

Ultrastructure of Hyaline, Border, and Vacuole Cells in Chick Inner Ear

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ABSTRACT

The sense organ for hearing in birds, the basilar papilla, is capable of replacing lost or damaged hair cells and supporting cells through regeneration. Potential candidates for precursor-cell populations include cells within the auditory receptor epithelium and nonsensory cells inferior to the sensory epithelium. Ultrastructural characteristics of hyaline cells, border cells, and vacuole cells, nonsensory cells which border or lie inferior to the receptor epithelium proper, were studied with transmission electron microscopy. Data were obtained from normal neonatal and adult chickens.

Several rows of epithelial cells separate hyaline cells from inferiorly located organ supporting cells and hair cells. Ultrastructural characteristics and location of these epithelial cells differentiate them from organ supporting cells and hyaline cells; consequently, we have termed them "border cells." Synaptic specializations are observed between neural elements and border cells, and gap junctions are found between adjacent border cells, between border cells and neighboring organ supporting cells, and between juxtaposed border and hyaline cells.

Hyaline cells, in contrast to border cells, are highly specialized. Dense bundles of filaments are present in hyaline cells from the basal one-half of the papilla, and an unusual structure, a rough tubular aggregate, is present in hyaline-cell cytoplasm. Pre- and postsynaptic specializations are observed between neural elements and hyaline cells, and gap-junctional complexes link neighboring hyaline cells.

Vacuole cells lie inferior to the hyaline cells and rest on the inferior fibrocartilaginous plate. They are unspecialized morphologically. Their only remarkable morphological feature is the abundance of spherical vacuoles within their cytoplasmic matrix.

Key words: bird, auditory, cochlea, basilar papilla, supporting cells

Hair cells, the sensory receptors of auditory, vestibular, and lateral-line end organs, transduce mechanical stimuli into electrical signals and are involved in the detection of sound, linear and angular accelerations, water motion and substrate vibration. They are damaged by disease, exposure to loud sound, treatment with ototoxic drugs, and processes associated with aging. In the ears and the lateral-line organs of amphibians and fish, hair cells are produced throughout life as well as in response to injury (Tester and Kendall, '69; Corwin, '81, '83, '85; Jørgensen, '81; Popper and Hoxter, '84; Barber et al., '85; Popper and Hoxter, '90; Presson and Popper, '90). Hair-cell production in mammals and birds, in contrast, was thought to cease shortly after birth (Ruben, '67; Rubel, '78), and postembryonic hair-cell loss was considered to be irreversible. Recent studies of avian inner ears demonstrated that the auditory receptor epithelium in the bird, the basilar papilla, is capable of replacing lost or damaged cells through regeneration (Cotanche, '87; Cruz et al., '87; Corwin and Cotanche, '88; Ryals and Rubel, '88; Girod et al., '89), and new hair cells

are added throughout life in avian vestibular organs (Jørgensen and Mathiesen, '88; Roberson et al., '92; Weisleder and Rubel, '91).

Regenerated hair cells in the chick basilar papilla are thought to arise from cells peripheral to the receptor organ (Girod et al., '89) and possibly from supporting cells within the receptor organ itself (Corwin and Cotanche, '88; Girod et al., '89). Little is currently known about these cell types. The present investigation was designed to study the ultrastructural characteristics of border, hyaline, and vacuole cells in the normal chicken cochlea with transmission electron microscopy. Border cells lie in the receptor organ near its inferior edge, and hyaline cells and vacuole cells are peripheral to the basilar papilla.

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Brief descriptions of some of our results have appeared (Oesterle et al., '90, '91).

METHODS

Morphological characteristics of border, hyaline, and vacuole cell types were investigated with transmission electron microscopy by standard techniques. Briefly, electron microscopic (EM) data were obtained from seven normal chickens (white leghorn) ranging from 10 days to 22 weeks in age. Animals were deeply anesthetized with an overdose of pentobarbital sodium and decapitated, and the bony capsule at the distal end near the lagena was widely opened. After a small opening was made in the round window, individual ears were fixed by intralabyrinthine perfusion with one of the following fixatives: 1) modified Karnovsky's fixative: a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) with 0.001% CaCl_2 ; 2) a mixture of 1% paraformaldehyde and 2.5% glutaraldehyde in 0.12 M phosphate buffer (pH 7.4) with 1.5% sucrose and 0.02% MgSO_4 ; or 3) 3.5% glutaraldehyde in 0.1 M Na/K phosphate buffer. Perfused inner ears were held in fixative for 4 or 24 hours (at 4°C) after which they were washed in buffer (buffers for fixatives 1, 2 and 3 are 0.1 M cacodylate, 0.01 M phosphate buffered saline [PBS], and 0.1 M Na/K phosphate buffer, respectively), and the cochlear ducts were dissected free from the temporal bone.

Inner ears were postfixed in 1% osmium tetroxide (in the appropriate buffer, pH 7.4) for 1 or 2 hours at room temperature. The ears were washed with buffer, dehydrated in a graded alcohol series, and embedded with Polybed 812 or Spurr epoxy resins. Series of semithin (1 μm) and ultrathin sections (90 nm) were collected from several regions along the cochlear duct. As illustrated in Figure 1, sections were taken from the low-, mid-, and high-frequency regions of the papilla. Specifically, sections were taken at distances 29–33, 70, and 90% from the basal (proximal) end of the basilar papilla. On the basis of the characteristic frequency data of Manley et al. ('89), the best frequencies (BFs) of individual hair cells located at a distance 90% from the basal end are estimated to be around 50 Hz or lower. Consequently, this region is referred to herein as the low-frequency region of the papilla. Similarly, hair cell BFs at a location 70% from the basal end are estimated to range between 300 and 400 Hz, and this region is referred to as the mid-frequency region of the papilla. Finally, the BFs of hair cells at a distance 29–33% from the basal end range from 1.5 to 1.9 kHz, and this region is referred to as the high-frequency region. Sections were stained with uranyl acetate and lead citrate before examination with a Philips EM 410 transmission electron microscope.

