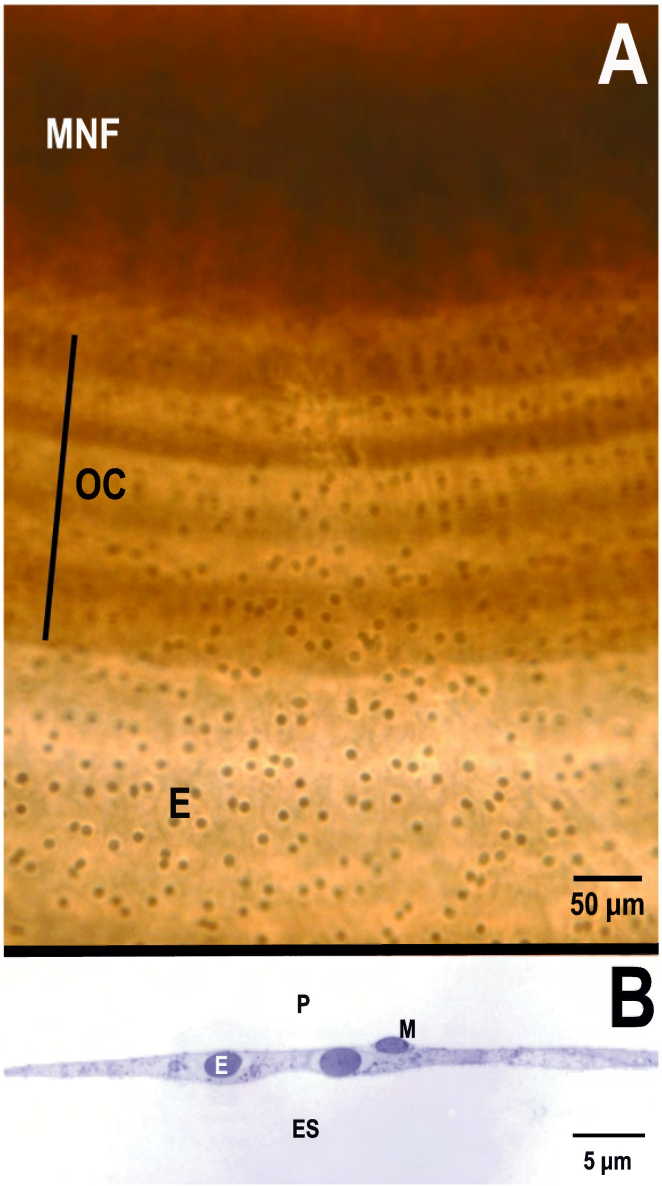
In the chinchilla, the VBM shuts down after birth, except in the third turn. Mesothelial cell nuclei (M) are visible on the scala tympani side of the basilar membrane. These cells are spindle-shaped and have attenuated cytoplasm that extends in an apical-basal direction. MNF - myelinated peripheral processes of the spiral ganglion neurons.

HORIZONTAL & RADIAL VIEWS of the STRIA VASCULARIS



The stria vascularis (flat preparation in A and B; stained radial section in C) forms the lateral wall of the cochlear duct (pp. 5 & 6), is responsible for the generation of the +80 to +100 mV positive potential (EP) in the endolymphatic space and for the maintenance of the high K^+ and low Na^+ concentrations in endolymph. A) As its name implies, the stria vascularis contains a dense network of capillaries (C) (filled here with red blood cells). The nuclei of some intermediate cells (I) are visible near the capillary; B) Melanin granules (arrows) are visible in the intermediate cells of the stria; C) The stria is composed of three poorly defined layers. Marginal cells (M; chromophils in older literature) abut the endolymphatic space (E) and are joined to one another by tight junctions (arrows). They have dark-staining cytoplasm, a smooth luminal border and numerous basal processes that extend deep into the epithelium. Intermediate cells (I; chromophobes in older literature) are deep to the marginal cells. They have pale-staining cytoplasm and often contain brown granules of melanin. Basal cells (B) form a continuous boundary between the stria vascularis and the underlying spiral ligament (SPL). The basal cell bodies are flattened but they send long processes toward the luminal surface of the stria. Cross-sectioned capillaries (C) are packed with red blood cells.

HORIZONTAL & RADIAL VIEWS of REISSNER'S MEMBRANE



Reissner's membrane (A - flat prep; B - stained radial section) is two cells thick that separates scala vestibuli from the endolymphatic space. The cells (E) facing the endolymphatic space (ES) are derived from the wall of the otocyst, and hence, are epithelial in origin. They are low cuboidal cells and have numerous short microvilli projecting from their free surface. Adjacent cells are joined by tight junctions. The cells facing the perilymphatic space (P) are derived from mesoderm. These cells are thin and elongated, contain few organelles and form an incomplete layer. A basal lamina is found between the two cell layers. MNF - myelinated nerve fibers in the osseous spiral lamina; OC - organ of Corti.

NOISE DAMAGE AND THE IDENTIFICATION OF SENSORY-CELL LOSS

Hair cells are held in the reticular lamina by the tight junctions between their apices and the phalangeal processes of the adjacent inner phalangeal cells (IHCs), or outer pillar cells and Deiters' cells (OHCs). If a hair cell is injured to the extent that it degenerates, the resulting gap or 'hole' in the reticular lamina is closed by the enlargement of adjacent supporting cell processes to form what is called a '**phalangeal scar**'. Phalangeal scars serve as markers for the location of degenerated hair cells. Although hair cells regenerate after noise exposure or treatment with ototoxic drugs in some animals (e.g., birds, amphibians), hair cells in the mammalian organ of Corti do not regenerate under normal conditions. Thus, in the mammalian cochlea, the extent of hair-cell loss resulting from exposure to a particular ototoxic agent can be determined after the traumatic event by counting phalangeal scars. In instances where a portion of the organ of Corti (i.e., all sensory and supporting cells) degenerates, the cells that form phalangeal scars also disappear. However, the bare region of the basilar membrane is eventually covered by squamous or low cuboidal epithelium (p. 67). The squamous cells appear to form by transdifferentiation of inner sulcus and Claudius' cells.

Phalangeal scars have different appearances in the 1st, 2nd and 3rd rows of outer hair cells (pp. 53, 54). Scars in the 1st row are formed by the enlargement of the phalangeal processes from two adjacent outer pillars. Scars in the 1st row have very similar appearances. Scars in the 3rd row are formed by the phalangeal processes from the 2nd row of Deiters' cells and also have a similar appearance. In the 2nd row, phalangeal scars can be quite variable in appearance because the four phalangeal processes (one from an outer pillar; two from 1st row Deiters' cells; one from a 2nd row Deiters' cell) that surround each 2nd row OHC may enlarge different amounts to form the scar. Phalangeal scars also form when IHCs degenerate. In this case, the phalangeal processes from the inner phalangeal cells and inner pillar head form the scar (p. 53).

Exposure to noise damages the cells of the organ of Corti by different mechanisms, depending on exposure parameters (i.e., frequency, intensity and duration of the exposure). High-intensity noise exposures, such as explosions, cause **acoustic trauma**. Immediately after exposure, the individual is aware of auditory symptoms including: difficulty hearing soft sounds, speech sounding muffled or far away, tinnitus and a sense of fullness in the ears. In the days following the exposure, some recovery of hearing takes place but the individual is usually left with a permanent hearing loss or **permanent threshold shift (PTS)**.

Moderate-level, long-duration noise exposures, such as those associated with the workplace, initially result in a **temporary threshold shift (TTS)**. Subjectively, the auditory symptoms are very similar to those described above. However, by 16-36 hours away from the noise, the individual's hearing returns to its pre-exposure level. Individuals who are exposed to workplace noise greater than 85 dBA for several years generally develop a PTS slowly compared to the PTS associated with acoustic trauma. This slowly developing hearing loss is termed **noise-induced permanent threshold shift (NIPTS)**.

Shortly after a damaging noise exposure, the cells of the inner ear are in dynamic states of injury, degeneration and/or repair. This phase can be termed the **acute phase of noise damage**. With acoustic trauma, a portion of the organ of Corti is displaced from its position

on the basilar membrane and is often found detached in the lumen of scala media (Lurie, 1942; p. 55). Large openings into the fluid spaces are created when the organ of Corti is detached from the basilar membrane. These openings allow intermixing of endolymph and the fluid with the organ of Corti and lead to degeneration of additional sensory and supporting cells. Swollen hair cells (pp. 56, 64) and swollen and fragmented nerve fibers are found within and adjacent to the detached portion of the organ of Corti (Bohne, 1976a). With workplace noise, a few hair cells may degenerate with each day's exposure. However, a permanent hearing loss is usually not evident until a moderate number of hair cells have degenerated in a restricted region of the organ of Corti.

The amount of structural damage that is present in a given cochlea depends on its auditory history. The longer the exposure, the greater the number of missing sensory cells (e.g., Bredberg, 1968; Bohne and Clark, 1990). Outer hair cells are far more likely to degenerate as a result of excessive exposure to noise than IHCs. As OHC loss increases, there is beginning loss of pillars and IHCs.

In the general pathology literature, two principal types of dying cells have been distinguished on the basis of their morphological appearances: 1) dark, shrunken cells with pyknotic nuclei that are termed 'apoptotic' (e.g., Kerr et al., 1972; Clarke, 1990) and 2) swollen cells with enlarged, pale nuclei that are termed 'necrotic'. Recently, the term 'oncotic' has been proposed to indicate swollen cells instead of the term 'necrotic' (i.e., meaning dead) (e.g., Majno & Joris, 1995, 1996; Jugdutt and Idikio, 2005). Although apoptosis and oncosis are death pathways that are common to many cell types, other death pathways are found in only one or a few cell types (e.g., Melino et al., 2005). Our recent work suggests that some hair cells in the organ of Corti follow death pathways that may be unique to the hearing organ (Bohne & Harding, 2012).

