MICROSCOPIC ANATOMY OF THE MOUSE INNER EAR

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PREFACE

This Atlas was developed for individuals who are using the mouse to study auditory processing, the genetics of hearing loss and/or the effect of noise or other ototoxic agents on the function and structure of the cochlea. There are a number of similarities among mammals with respect to cochlear structure. This Atlas includes illustrations of some cell types in the mouse cochlea. For illustrations of cell types that are not included here, the reader is referred to the 3rd edition of the chinchilla Atlas ('Microscopic Anatomy of the Inner Ear', B.A. Bohne, 2002).

COMMON ABBREVIATIONS

A - afferent nerve ending (TEM)
B - Boettcher cell (or basal cell if referring to the stria vascularis)
BM - basilar membrane
BV - blood vessel
C - Claudius cell (or capillary if referring to stria vascularis)
Co - cochlear bone
D1 - 1st row Deiters' cell
d1 - phalangeal process from 1st Deiters' cell
D2 - 2nd row Deiters' cell
d2 - phalangeal process from 2nd Deiters' cell
D3 - 3rd row Deiters' cell
d3 - phalangeal process from 3rd Deiters' cell
DC - Deiters' cup
E - efferent nerve ending (TEM)
ES - endolymphatic space
GER - greater epithelial ridge (in developing otocyst)
HC - Hensen cell
HS - Hensen's stripe
I - intermediate cell of the stria vascularis
IB - inner border cell
IHC(s) - inner hair cell(s)
IP - inner pillar cell
IP_f - inner pillar foot
IS - inner sulcus or inner sulcus cell
ISB - inner spiral bundle
L - limbus
LC - lateral crista
LSC - lateral semicircular canal
M - mesothelial cell (or marginal cell if referring to the stria vascularis)
MNF(s) - myelinated peripheral process(es) of spiral ganglion cells
MS - macula saccule
MU - macula utricle
N - Nuel space
O1 - 1st row outer hair cell
O2 - 2nd row outer hair cell
O3 - 3rd row outer hair cell
OC - organ of Corti
OHC(s) - outer hair cell(s)
OP - outer pillar cell
OP_b - outer pillar body
OSL - osseous spiral lamina
OW - oval window
P - perilymphatic space
PC - posterior crista
pop - phalangeal process from outer pillar head
PSC - posterior semicircular canal
RBC - red blood cell
RC - Rosenthal's canal
RM - Reissner's membrane
RTF - radial tunnel fiber
RW - round window
SC - superior crista
SG - spiral ganglion
SGC - spiral ganglion cell
SPL - spiral ligament
SSC - superior semicircular canal
st - stereocilia
ST - scala tympani
StV - stria vascularis
SV - scala vestibuli
T - tunnel
TM - tectorial membrane
VBM - vessel of the basilar membrane
VTL - vessel of the tympanic lip of the osseous spiral lamina

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INTRODUCTION

The petrous portion of the temporal bone houses the organs for hearing, equilibrium and motion detection. The cochlea, which houses the sensory organ for hearing, 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 vestibulocochlear nerve (i.e., 8th cranial) 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 (i.e., perilymph).

The vestibule houses the two static organs of equilibrium (saccule and utricle) as well as the cristae in the semicircular canals. The vestibular sensory areas contain sensory (hair) cells and supporting cells. Hair cells have a bundle of elongated microvilli called stereocilia that project from the apical membrane into an extracellular gelatinous material that overlies the sensory area in each vestibular organ. Within the utricle and saccule, the sensory cells are arranged in a flat plate of cells called a macula. The maculae of the utricle and saccule are nearly perpendicular to one another. The otolithic membrane overlies each macula and is composed of numerous filaments and has a high content of acid mucopolysaccharides. Embedded in the otolithic membrane are numerous crystalline bodies, otoliths or otoconia, that are composed of a protein core and calcium carbonate.

There are three semicircular canals that are oriented at nearly right angles to one another and are named the anterior (superior), lateral (horizontal) and posterior canals. Each canal has one enlarged or ampullated end that contains the crista, a crest of sensory and supporting cells that is oriented perpendicular to the axis of its canal. The stereocilia on the hair cells project into the overlying gelatinous material called the cupula.

The organ of Corti (hearing organ) is unique in that it need not be sectioned in order to be examined microscopically. Portions of the cochlear duct can be dissected free from the otic capsule, placed flat on microscope slides in a liquid medium and cover-slipped (e.g., Engström et al., 1966). The cochlea can also be embedded in plastic before dissection of the cochlear duct (Bohne and Harding, 1997). The various cells in the organ of Corti can be examined by 'optically sectioning' or using the z-axis (fine focus) of a microscope to focus at successively deeper layers within the epithelium. Tissues prepared this way are called 'surface' or 'flat' preparations.

Because of its small size, the mouse cochlea presents some advantages and disadvantages for cochlear preparation and microscopic evaluation.

Advantages: 1) The soft tissue in the temporal bone can readily be preserved by vascular perfusion because of the short diffusion distances from the cochlear vasculature to the organ of Corti; 2) The bony labyrinth is joined to the rest of the skull by fibrous tissue only. Thus, the cochleas and vestibules can be easily removed from the skull without damage;

3) The cochlear bone is extremely thin. Removal of the bony labyrinth from the membranous labyrinth requires a small sharp pick and manual dexterity only; 4) The mouse organ of Corti averages about 6 mm in length and contains about 700 inner hair cells and 2400 outer hair cells. Thus, the collection of quantitative data in a given cochlea requires much less time than for mammals with longer cochleas.

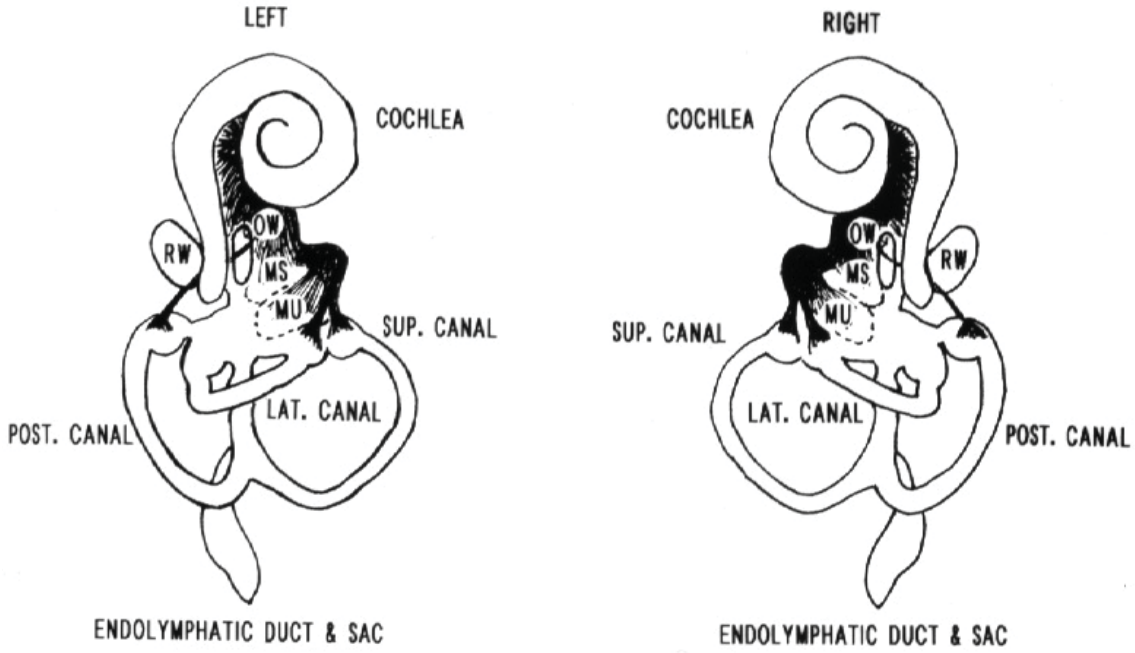
Disadvantages: 1) The hair cells and supporting cells in the organ of Corti are very small. The small size makes it difficult to evaluate intracellular detail in surface preparations; 2) The frequency range of mouse hearing is about 1-100 kHz (Ehret, 1974; Mikaelian et al., 1974). The functional testing of frequencies above 50 kHz requires expensive, specialized equipment and, therefore, is rarely performed. In most laboratories, 32, 40 or 50 kHz is the highest frequency that is routinely tested (e.g., Davis et al., 1999; Jimenez et al, 2001; Ohlemiller et al., 2000; Ou et al, 2000a; Vazquez et al., 2004). Based on the frequency-place map of the mouse cochlea by Ou et al. (2000b), 32 kHz and 50 kHz are located about 76% and 85% distance from the apex, respectively. Thus, beginning damage in the basal 15-24% of the organ of Corti cannot be detected if 32-50 kHz is the highest frequency that is functionally tested; 3) It is more difficult to perform survival surgery on the inner ear of a mouse compared to other rodents (e.g., chinchilla, guinea pig) (Bohne et al., 2001).

Fixation and preparation of the specimens illustrated in this Atlas was as follows. The heads of the 18.5-day embryonic mice were fixed by immersion in 4% buffered paraformaldehyde for several days. After washing in buffer, the heads were bisected, the brain removed and the two halves of the skull immersed for one hour in a buffered solution of cold 1% osmium tetroxide. After washing out the osmium, the temporal bones were separated from the remainder of the skull, dehydrated in a graded series of ethanol and propylene oxide then infiltrated with plastic. Each infiltrated temporal bone was carefully held in a small pool of liquid plastic and divided with razor blades into 4-5 thick sections. The thick sections were re-embedded flat in thin layers of plastic for subsequent microscopic examination (Nikon dissection and Wild phase-contrast microscopes).

The temporal bones of the 9-day-old and adult mice were fixed by perfusing buffered 1% osmium tetroxide through the vascular system. The temporal bones were removed and immersed in cold fixative for two hours. The specimens were washed in Hank's balanced salt solution, dehydrated in ethanol and propylene oxide then embedded in plastic. After the plastic polymerized, the cochleas were dissected as surface preparations. The dissected segments of the cochlear duct were re-embedded in thin layers of plastic for examination by phase-contrast microscopy (Wild, M-20) (Bohne and Harding, 1997). Selected organ-of-Corti segments were sectioned radially with an RMC ultramicrotome. One-micrometer-thick sections were mounted on glass slides and either stained with methylene blue-azure II (Richardson et al., 1960) or left unstained, cover-slipped and examined by phase-contrast microscopy. Thinner sections were mounted on grids and examined by transmission electron microscopy (Hitachi H7500).

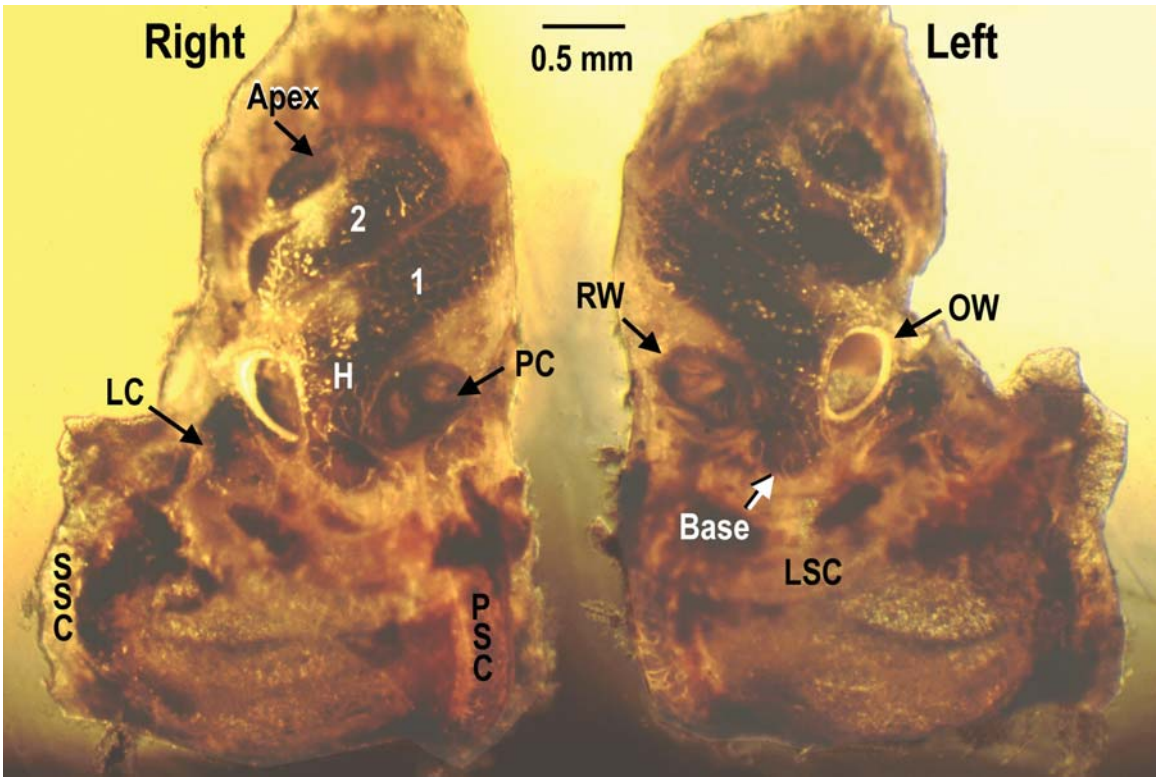
The thick and semi-thick sections of inner ear and organ of Corti and flat preparations of the cochlear duct were photographed with a Micropublisher digital camera, then color-balanced, cropped and labeled using Adobe Photoshop (version 7).

MOUSE MEMBRANOUS LABYRINTH (SCHEMATIC)



Schematic drawings of the membranous labyrinth in the left and right temporal bones (bone not shown) of the mouse show the organs for hearing (i.e., cochlea) and balance (MU - utricle; MS - saccule; lateral (lat.), posterior (post.) and superior (sup.) semicircular canals). The endolymphatic sac, that serves as a reservoir for endolymph, is connected to the endolymphatic spaces in the utricle and saccule via the endolymphatic duct (partly visible here). The different organs contain sensory (hair) cells for the detection of sound (i.e., the organ of Corti in the cochlea), the detection of gravity (i.e., macula of utricle and saccule) or the detection of angular acceleration (i.e., crista of the lateral, posterior and superior semicircular canals). The two openings in the bone that surrounds the membranous labyrinth that allow communication with the middle ear are the round window (RW) and oval window (OW). The round window, closed by the round window membrane, is located at the basal end of scala tympani. The oval window, closed by the footplate of the stapes, is located inferior to vestibule, over the macula of the saccule. Note that the two ears are mirror images of one another.

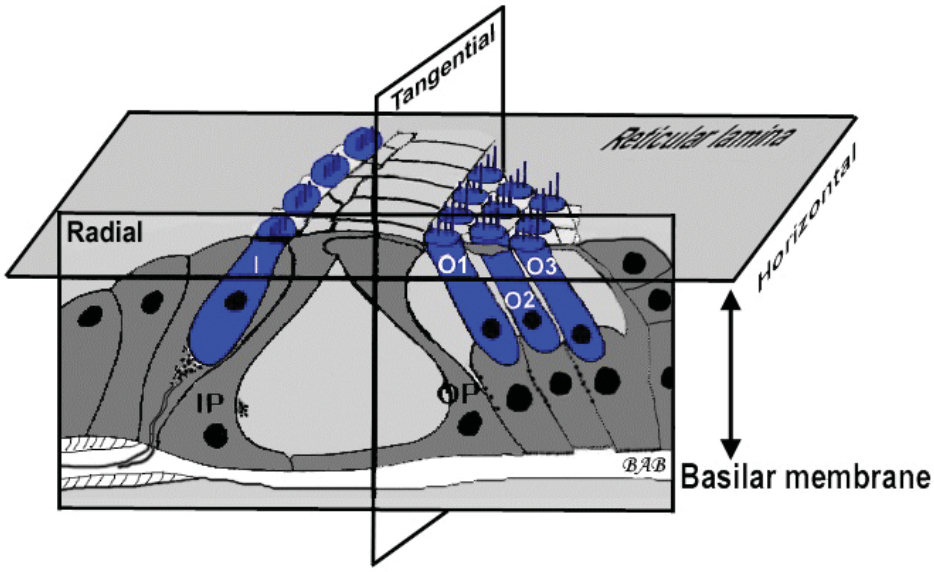
MOUSE MEMBRANOUS LABYRINTH



Right and left temporal bones viewed with a dissection microscope from the ventral side of an adult mouse. Once dehydrated, the bone becomes transparent and the membranous labyrinth can be visualized through the bone. The apex and base of the cochlear duct are visible, along with the second (2) and first (1) turns of the cochlea. H - hook of the cochlear duct; LC - lateral crista; LSC - lateral semicircular canal; OW - oval window; PC - posterior crista; PSC - posterior semicircular canal; RW - round window; SSC - superior semicircular canal.

To understand anatomical relations in the cochlea, examine a surface preparation of the undamaged organ of Corti under a high-power microscope and simultaneously have available for observation a photomicrograph of a radial section through the organ. In your mind, draw lines across the radial section and observe how a section cut parallel to the basilar membrane would pass through the various cells. Compare what is visible in the radial section to the view that is seen in the surface preparation.

VIEWING ANGLES FOR THE ORGAN OF CORTI

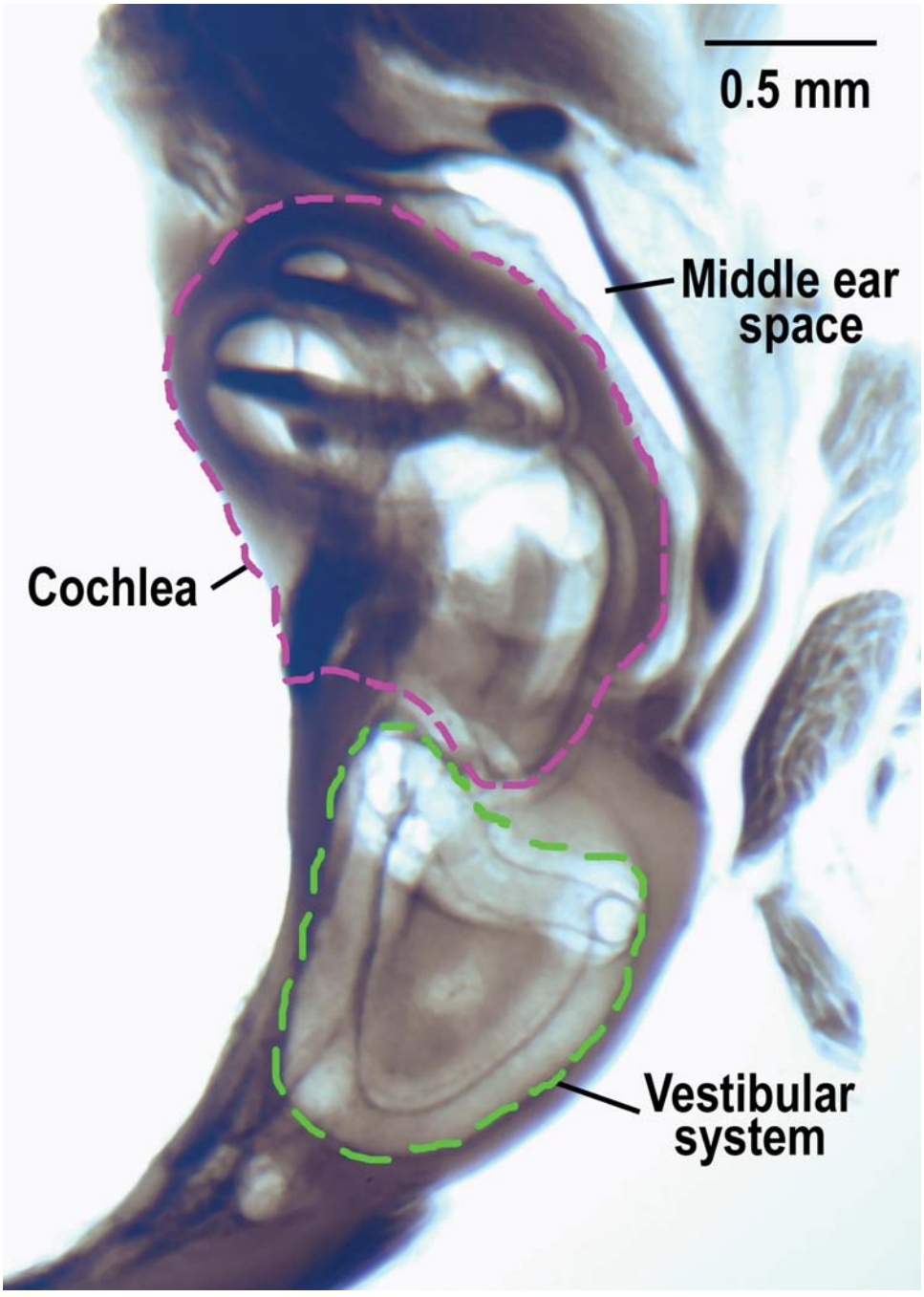


This drawing of the organ of Corti shows the three planes in which it can be viewed: **Radial** - perpendicular to both the inner (IP) and outer pillar (OP) heads and the basilar membrane, passing through one cell in each sensory-cell row [i.e., inner hair cell (I) and three rows of outer hair cells (1, 2, 3)] (see pp. 26 & 28); **Horizontal** - parallel to the surface of the organ of Corti and the basilar membrane, passing through a number of hair cells in each row simultaneously. This view can be either a sectioning plane or a focal plane in a surface preparation (see pp. 13-18, 29-35, 37-41); **Tangential** - parallel to the pillar heads and perpendicular to the basilar membrane, passing through one row of sensory cells at a time.