RESULTS

Overview

A cross section through the cochlear duct of the chicken is shown in Figure 2. Detailed descriptions of the structural organization of the cochlear duct in the mature chicken ear have been published elsewhere (Jahnke et al., '65; Tanaka and Smith, '78; Smith, '81, '85). The basilar papilla lies on the basilar membrane and is composed of hair cells, supporting cells, and unmyelinated terminal portions of cochlear nerve fibers. The clear cells and the hyaline cells abut the

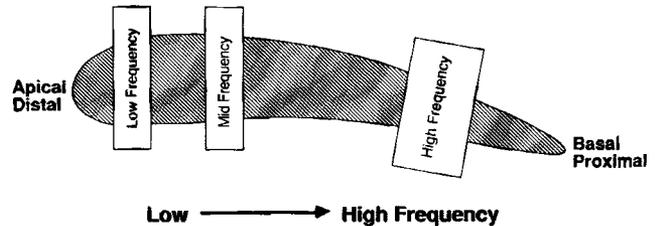


Fig. 1. Schematic of the basilar papilla of the chicken. The broad apical end, the distal end, is tuned to low frequencies and the other end, the basal or proximal end, is tuned to high frequencies. Sections were taken at distances 29–33, 70, and 90% from the basal end—the areas delineated by boxes. Sections were taken from the transverse axis of the papilla.

superior and inferior edges of the basilar papilla, respectively. Descriptions of the ultrastructure of the basilar papilla in the adult chicken can be found in Vinnikov et al. ('65), Jahnke et al. ('69), Hirokawa ('78), Tanaka and Smith ('78), and Smith ('85). At the inferior edge of the papilla, a few rather unspecialized epithelial cells separate the sensory region from the hyaline cells. As will be demonstrated shortly, the epithelial cells are morphologically distinct from the rest of the papilla supporting-cell population, and consequently, will be referred to herein as "border cells." The remainder of the papilla supporting-cell population, namely, the supporting cells in the basilar papilla superior to the border cells, will be referred to as the "organ supporting cells." Hyaline cells rest on the basilar membrane and extend from the border-cell region to the inferior fibrocartilaginous plate (IFP). A rather unspecialized series of cells, which we will refer to as "vacuole cells," lies above the IFP inferior to the hyaline cells. Vacuole cells have formerly been referred to as cuboidal cells (Takasaka and Smith, '71), epithelial cells (Retzius, 1884), or hyaline cells (Fermin and Cohen, '84). Cuboidal cells rest on the IFP inferior to the vacuole cells. The thickness of the basilar membrane decreases under the border cells, and, as will be described in more detail later, the structure of the basilar membrane under the receptor epithelium differs from that under the hyaline cells.

Our primary objective was to characterize the ultrastructural properties of border cells and hyaline cells in the normal chicken cochlea and to provide preliminary information regarding the ultrastructural characteristics of vacuole cells. Findings regarding the ultrastructural characteristics of border cells will be presented first and followed by discussions of the morphological features of hyaline cells and vacuole cells.

Border cells

The inferior region of the basilar papilla in the chicken cochlea and several nearby hyaline cells is shown in Figure 3. This micrograph is taken from the high-frequency region of the cochlea. The inferior hair cell, a short hair cell, abuts an organ supporting cell. Several rows of cells, which we have termed border cells, separate the organ supporting cell from nearby hyaline cells. Border cells are irregular in shape with an expanded, sloping, luminal surface. Border-cell height decreases rapidly in the radial direction. Average heights of the tallest (superior) and shortest (inferior) parts of the border cell are 20 and 15 μm , respectively, for border cells from the high-frequency region of the cochlea ($n = 7$). Bottom and central portions of border cells tend to be