In our studies of cochlear damage from noise, we have found a large number of damaged OHCs dying by oncosis (p. 56) and far fewer cells dying by apoptosis (p. 57). Bohne et al. (2007) and Lee et al. (2008) identified OHCs that appeared to be dying by a unique death pathway that is non-apoptotic and non-oncotic (p. 58). In dying OHCs, each of these pathways has a distinct histological appearance and is predominantly associated with a specific type of noise exposure. We have found rare oncotic IHCs (p. 60) and some apoptotic IHCs (p. 61) in noise-damaged cochleae. More IHCs have been found following a newly identified death pathway termed 'disconnected' (pp. 62, 63). However, the death pathway followed by many IHCs is still unknown. The same is true for the supporting cells of the organ of Corti. It should be noted that there are discrepancies in the literature as to which death pathway is most active following exposure to a damaging noise (e.g., Hu et al., 2000, 2006).

After a recovery period of a few days (for moderate exposures) to 1-2 months (for severe exposures), the histological appearance of the organ of Corti is considerably different from that seen acutely. The recovery period allows damaged cells to complete the process of degeneration. Remaining supporting cells participate in the formation of scars in the reticular lamina and on the basilar membrane. This period can be termed the **chronic phase of noise damage** because hearing thresholds have stabilized and most degeneration in the organ of Corti resulting from the exposure has been completed. The exact appearance of a noise-

damaged cochlea long after the exposure has terminated depends on the initial apex-to-base extent of the damage and the rapidity with which the reticular lamina and basilar membrane scar over. Some intraspecies variation in the size and location of lesion(s) in chronic noise-damaged cochleae appears to have a genetic component.

With acoustic trauma, the large portion of missing organ of Corti is replaced on the basilar membrane by an undifferentiated squamous epithelium (p. 67). The open ends of the tunnel space are sealed by Hensen's, Deiters' Claudius' and inner sulcus cells (Bohne, 1976b; Fried et al., 1976). Most nerve fibers that originally innervated hair cells in the degenerated portion of the organ of Corti also degenerate (p. 67). Some debris from the detached portion of the organ of Corti may still be visible in the endolymphatic space months after the traumatic event.

With NIPTS, sensory cells are lost very gradually. Degenerated hair cells are replaced by phalangeal scars (pp. 53, 54). Because the endolymphatic surface of the outer pillar heads is covered by the inner pillar headplates, scattered loss of outer pillars probably does not affect the continuity of the reticular lamina. Isolated losses of inner pillar cells are repaired by the inner phalangeal, inner border and inner sulcus cells.

Phalangeal scars do not form immediately after a hair cell degenerates. For a period of time 'holes' or discontinuities (pp. 64, 65) may exist in the reticular lamina, thus allowing endolymph and the fluid in the organ of Corti to intermix. Holes are especially apparent when a large number of outer hair cells have been destroyed simultaneously. However, holes can occasionally be seen when isolated cells have degenerated. Holes are visible for a finite period of time after hair cells degenerate and before phalangeal scars form. Thus, in order to see holes, the cochlea must be fixed shortly after the traumatic event that precipitated hair cell death.

Two types of phalangeal scars, termed 'mature' and 'immature' (p. 66), can be distinguished in the reticular lamina following a damaging noise exposure (Bohne, 1976b; Bohne et al, 1976; 2007). Often, cellular debris can be seen in the fluid spaces of the organ of Corti beneath and near immature scars but not mature scars (Bohne, 1976c). These observations suggest that immature scars are formed during or shortly after a damaging exposure and that they mature with longer recovery times. Part of the maturation process seems to involve the removal of cellular debris from the fluid spaces of the organ of Corti.

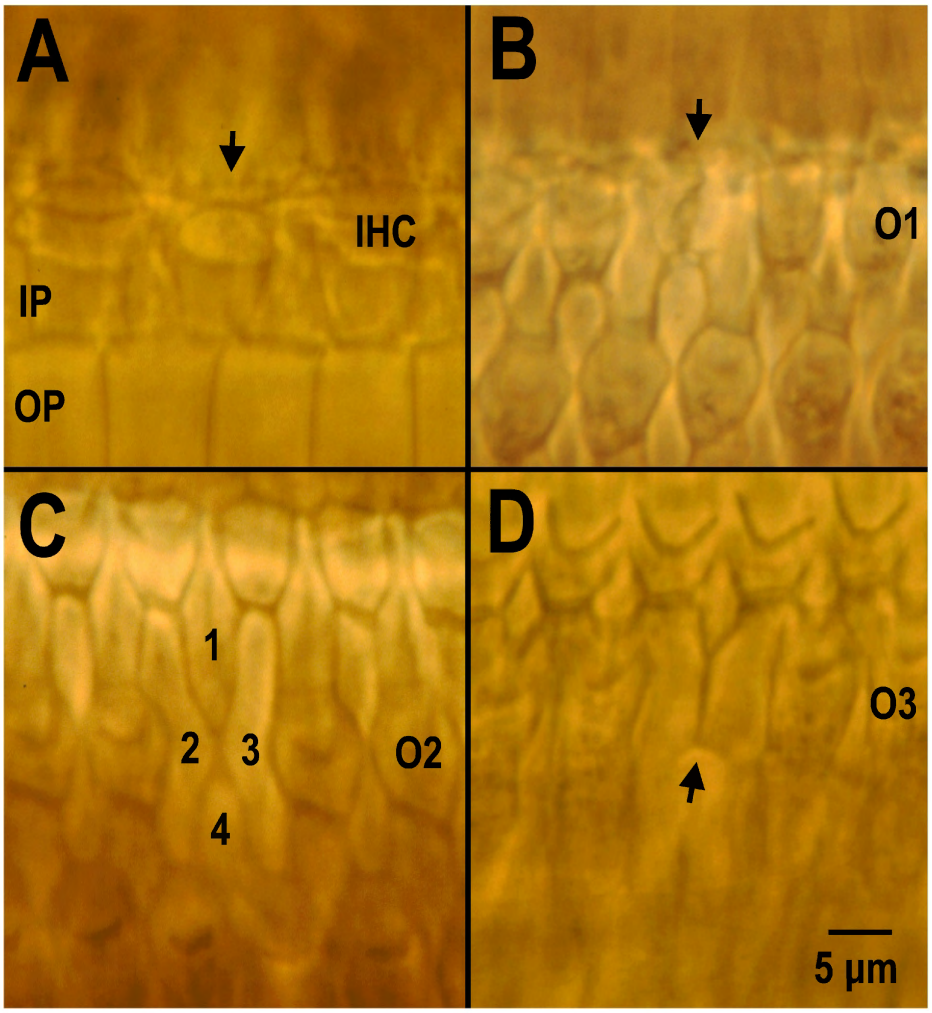
Controversy exists as to when, in the time course of acoustical injury, the non-myelinated nerve fibers in the organ of Corti first show signs of injury. Some investigators have stated the changes in these fibers are secondary to hair-cell degeneration and/or changes in the ionic composition of the organ of Corti fluid (Bohne, 1976a, Lim and Dunn, 1979); other investigators report that the non-myelinated nerve fibers are directly affected by overexposure to noise (Fredelius, 1988; Liberman et al., 1986; Pujol, 1992; Spoendlin, 1971; 1976).

Further loss of outer hair cells, inner hair cells, outer and inner pillars over a narrow extent of the organ of Corti lead to what Bohne and Harding term a 'focal lesion' (Harding and Bohne, 2006; 2009). These lesions are defined as regions in which 50% or more of the outer hair cells and/or inner hair cells are missing over a distance of at least 0.03 mm (i.e., 3 inner

hair cells). Once inner hair cell loss reaches moderate numbers, degeneration of myelinated nerve fibers (i.e., the peripheral processes of the spiral ganglion cells) in the osseous spiral lamina (OSL) is evident (Bohne et al., 1987). This degeneration proceeds from the peripheral to the medial region of the OSL.

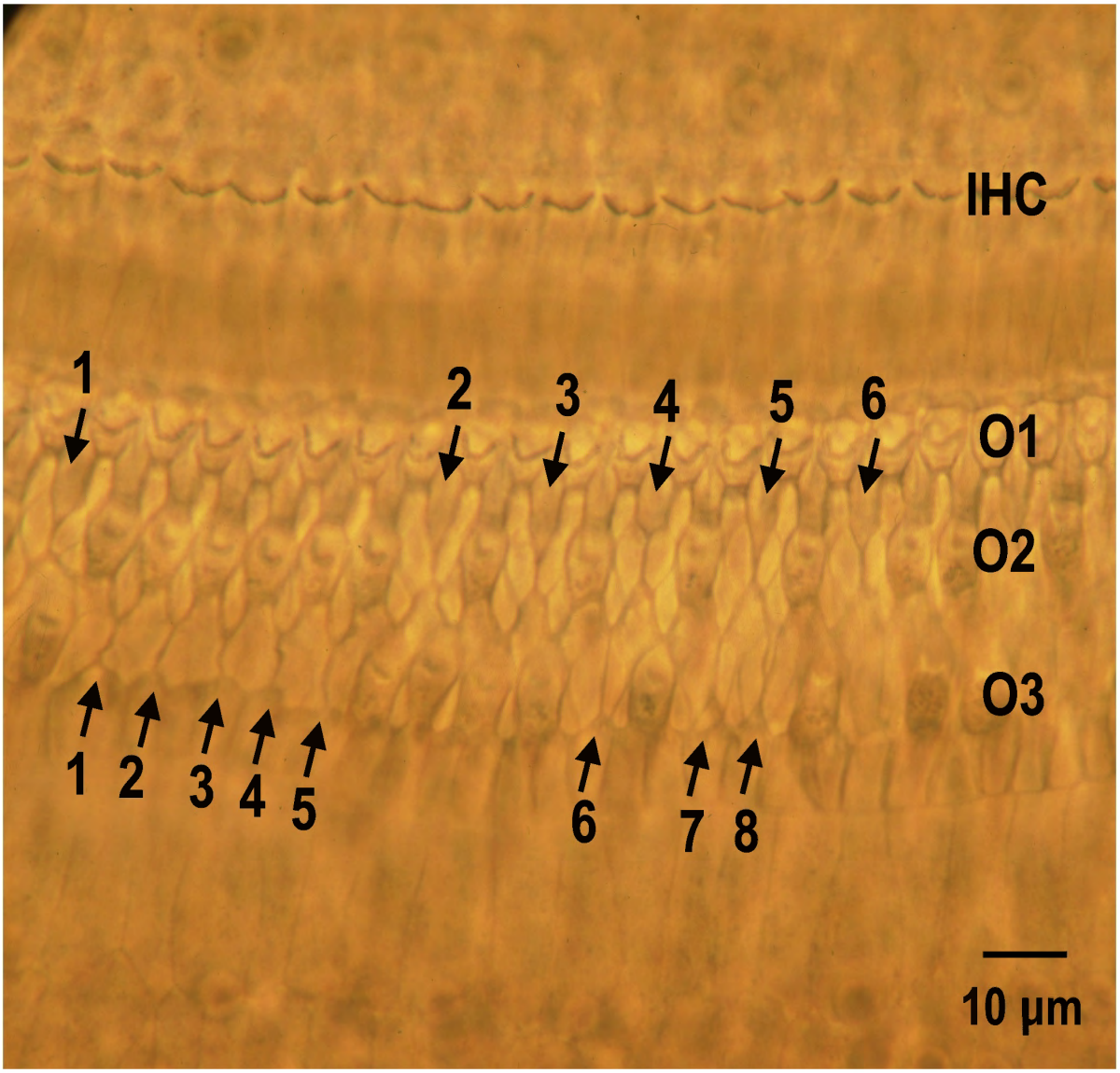
Damage within focal lesions can progress to total loss of all sensory and supporting cells of the organ of Corti over a variable distance on the basilar membrane. Bohne and Clark (1982) termed this lesion an 'organ of Corti (OC) wipeout'. Some scientists term this type of lesion a 'cookie-bite' defect (e.g., Johnsson, 1974). Eventually, many spiral ganglion neurons that originally innervated the degenerated portion of the organ of Corti also degenerate, including their central (axonal) processes that form the auditory portion of the eighth nerve (Nadol and Xu, 1992).