18.5-day-old embryo

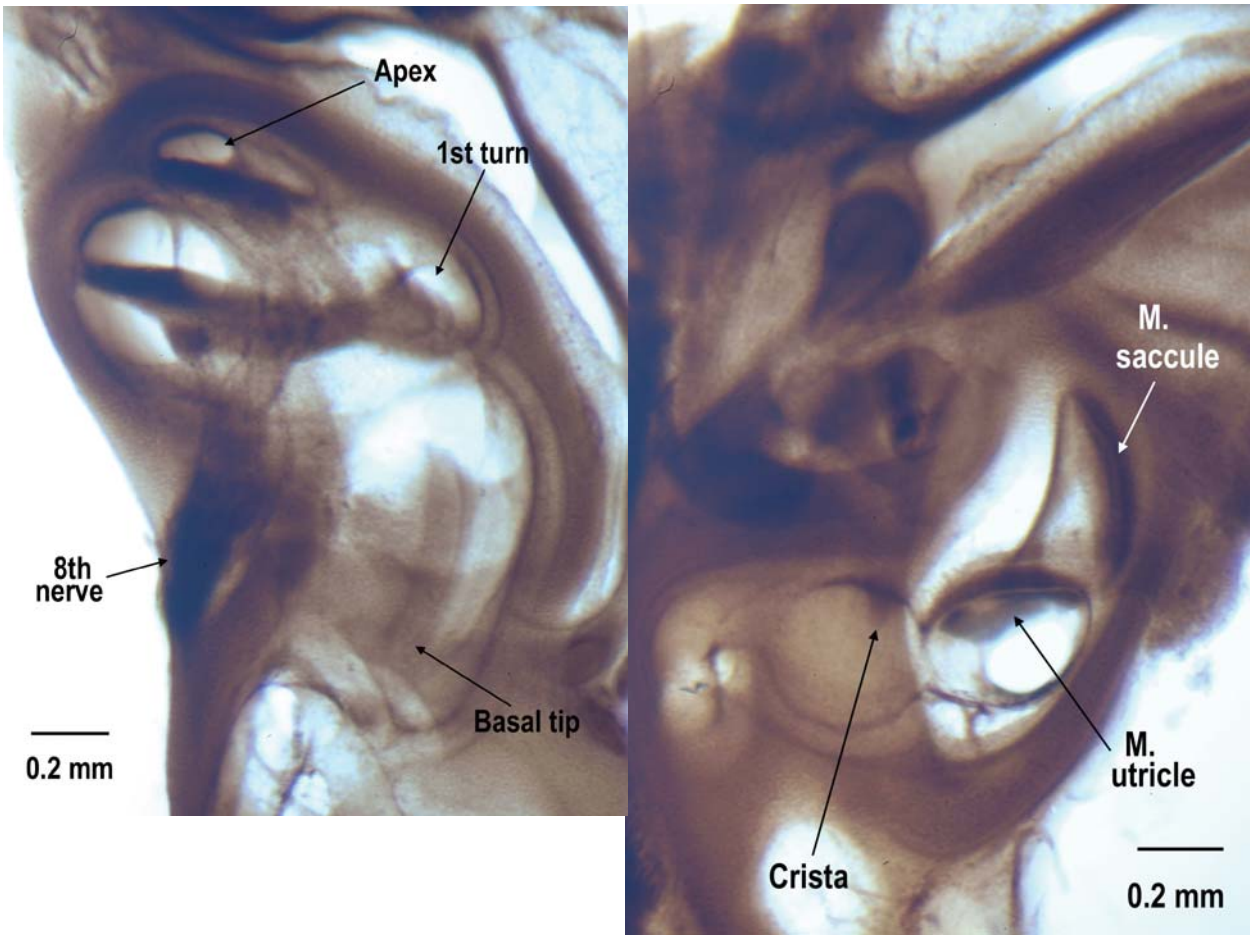
The gestation period for mice is 21 days. At 18.5 days of gestation, the cochlea has finished coiling, the gravity-sensing organs (i.e., maculae of the saccule and utricle) and semicircular canals and their cristae have developed. Although hair cells are present in the organ of Corti, the hearing organ has not yet matured and the animal cannot hear.

THICK, RADIAL SECTION OF EMBRYONIC MOUSE TEMPORAL BONE



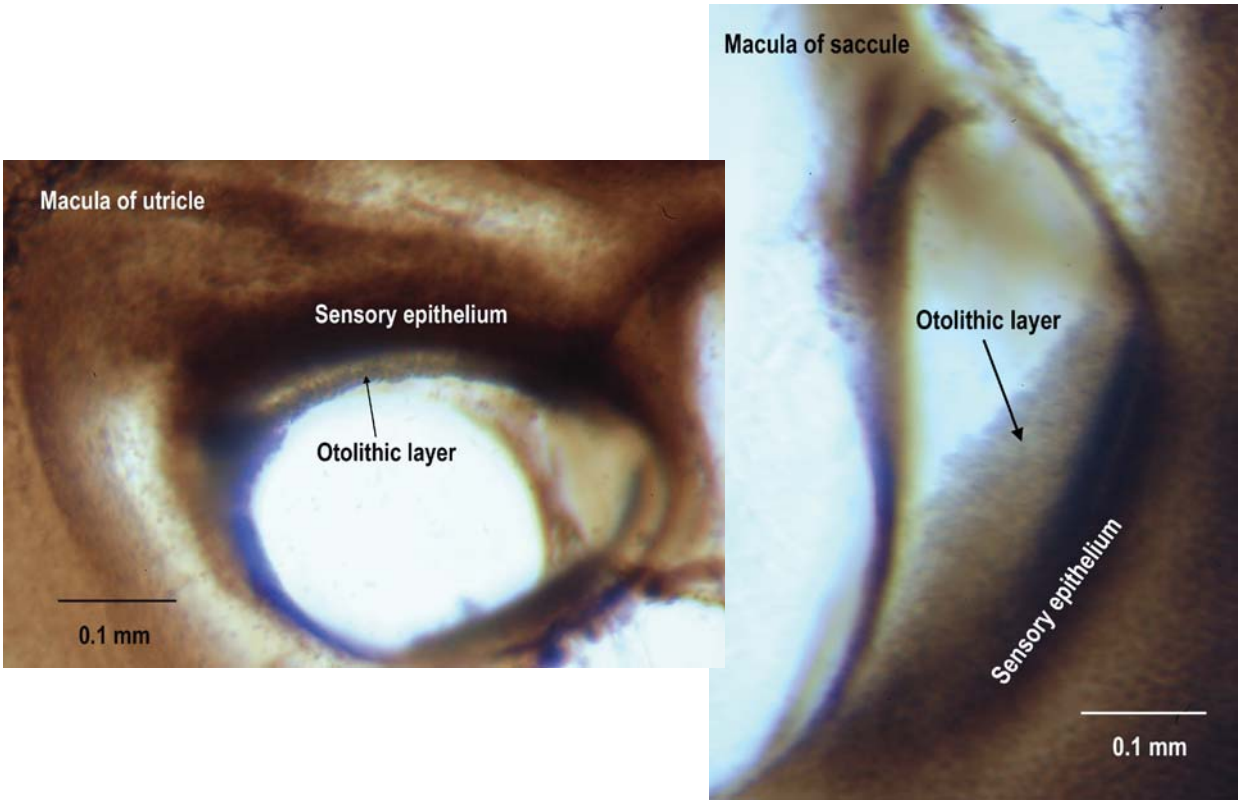
Thick section of a plastic-embedded 18.5-day-old embryonic mouse ear as viewed under a dissection microscope. The cochlea is outlined in pink; the vestibular system is outlined in green. The middle ear space is not fully developed at this age.

COCHLEA & VESTIBULAR END ORGANS IN EMBRYONIC MOUSE



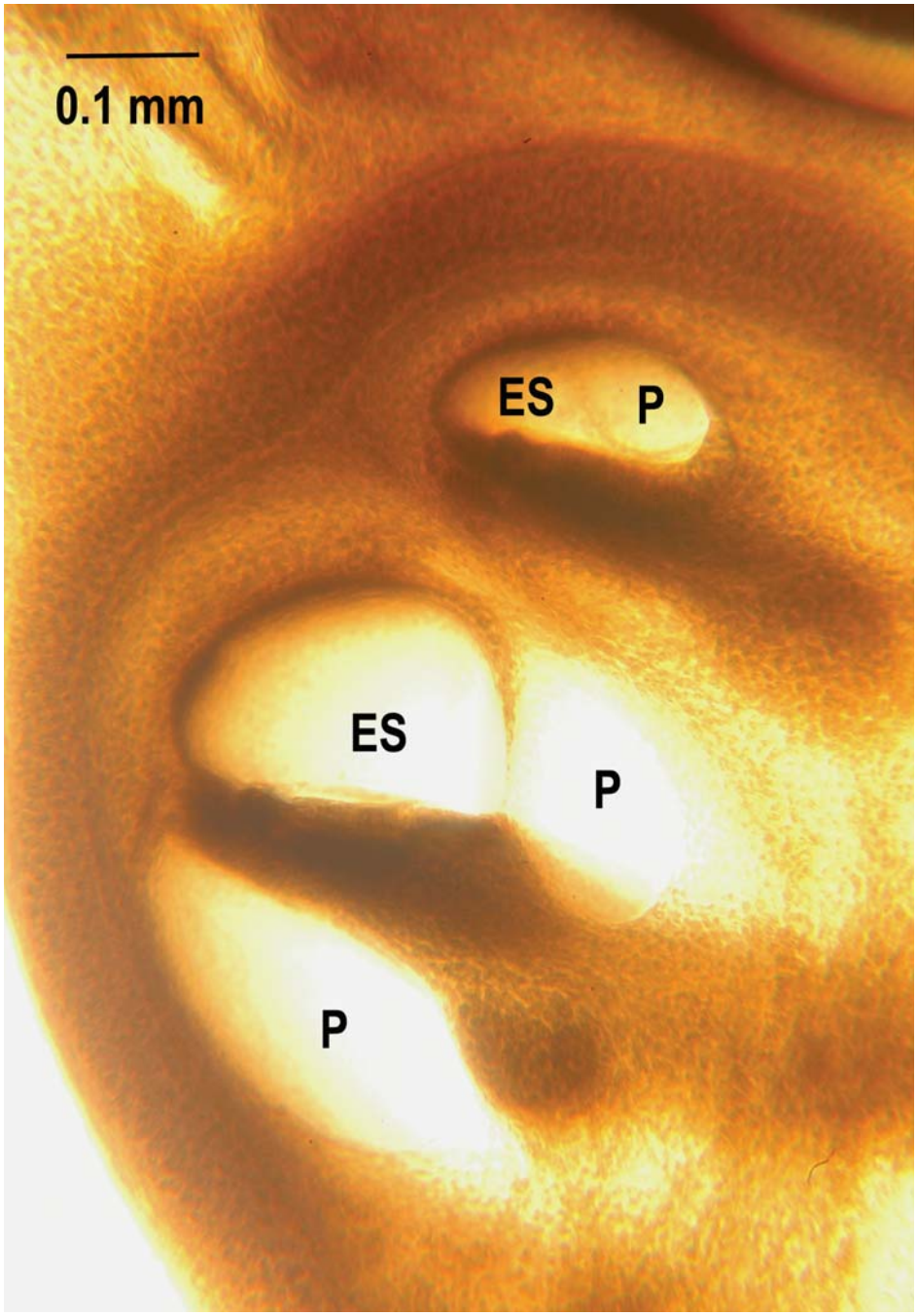
An overview of the relations among the sensory regions in the cochlea (left) and vestibule (right) can be gained using a dissection microscope. In the cochlea, the apex, first turn and round window hook that ends at the basal tip are visible along with the eighth nerve. In the vestibule, the maculae of the saccule and utricle and the crista of one of the semicircular canals are visible.

MACULAE IN UTRICLE & SACCULE OF EMBRYONIC MOUSE



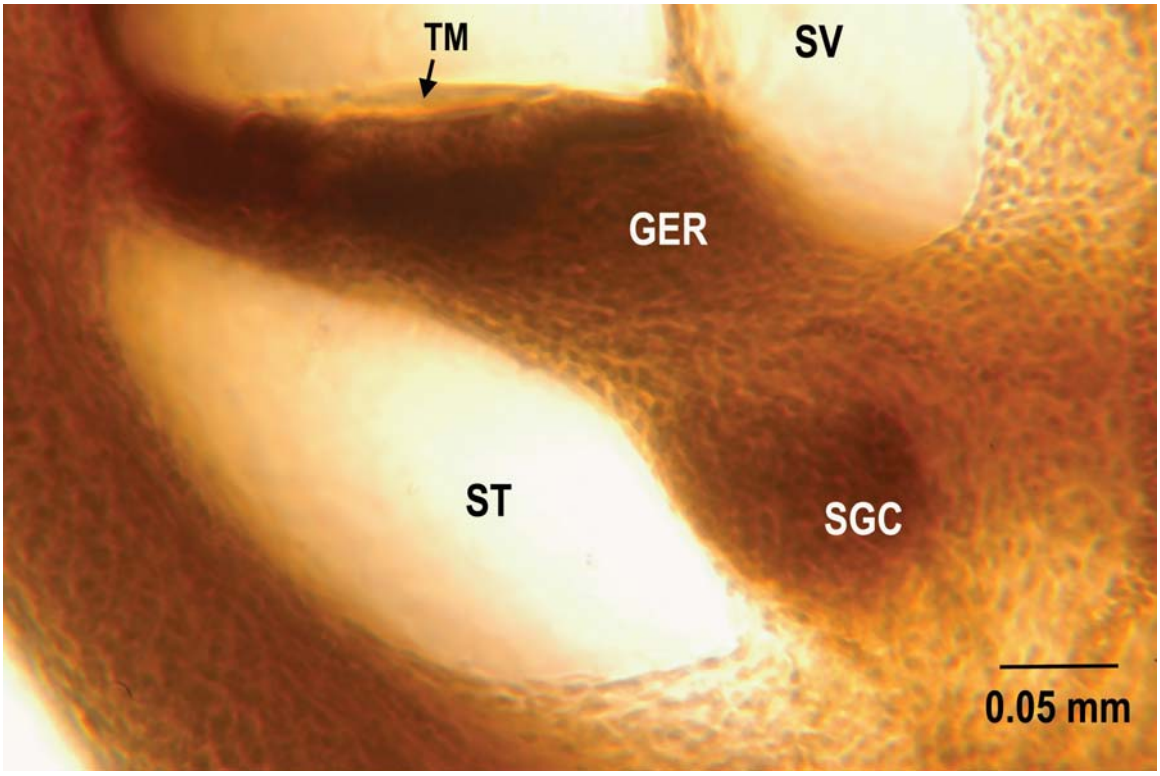
The maculae of the utricle (left) and saccule (right) both have a fully developed otolithic layer overlying the sensory epithelium.

COCHLEA IN EMBRYONIC MOUSE

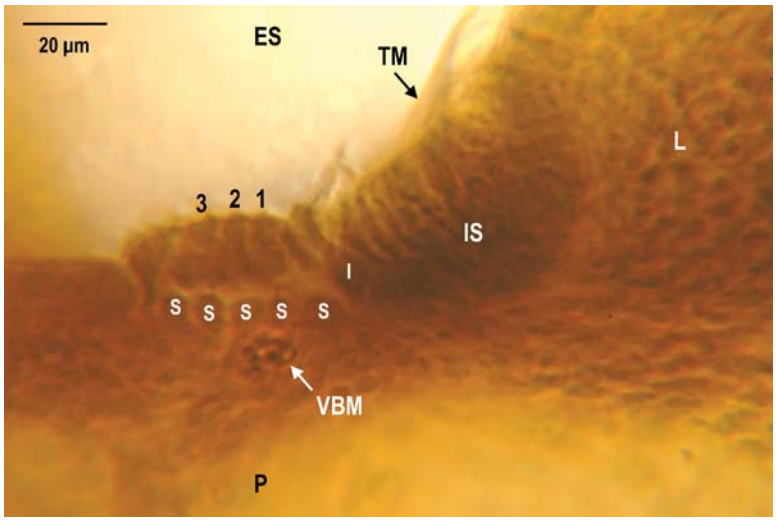


Thick, radial section of the cochlea viewed through a phase-contrast microscope. The endolymphatic space (ES) and perilymphatic spaces (P) can be seen. The cells of the organ of Corti have not yet differentiated.

DEVELOPING ORGAN OF CORTI



Developing cochlea shows a clump of cells (SGC) that represents the future spiral ganglion near the cells of the greater epithelial ridge (GER) that represents the future limbus. The future organ of Corti is located on the basilar membrane lateral to the GER. TM - tectorial membrane; ST - scala tympani; SV - scala vestibuli.

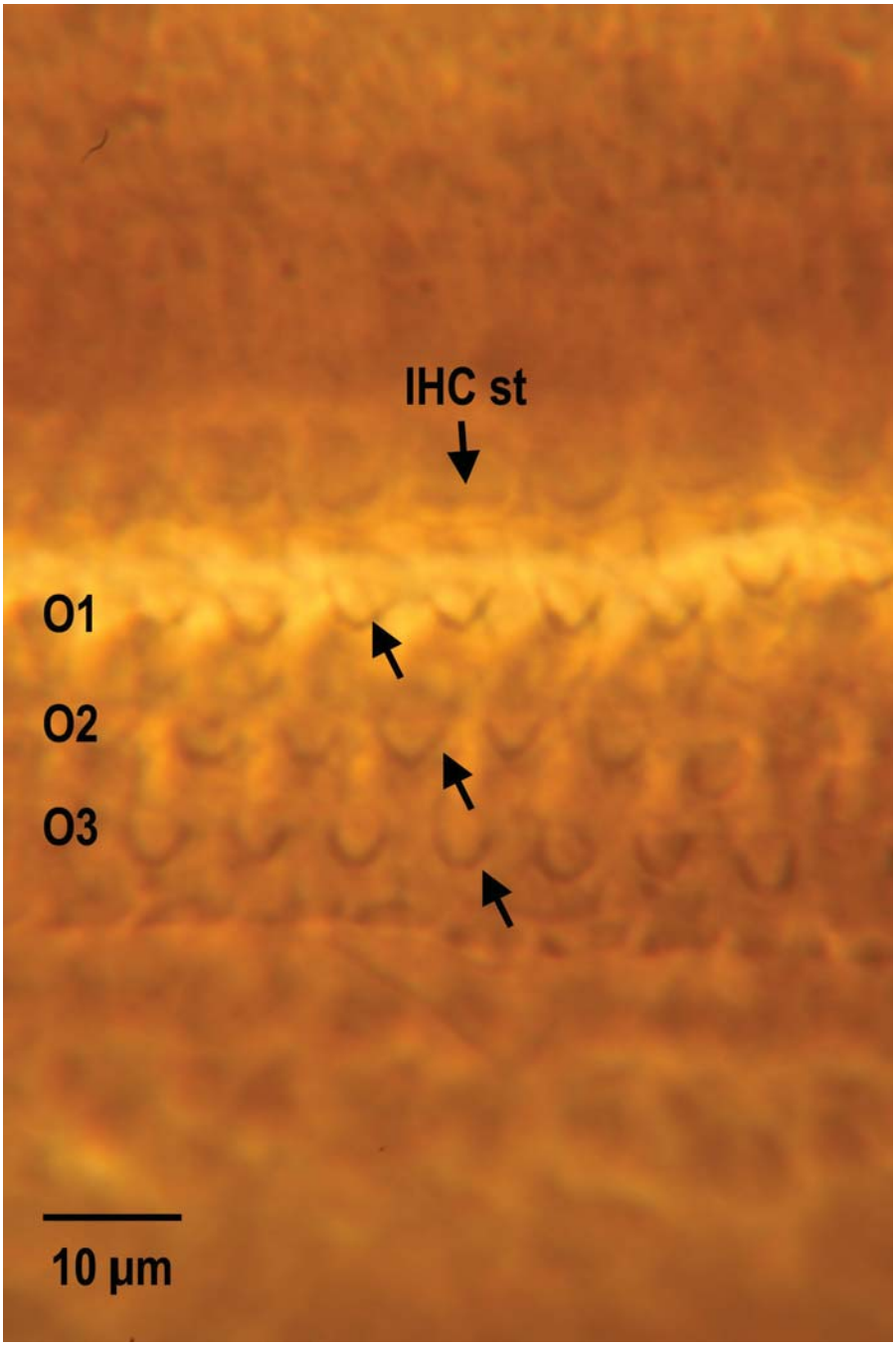


Developing organ of Corti contains identifiable inner (I) and outer hair cells (1, 2, 3). Supporting cells (S), with their nuclei near the basilar membrane, have not yet matured. Neither the tunnel nor the Nuel spaces have formed. The vessel of the basilar membrane (VBM) is quite prominent at this stage. ES - endolymphatic space; IS - inner sulcus; L- limbus; P - perilymphatic space; TM - tectorial membrane.

9-day-old mouse cochlea

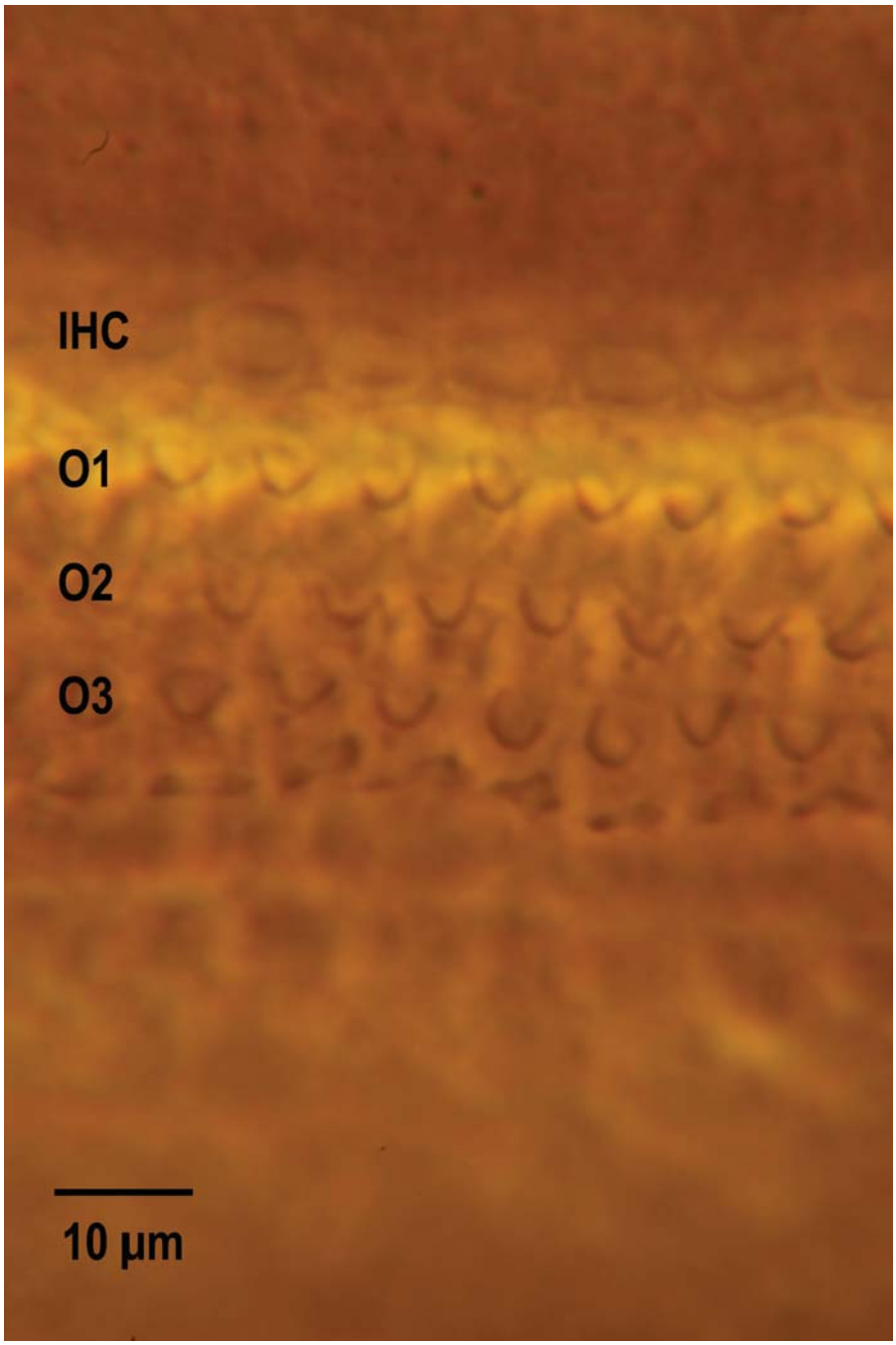
The hair cells and their stereocilia are differentiated. However, the supporting cells have not completely differentiated. The large blood vessel that runs beneath the tunnel space during development of the organ of Corti shuts down after birth. The blood elements that are trapped in the vessel when flow ceases are phagocytized by macrophages.