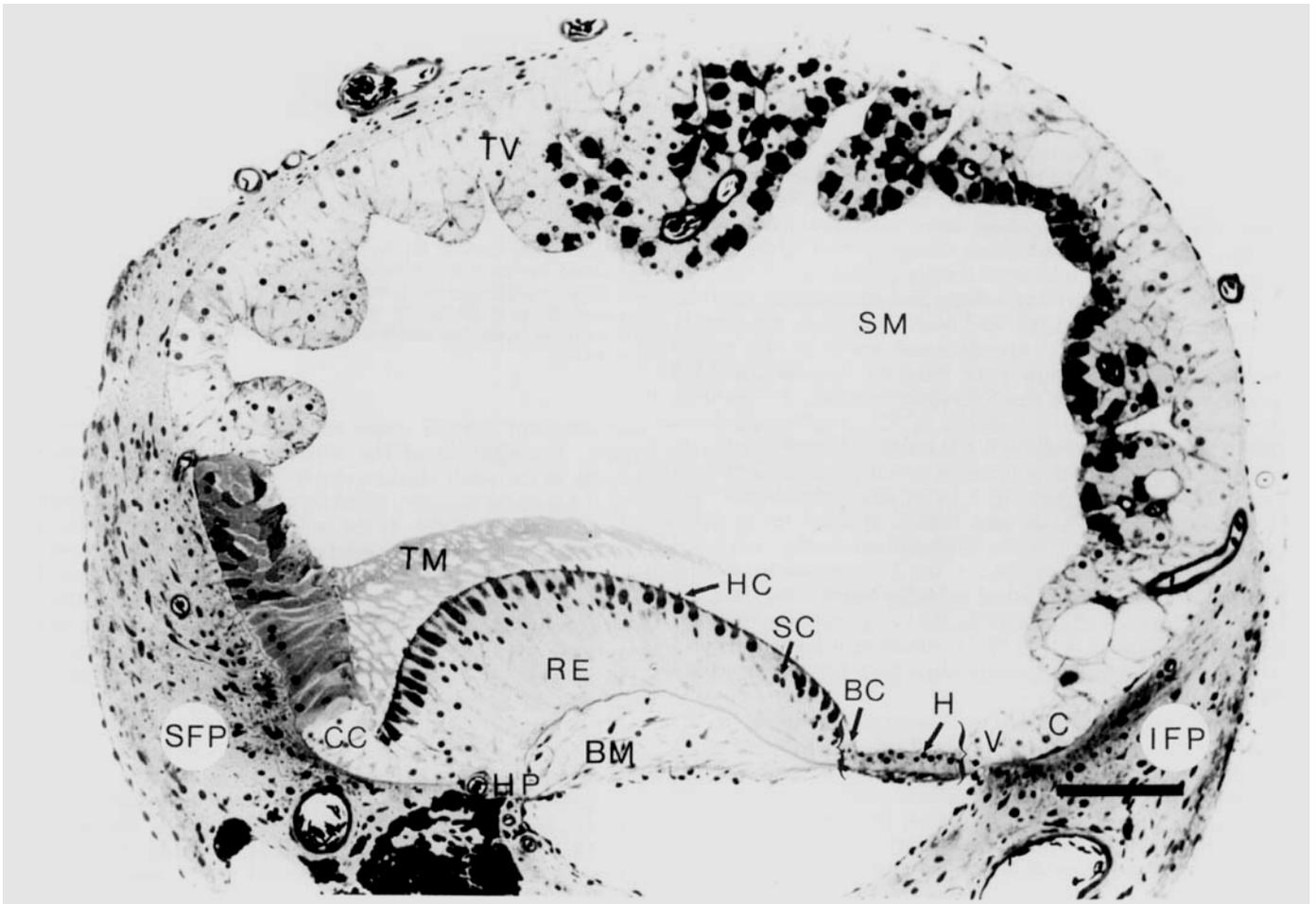


Fig. 2. Light microscope picture of a transverse section through the inner ear of the chicken. The receptor epithelium (RE; also called the basilar papilla) lies on the basilar membrane (BM) and is composed of hair cells (HC), supporting cells and unmyelinated terminal portions of cochlear nerve fibers. Two types of supporting cells are present in the receptor epithelium: organ supporting cells (SC) and border cells (BC). Hyaline cells lie inferior to the receptor epithelium (H) and extend inferiorly towards the inferior fibrocartilaginous plate (IFP). Vacuole

cells (V) and cuboidal cells (C) rest on the inferior fibrocartilaginous plate. The inferior region of the papilla and juxtaposed supporting cells, the bracketed region, is depicted in Figure 3 at higher magnification. CC, clear cells; TM, tectorial membrane; SM, scala media; TV, tegmentum vasculosum; HP, habenula perforata; SFP, superior fibrocartilaginous plate. Scale bar = 100 μm . Figure modified from Figure 1 of Rubel and Ryals ('82, p. 33).

roughly equal in width (average widths of bottom and central regions of border cells from the high-frequency region of the cochlea are 5.5 and 4.0 μm , respectively; $n = 15$), and both are smaller than the luminal surface (7.1 μm in width). Luminal surfaces of border cells are considerably wider than those of nearby organ supporting cells (e.g., Fig. 3). To illustrate, the average width of the luminal surface of border cells located in the high-frequency region of the cochlea is 7.1 μm , whereas the average width of the luminal surface of nearby organ supporting cells is 0.9 μm ($n = 15$). Thus, in the high-frequency region of the cochlea, apical surfaces of border cells are roughly 20 times those of nearby organ supporting cells.

Scattered microvilli arise from the border-cell luminal surface (Fig. 4). The microvilli are short and stubby (mostly less than 0.7 μm in length and 80–110 nm in diameter for cells in the high-frequency region). Their dimensions and content approximate those of nearby organ supporting cells. They appear to contain a fibrous material similar to that in the organ supporting cells. On the other hand, fine

rootlet filaments extend from the base of microvilli atop the organ supporting cells into the apical cytoplasm but are absent in border cells. In addition, the density of the microvilli is markedly less for border cells than organ supporting cells (Fig. 4b), and the filamentous part of the tectorial membrane typically extends to the microvilli of the inferiormost organ supporting cell but not to nearby border-cell microvilli (Fig. 4a).

Ultrastructurally the cytoplasm of border cells, like that of other nonsensory cells in the cochlear duct, is more electron-lucent than that of the hair cells and contains fewer organelles. Cytoplasmic content of the border cells is not remarkable. Mitochondria, rough endoplasmic reticulum, and polyribosomes are prominent. A few microtubules, lysosomes, and multivesiculated bodies are present. Golgi apparatus appears near the luminal surface or the perinuclear region. The nucleus is oval in shape and is often positioned in the middle or lower one-third of the cell. Diplosomes are observed infrequently near the luminal surface.