HORIZONTAL VIEW of PHALANGEAL SCARS in the RETICULAR LAMINA



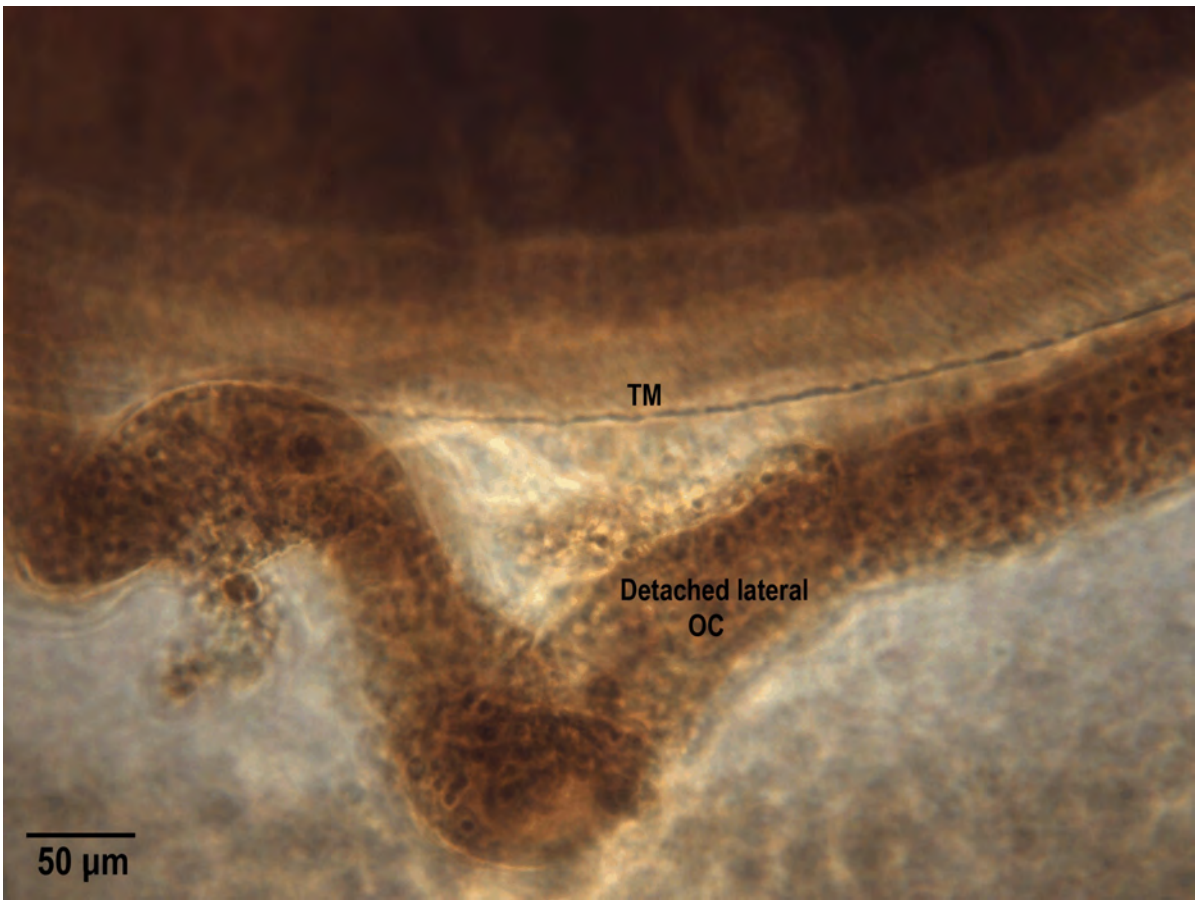
Typical 'mature' phalangeal scars in the reticular lamina. The tight junctions (zonulae occludentes), originally found between hair cell apices and phalangeal processes of the supporting cells, must be replaced when the hair cells degenerate in order to maintain (or reestablish) the endolymphatic boundary. After a hair cell degenerates, new tight junctions form between the phalangeal processes that make up the scar. In these mature scars, there is a dense line of union between adjacent phalangeal processes. A) Phalangeal processes from two inner phalangeal cells and one inner pillar cell (IP) form the scar (arrow) replacing a degenerated inner hair cell (IHC); B) Phalangeal processes from two adjacent outer pillar heads (OP) form the scar (arrow) replacing a degenerated 1st row outer hair cell (O1); C) Phalangeal processes from one outer pillar head (1), two 1st row Deiters' cells (2 & 3), and one 2nd row Deiters' cell (4) have enlarged in nearly equal amounts to form the scar replacing a degenerated 2nd row outer hair cell (O2); D) Phalangeal processes from two 2nd row Deiters' cells form the scar (arrow) replacing a degenerated 3rd row outer hair cell (O3).

VARIABLE APPEARANCE of PHALANGEAL SCARS



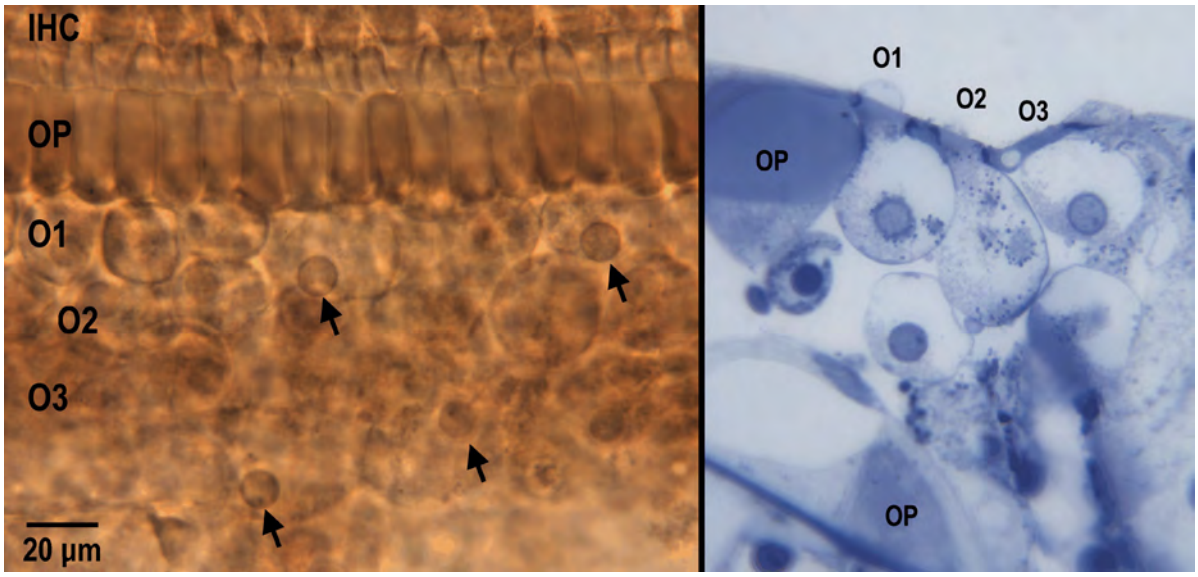
When several OHCs in a row are missing, the phalangeal scars that replace them may not have the classical appearances as shown on p. 51. This is especially true of scars in the second row. Six scars are in focus in the second row (O2) of OHCs. Scars 1, 2, 3 and 5 are fairly similar in appearance while scars 4 and 6 are different due to the variable enlargement of the four processes that make up these scars. Likewise, eight scars are in focus in the third row (O3) of OHCs. Scars 1, 2, 3, 4 and 5 are similar while scars 6, 7 and 8 have a different appearance. Note that the inner hair cells (IHC) and the 1st row OHCs (O1) are intact.