HAIR-CELL STEREOCILIA



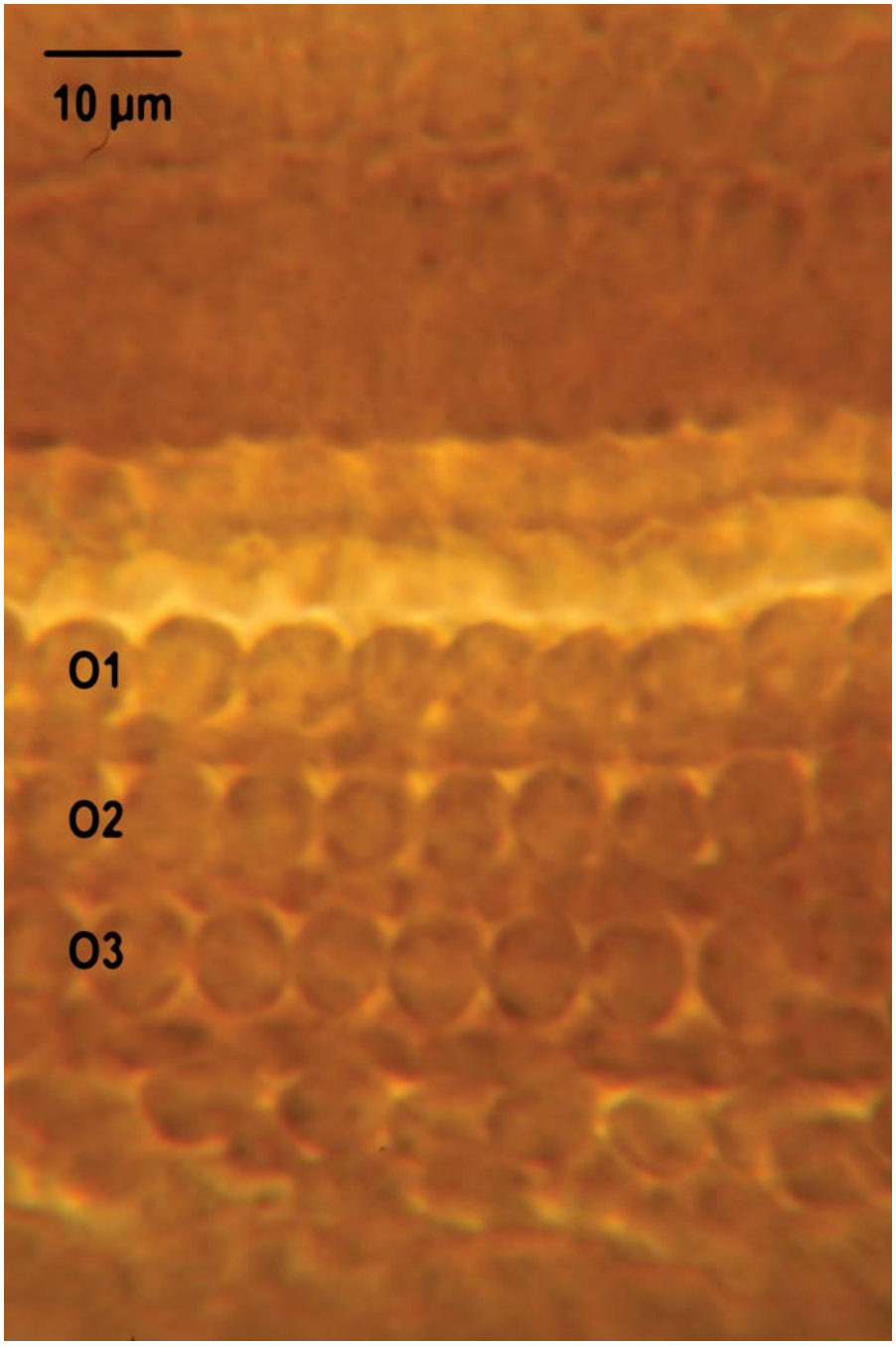
Phase-contrast photomicrograph of the endolymphatic surface of the organ of Corti. Stereocilia form a nearly straight line on the surface of the inner hair cells (IHC st) while they form a 'U'-shaped pattern (arrows) on the surface of the outer hair cells (O1, O2, O3).

HAIR-CELL STEREOCILIA



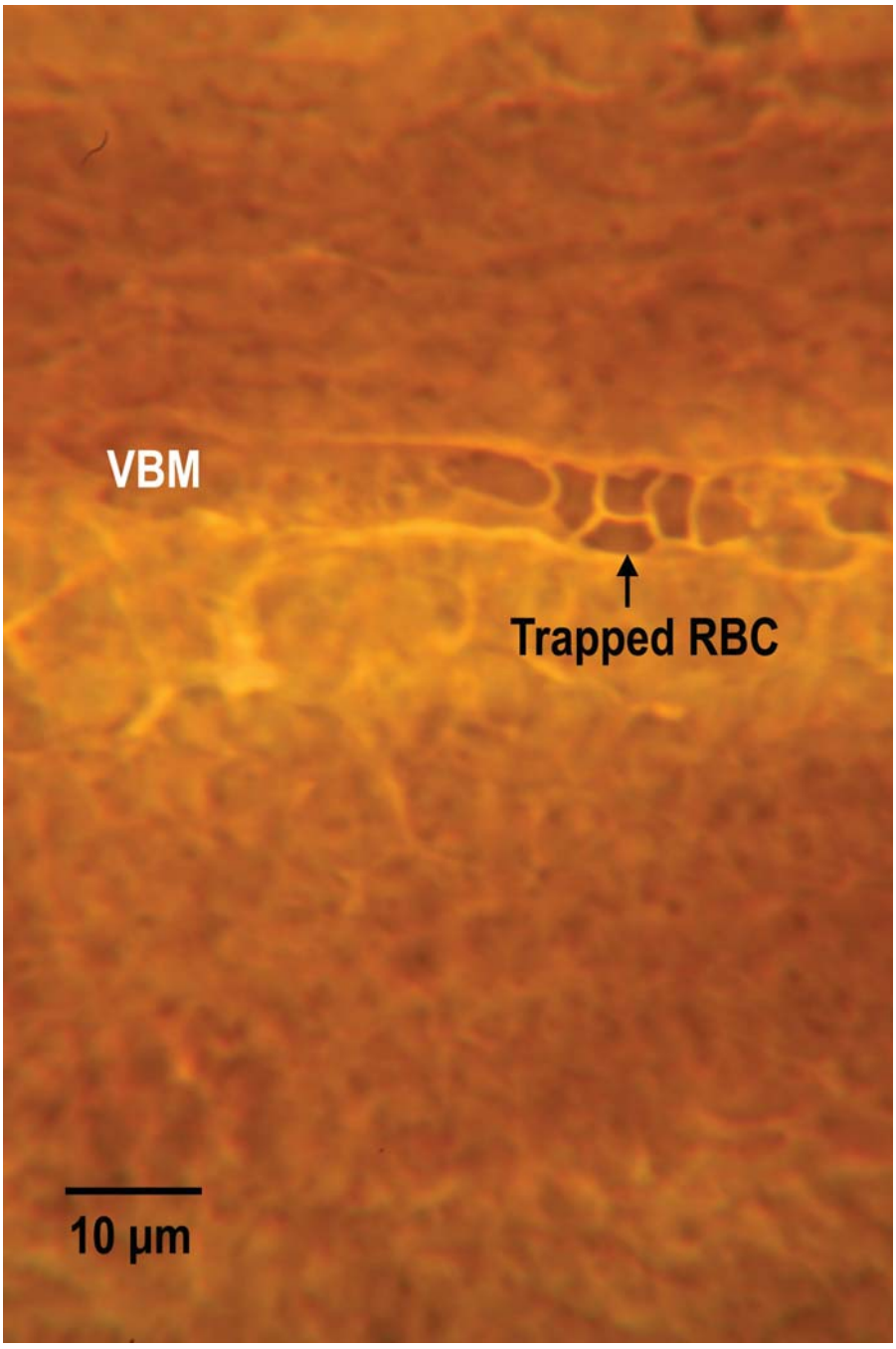
Stereocilia on inner hair cells (IHC) are located fairly close to the three rows of outer hair cells (O1, O2, O3) because the heads of the pillar cells have not yet fully developed.

OUTER HAIR CELL BODIES



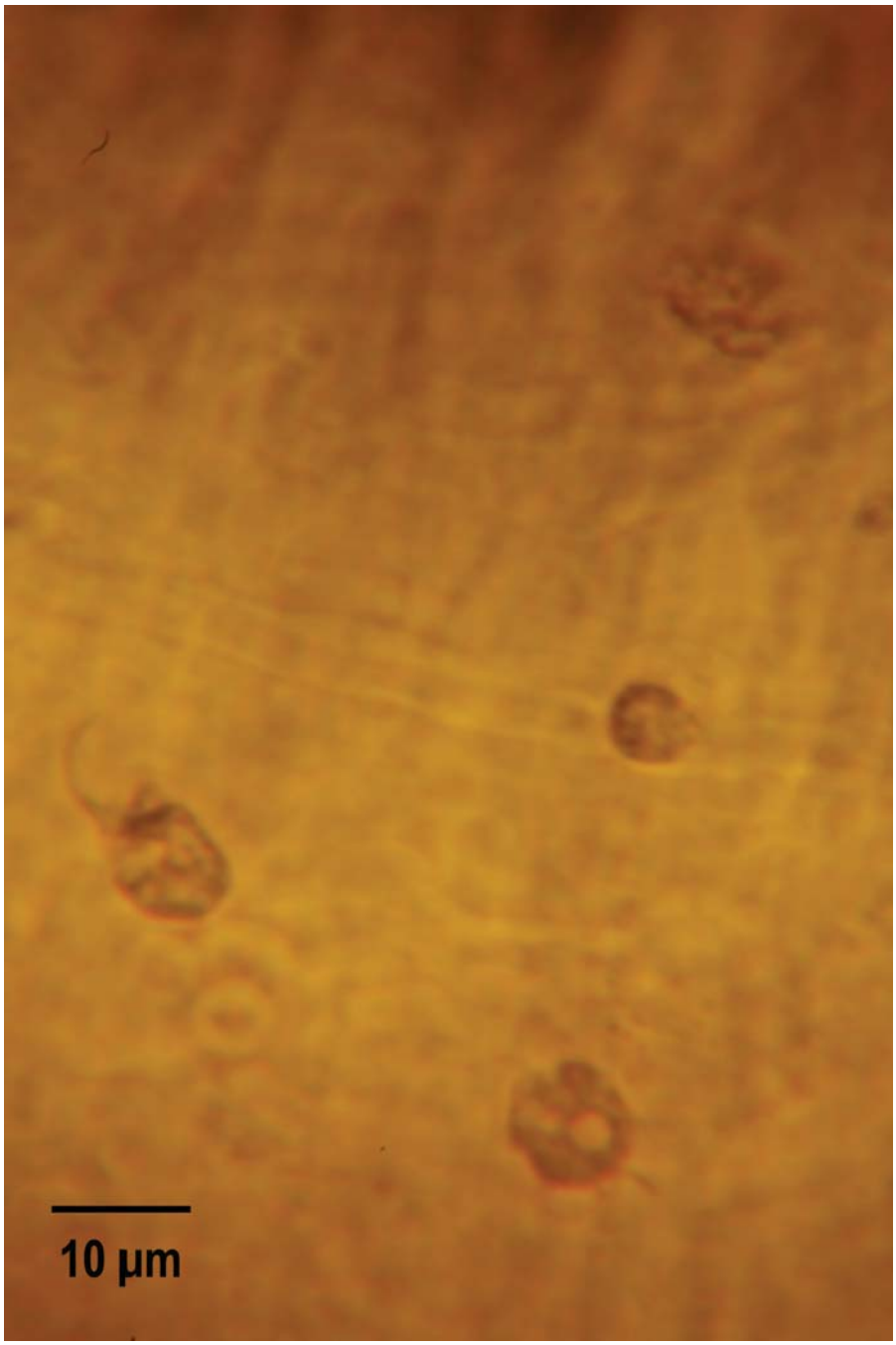
Outer hair cell bodies (O1, O2, O3) have larger diameters than in adult cochleas so they touch side-to-side. The Nuel spaces (i.e., fluid space surrounding OHC bodies) are barely visible.

VESSEL OF THE BASILAR MEMBRANE



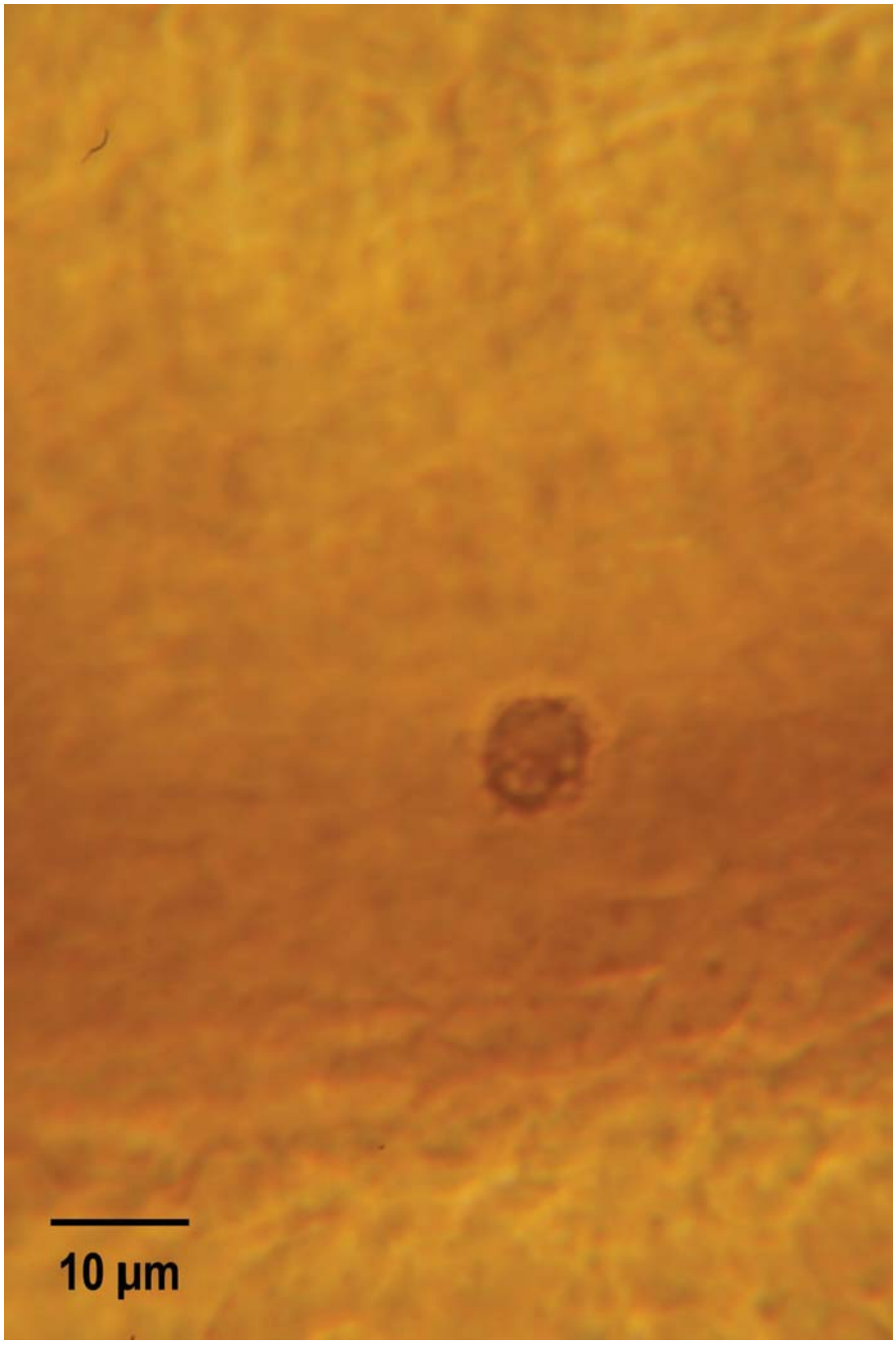
Vessel of the basilar membrane runs beneath the tunnel space. In some species, such as the mouse, this vessel shuts down shortly after birth. Red blood cells and other blood elements that were in the vessel when it shut down become trapped and must be phagocytized.

SCALA-TYMPANI SIDE OF BASILAR MEMBRANE



Scala-tympani side of the basilar membrane. Three macrophages are visible. Presumably these cells came from the blood stream and are phagocytizing the red blood cells that were trapped in the vessel of the basilar membrane when it shut down.

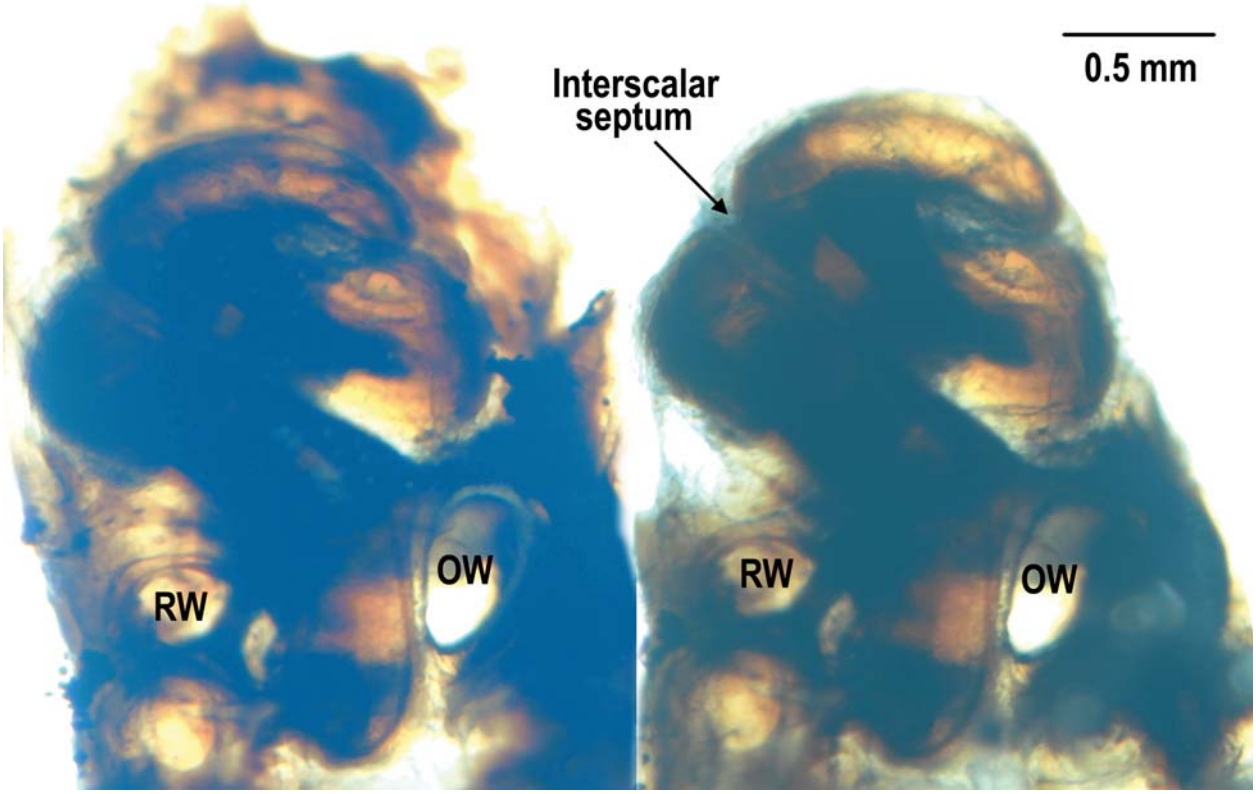
SCALA-TYMPANI SIDE OF BASILAR MEMBRANE



Another macrophage on the basilar membrane. In the non-damaged adult cochlea, this type of cell is rarely seen on the basilar membrane.