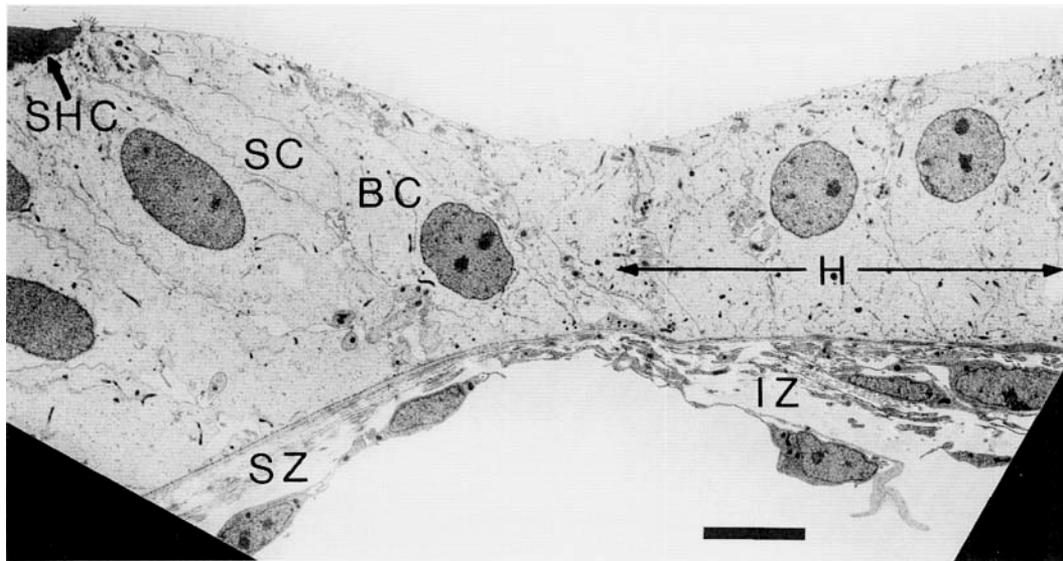


Fig. 3. Inferior region of the chick basilar papilla and the neighboring supporting cells. Section is taken from the high-frequency region of the cochlear duct from a 17 day old chick. A short hair cell (SHC) is on the left. At the inferior edge of the papilla, a few cells—the border cells

(BC)—separate the sensory region from the hyaline cells (H). SC, organ supporting cells; SZ, superior zone of the basilar membrane; IZ, inferior zone of the basilar membrane. Scale bar = 5 μm .

Density of cytoplasmic organelles in organ supporting cells is increased relative to that in the border cells. Secretory vesicles, golgi complexes, endoplasmic reticulum, mitochondria, and electron-dense bodies are numerous and congregated in apical regions of organ supporting cells in contrast to the border cells. Microtubules extend into the neck regions of organ supporting cells and often appear to be associated with the microvilli complex. Microtubules, in contrast, are typically absent from apical regions of border cells and are not associated with border-cell microvilli. Longitudinally oriented microtubules are present in central and bottom regions of organ supporting cells and are typically absent from corresponding regions of border cells. Long strands of rough endoplasmic reticulum encircle the nucleus of organ supporting cells, and organ supporting cells often have a denser filamentous appearance to the cytoplasm in general, and more polyribosomes than border cells.

Border-cell interdigitations and unmyelinated nerve fibers are present in extracellular spaces adjacent to border cells (Figs. 4, 5). Some neural elements are filled with small round vesicles and mitochondria and look much like efferent nerves. As shown in Figure 5, small presynaptic densities with associated synaptic vesicles are present inside some nerve fibers and are attached to the cytoplasmic face of the presynaptic membrane. The synaptic vesicles are typically 30–45 nm in diameter, have clear centers, and are spherical in shape. A few dense-cored vesicles, 60–90 nm in diameter, are seen in some neural elements interspersed among the numerous clear synaptic vesicles. The border cell is often separated from the neural element by a cleft 20–30 nm in width.

Gap junctions are found between adjacent border cells, between border cells and neighboring organ supporting cells, and between juxtaposed border and hyaline cells. To illustrate, a gap junction between neighboring border cells is visible in Figure 5. In a high magnification of the junction (insert of Fig. 5), two parallel membranes are apposed with

an intercellular gap of about 4 nm in width. Such gap junctions are commonly observed between apical, central, and bottom regions of the border cell and adjoining cells.

The basilar membrane can be divided into a superior (inner) zone that is covered on its endolymphatic surface by the basilar papilla and an inferior (outer) zone that is covered on the endolymphatic surface by the hyaline cells (Fig. 3). The transition from superior to inferior zones occurs under the border cells. The superior zone (Fig. 6a) consists of several layers which are separated from the organ supporting cells by a well defined basal lamina. The layers are largely acellular in nature, and dense bundles of closely packed fibers with the longitudinal dimension for the most part positioned in a radial direction are embedded in a fine filamentous matrix in central and lower (towards scala tympani) regions of the membrane. The individual fibers are roughly 5–10 nm in diameter. In contrast, the inferior zone of the basilar membrane (Fig. 6b) has fewer layers, is more cellular in nature (numerous fibroblasts lie within the basilar membrane), and has fewer dense bundles of filaments, and the fibers comprising the filament bundles are larger in diameter (40–80 nm in diameter). There is a distinct thinning of the basilar membrane under the border cells, where its structure changes.

Hyaline cells

Hyaline cells are a row of epithelial cells which lie inferior to the basilar papilla and extend towards the inferior fibrocartilaginous plate (Figs. 2, 3, 7). Their basal surfaces rest on a basal lamina atop the basilar membrane, and their apical surfaces contact the endolymphatic fluids of the scala media space. Inferior and superior neighbors to the hyaline cells are the vacuole cells and border cells, respectively.