ORGAN of CORTI DAMAGED by ACOUSTIC TRAUMA



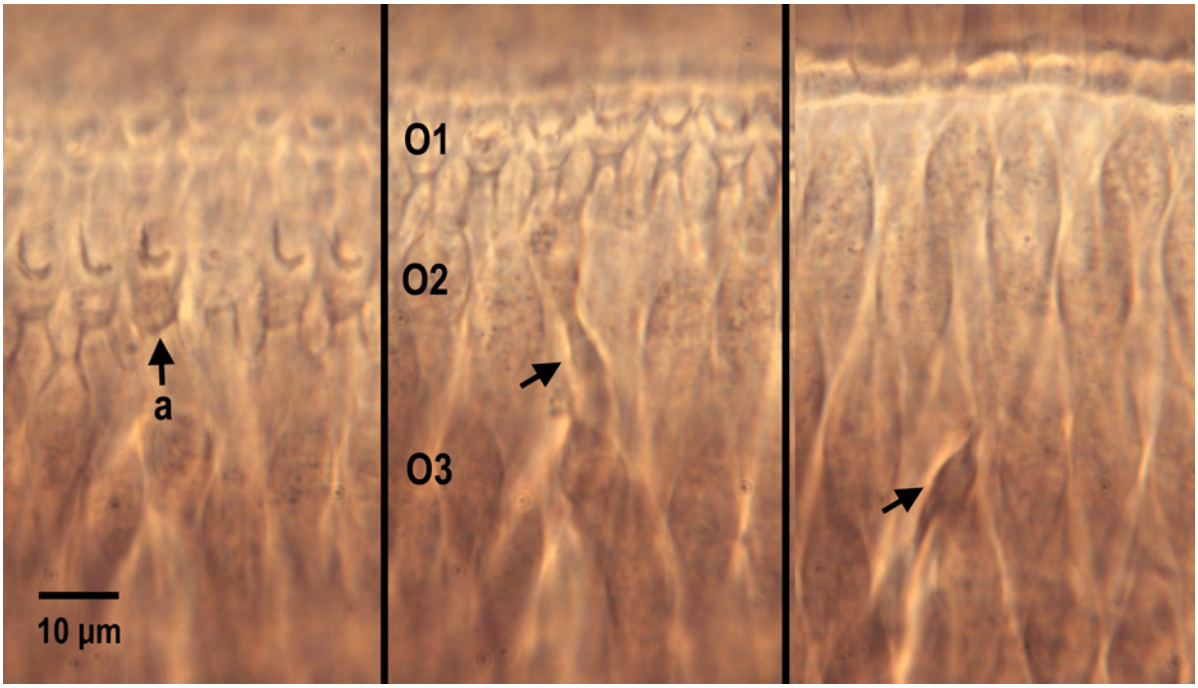
Low-power view of the cochlear duct from an ear that was exposed to a 0.5-kHz OBN at 120 dB SPL for 6 hours. The ear was fixed for microscopic examination within an hour post-exposure. In the 2nd turn, the lateral part of the organ of Corti is detached from the basilar membrane and is coiling in the endolymphatic space. Most OHCs in the detached portion of the organ of Corti were already degenerated; a few were grossly swollen. The tectorial membrane (TM) remained in its typical position and the myelinated nerve fibers (dark area at top of the photomicrograph) are intact at this time.

NOISE-DAMAGED OHCs DYING by the ONCOTIC DEATH PATHWAY



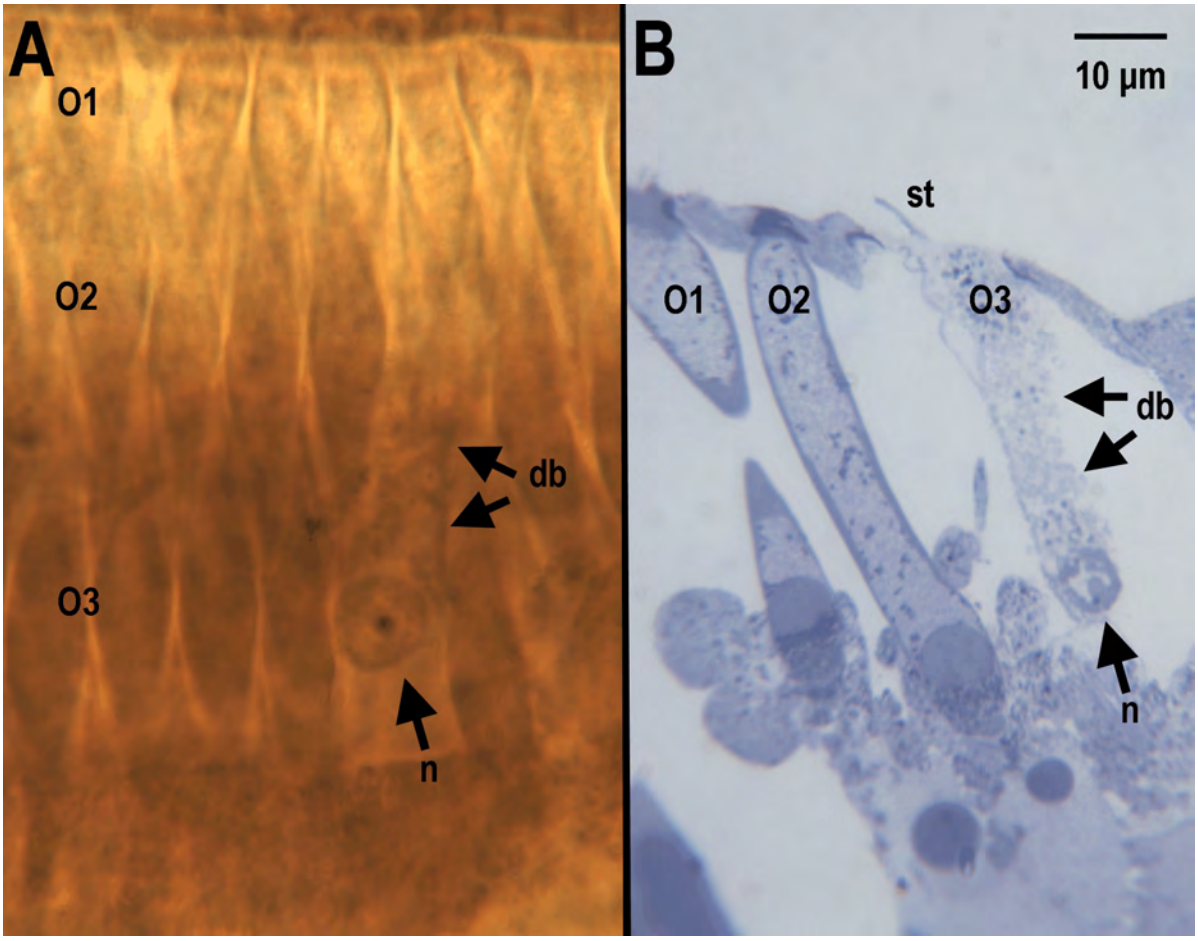
Animal was exposed to a 4-kHz OBN at 108 dB SPL for 1 hour; cochlea was fixed for microscopic examination within 2 hours post-exposure.. At left is a horizontal view (phase contrast of a flat preparation) of the organ of Corti in the basal half of the cochlea. Many of the outer hair cells in the 1st (O1), 2nd (O2) and 3rd (O3) rows are grossly swollen. Arrows point to enlarged, pale-staining nuclei that belong to the dying OHCs. These cells have the typical appearance of cells dying by the oncotic death pathway. At right is a stained, one-micrometer- thick radial section of the same area. Note that OHCs (O1, O2, O3) are grossly swollen, have lost cytoplasm and have fused stereocilia. IHC - inner hair cells; OP - outer pillars.

OHC DYING by the APOPTOTIC DEATH PATHWAY



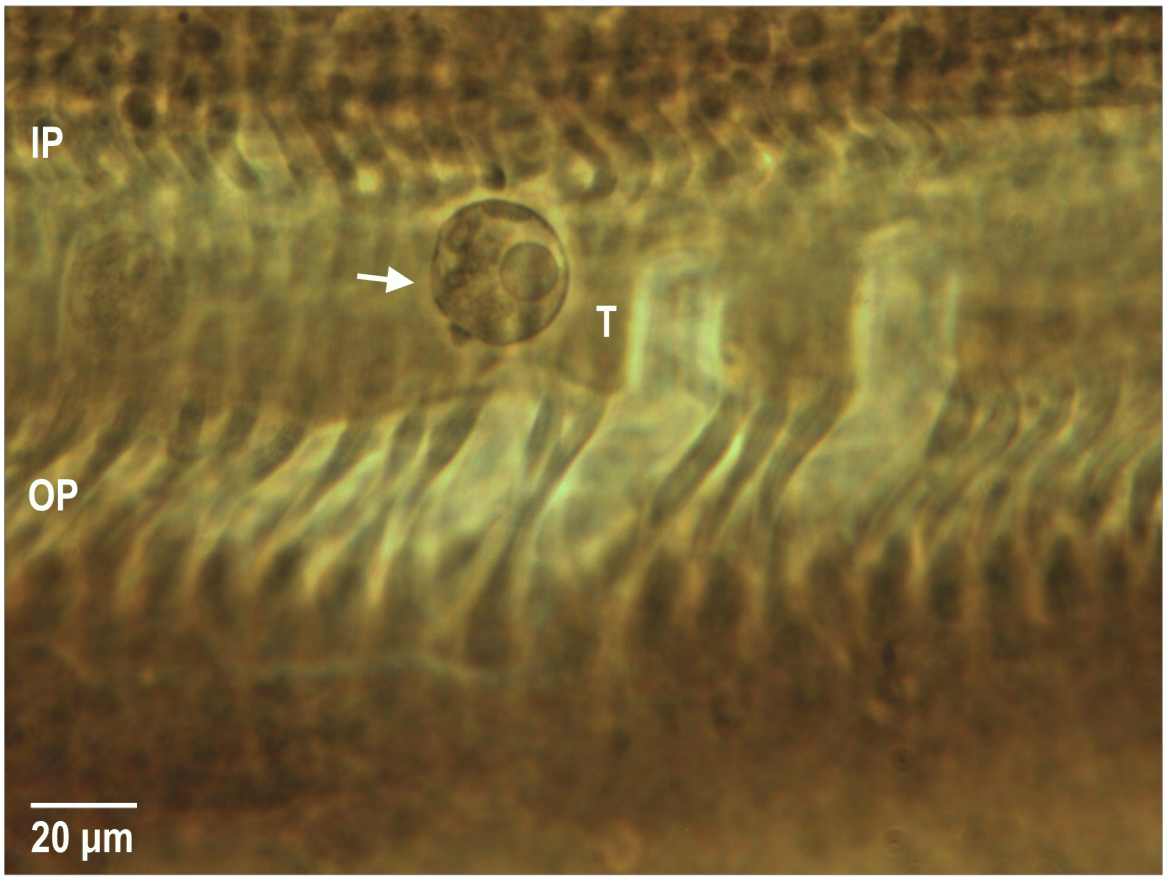
A noise-damaged OHC (arrows indicate same cell in all three photomicrographs) in the 2nd row is dying by the apoptotic death pathway. At left, the focus is on the reticular lamina where the cell's apical surface is slightly darker then usual. In center, the focus is on the OHC bodies. The apoptotic hair cell has a shrunken, dark-staining body. At right, the focus is on the OHC nucleus. The nucleus of the apoptotic cell is shrunken and darkly stained (i.e., pyknotic).