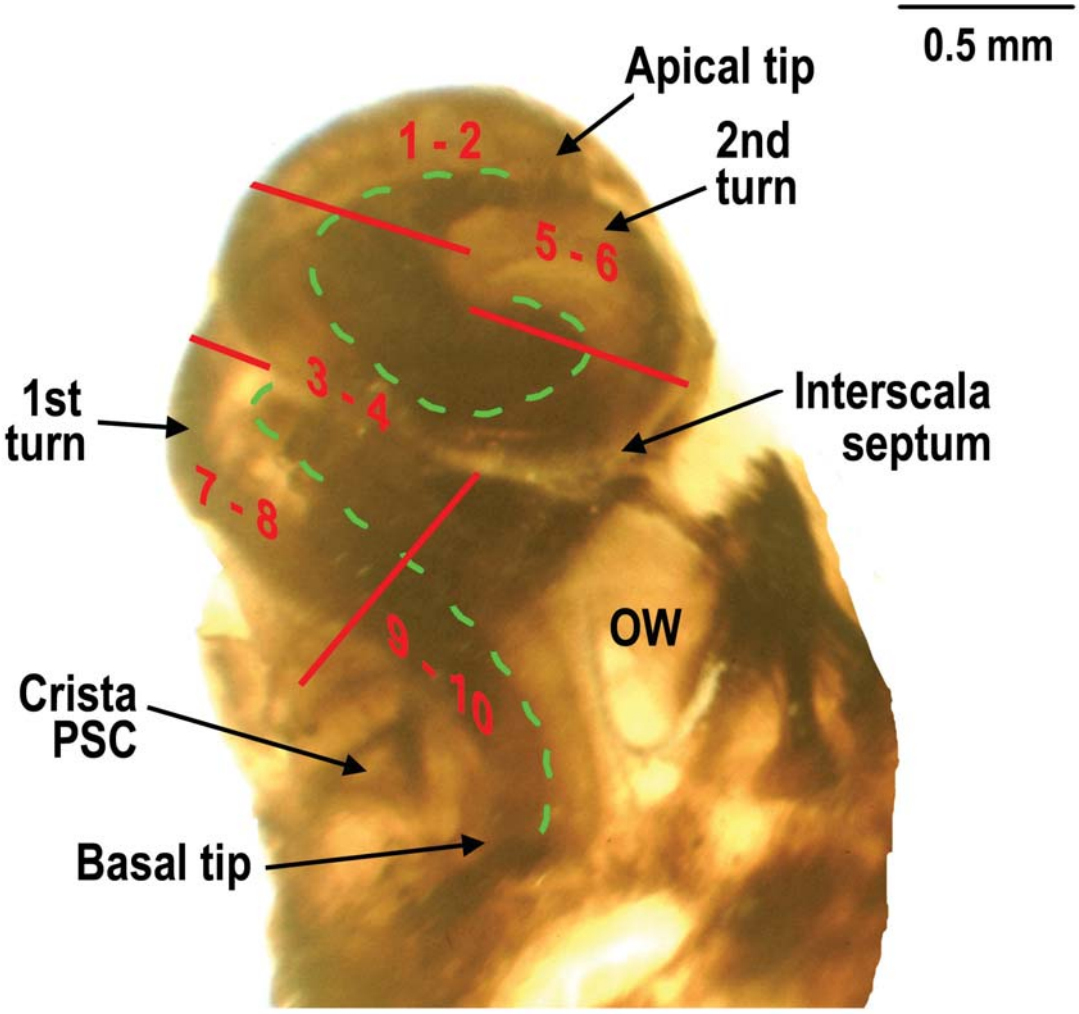
The adult mouse organ of Corti

INTACT MOUSE COCHLEA WITH & WITHOUT COCHLEAR BONE



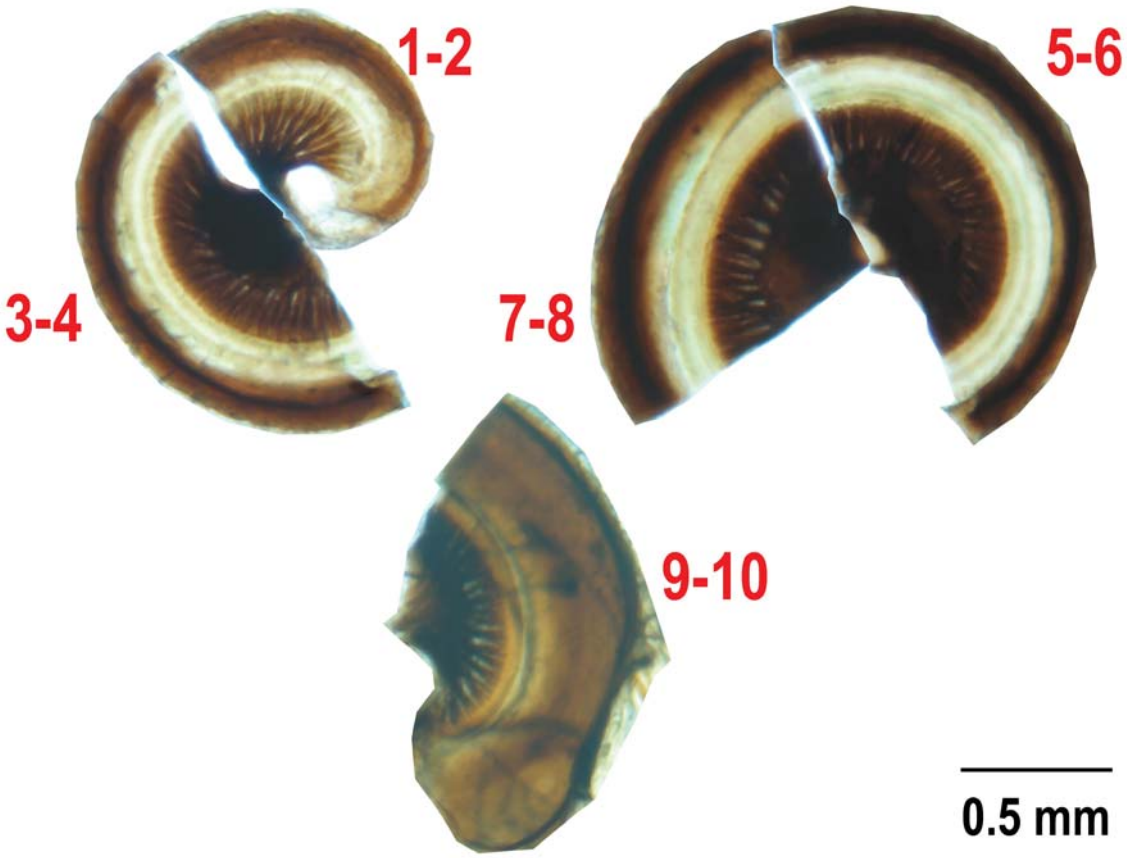
View of left temporal bone (left) from the ventral side after it was fixed with a buffered solution of 1% osmium tetroxide. The temporal bone was removed from the skull, dehydrated then infiltrated with plastic. The thin cochlear bone becomes transparent when the specimen is dehydrated and the entire cochlear duct is visible through the transparent bone. Cochlear bone and marrow spaces can be seen surrounding the cochlear duct. The round window (RW) and oval window (OW) are also visible. At the right, the same cochlea is shown after the cochlear bone was removed with a sharp steel pick. (Photographed with a dissection microscope while specimen was immersed in dish of liquid plastic.)

MOUSE COCHLEA READY FOR DISSECTION INTO FIVE SEGMENTS



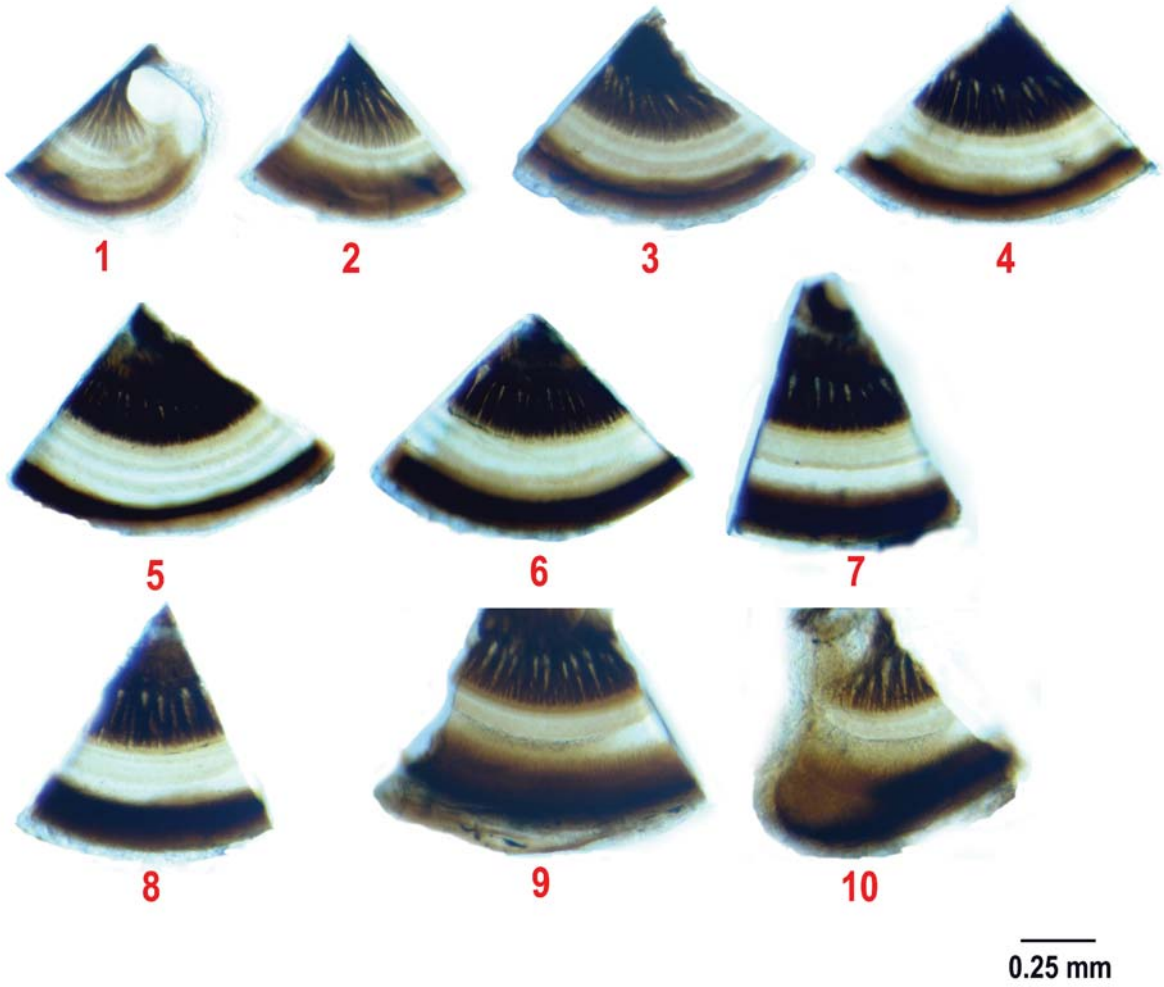
Plastic-embedded left cochlea from which the cochlear bone was removed. The crista of the posterior semicircular canal (PSC) can be seen through the round window membrane (membrane not visible here). The green line indicates the approximate location of the osseous spiral lamina as it spirals from the basal tip to the apical tip of the organ of Corti. The red lines show the locations for making razor blade cuts perpendicular to the basilar membrane to divide the cochlear duct. Cuts parallel to the basilar membrane are made through the interscalar septum to separate five half-turn segments (i.e., 1-2, 3-4, 5-6, 7-8, 9-10) from the remainder of the temporal bone during the first dissection.

OC SEGMENTS OBTAINED FROM 1ST DISSECTION OF MOUSE COCHLEA



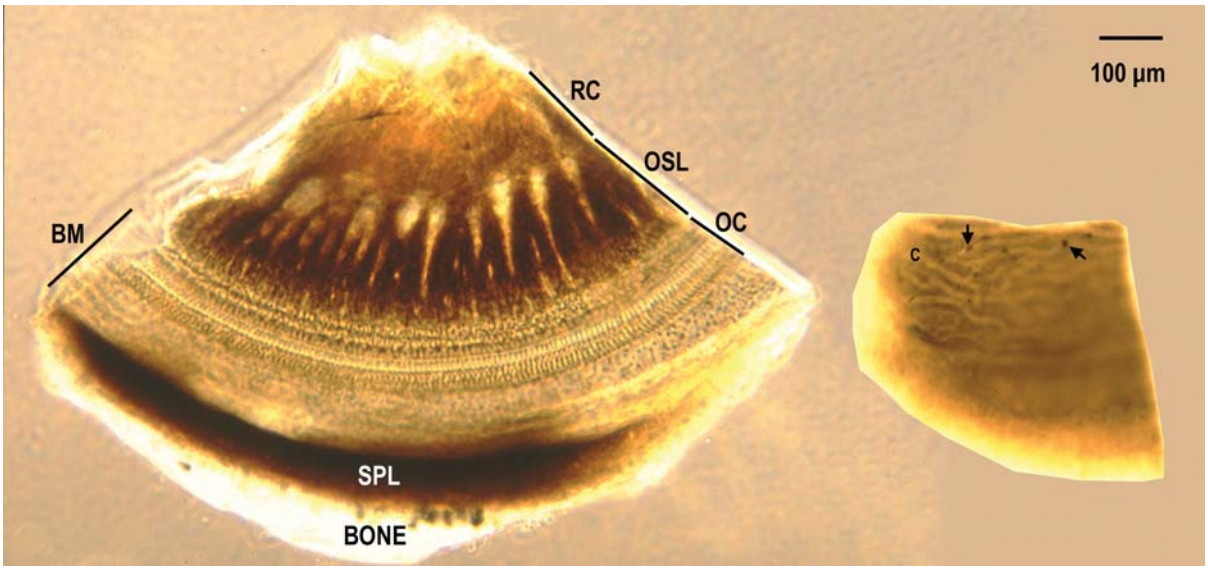
A first dissection of the cochlea shown on the preceding page was performed to divide the cochlear duct into five segments. The segments were removed in numerical order from the apical tip (i.e., 1-2) to the basal tip (i.e., 9-10). (Dissection microscope; photographed in liquid plastic).

OC SEGMENTS OBTAINED FROM 2ND DISSECTION OF MOUSE COCHLEA



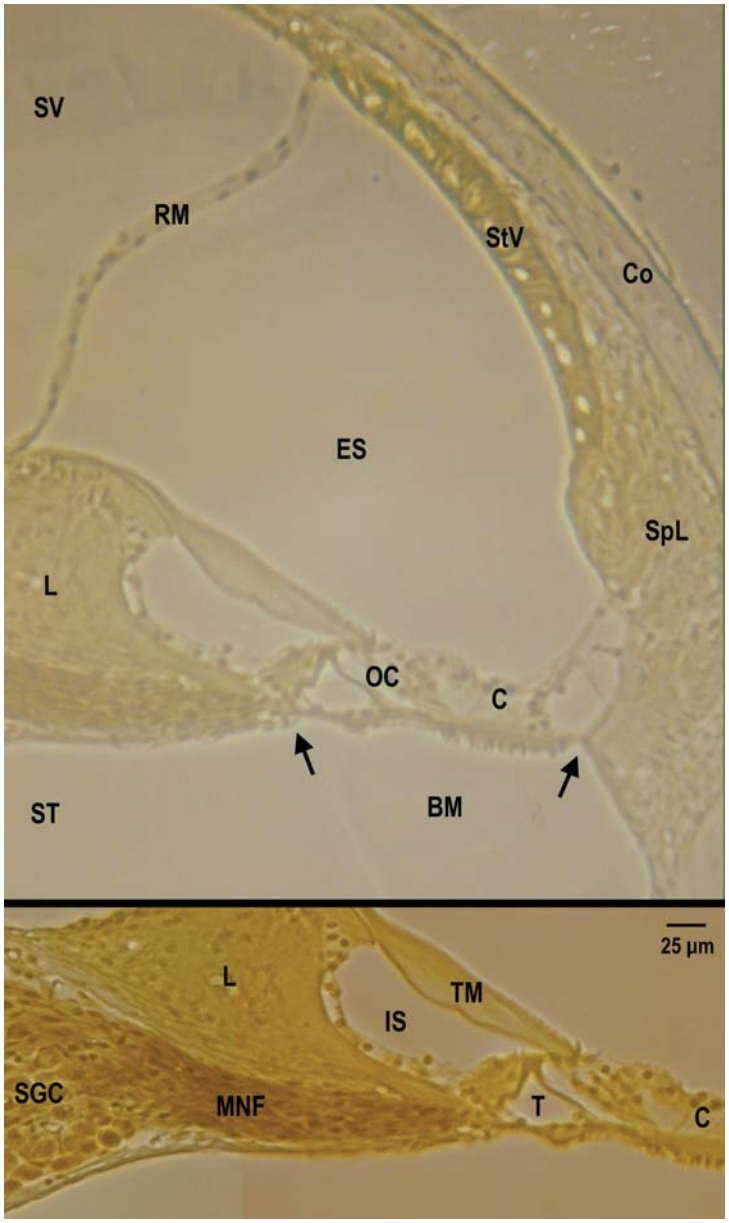
Each segment of cochlear duct shown on the preceding page was divided in half during the second dissection to obtain 10 segments of the cochlear duct (#1 - apex; #10 - base). Each segment is not a standard length. After the second dissection, the length of each segment is accurately measured. Thus, if one segment is shorter than usual, another one will be longer than usual but the total length of the cochlear duct will be the same. The guiding principles for dissection are to be certain that the segments lie flat after the second dissection and to avoid cutting through any focal lesions in the organ of Corti. (Dissection microscope; photographed in liquid plastic).

DISSECTION MICROSCOPE VIEW OF ONE SEGMENT OF COCHLEAR DUCT



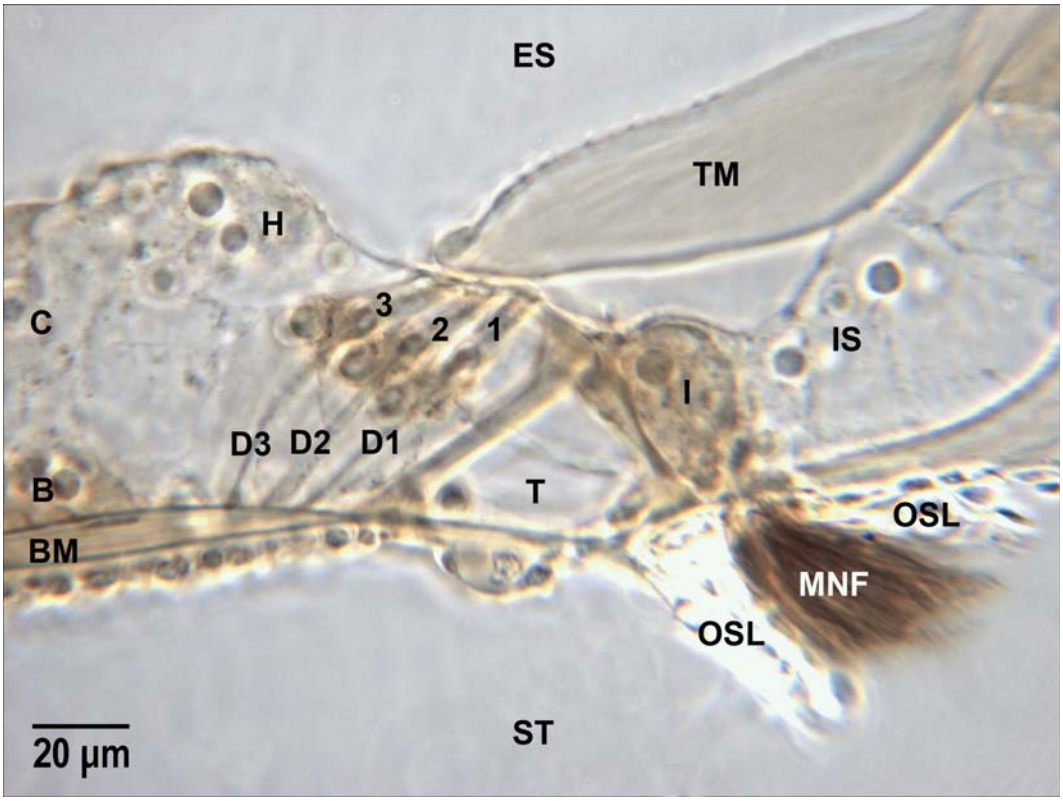
Low-power phase-contrast photomicrograph of a dissected quarter-turn segment of the cochlear duct (to left), viewed from scala vestibuli. Primary auditory neurons or spiral ganglion cells are located in Rosenthal's canal (RC). The peripheral processes of these cells are myelinated and form small bundles as they pass through the osseous spiral lamina (OSL). In this specimen, the fibers appear dark because osmium turns lipids black (myelin has a high content of lipid). These nerve fibers lose their myelin sheaths before crossing the basilar membrane (BM) and entering the organ of Corti (OC). The stria vascularis and spiral ligament (SPL) that form the lateral wall of the cochlear duct are oriented perpendicular to the basilar membrane. A small portion of the stria vascularis and spiral ligament was removed from the right edge of the segment during the second dissection. It was placed flat in a dissection dish (stria side up) for the photograph. The dense capillary network (C) in the stria vascularis appears as light, irregular bands because the blood was washed out of the vessels during the vascular perfusion. The arrows point to melanocytes near the capillary network (unstained surface preparation; dissection microscope).

RADIAL SECTION OF COCHLEAR DUCT & ORGAN OF CORTI



Thick, unstained radial section of the cochlear duct. The endolymphatic space (ES) is bounded by Reissner's membrane (RM), the stria vascularis (StV), and the organ of Corti (OC) attached to the basilar membrane (BM). The primary auditory neurons or spiral ganglion cells (SGC) are found in Rosenthal's canal, located at the periphery of the modiolus. Their peripheral processes are myelinated (MNF) as they extend through the osseous spiral lamina to innervate hair cells. C - Claudius cell; Co - cochlear bone; IS - inner sulcus; L - limbus; SpL - spiral ligament; T - tunnel; TM - tectorial membrane phase contrast).

THICK, RADIAL SECTION OF ORGAN OF CORTI



Phase-contrast photomicrograph of a thick radial section through the organ of Corti in the second turn. The organ of Corti is attached to the basilar membrane (BM) and projects into the endolymphatic space (ES). Scala tympani (ST) is separated from the endolymphatic space by the basilar membrane (BM). The fluid spaces of the organ of Corti (e.g., T - tunnel) are filled with a perilymph-like fluid rather than endolymph.