Numbers of hyaline cells and hyaline-cell height vary as a function of cochlear location. Three to five hyaline cells are typically present in transverse sections from the high-frequency region of the chick cochlea, whereas up to 12

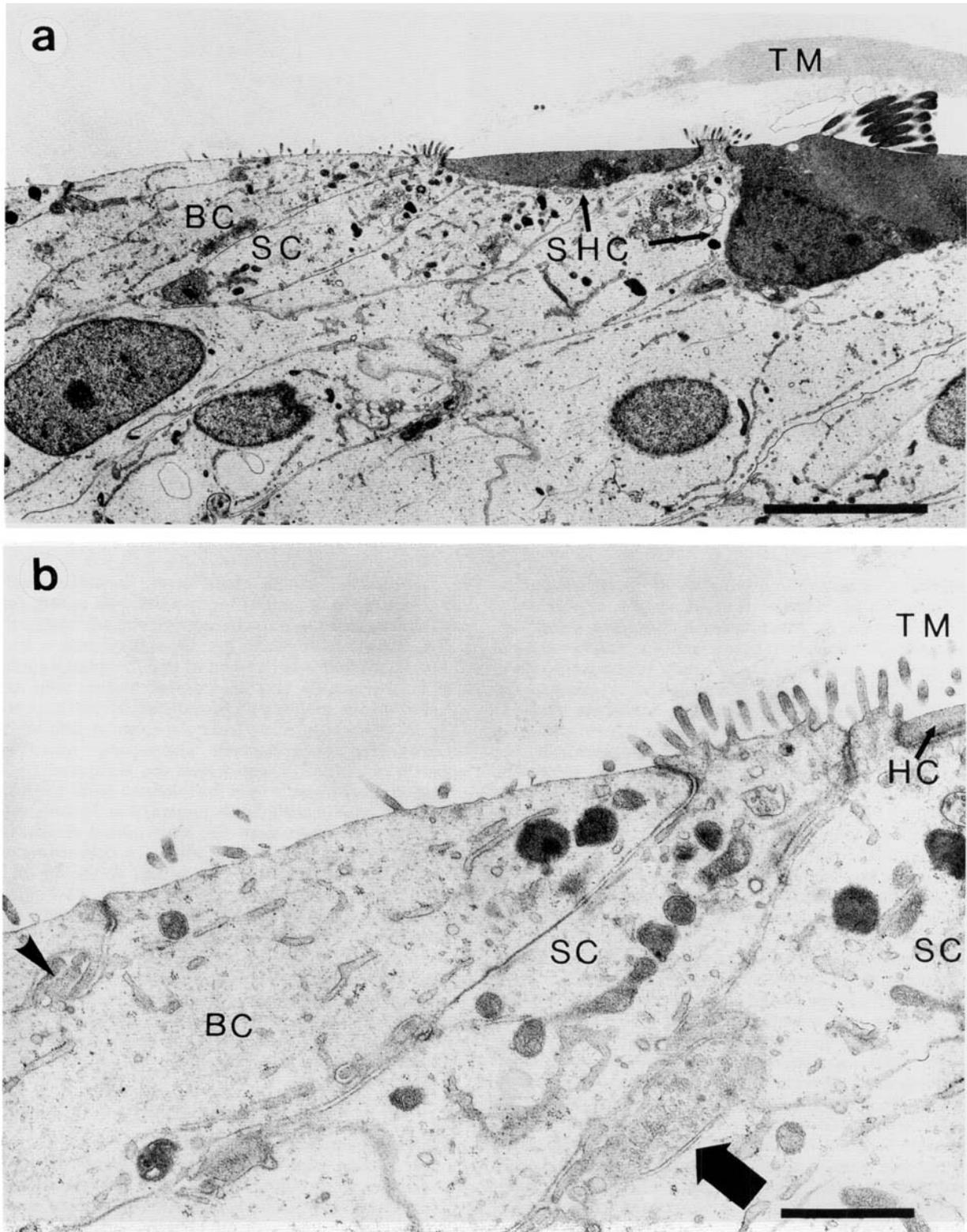


Fig. 4. Border-cell luminal surface. **a:** Surfaces of several border cells (BC) juxtaposed to an organ supporting cell (SC). Short hair cell (SHC). Section taken from the high-frequency region of the cochlear duct from an 11 day old chicken. TM, tectorial membrane. Scale bar = 5 μ m. **b:** Higher magnification of an organ supporting cell (SC) juxta-

posed to a border cell (BC). Note the presence of a vesicle-filled neural element between the organ supporting cells (arrow) and interdigitations between adjacent border cells (arrowhead). HC, hair cell; TM, tectorial membrane. Scale bar = 1 μ m.