OHCs DYING by NON-APOPTOTIC, NON-ONCOTIC DEATH PATHWAY



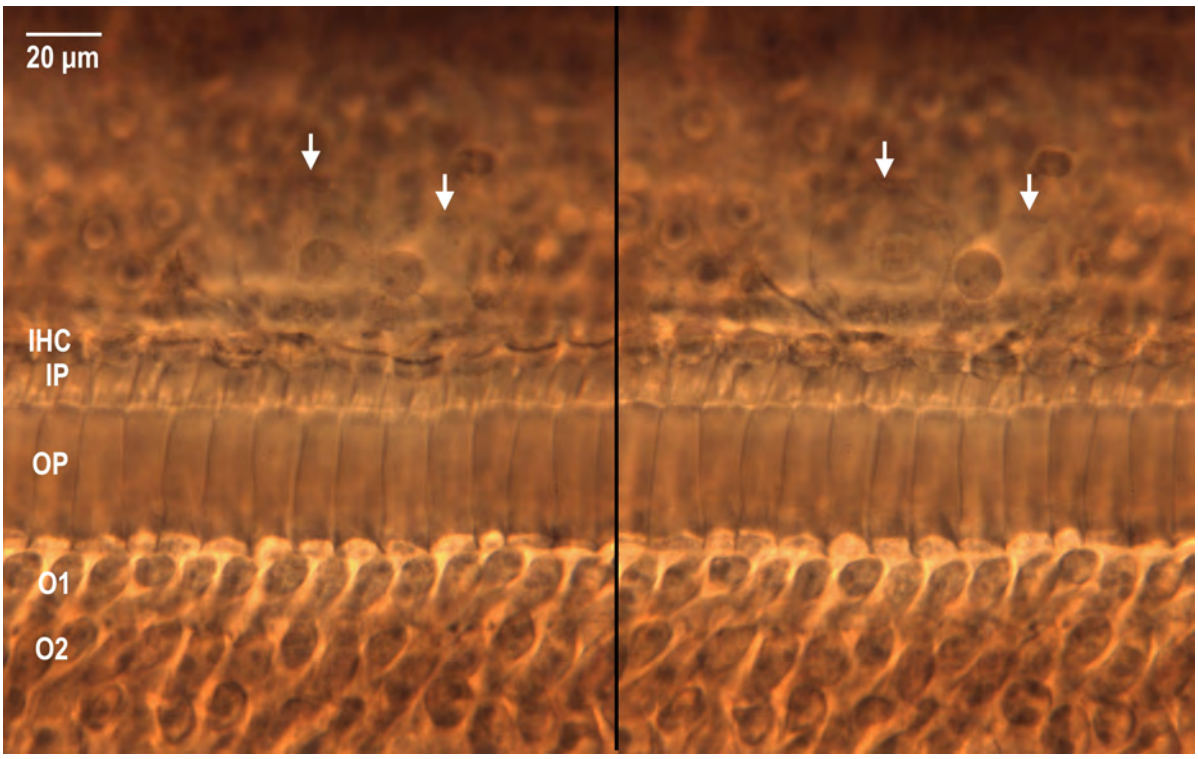
An OHC dying by a non-apoptotic, non-oncotic death pathway [or 3rd death pathway (Bohne et al., 2007)] in apical half of the cochlea after exposure to a 0.5-kHz OBN at 95 dB SPL for 2d. This cell has a deficient basolateral membrane, a pale nucleus (n) with clumped chromatin and cellular debris (db) arranged in the typical shape of an OHC. Stereocilia are present, but decreased in number. A) Phase contrast view of a flat preparation shows a 2nd row OHC (O2) dying by non-apoptotic, non-oncotic death pathway; B) A stained, radial section shows a 3rd row OHC (O3) following this death pathway. O1 - 1st row OHCs.

DISCONNECTED OHC



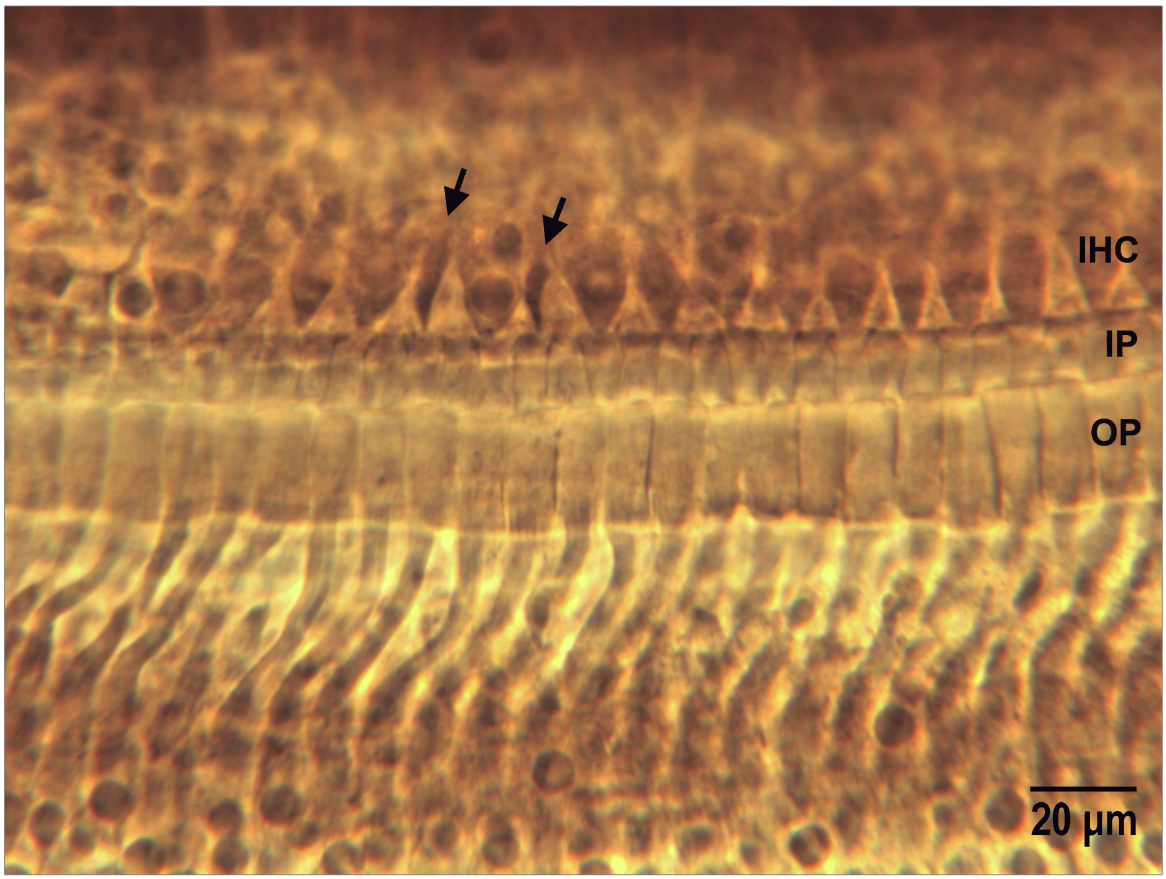
Focused on the tunnel space (T) in the 2nd turn where an OHC (arrow) appears disconnected from the reticular lamina. The cell can be identified by the Hensen bodies (i.e., membrane whorls) in its cytoplasm. This ear was exposed to a 0.5-kHz OBN, at 120 dB SPL for 3.5 h and was fixed less than 1 h post-exposure. IP - inner pillar bodies; OP - outer pillar bodies.