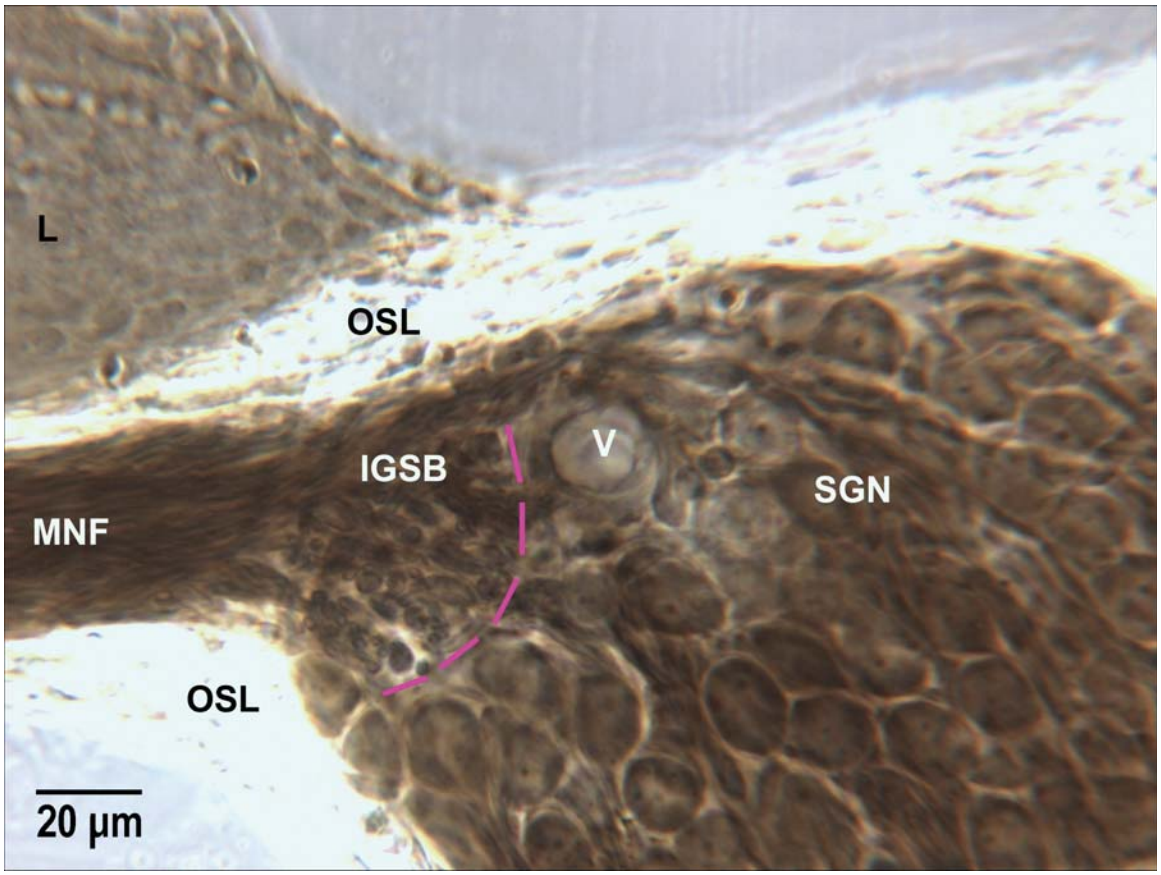
The inner hair cells (I) form a single row near the lateral edge of the osseous spiral lamina (OSL). The outer hair cells (1, 2, 3) are arranged in 3 rows over the middle of the basilar membrane. Inner pillars, outer pillars and the 3 rows of Deiters' cells (D1, D2, D3) contain compact bundles of intracellular microtubules that appear as dark streaks which run at an angle to the BM. These supporting cells help maintain the hair cells in their proper orientation in the organ of Corti. IPs are adjacent to the IHCs; OPs are adjacent to the 1st row OHCs. The bodies of 1st row Deiters' cells (D1) are below the 1st row of OHCs. The bodies of 2nd row Deiters' cells (D2) are below the 2nd row of OHCs. The bodies of 3rd row Deiters' cells (D3) are below the 3rd row of OHCs. Supporting cells without intracellular microtubules that are visible in this photomicrograph are inner sulcus (IS), Hensen (H), Claudius (C) and Boettcher (B) cells. The inner phalangeal cells between adjacent IHCs are not visible. Myelinated peripheral processes (MNF) of the spiral ganglion cells enter the organ of Corti by passing through a series of small holes in the spiral lamina called 'habenulae perforata'. There is approximately one habenula per IHC. (cont p 27)

Within the organ of Corti, the nerve fibers form either spiral bundles (i.e., inner spiral bundle, tunnel spiral bundle, outer spiral bundles) or run in a radial direction to the IHCs or across the tunnel to the OHCs.

Projecting from the apical surface of each hair cell is a group of elongated microvilli called stereocilia. The tallest OHC stereocilia project into the inferior surface of the tectorial membrane (TM) while the tallest IHC stereocilia abut on the lateral side of Hensen's stripe, located on the inferior surface of the tectorial membrane. Motion of the basilar membrane/organ-of-Corti complex relative to the tectorial membrane deflects the hair-cell stereocilia, opens transduction channels in their tips, depolarizes the cells which then release packets of neurotransmitter at their basal poles. The transmitter depolarizes the afferent nerve terminals applied to the basal poles of the hair cells.

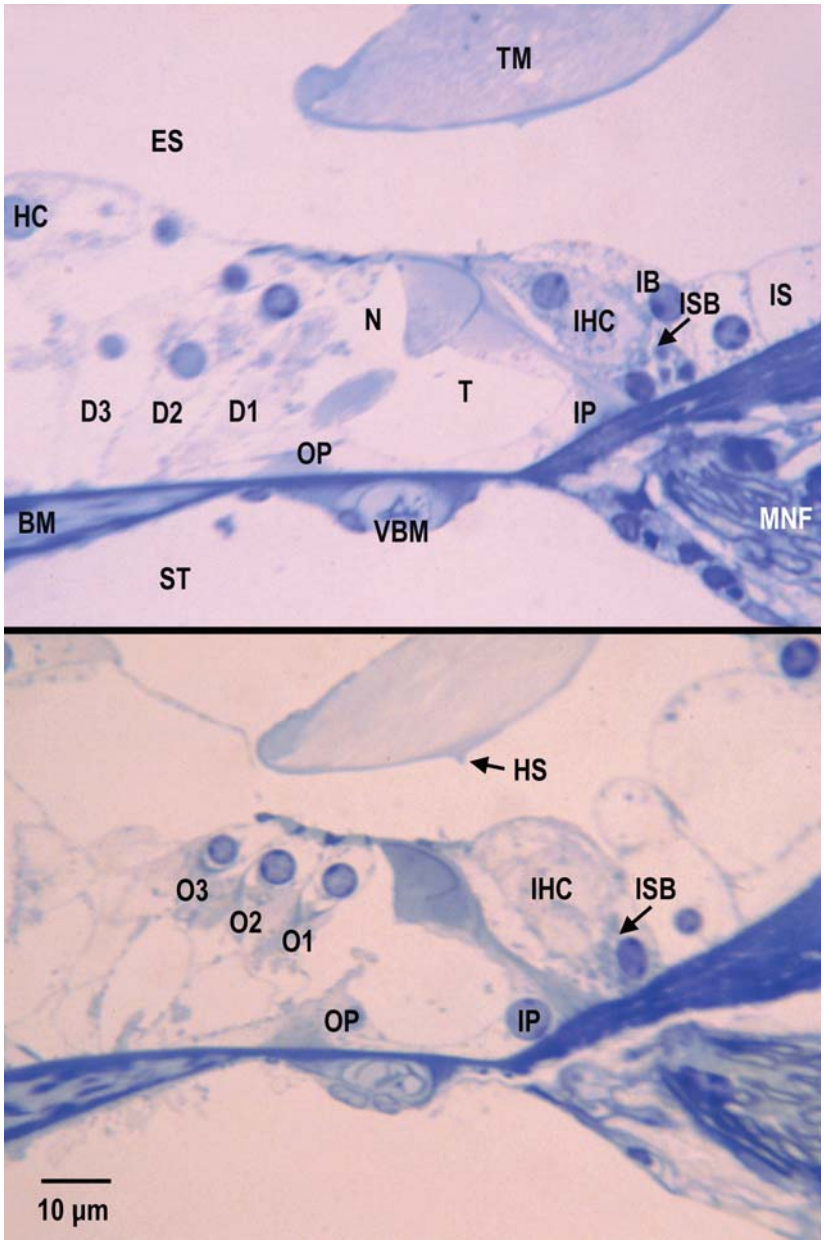
There are no blood vessels within the organ of Corti. The blood vessels nearest the organ of Corti are found on scala-tympani side of the basilar membrane - the vessel of the basilar membrane located directly below the tunnel space and the vessel of the tympanic lip of the osseous spiral lamina located beneath the IHC. According to Kraus and Aulbach-Kraus (1981), the vessel of the basilar membrane in the mouse shuts down around the tenth postnatal day. Mesothelial cells cover the scala-tympani side of the basilar membrane.

SPIRAL GANGLION CELLS IN ROSENTHAL'S CANAL



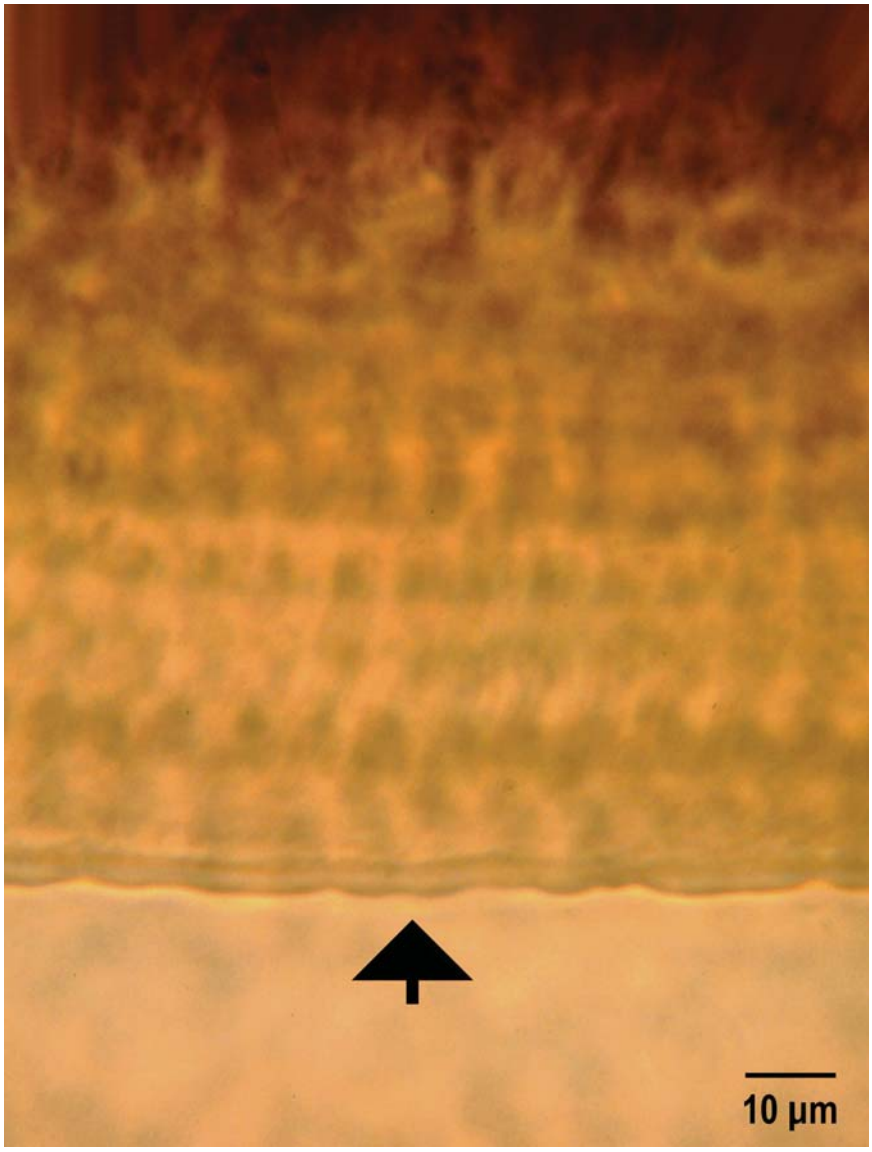
Focused on Rosenthal's canal which is located at the periphery of the modiolus. The bodies of the spiral ganglion cells (SGN) interspersed with myelinated nerve fibers and capillaries (V) fill most of the canal. The medial edge of the osseous spiral lamina (OSL) originates from the bone of the modiolus. The myelinated peripheral processes (MNF) of the spiral ganglion cells fill the OSL. The purple line indicates where the intraganglionic spiral bundle of nerve fibers (efferent) is located.

ONE-MICRON RADIAL SECTIONS OF THE ORGAN OF CORTI



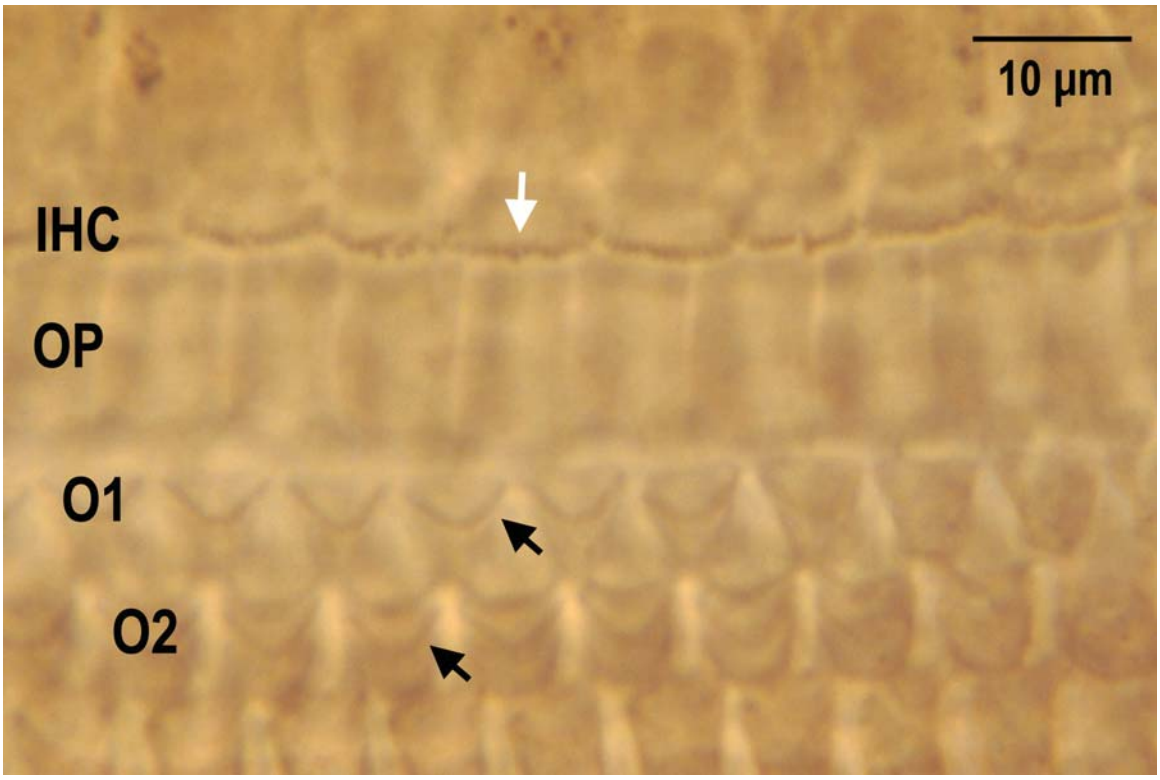
Stained, one-micrometer-thick radial sections of the organ of Corti from an adult mouse. BM - basilar membrane; D1 - first row Deiters' cell; D2 - second row Deiters' cell; D3 - third row Deiters' cell; ES - endolymphatic space; HC - Hensen cell; HS - Hensen's stripe; IB - inner border cell; IHC - inner hair cell; IP - inner pillar; IS - inner sulcus cell; ISB - inner spiral bundle; MNF - myelinated peripheral processes of spiral ganglion cells; N - Nuel spaces; O1, O2, O3 - outer hair cells; OP - outer pillar; ST - scala tympani; T - tunnel space; TM - tectorial membrane; VBM - vessel of the basilar membrane.

TECTORIAL MEMBRANE



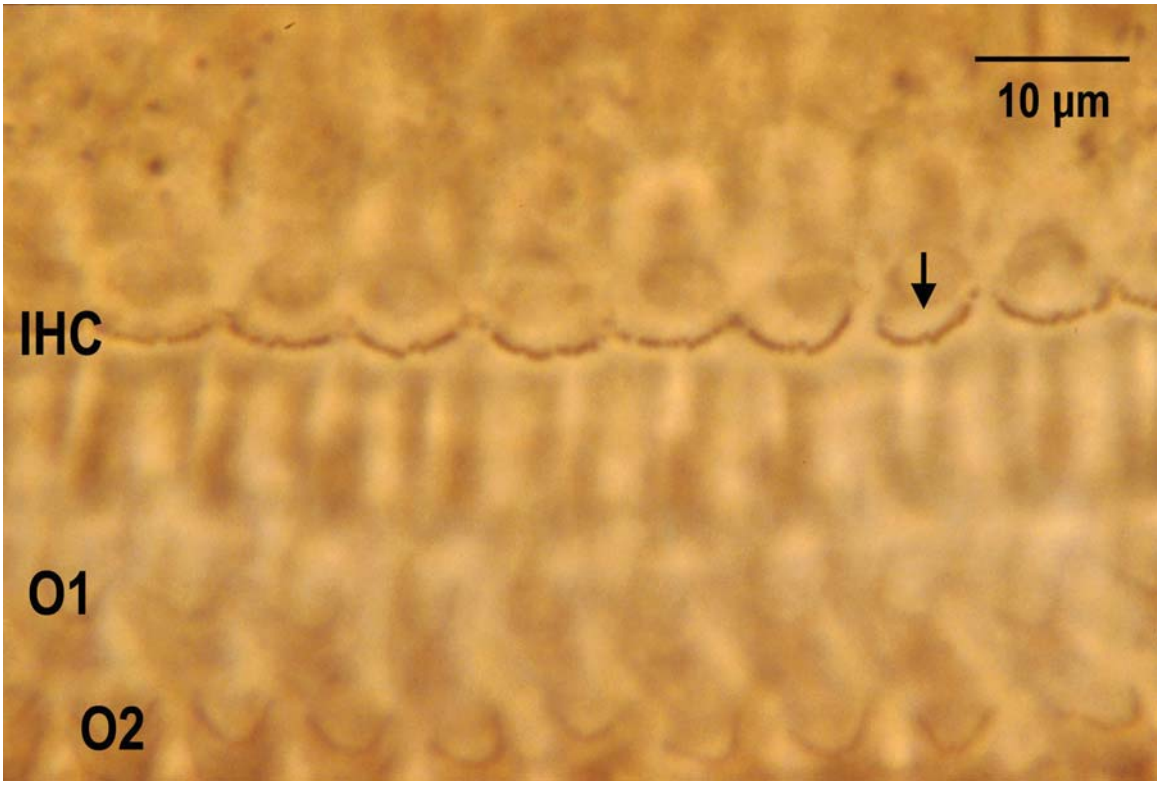
Phase-contrast photomicrograph of a surface preparation from a plastic-embedded segment of the cochlear duct. Because the specimen was embedded in plastic prior to dissection, the tectorial membrane (TM) is visible in the endolymphatic space near the focal plane of the organ of Corti. The arrow points to the rolled-up lateral edge of the TM.

HAIR-CELL STEREOCILIA



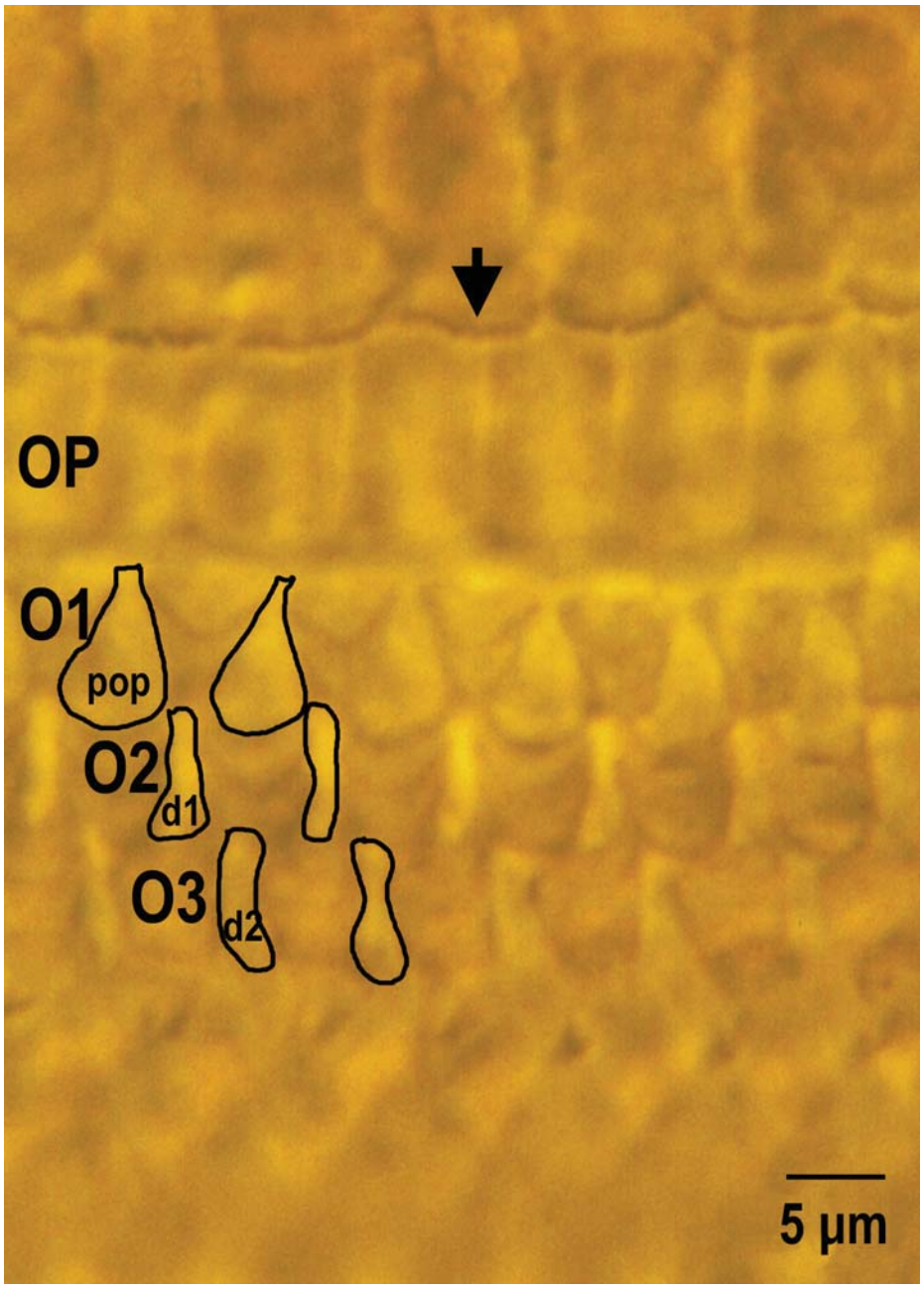
By phase-contrast microscopy, the inner hair cell (IHC) stereocilia (white arrow) appear as dark dots arranged in a nearly straight line across the cell's apical surface. The stereocilia on the outer hair cells (e.g., O1, O2) are smaller in diameter than the IHC stereocilia and cannot be resolved individually by light microscopy. Under normal conditions, OHC stereocilia are arranged in a smooth 'U' or 'V-shape' (black arrows). OP - outer pillar heads.