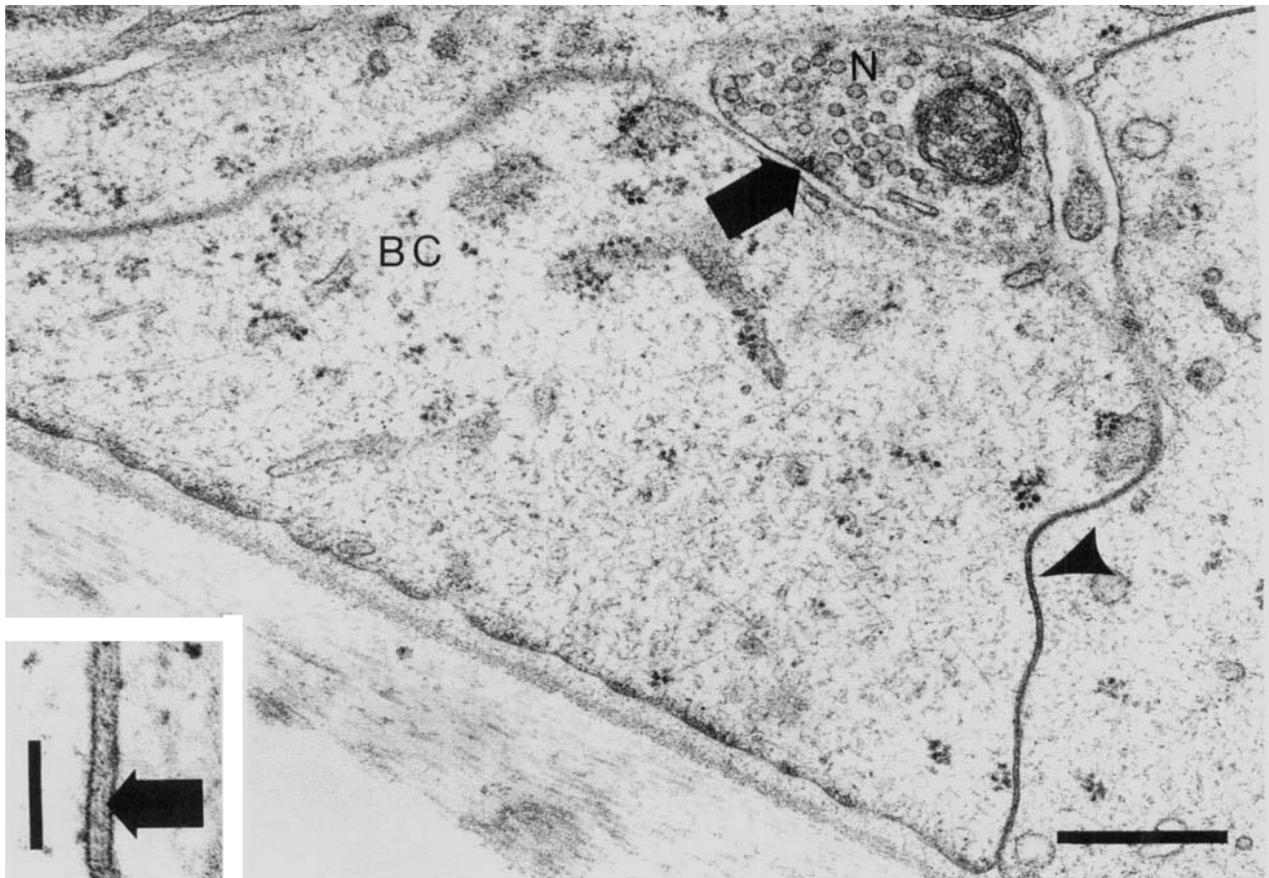


Fig. 5. Border-cell junctions. Section from the high-frequency region of the cochlea from a 17 day old chick. Junctional complex between a nerve fiber (N) and a border cell (BC). Synaptic specializations are present: a small presynaptic density in the neural element (arrow) is associated with numerous clear synaptic vesicles. Arrowhead points to

a low magnification of a gap junction between adjacent border cells. Scale bar = 0.5 μm . **Inset:** Higher magnification of the gap junction. The two plasma membranes are separated by an intercellular gap (arrow) of about 4 nm width. Scale bar = 0.05 μm .

hyaline cells may be seen in cross sections from low-frequency regions. Hyaline cells from high-frequency regions of the cochlea range between 10.5 to 15.6 μm in height¹ (average = 13.2 μm ; n = 17), whereas hyaline cells located in low-frequency regions are taller, ranging from 17.9 to 21.7 μm in height (average = 19.6 μm ; n = 10). Hyaline-cell width, in contrast, remains relatively constant across the cochlea. For example, average widths of basal and luminal surfaces for hyaline cells located in the high-frequency region are 6.9 and 6.0 μm , respectively, and average widths of basal and luminal surfaces of hyaline cells located in the low-frequency region are 6.3 and 6.1 μm , respectively.

Mitochondria, smooth and rough endoplasmic reticulum, ribosomes, and polyribosomes are prominent in hyaline-cell cytoplasm (Fig. 8). Lipid vacuoles, lysosomes, and microtubules are present occasionally. Golgi apparatus appears in apical regions of the cell.

Striated rough tubular aggregate in hyaline cells. An unusual structure is frequently observed in hyaline cells. The structure, referred to herein as a striated rough

tubular aggregate, lies in the cytoplasmic matrix of hyaline cells (e.g., Figs. 7–9). Its location is not restricted to any particular region, and it has been observed in apical, basal, and perinuclear regions of hyaline cells in animals ranging from 10 days to 22 weeks in age. Most often, one rough tubular aggregate is observed in a cross section of a cell, but sometimes several aggregates are observed in separate regions of a cell (e.g., apical and basal regions). The organelle may reach up to 3–4 μm in length and 0.5 μm in diameter.

The structure is shown at a high magnification in Figure 9. A well developed tubular aggregate consists of stacks of up to 31 tubules arranged in parallel. The bundles of tubules may all run in one direction or they may run at various angles up to about 90° to each other (e.g., Fig. 9a). In cross section (Fig. 9b) each tubule appears circular in shape with a diameter of 85–95 nm. The tubules show a striated appearance (Fig. 9a). The striations run at regular intervals, perpendicular to the longitudinal axis of the tubule. Only fine granules and amorphous material of low electron density are seen in the tubule lumen. Ends of the tubules are often contiguous with rough-surfaced endoplasmic reticulum (Fig. 9a,c).

Tubular aggregates are seen in hyaline cells only; they do not occur in other cell types in or adjacent to the basilar

¹Hyaline-cell height is defined as the distance from the hyaline-cell plasma membrane abutting the underlying basal lamina to the hyaline-cell plasma membrane contacting the endolymphatic fluids of the scala media space.