IHCs DYING by the ONCOTIC DEATH PATHWAY



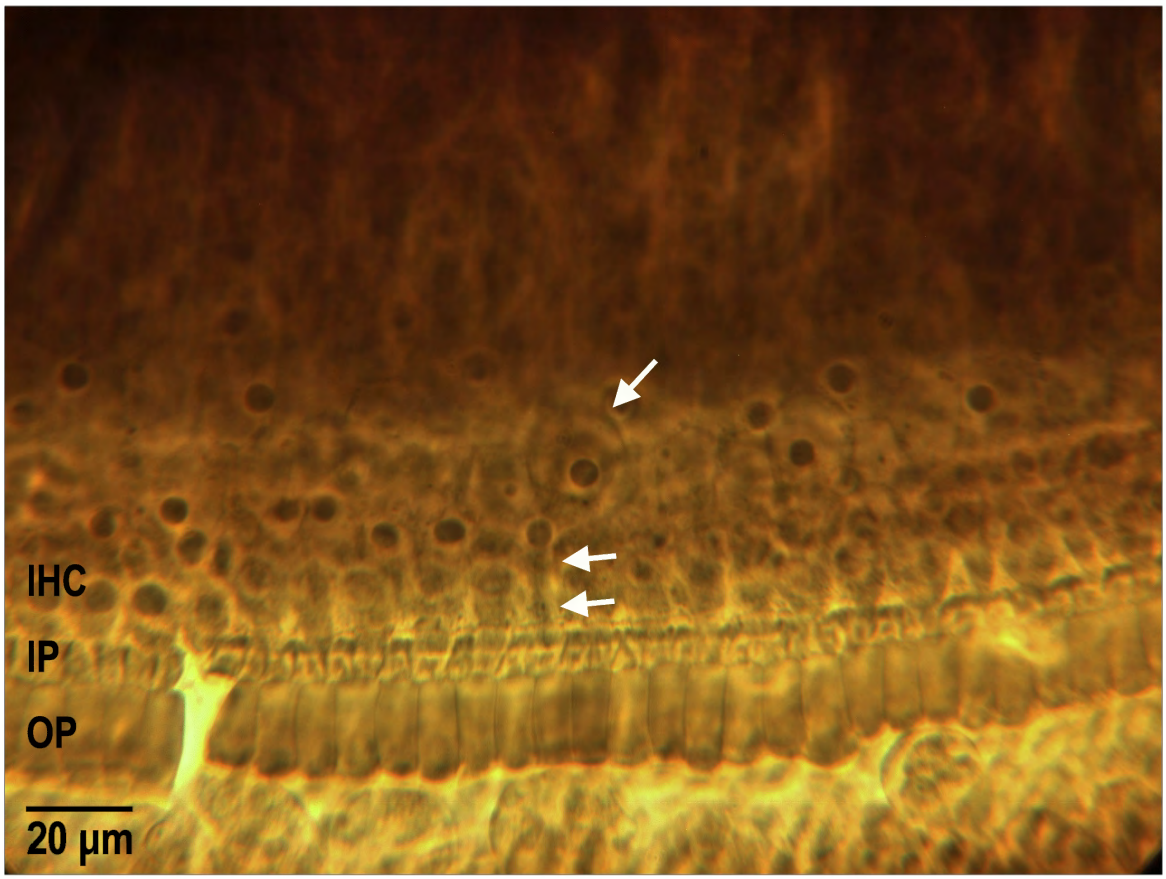
Phase-contrast appearance of 1st turn organ of Corti after exposure to 4-kHz OBN at 108 dB SPL for 1 h with less than 1 h recovery. Two IHCs (arrows) are degenerating by the oncotic death pathway. At left, the stereocilia on most IHCs appear normal, including one of the degenerating IHCs. At right, the degenerating IHCs are swollen, pale-staining and have enlarged nuclei. These cells would have disappeared entirely given a longer recovery time.

APOPTOTIC IHCS



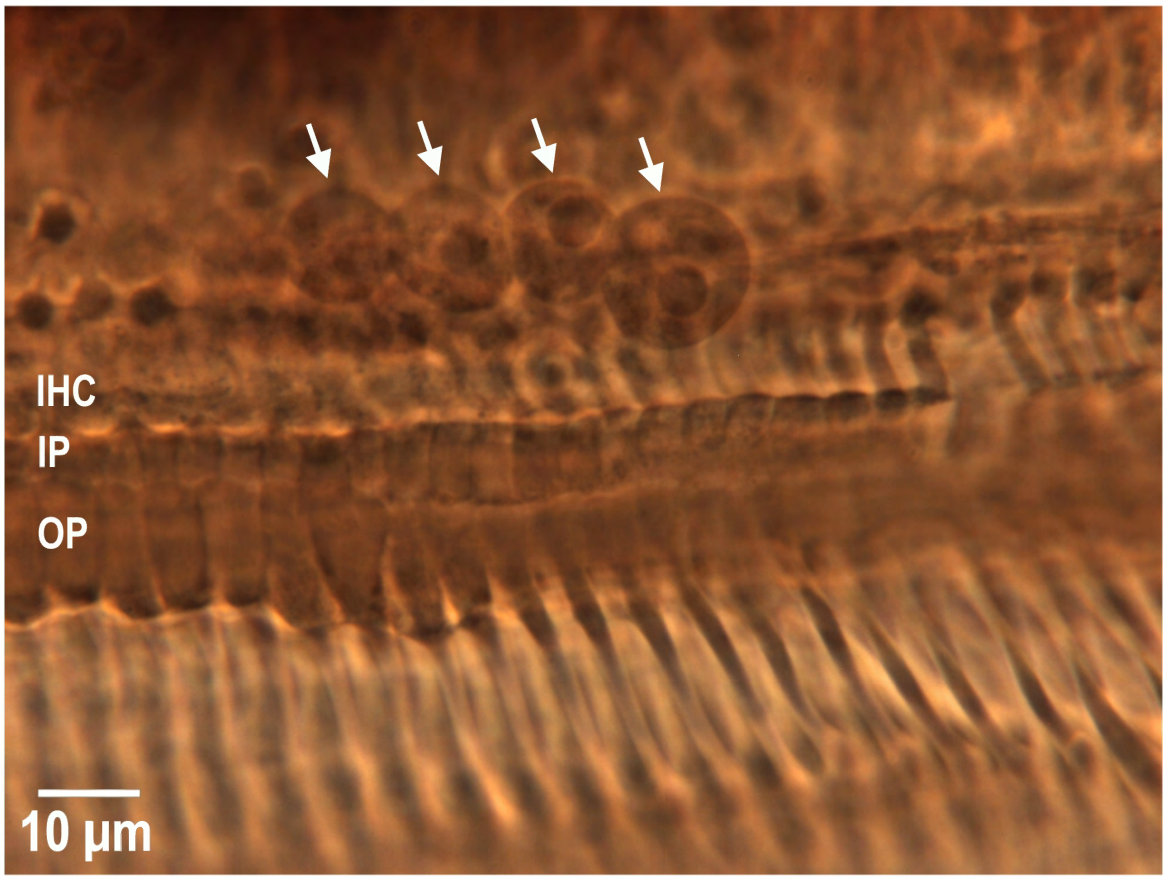
Two apoptotic IHCs (arrows) in the first turn. These cells are shrunken, darkly stained and the nuclei are not visible. This ear was exposed to the 4-kHz OBN at 108 dB SPL for 1 h and was fixed less than 1 h post-exposure.

NEARLY DISCONNECTED IHC



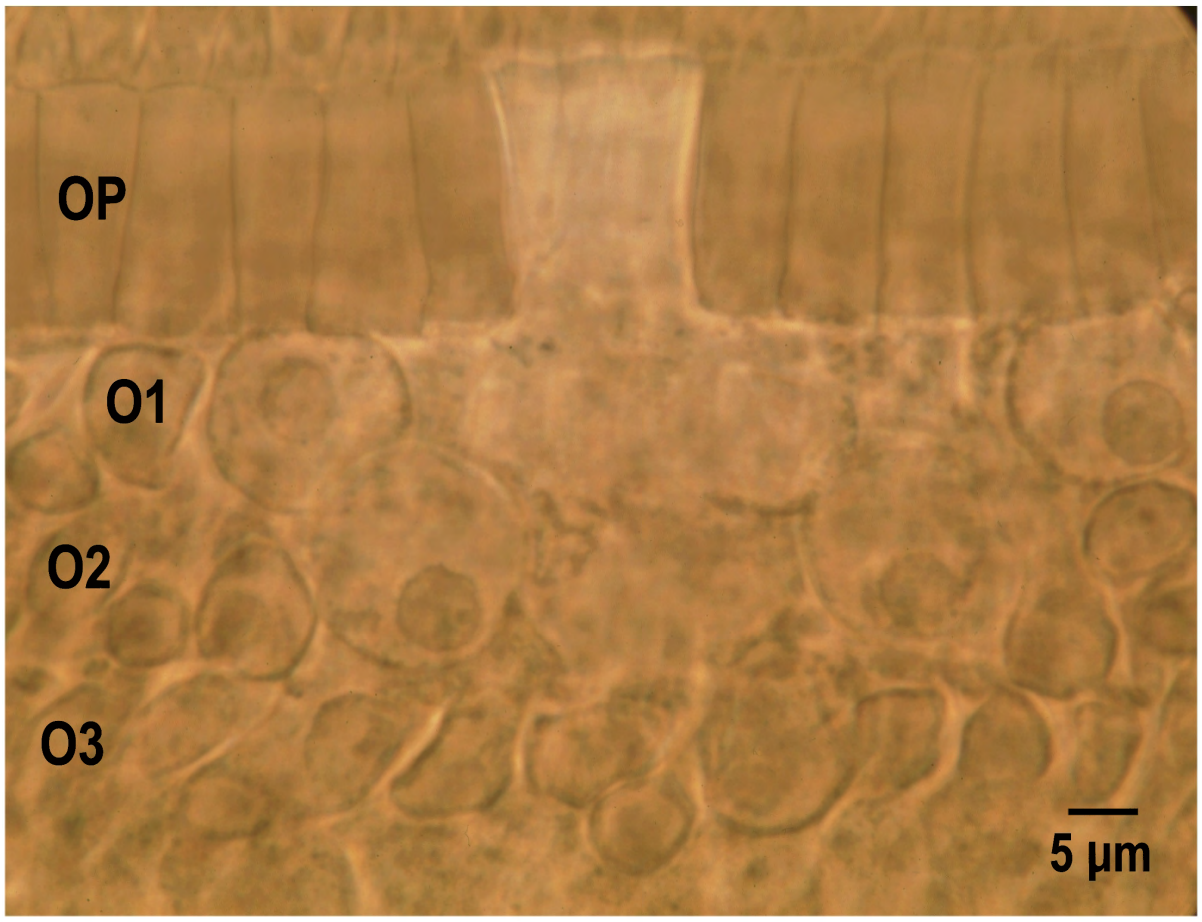
An inner hair cell (IHC) has been nearly disconnected from its apical surface in the reticular lamina. The angled arrow points to the cell body while the left-pointing arrows point to a thin neck that connects the cell body to the reticular lamina. This ear was exposed to a 0.5-kHz OBN at 120 dB SPL for 3.5 h. IP - inner pillar heads; OP - outer pillar heads.