IHC STEREOCILIA



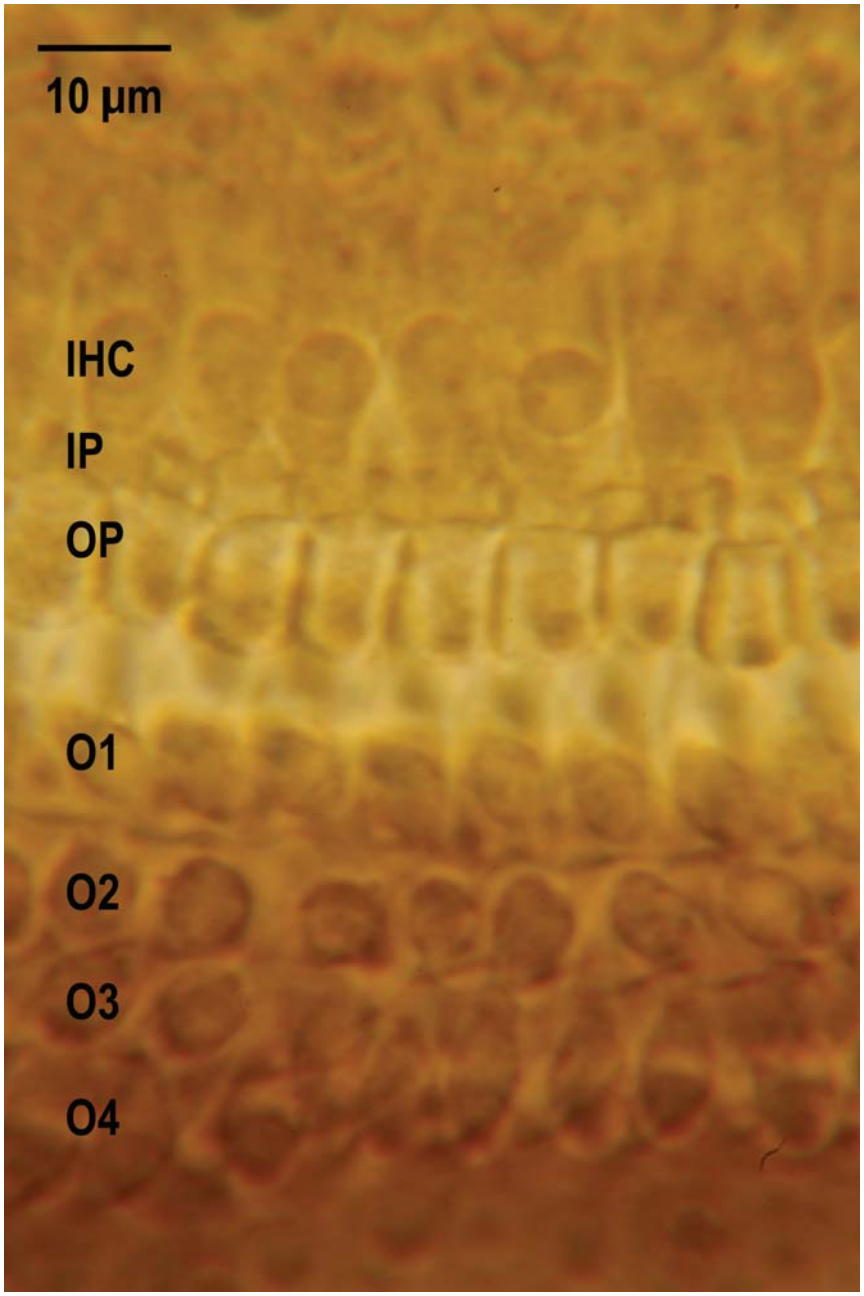
In control cochleae, there may be some variation in the stereocilia arrangement on the inner hair cells (IHC). The stereocilia patterns shown here were graded 0 (i.e., normal). Grade 0 stereocilia are oriented perpendicular to the cell surface and are arranged in a straight or slightly curved line across the cell. Some grade 0 stereocilia may be slightly irregular in position or the pattern more rotated (arrow). These anatomical variations are thought to have no functional significance because the stereocilia links (i.e., side, tip) are mostly intact. O1, O2 - first and second row outer hair cells, respectively.

RETICULAR LAMINA



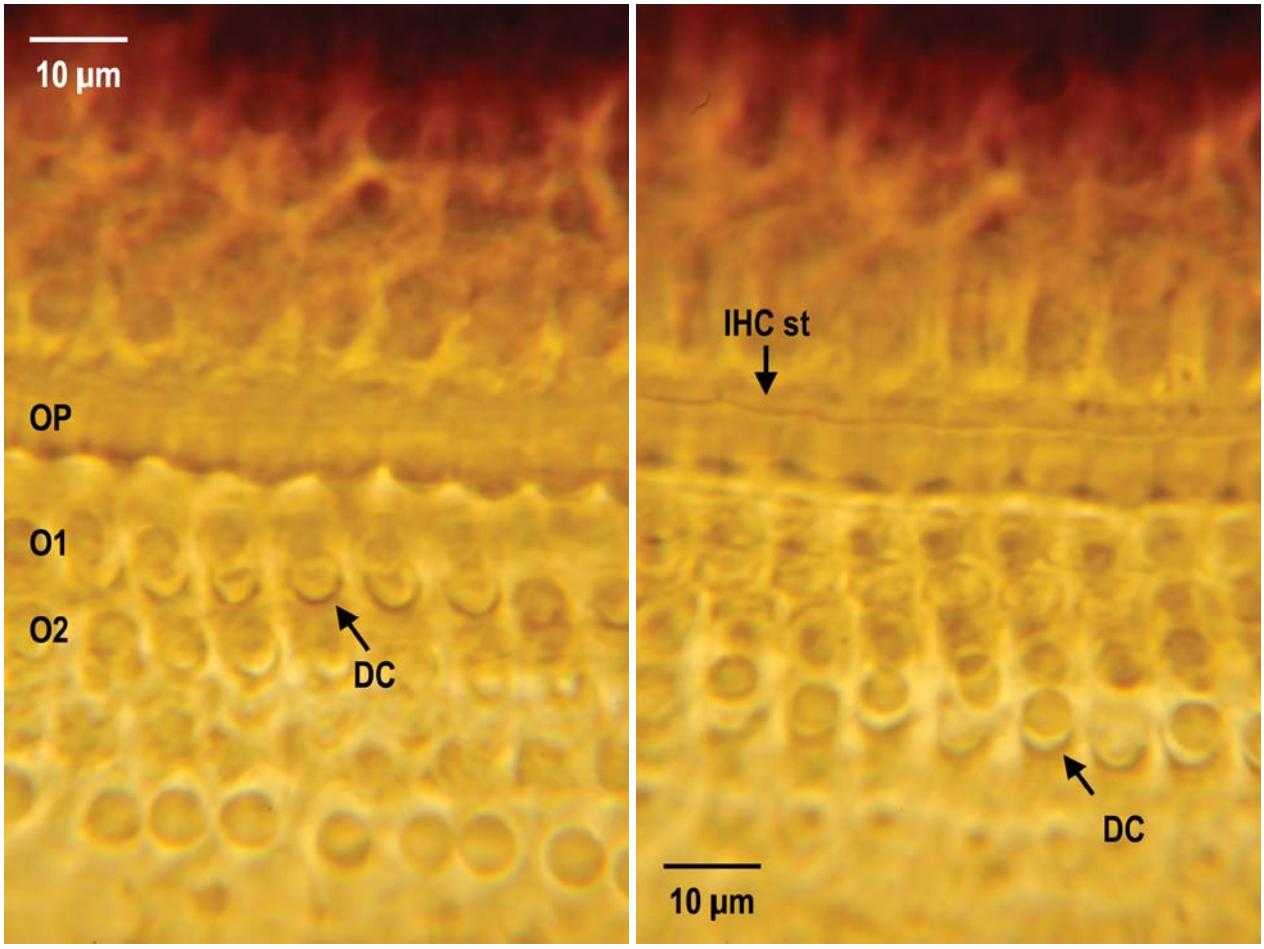
The reticular lamina by the outer hair cells (O1, O2, O3) is formed by the apical surface of the hair cells and phalangeal processes (pop) from the outer pillar head (OP) and the three rows of Deiters' cells (1st row - d1; 2nd row - d2). Not visible here is that portion of the reticular lamina formed by the apical surface of the inner hair cells (IHC), inner phalangeal cells and inner pillar heads. The arrow points to the stereocilia arrangement on the IHCs.

INNER & OUTER HAIR CELL BODIES



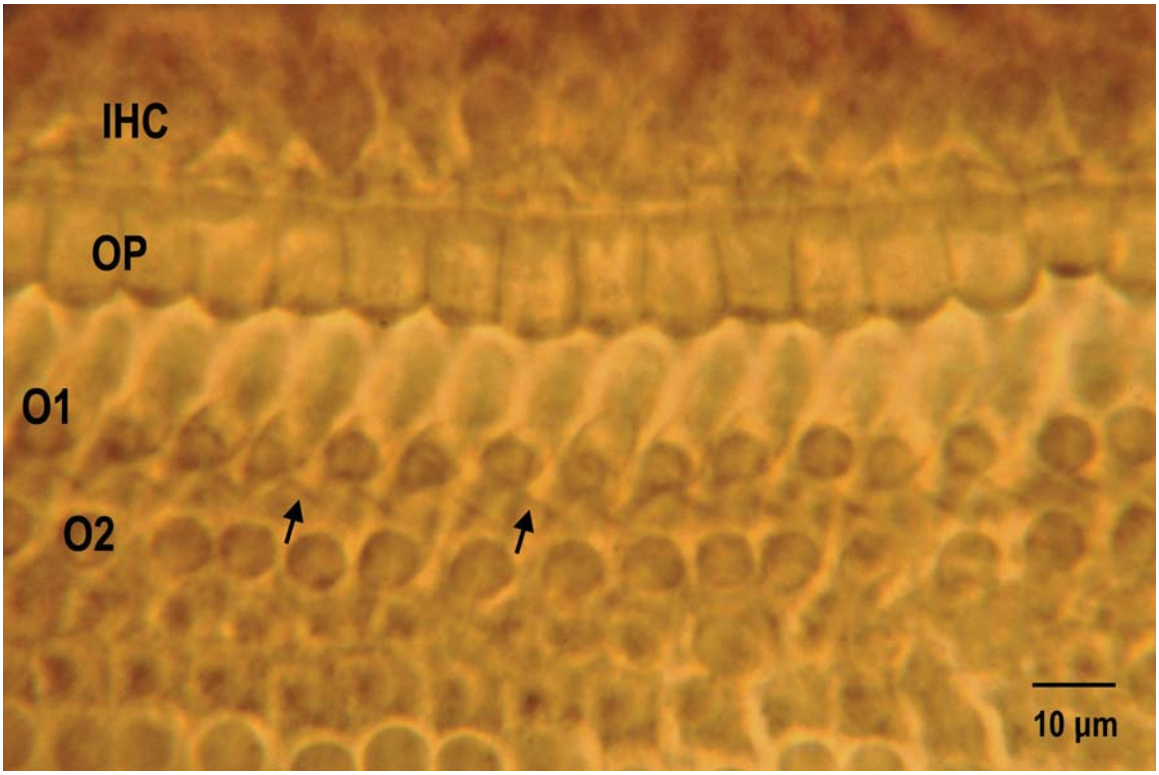
Inner hair cell bodies (IHC) are flask-shaped and have a large central nucleus. Side-to-side, these cells nearly contact one another. Outer hair cells (O1, O2, O3) are generally arranged in three rows, although occasional 4th row OHCs (O4) can be found. The heads of the inner pillars (IP) and outer pillars (OP) separate the row of IHCs from the three rows of OHCs.

DEITERS' CUPS



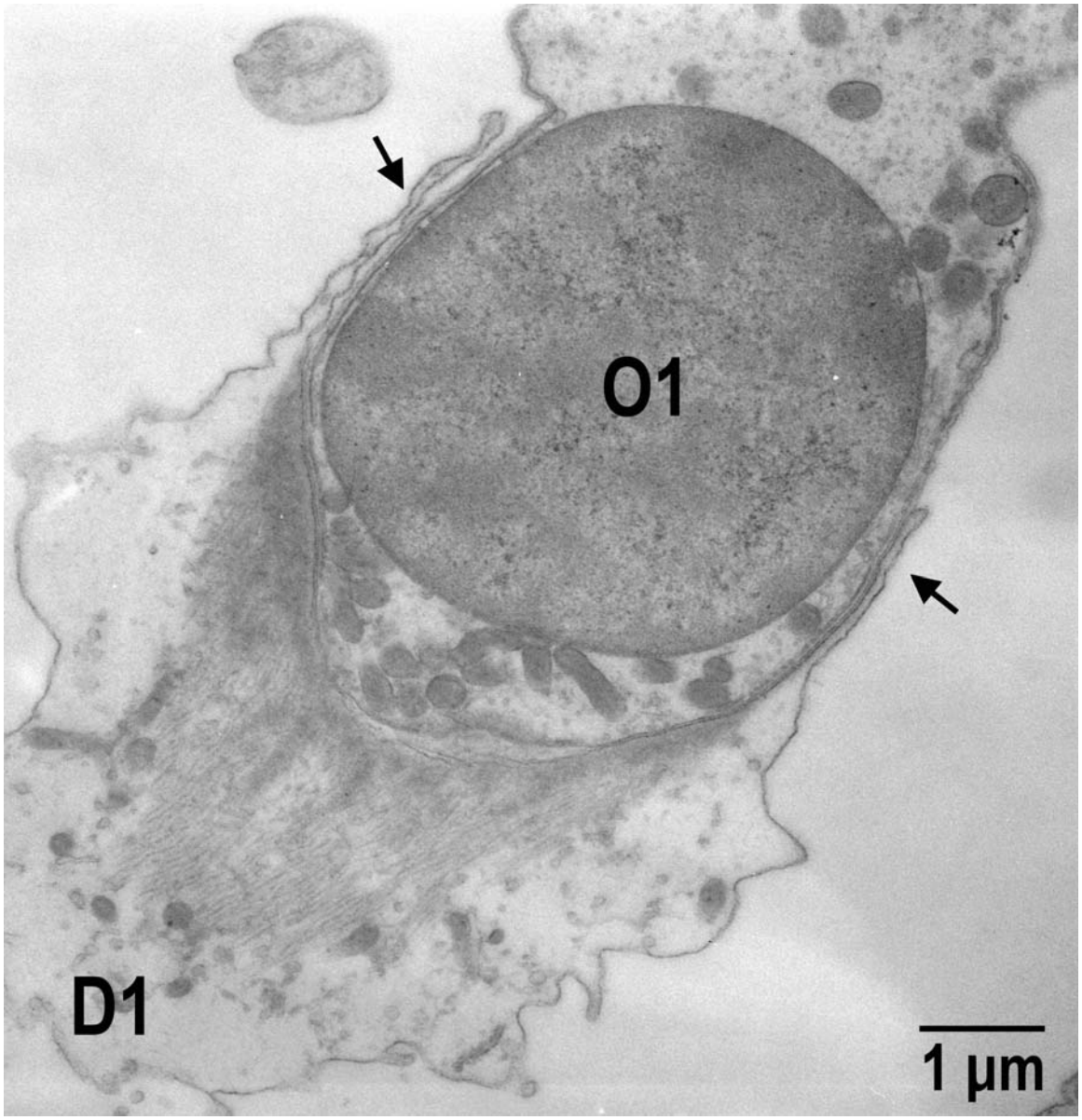
The base of each outer hair cell sits in the cup area of a Deiters cell (DC). Microtubules in each Deiters cell form a dark, crescent-shape beneath each outer hair cell nucleus. IHC st - inner hair cell stereocilia; O1 - first row outer hair cells; O2 - second row outer hair cells; OP - outer pillar heads.

SLENDER STALKS OF DEITERS' CELLS



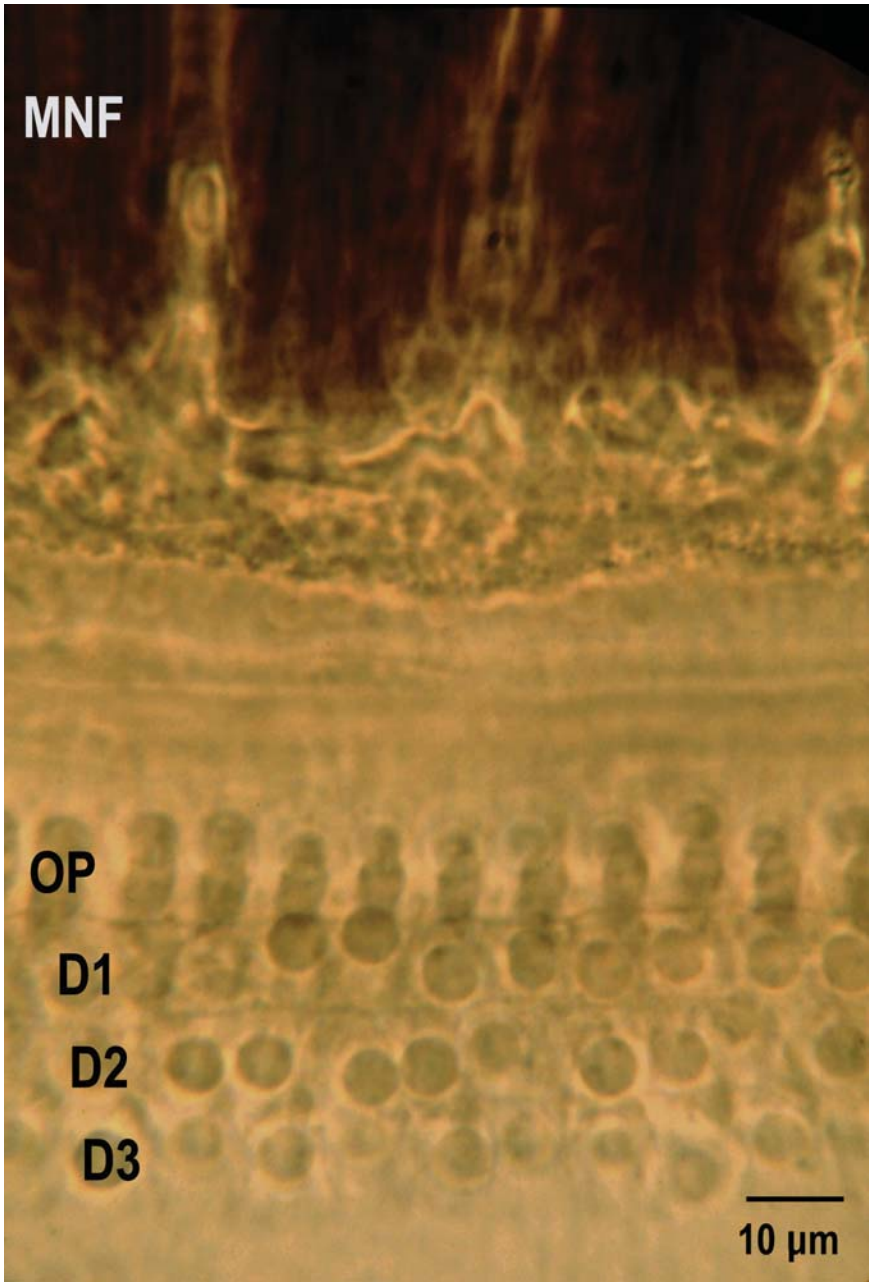
Phase-contrast photomicrograph focused on the bodies of the inner hair cells (IHC) and outer hair cells in the first (O1) and second (O2) rows. The arrows point to the slender processes of the Deiters cells as they run apically across 3-4 OHC bodies while extending from the cell bases on the basilar membrane to the phalangeal processes in the reticular lamina. OP - outer pillar heads.

BASE OF OHC & DEITERS' CUP



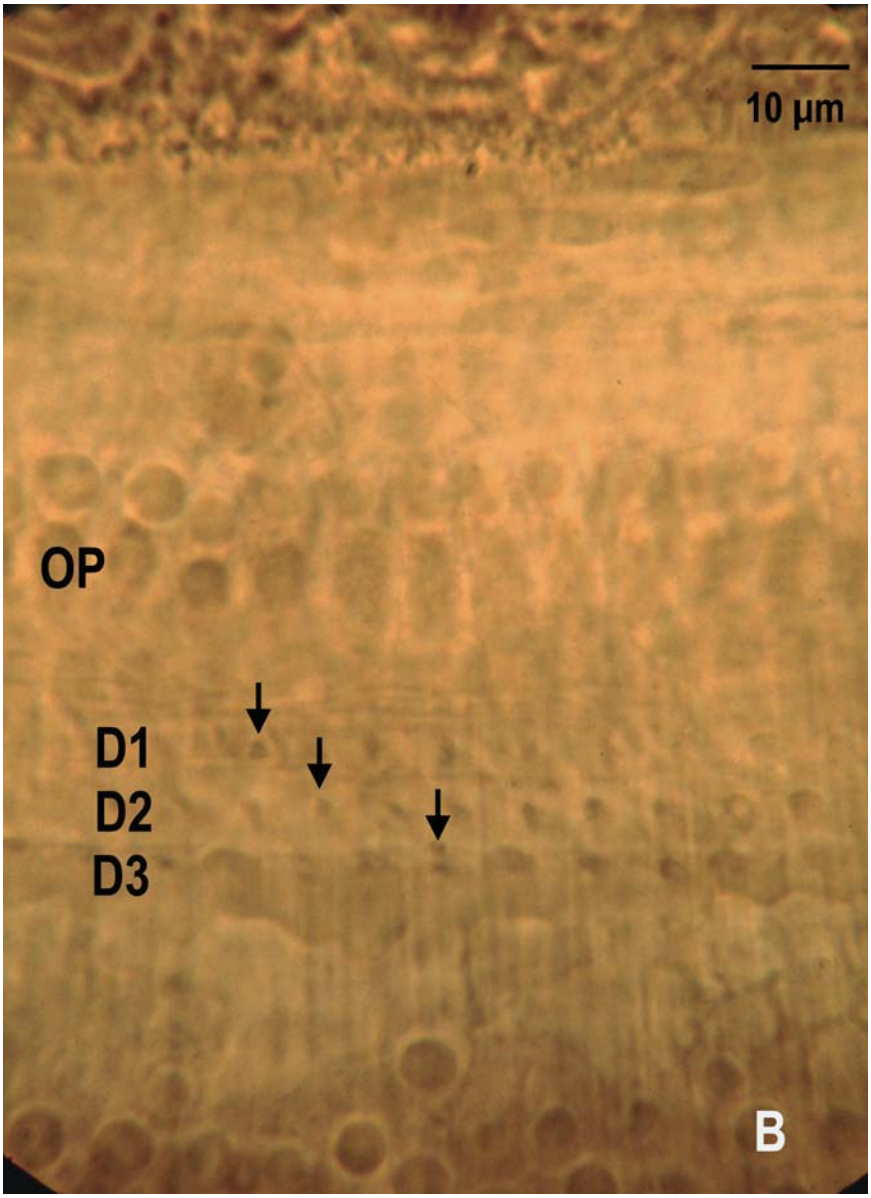
Transmission electron micrograph through the base of a first row outer hair cell (O1) and its associated Deiters' cup (D1). The microtubules in the Deiters cell form a 'V' around the infranuclear region of the hair-cell base. Thin extensions (arrows) of the Deiters cell enclose the hair-cell base as far as its mid-nuclear region.