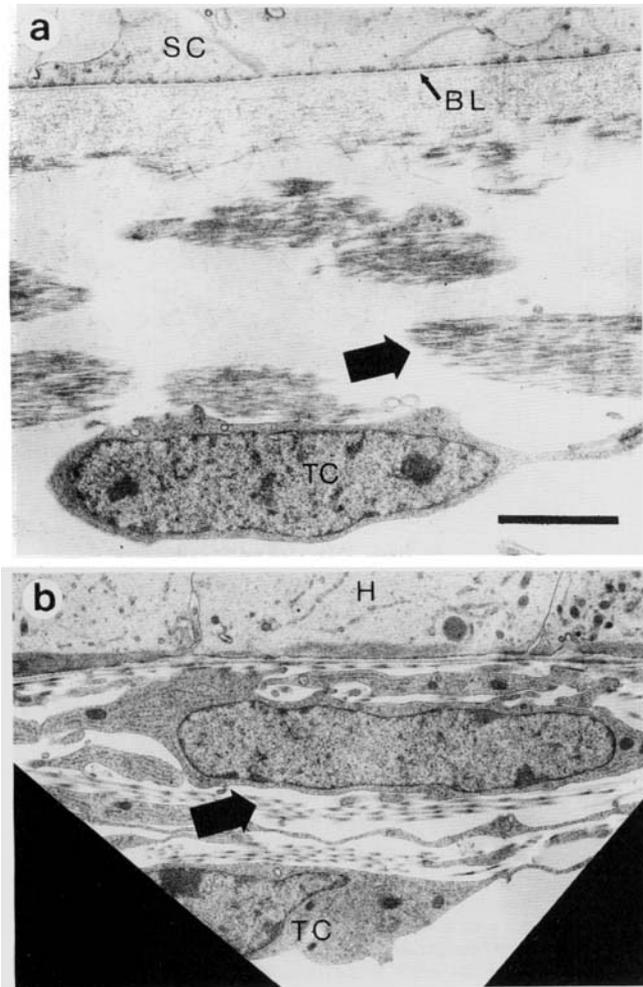


Fig. 6. Basilar membrane characteristics. **a:** Part of the inner zone of the basilar membrane which lies under the receptor epithelium. Dense bundles of closely packed fibers (arrow) are embedded in a fine filamentous matrix. Section from the high-frequency region of the cochlea from an 11 day old chick. BL, basal lamina; SC, organ supporting cell; TC, tympenic border cell. Scale bar = 2 μ m. **b:** Part of the outer zone of the basilar membrane which lies under the hyaline cell region. Arrow points to bundles of collagen fibers 40–80 nm in diameter. Section from the high-frequency region of the cochlea from a 12 week old chicken. H, hyaline cell; TC, tympenic border cell. Figure magnification is the same as that for part a.

papilla. The aggregates are observed in all regions of the cochlear duct examined, in hyaline cells located in low-, mid-, and high-frequency regions of the cochlea.

Filament networks in basal regions of hyaline cells. A dense network of fine filaments is present in most hyaline cells, lying directly adjacent to the basal lamina (e.g., Figs. 7, 8). A high magnification of the hyaline-cell basal area is shown in Figure 10. Individual filaments are approximately 8 nm in diameter. Immunocytochemical studies of Cotanche et al. ('90) have demonstrated that filament bundles in basal regions of hyaline cells label heavily with antibodies to actin. Hence, the 8 nm fibers observed in this study appear to be actin filaments. Interspersed among the filaments are numerous dense plaques, and dense plaques also occur along the basal plasma membrane. The presence

of a dense filament network in hyaline cells is dependent on cochlear location; they are prominent in hyaline cells located in high-frequency regions of the cochlea, sparse in mid-frequency regions, and absent in low-frequency regions. Hyaline cells from the low-frequency region of the papilla are shown in Figure 11. Note the presence of the tubular aggregate described earlier and observe the absence of filaments in the basal regions of these cells. A higher magnification of one hyaline cell is shown in Figure 11b. A filament network is not discernible in its basal region.

Neural-hyaline contacts. Hyaline-cell interdigitations and neural elements are present in extracellular spaces between hyaline cells (Figs. 8, 11). As shown in Figure 12, synaptic specializations are observed in nerve fibers juxtaposed to hyaline cells. A nerve fiber opposite a hyaline cell from the mid-frequency region of the papilla is shown in Figure 12a. Small presynaptic densities surrounded by associated synaptic vesicles are present inside the nerve fiber and are attached to the cytoplasmic face of the presynaptic membrane. Single densities may be visible, or as shown in Figure 12a, several densities may be clustered together. Synaptic vesicles are 30–40 nm in diameter, have clear centers, and are roughly spherical. Mitochondria are typically found in these nerve endings, and a few dense-core vesicles are often present. The hyaline cell is separated from the neural element by a synaptic cleft about 20–50 nm in width. Slender filamentous material extends across the entire width of the cleft and appears to bridge the gap. Postsynaptic thickenings are not apparent on the hyaline cell.

Presynaptic specializations between neural elements and hyaline cells are observed in all regions of the cochlea; they appear to be more numerous in high-frequency regions of the papilla. Interestingly, presynaptic specializations are observed on hyaline cells in the low-frequency region of the cochlea where there is no apparent filament network in the basal regions of the cells.

Postsynaptic specializations are also occasionally observed between neural elements and hyaline cells. A neural element adjacent to a hyaline cell located in the high-frequency region of the cochlea is shown in Figure 12b. Spherical synaptic vesicles, 30–50 nm in diameter, with clear centers are present in the neural element. Few if any mitochondria are present. The synaptic gap (11–30 nm) is regular and bridged by fine strands. Neither of the apposed membranes is thickened. A subsynaptic cisterna is present in the hyaline cell. It is separated from the postsynaptic membrane by a narrow 9–11 nm space. This type of nonclassical synapse is observed less frequently than the type described previously.

Puncta adhaerens are adhesive junctions observed between hyaline cells and neural elements (Fig. 12c). Dense material is present symmetrically on the plasma membranes of the hyaline cell and neural element, and synaptic vesicles are not intimately associated with the complex.