DISCONNECTED IHCS



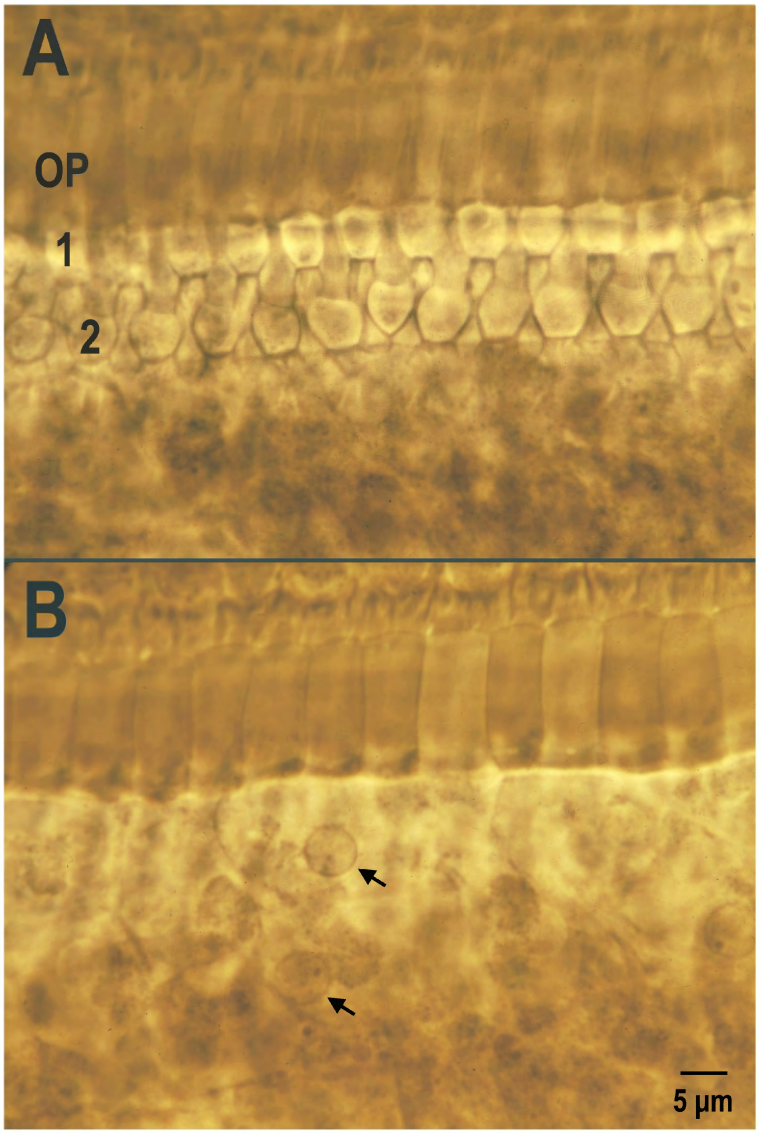
Four IHCs (arrows) have been disconnected from their apices in the reticular lamina in the round window hook portion of the organ of Corti. These disconnected cells are situated beneath the epithelial surface. It remains to be determined if disconnected hair cells reattach or can be stimulated to reattach to the reticular lamina. This ear was exposed to a 4-kHz OBN at 108 dB SPL for 18 h and was fixed less than 1 h post-exposure. IP - inner pillar heads; OP - outer pillar heads.

BEGINNING FORMATION of a NOISE-INDUCED FOCAL OHC LESION



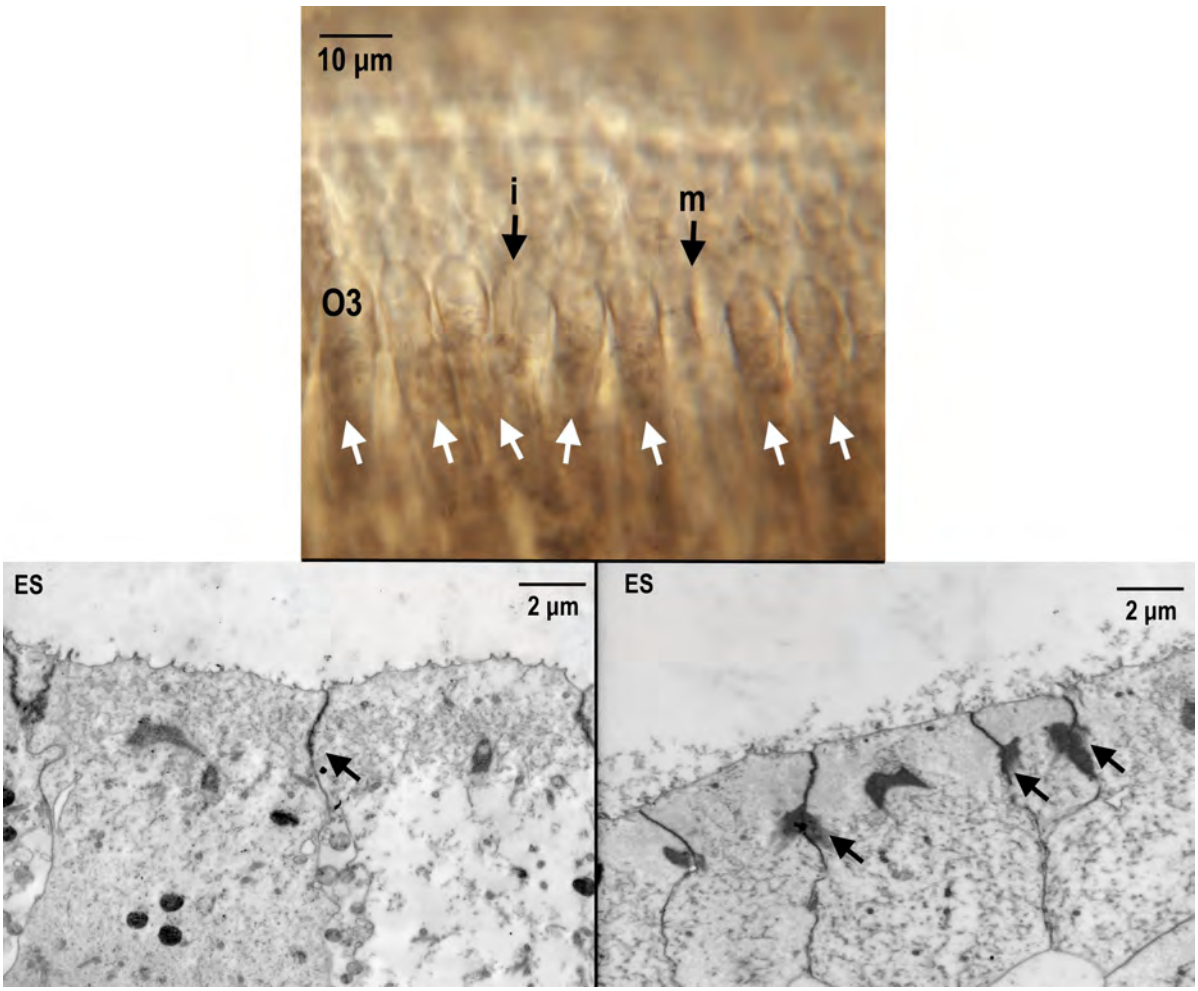
Phase-contrast photomicrograph of the organ of Corti shortly after exposure to a damaging noise. In the center, several outer hair cells in the 1st (O1) and 2nd (O2) rows have degenerated. Two outer pillar cells (OP) have also degenerated. Third row outer hair cells (O3) are misshapen but none have degenerated. Because of the short recovery time, the holes in the reticular lamina had not yet begun to close (reticular lamina is out-of-focus). Note that the outer hair cells closest to the degenerated cells are grossly swollen whereas those further away have more normal shapes. It is hypothesized that endolymph diffused into the organ of Corti fluid spaces through the damaged reticular lamina, resulting in damage to additional OHCs that were not initially injured by the noise exposure.

RETICULAR LAMINA SHORTLY after a DAMAGING NOISE EXPOSURE



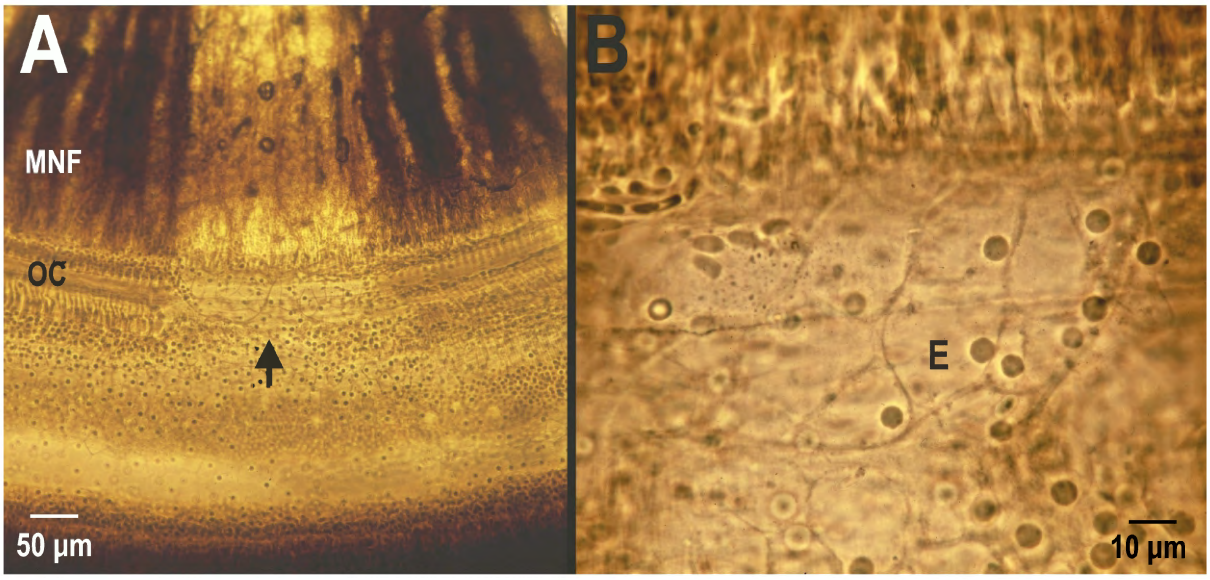
Phase-contrast view of an acutely noise-damaged organ of Corti. A) This specimen, fixed 2 h after exposure to a 4-kHz OBN at 108 dB SPL for 1 h, shows that phalangeal scars have not yet formed to replace the degenerated OHCs. When focusing on the reticular lamina, light-staining regions (i.e., holes) instead of hair-cell apices are seen in the 1st (1) and 2nd (2) rows. Most phalangeal processes adjacent to the holes still have their typical shapes; B) Focused below reticular lamina at same location as 'A'. No OHC bodies are visible. All that remains of the OHCs are fragments of plasma membrane and an occasional enlarged, pale nucleus (arrows). Based on the arrangement of cellular debris in the organ of Corti, most of the missing OHCs died via the oncotic death pathway. OP - outer pillar heads.