DEITERS' CELL NUCLEI



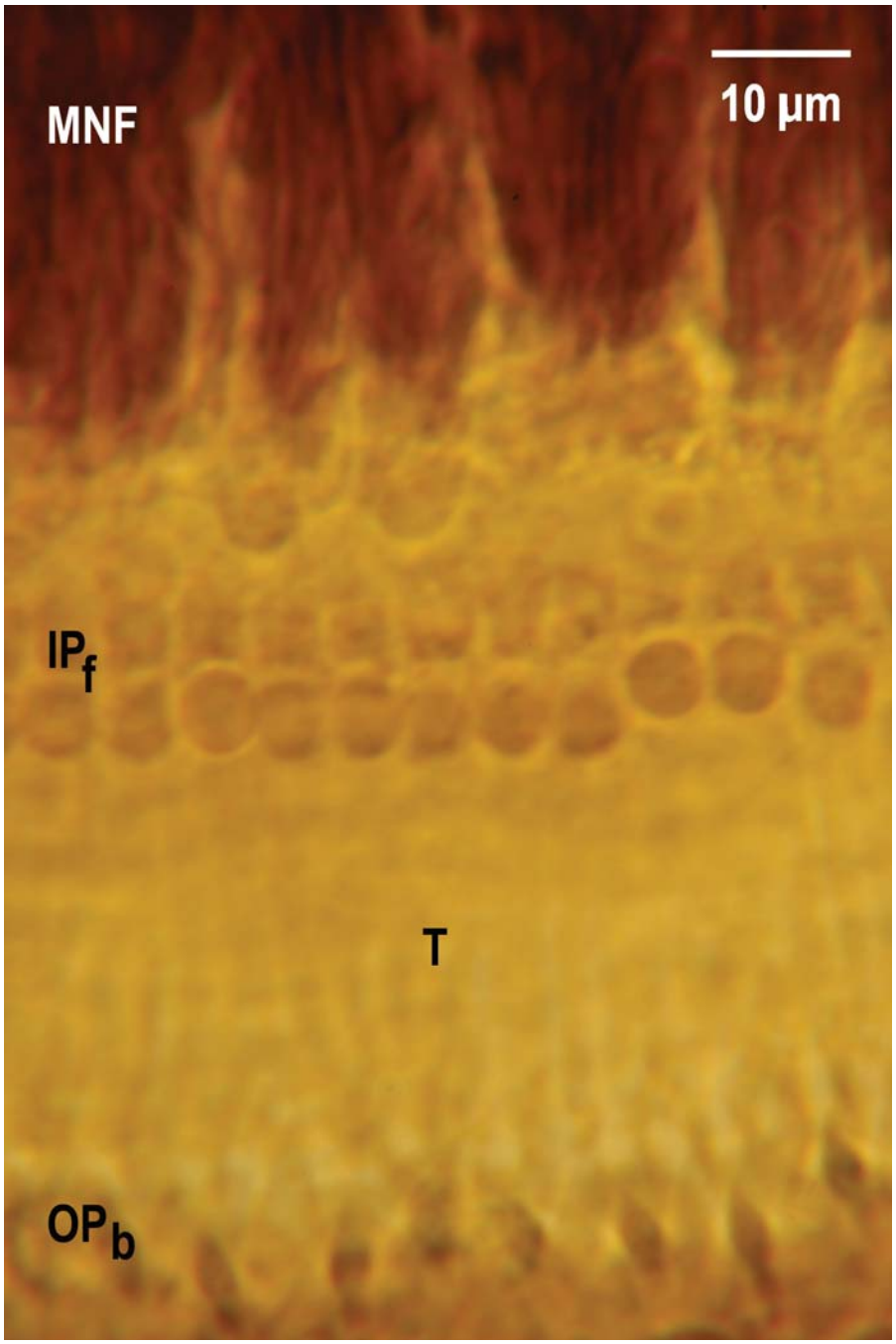
Phase-contrast photomicrograph focused on the organ of Corti approximately midway between the reticular lamina and the basilar membrane. Three rows of Deiters cell nuclei (D1, D2, D3) are visible beneath the corresponding rows of OHCs. Fibers in the outer spiral bundles appear as a faint line running from left to right between the outer pillar bodies (OP) and rows of Deiters' nuclei. MNF - myelinated peripheral processes of spiral ganglion cells.

DEITERS' CELL BASES



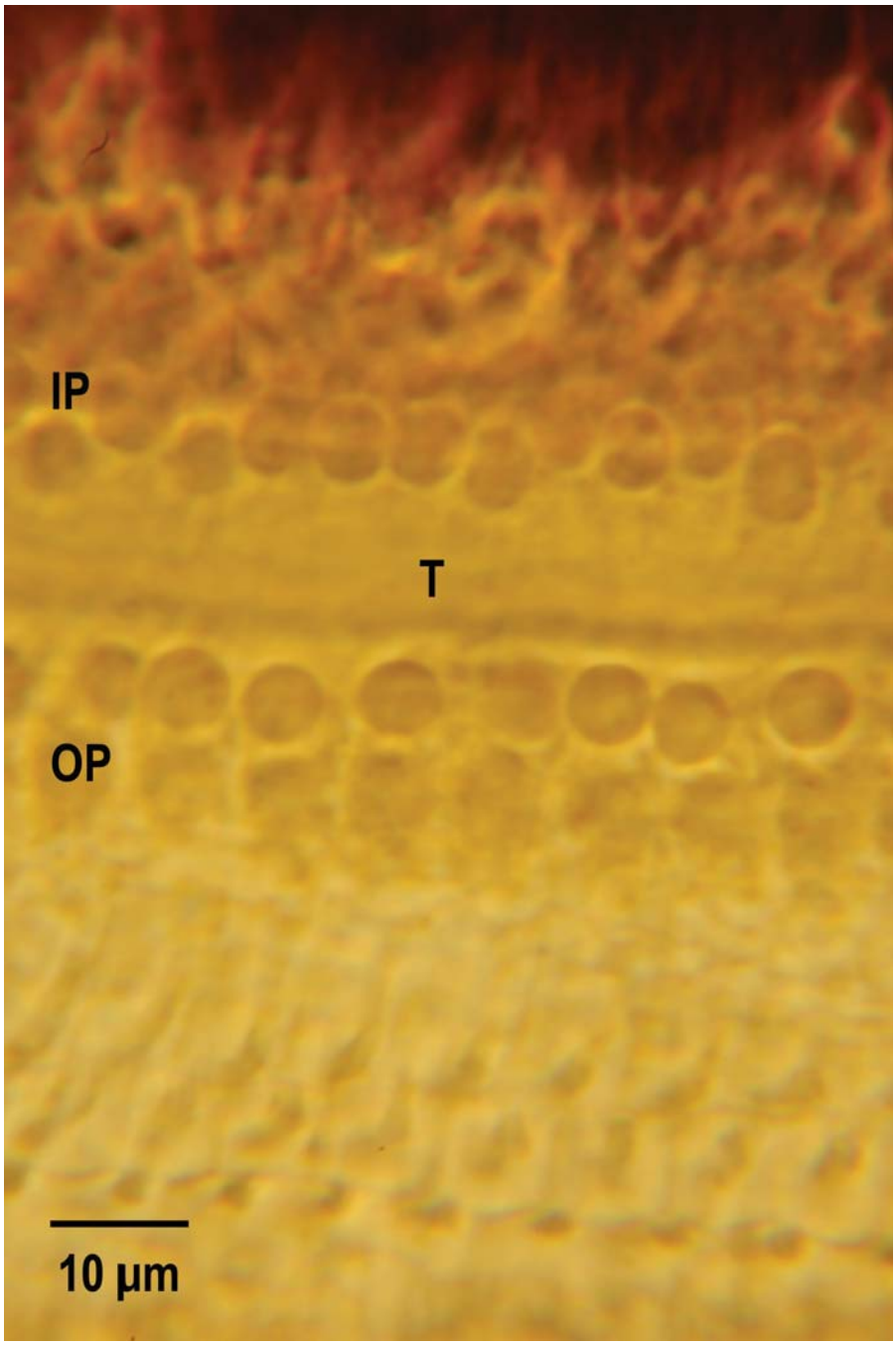
The bases of the Deiters' cells (D1, D2, D3) are in contact with the basilar membrane lateral to the bases of the outer pillars (OP). Each cell contains a small, compact bundle of microtubules (arrows) that extent from the cell base, through the body and slender process, to the phalangeal process in the reticular lamina. The striations running vertically in the lower third of the photomicrograph are fibers in the basilar membrane. B - Boettcher cells.

FEET & BODIES OF PILLAR CELLS & TUNNEL SPACE AT APEX



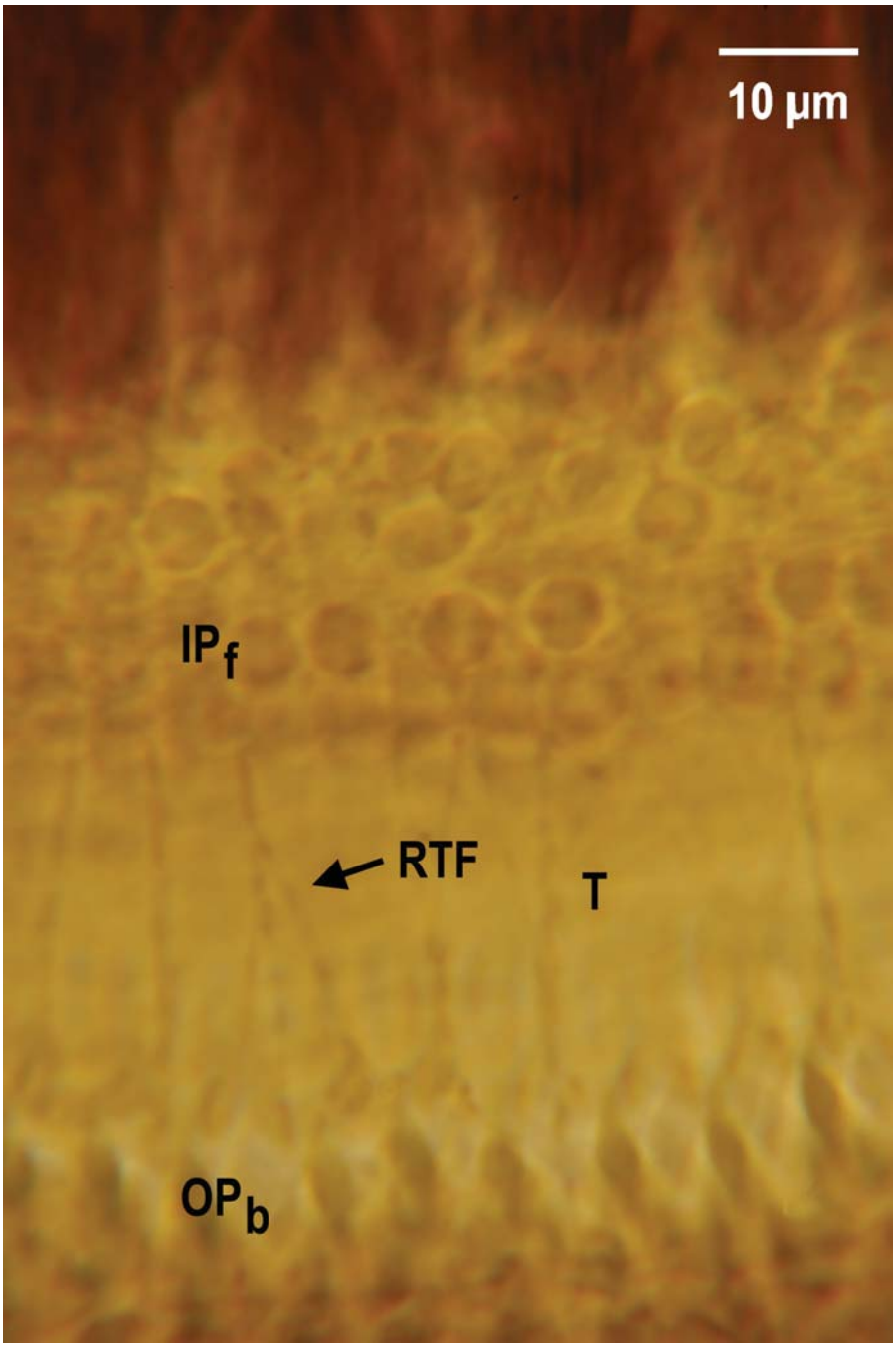
The feet of the inner pillars (IP_f) and bodies of the outer pillars (OP_b) are visible medial and lateral to the tunnel space (T) in the second turn. MNF - myelinated peripheral processes of spiral ganglion cells within osseous spiral lamina.

PILLAR FEET & TUNNEL SPACE AT COCHLEAR BASE



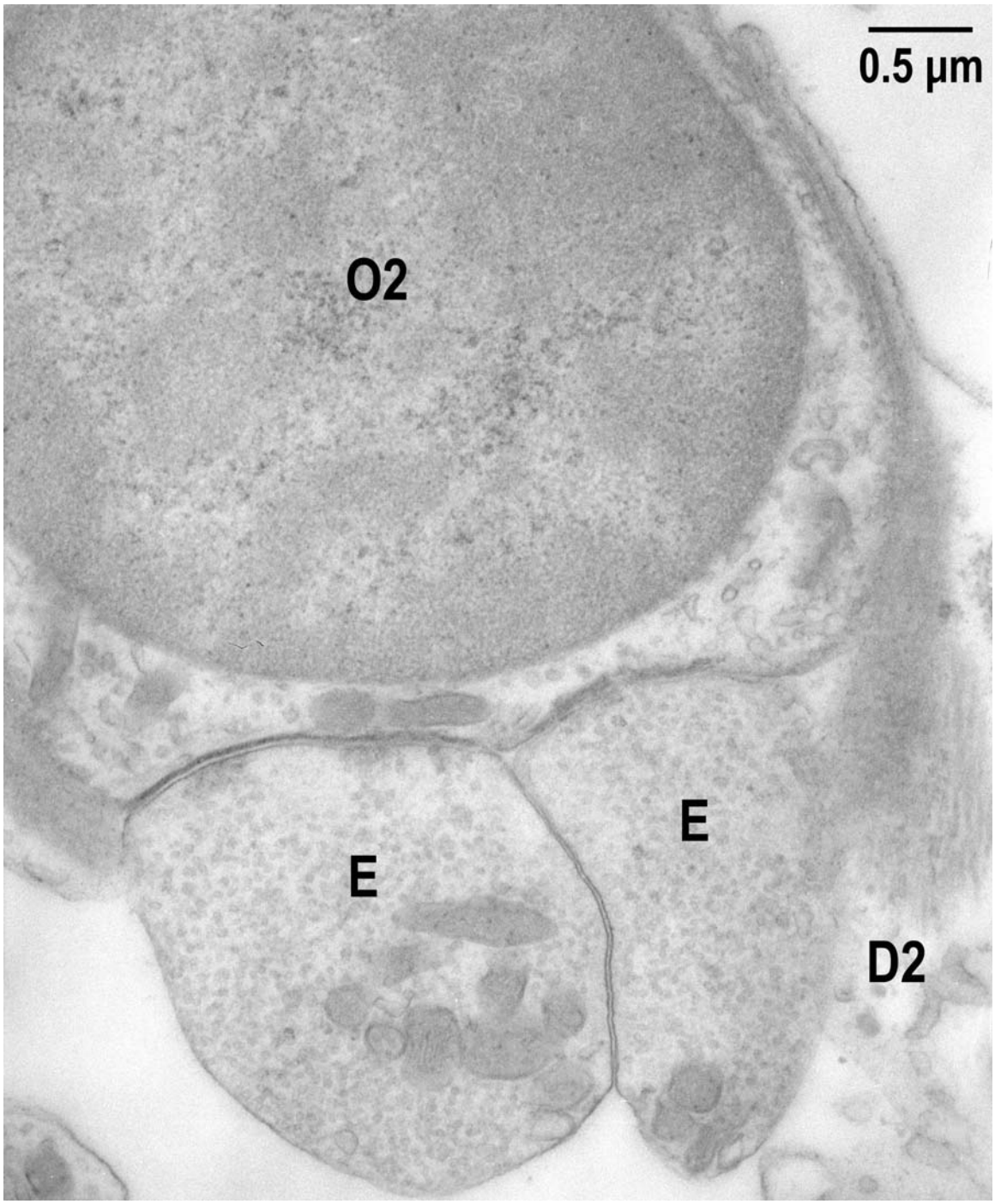
In the cochlear base, the tunnel space (T) is considerably narrower than in the second turn. IP - inner pillar feet; OP - outer pillar feet.

RADIAL TUNNEL FIBERS CROSSING TUNNEL SPACE



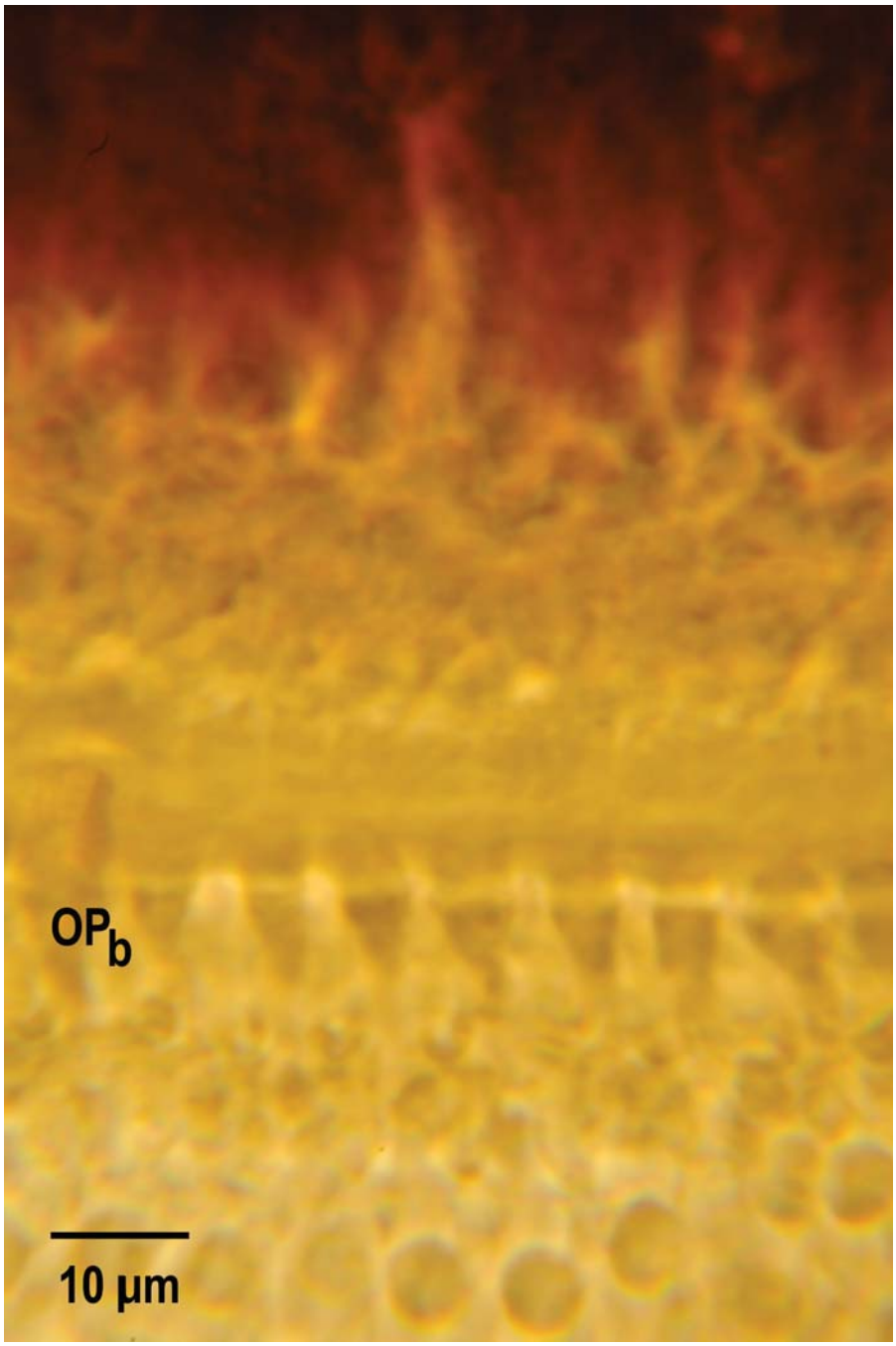
Non-myelinated nerve fibers [i.e., radial tunnel fibers (RTF)] enter the tunnel space (T) between adjacent inner pillar feet (IP_f), exit the tunnel between adjacent outer pillar bodies (OP_b) and then turn basally to join one of the outer spiral bundles of nerve fibers.

NERVE ENDINGS ON OHC BASE



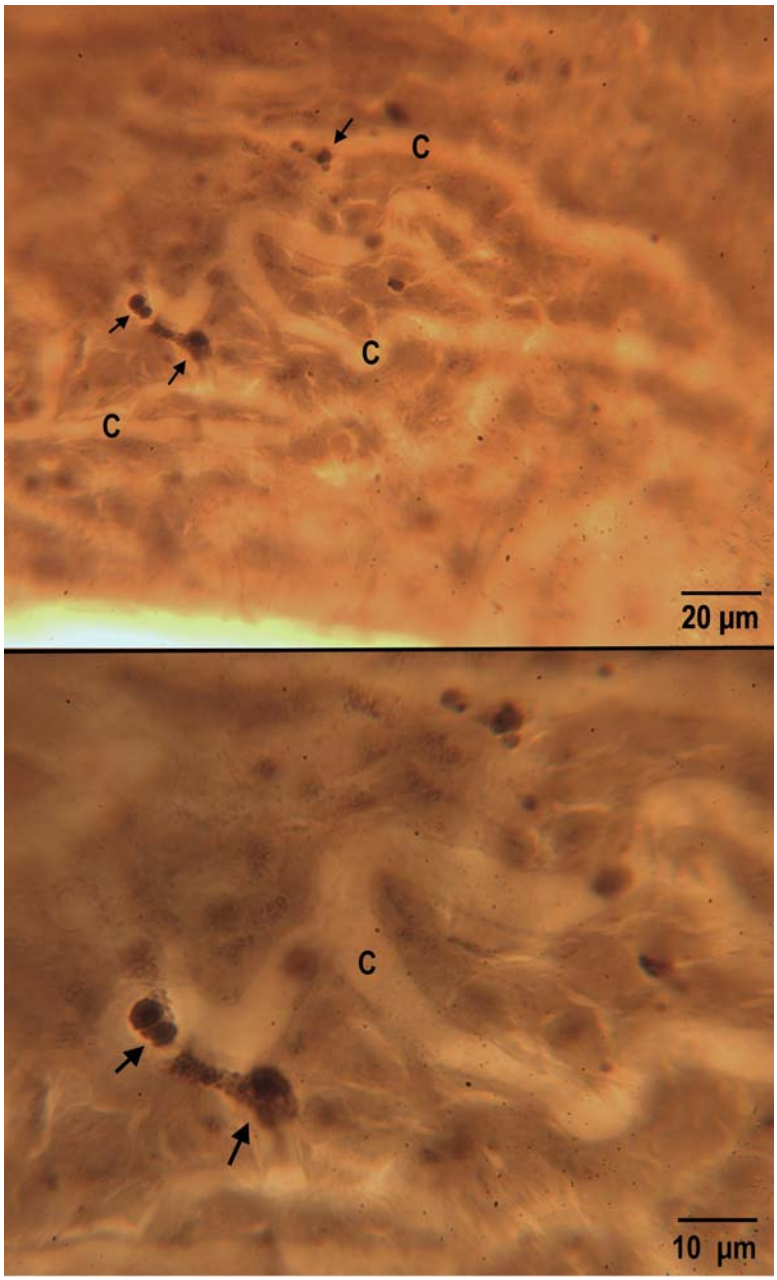
Transmission electron micrograph of a radial section through the base of a second row outer hair cell (O2). Two efferent nerve endings (E) indent the cell's base and are partly enclosed in the adjacent Deiters cup (D2).