IMMATURE & MATURE PHALANGEAL SCARS in the RETICULAR LAMINA



Two different types of phalangeal scars can be found in the reticular lamina shortly after a damaging noise exposure. These scars have distinct features when viewed as a flat preparation (upper photomicrograph) or in tangential sections by TEM (lower photomicrographs). By phase-contrast microscopy, a 'mature' phalangeal scar (m) has a dense line of union between adjacent phalangeal processes that form the scar; an 'immature' scar (i) has a very pale line of union. The white arrows point to the bodies of surviving 3rd row OHCs (O3). By TEM, the phalangeal processes forming an immature scar (arrow, lower left) abut each other but lack electron-dense material adjacent to the plasma membrane of the processes as is seen in mature scars (arrows, lower right).

ORGAN of CORTI WIPEOUT as a RESULT OF NOISE DAMAGE



Phase contrast view of the organ of Corti in the basal turn several months after a exposure to a 0.5-kHz OBN at 95 dB SPL for 9 days. A) The organ of Corti (OC) is visible at the left and right edges of the photomicrograph, along with the myelinated peripheral processes (MNF) of the spiral ganglion cells. At the arrow, all sensory and supporting cells of the organ of Corti have degenerated. This lesion is called an OC wipeout. Note that most MNFs that originally innervated the degenerated region of the organ of Corti have also degenerated (i.e., no staining); B) Undifferentiated squamous epithelial cells (E) cover the basilar membrane at the OC wipeout.

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BIBLIOGRAPHY

Bohne, B.A. (1972): Location of small cochlear lesions by phase contrast microscopy prior to thin sectioning. Laryngoscope, 82:1-16.

Bohne, B.A. (1976a): Mechanisms of noise damage in the inner ear. In: The Effects of Noise on Hearing. Eds.: D. Henderson, R.P. Hamernik, D.S. Dosanjh, J.H. Mills. Raven Press, New York, pp. 41-68.

Bohne, B.A. (1976b): Healing of the noise-damaged inner ear. In: Hearing and Davis: Essays Honoring Hallowell Davis. Eds.: S.K. Hirsh, D.H. Eldredge, I.J. Hirsh, S.R. Silverman. Washington University Press, St. Louis, pp. 85-96.

Bohne, B.A. (1976c): Safe level for noise exposure? Ann. Otol. Rhinol. Laryngol. 85: 711-724.

Bohne, B.A., Clark, W.W. (1982): Growth of hearing lesion and cochlear lesion with increasing duration of noise exposure. In: New Perspective on Noise-Induced Hearing Loss. Eds.: R.P. Hamernik, D. Henderson, R. Salvi, Raven Press, New York, pp. 283-301.

Bohne, B.A., Clark, W.W. (1990): Studies of noise-induced hearing loss using an animal model. Hear. Instrmts., 41:13-16 & 58.

Bohne, B.A., Harding, G.W. (1993): Combined organ of Corti/modiolus technique for preparing mammalian cochleas for quantitative microscopy. Hear. Res., 71:114-124.

Bohne, B.A., Harding, G.W. (2012): Different cells in the organ of Corti follow distinct death pathways after noise exposure. Abst. of the 35th Mid-Winter Research Meeting of the Association for Research in Otolaryngology, #955, p. 335.

Bohne, B.A., Ward, P.H., Fernandez, C. (1976): Irreversible inner ear damage from rock music. Trans. Am. Acad. Ophthamol. Otolaryngol. 82:59-59.

Bohne, B.A., Yohman, L., Gruner, M.M. (1987): Cochlear damage following interrupted exposure to high-frequency noise. Hear. Res., 29:251-264.

Bohne, B.A, Harding, G.W., Lee, S.C. (2007): Death pathways in noise-damaged outer hair cells. Hear. Res. 223:61-70.

Bredberg, G. (1968): Cellular pattern and nerve supply of the human organ of Corti. Acta Otolaryngol. Suppl. 236, pp. 1-135.

Clarke, P.G.H. (1990): Developmental cell death: Morphological diversity and multiple mechanisms. Anat. Embryol. 181:195-213.

Engström, H., Ades, H.W. and Andersson, A. (1966): Structural Pattern of the Organ of Corti. Almquist & Wiksell, Stockholm, Sweden.

Fredelius, L. (1988): Time sequency of degeneration pattern of the organ of Corti after acoustic overstimulation. Acta Otolaryngol., 106:373-385.

Fried, M.P., Dudek, S.E., Bohne, B.A. (1976): Basal turn cochlear lesions following exposure to low frequency noise. Trans. Amer. Acad. Ophthalmol. Otolaryngol., 82:285-298.

Harding, G.W., Bohne, B.A. (2007): Distribution of focal lesions in the chinchilla organ of Corti following exposure to a 4-kHz or a 0.5-kHz octave ban noise. Hear. Res. 225:50-59.

Harding, G.W., Bohne, B.A. (2009): Relation of focal hair-cell lesions to noise-exposure parameters from a 4- or a 0.5-kHz octave band of noise. Hear. Res. 254:54-63.

Hu, B.H., Guo, W., Wang, P.Y., Henderson, D., Jiang, S.C. (2000): Intense noise-induced apoptosis in hair cells of guinea pig cochleae. Acta Otolaryngol. 120:19-24.

Hu, B.H., Henderson, D., Nicotera, T.M. (2006): Extremely rapid induction of outer hair cell apoptosis in the chinchilla cochlea following exposure to impulse noise. Hear. Res. 211:16-25.
Johnsson, L.-G. (1974): Sequence of degeneration of Corti's organ and its first order neurons. Ann. Otol. Rhinol. Laryngol., 83:294-303.
Jugdutt, B.I., Idikio, H.A. (2005): Apoptosis and oncosis in acute coronary syndromes: Assessment and implications. Mol. Cell. Biochem. 270:177-200.
Kaasik, A., Rikk, T., Piirsoo, A., Zharkovsky, T., Zharkovsky, A. (2005): Up-regulation of lysosomal cathepsin L and autophagy during neuronal death inducd by reduced serum and potassium. Eur. J. Neurosci. 22:1023-1031.
Kerr, J.F.R., Wyllie, A.H., Currie, A.R. (1972): Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. Br. J. Cancer 26:239-257.
Kessel, D., Reiners, J.J. Jr., Hazeldine, S.T., Polin, L., Horwitz, J.P. (2007): The role of autophagy in the death of L1210 leukemia cells initiated by the new antitumor agents, XK469 and SH80. Mol. Cancer Ther. 6:370-379.
Lee, S.C., Bohne, B.A., Harding, G.W. (2008): Cochlear base-apex differences in cell-death pathways following exposure to low-frequency noise. The Open Otorhinolaryngol. J. 2:29-43.
Liberman, M.C., Dodds, L.W. and Learson, D.A. (1986): Structure-function correlation in noise-damaged ears: A light and electron microscopic study. In: Basic and Applied Aspects of Noise-Induced Hearing Loss. Eds.: R.J. Salvi, D. Henderson, R.P. Hamernik and V. Colletti. Plenum Publishing Corporation, pp. 163-177.
Lim, D.J. and Dunn, D.E. (1979): Anatomic correlates of noise induced hearing loss. Otolaryngol. Clin. N. Am., 12:493-513.
Lurie, M.H. (1942): The degeneration and absorption of the organ of Corti in animals. Ann. Otol. Rhinol. Laryngol., 51:712-717.
Majno, G., Joris, I. (1995): Apoptosis, oncosis and necrosis. An overview of cell death. Am. J. Pathol. 146:3-15.
Majno, G., Joris, I. (1996): Cells, Tissues, and Disease. Blackwell Science, Cambridge, MA, pp. 175-227.
Melino, G., Knight, R.A., Nicotera, P. (2005): How many ways to die? How many different models of cell death? Cell Death Differ. 12:1457-1462.
Nadol, J.B. Jr. and Xu, W.-Z. (1992): Diameter of the cochlear nerve in deaf humans: Implications for cochlear implantation. Ann. Otol. Rhinol. Laryngol., 101:988-993.
Pujol, R. (1992): Sensitive developmental period and acoustic trauma: Facts and hypotheses. In: Noise-Induced Hearing Loss. Eds.: A.L. Dancer, D. Henderson, R.J. Salvi and R.P. Hamernik. Mosby Year Book, St. Louis, Baltimore, Boston et al., pp. 196-203.
Richardson, K.C., Jarett, L, Finke, E.H. (1960): Embedding in epoxy resin for ultrathin sectioning for electron microscopy. Stain Tech., 35:303-323.
Schuknecht, H.F. (1993): Pathology of the Ear, 2nd Edition, Lea and Febiger, Philadelphia.
Spoendlin, H. (1966): The Organization of the Cochlear Receptor, S. Karger, Basal, SZ, p. 117.
Spoendlin, H. (1971): Primary structural changes in the organ of Corti after acoustic overstimulation. Acta Otolaryngol., 71:166-176.
Spoendlin, H. (1976): Anatomical changes following various noise exposures. In: The Effects of Noise on Hearing. Eds.: D. Henderson, R.P. Hamernik, D.S. Dosanjh and J.H. Mills. Raven Press, New York, pp. 69-89.