OUTER PILLAR BODIES AT COCHLEAR BASE



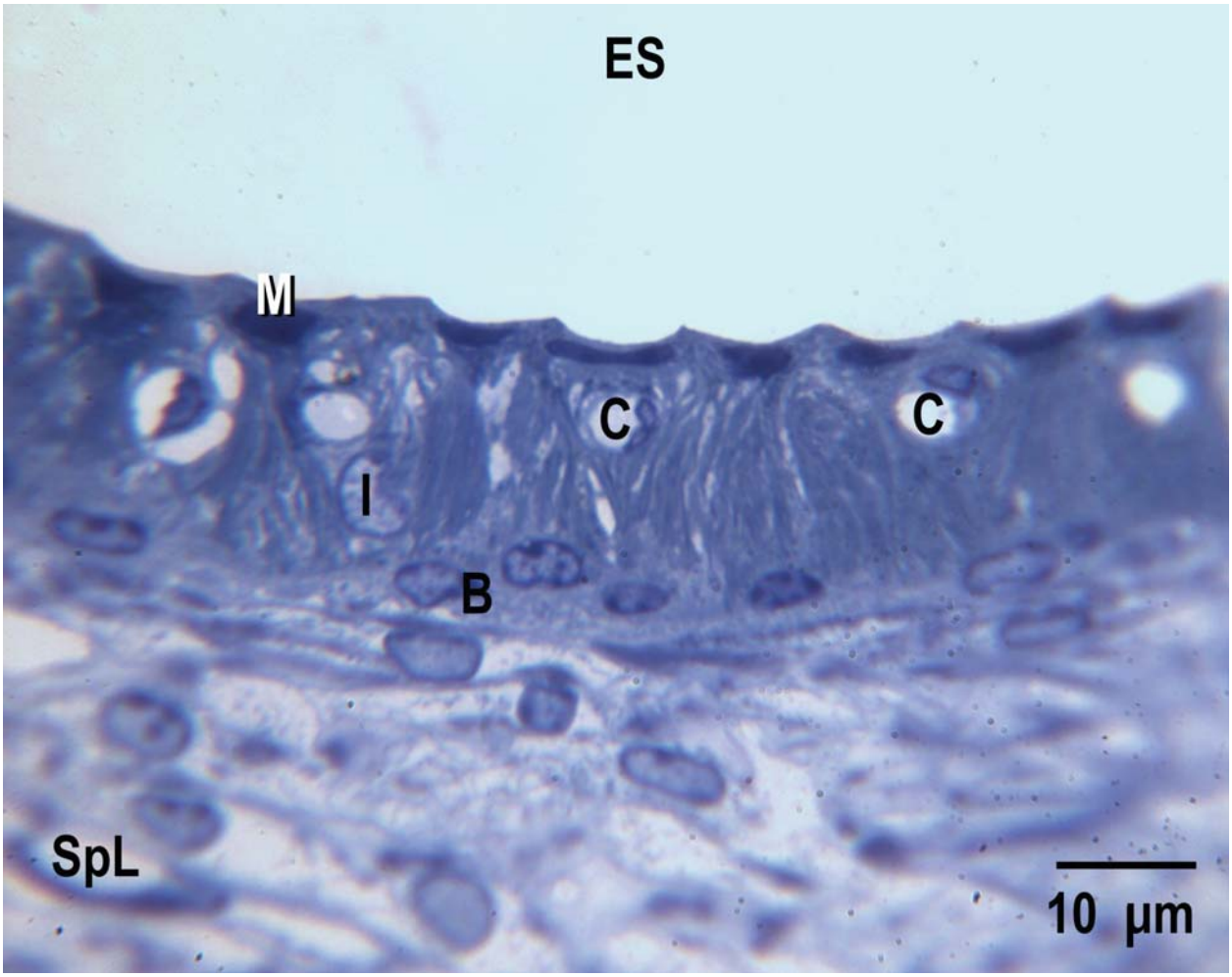
In the cochlear base, the bodies of the outer pillars (OP_b) are shorter and thicker than in the second turn.

CAPILLARIES & MELANOCYTES IN STRIA VASCULARIS



The stria vascularis forms the lateral wall of the cochlear duct. It generates the +100 mV positive potential (EP) in the endolymphatic space and maintains the high K^+ concentration and low Na^+ concentration in endolymph. These photomicrographs are of flat preparations of the stria viewed from its endolymphatic surface. A dense capillary network (C) is found within the stria. Because the cochlea was fixed by vascular perfusion, the capillaries appear as crisscrossing pale channels that are devoid of red blood cells. Melanin (arrows) is found in the intermediate cells of the stria. The intermediate cells are closely applied to capillaries.

ONE-MICRON RADIAL SECTION OF THE STRIA VASCULARIS



Stained, radial section of the stria vascularis. The stria is composed of three cell types: a) Marginal (M) cells form its endolymphatic boundary. These cells are joined at their apical borders by tight junctions; b) Deep to the marginal cells are the intermediate (I) cells and the intraepithelial capillary bed (C). The intermediate cells stain very lightly and often contain clumps of melanin; c) Basal (B) cells, joined by tight junctions, form a continuous boundary between the interior of the stria and the spiral ligament (SpL). The spiral ligament consists of fibrocytes, blood vessels and fluid-filled spaces. ES - endolymphatic space.

IDENTIFICATION OF NOISE DAMAGE IN THE MOUSE COCHLEA

In recent years, the mouse has become popular for studies of noise-induced hearing loss (NIHL), primarily because of the availability of different mouse strains having differing susceptibilities to noise trauma (e.g., Li, 1992; Erway et al., 1996; Davis et al., 1999; Liberman et al., 2000; Jimenez et al., 2001). Comparison of the effects of noise on different mouse strains should shed light on the role of genetics in the development of NIHL and cochlear damage.

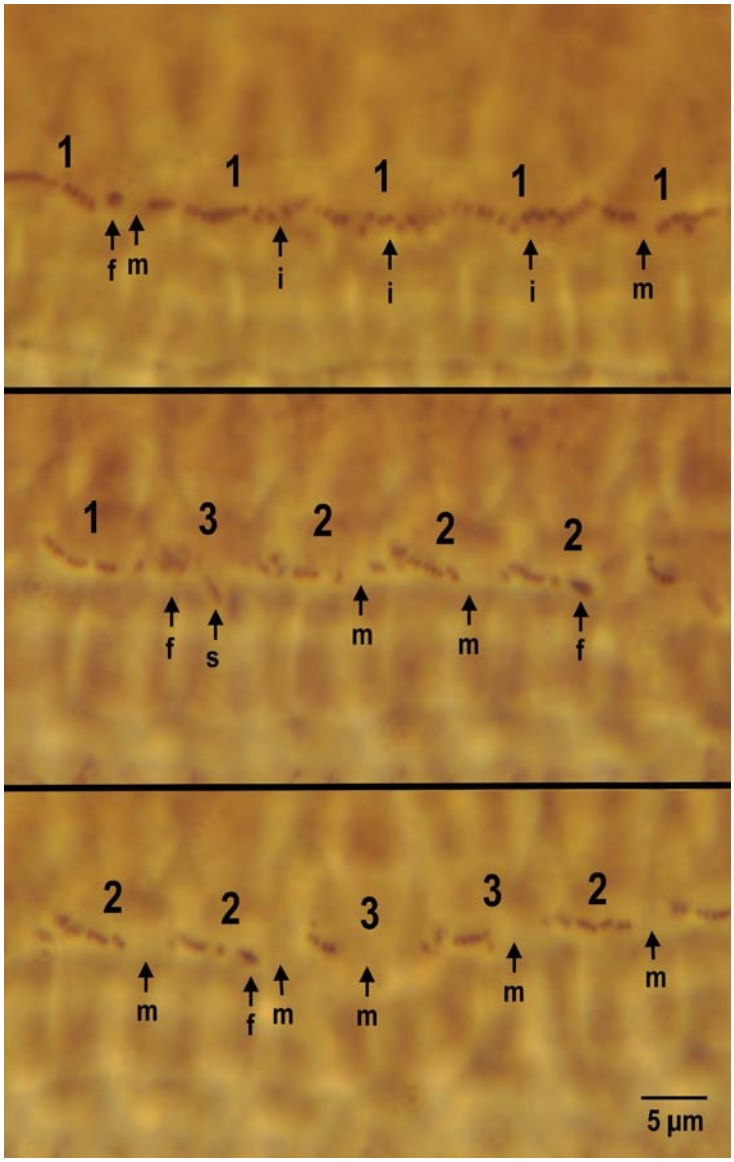
Although the magnitude of hearing loss, hair-cell loss and other signs of cochlear damage may differ across mouse strains after a damaging noise exposure, the general histopathological appearance the noise-damaged mouse cochlea seems to be similar among the strains. Thus, the illustrations included here should be considered representative of noise damage in the mouse. The following photomicrographs are of inbred C57BL and CBA/C57BL F1 hybrid mice (Ou et al. 2000a & 2000b) that had been exposed to octave bands of noise that resulted in permanent threshold shifts of different magnitudes.

Damage to the stereocilia bundle on individual hair cells correlates with permanent deficits in auditory function (e.g., ABRs; Ou et al., 2000a). A 3-point scale (i.e., 1 - slight; 2 - moderate; 3 - severe) is used to grade stereocilia damage. The scale is roughly based on the approximate numbers of tip and side links that are thought to be missing or disrupted.

Degenerated hair cells are replaced by phalangeal scars. Scars in the first row OHCs are formed by the enlargement of the phalangeal processes from the outer pillar heads. In the second row, scars are primarily formed by phalangeal processes from the first row of Deiters' cells. In the third row, scars are formed by phalangeal processes from the second row of Deiters' cells.

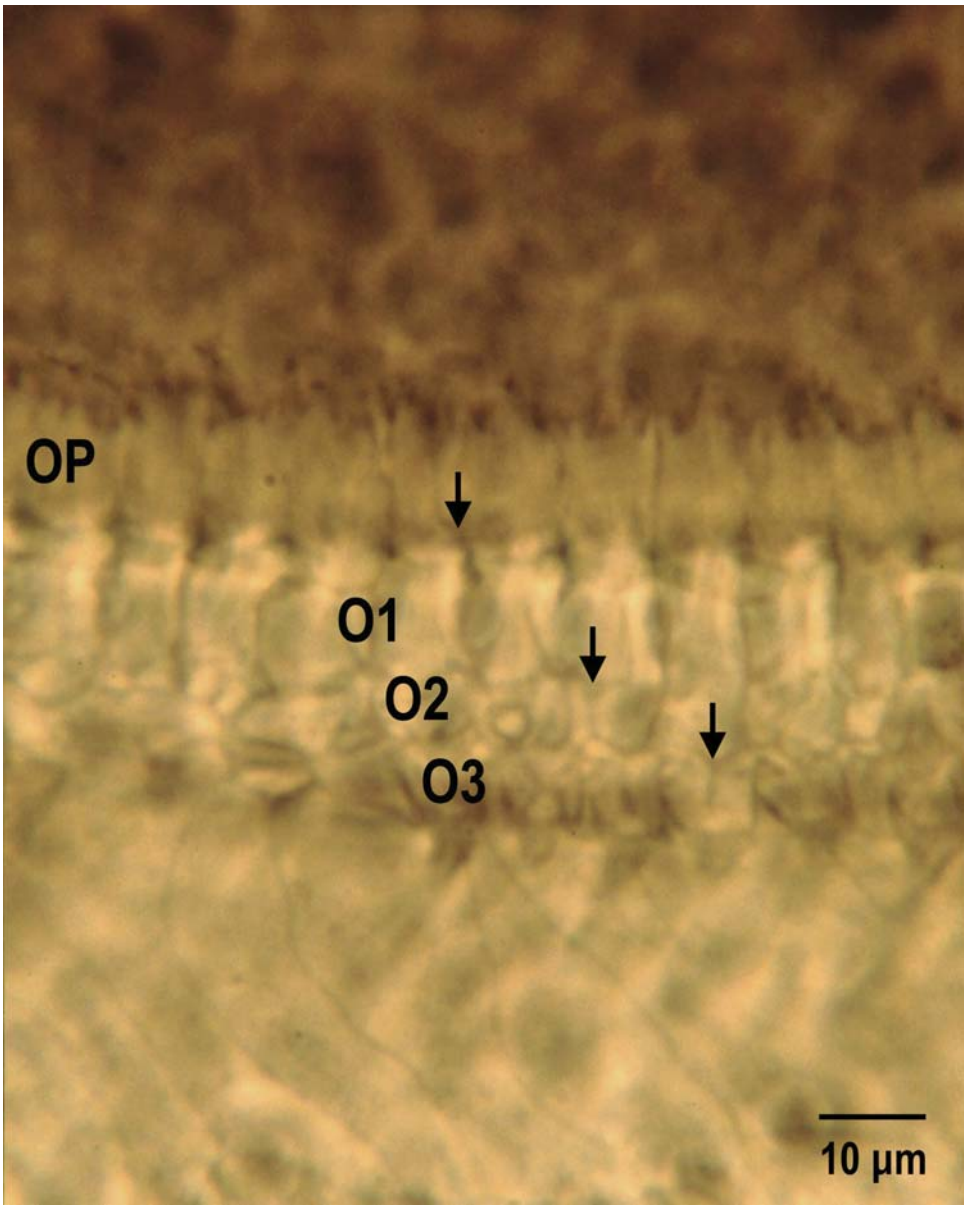
With more severe damage, both sensory and supporting cells degenerate. When all recognizable cells of the organ of Corti degenerate, an organ-of-Corti (OC) wipeout is formed. Undifferentiated, squamous epithelial cells replace the degenerated organ of Corti on the basilar membrane.

IHC STEREOCILIA PATHOLOGY



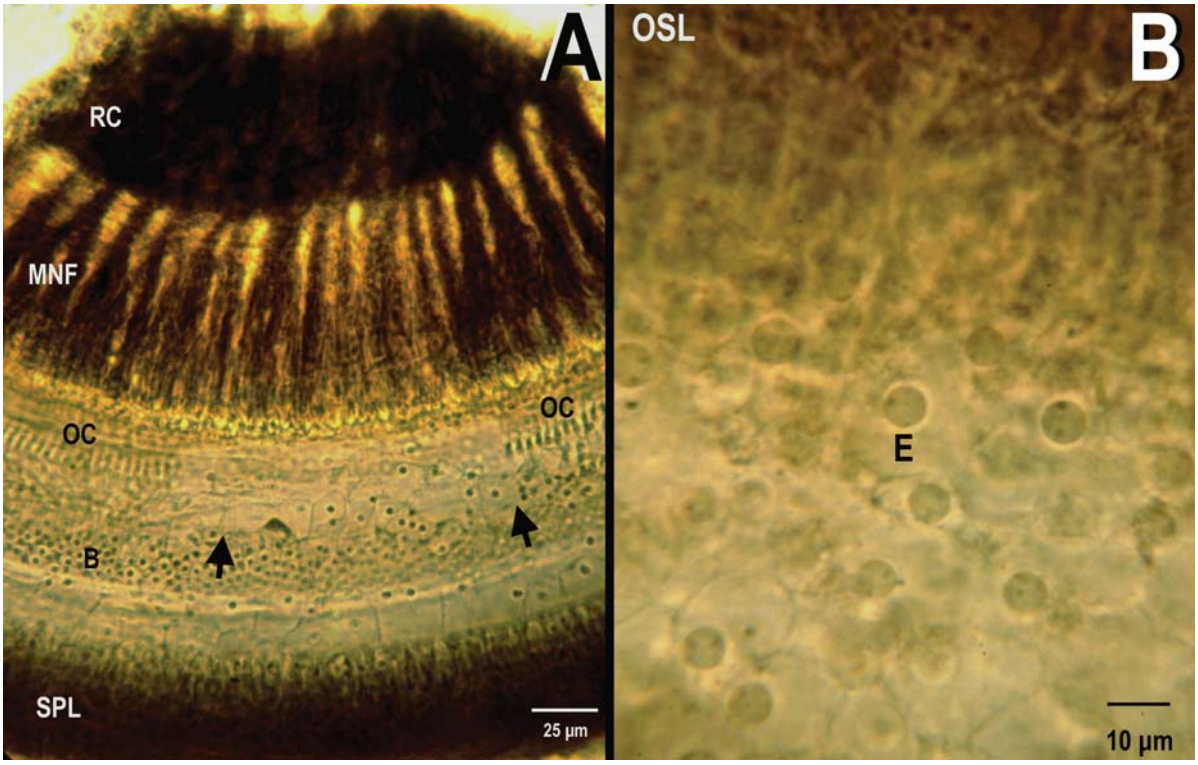
Evaluation of noise-induced damage to the IHC stereocilia bundle. Abnormalities in the stereocilia bundle on individual cells are graded (i.e., Grades 1-3) as follows: 1 - slight; 2 - moderate; or 3 - severe. Abnormalities include: fusion (f) of several adjacent stereocilia; irregular (i) or ruffled arrangement of stereocilia; missing (m) stereocilia; and splayed (s) stereocilia. Missing, fused and splayed stereocilia likely indicate that some stereocilia links (i.e., tip and side) have been disrupted. The more stereocilia on a particular cell that are abnormal, the higher grade the cell is given.

PHALANGEAL SCARS IN THE RETICULAR LAMINA



Outer hair cells in all three rows (O1, O2, O3) have degenerated in this region following severe noise trauma. The phalangeal processes that originally surrounded the hair cells have enlarged to form phalangeal scars, thus, re-establishing the endolymphatic boundary of the organ of Corti. The arrows point to new tight junctions between phalangeal processes comprising individual scars in each row of OHCs.

OC WIPEOUT & EPITHELIAL SCAR ON BASILAR MEMBRANE



A) A severe noise exposure can eventually lead to degeneration of all sensory and supporting cells in a particular segment of the organ of Corti. This type of lesion (between arrows) has been termed an 'OC wipeout' by Bohne and Clark (1982). Note that a portion of the organ of Corti (OC) is visible on either edge of the wipeout. Some spiral ganglion cells in Rosenthal's canal (RC) along with their myelinated peripheral processes (MNF) appear to be lighter stained. The lack of staining with osmium indicates that these cells and processes have also degenerated; B) Higher power view of the cells covering the basilar membrane where the organ of Corti has degenerated. These cells are flattened, undifferentiated epithelial (E) cells that serve to seal off the open ends of the tunnel and Nuel spaces and re-establish the endolymphatic boundary. B - Boettcher cells; OSL - osseous spiral lamina; SPL - spiral ligament and stria vascularis.

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BIBLIOGRAPHY

Bohne, BA, Harding, GW (2010): Microscopic Anatomy of the Inner Ear, 4th Edition, Washington University Press, St. Louis, MO, 65 pp.

Bohne, BA, Clark, WW (1982): Growth of hearing loss and cochlear lesion with increasing duration of noise exposure. In: New Perspectives on Noise-Induced Hearing Loss, edited by RP Hamernik, D Henderson, R Salvi, Raven Press, New York, pp. 283-302.

Bohne, BA, Harding, GW (1997): Processing and analyzing the mouse temporal bone to identify gross, cellular and subcellular pathology. Hear. Res. 109:34-45.

Bohne, BA, Harding, GW, Ou, HC (2001): Preparation and evaluation of the mouse temporal bone. In: Handbook of Mouse Auditory Research, edited by JF Willott, CRC Press, Boca Raton, London, New York, Washington, D.C., pp. 171-187.

Davis, RR, Cheever, ML, Krieg, EF, Erway, LC (1999): Quantitative measure of genetic differences in susceptibility to noise-induced hearing loss in two strains of mice. Hear. Res. 134:9-15.

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

Ehret, G (1974): Age-dependent hearing loss in normal hearing mice. Naturwissenschaften 61:506.

Ehret, G (1979): Quantitative analysis of nerve fibre densities in the cochlea of the house mouse *(Mus musculus)*. J. Comp. Neurol. 183:73-88.

Erway, LC, Shiau, Y-W, Davis, RR, Krieg, EF (1996): Genetics of age-related hearing loss in mice. III. Susceptibility of inbred and F1 hybrid strains to noise-induced hearing loss. Hear. Res. 93:181-187.

Jimenez, AM, Stagner, BB, Martin, GK, Lonsbury-Martin, BL (2001): Susceptibility of DPOAEs to sound overexposure in inbred mice with AHL. J. Assoc. Res. Otolaryngol. 2:233-245.

Kraus, H-J, Aulbach-Kraus, K (1981): Morphological changes in the cochlea of the mouse after the onset of hearing. Hear. Res. 4:89-102.

Li, H-S (1992): Influence of genotype and age on acute acoustic trauma and recovery in CBA/Ca and C57BL/6J mice. Acta Otolaryngol. 112:956-967.

Mikaelian, DO, Warfield, D, Norris, O (1974): Genetic progressive hearing loss in the C57/bl6 mouse. Acta Otolaryngol. 77:327-334.

Ohlemiller, KK, Wright, JS, Heidbreder, AF (1999): Vulnerability to noise-induced hearing loss in 'middle-aged' and adult mice: A dose-response approach in CBA, C57BL, and BALB inbred strains. Hear. Res. 149:239-247.

Ou, HC, Bohne, BA, Harding, GW (2000a): Noise damage in the C57BL/CBA mouse cochlea. Hear. Res, 145:111-122.

Ou, HC, Harding, GW, Bohne, BA (2000b): An anatomically based frequency-place map for the mouse cochlea. Hear. Res. 145:123-129.

Richardson, KC, Jarrett, L, Finke, EH (1960): Embedding in epoxy resin for ultrathin sectioning for electron microscopy. Stain Tech. 35:303-323.

Spoendlin, H (1966): The Organization of the Cochlear Receptor, S Karger, Basal, SZ.

Vazquez, AE, Jimenez, AJ, Martin, GK, Lubke, AE, Lonsbury-Martin, BL (2004): Evaluating cochlear function and the effects of noise exposure in the B6.CAST+*Ahl* mouse with distortion product otoacoustic emissions. Hear. Res. 194:87-96.

Wang, Y, Hirose, K, Liberman, MC (2002): Dynamics of noise-induced cellular injury and

repair in the mouse cochlea. J. Assoc. Res. Otolaryngol. 3:248-268.
Yoshida, N, Hequembourg, SJ, Atencio, CA, Rosowski, JJ, Liberman, MC (2000): Acoustic injury in mice: 129/SvEv is exceptionally resistant to noise-induced hearing loss. Hear. Res. 141:97-106.