Gap junctions are found between adjacent hyaline cells, and between hyaline and border cells. They appear in apical, central, and bottom portions of hyaline cells in all regions of the cochlea examined.

Vacuole cells

Vacuole cells, whose most distinguishing feature is the abundance of osmiophilic spherical vacuoles in the cytoplasmic matrix, lie inferior to the hyaline cells (Fig. 2). Their basal surfaces rest on the inferior fibrocartilaginous plate,

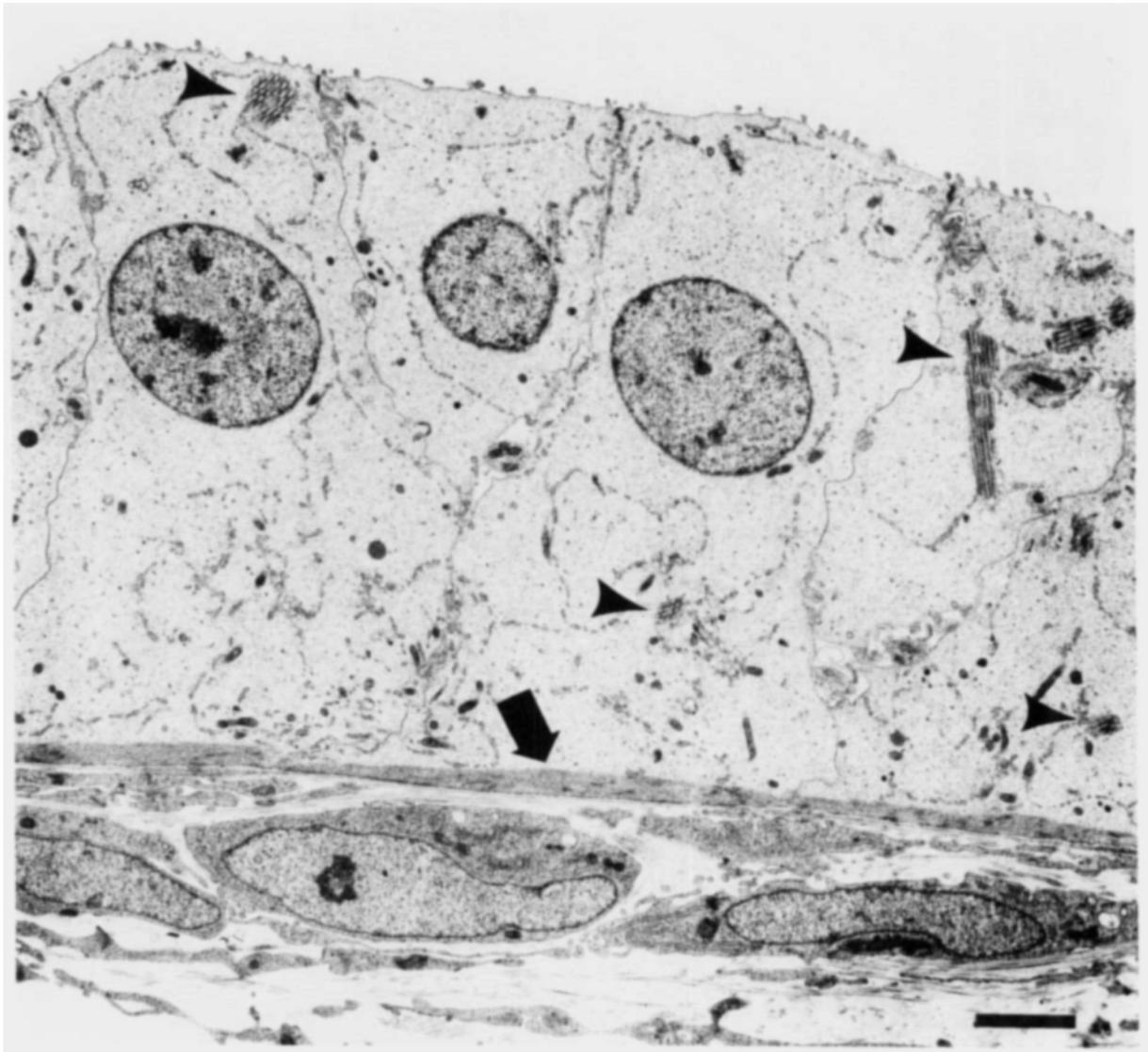


Fig. 7. Hyaline cells from the high-frequency region of the cochlea from an 11 day old chick. Dense networks of fine filaments appear directly adjacent to the basal lamina (arrow). An unusual structure

consisting of regular arrays of tubular membrane—a striated rough tubular aggregate—is often observed in apical and/or basal regions of hyaline cells (arrowheads). Scale bar = 2 μ m.

and their apical surfaces contact the endolymphatic fluids of the scala media space. The cells are rectangular in shape, and a few short, stubby microvilli protrude from the apical surfaces (Fig. 13).

Electron-dense vacuoles are prominent in the cytoplasmic matrix of the vacuole cells (Fig. 14a). Numbers of vacuoles vary from cell to cell, and they can be extensive in some cells (e.g., Fig. 14b). Golgi apparatus is present in apical and nuclear regions. Mitochondria, polyribosomes, a few lysosomes, a few microtubules, rough ER, and a few multivesiculated bodies are present. Diplosomes appear near the apical surface. In general, there is a sparsity of intracellular organelles with the exception of the abundant spherical vesicles. Unlike neighboring hyaline cells, dense filament networks are not present in the basal regions of these cells. Neural elements are not observed in the extracellular spaces between adjacent vacuole cells but a few

interdigitations between neighboring vacuole cells are present.

DISCUSSION

Held detected and described hyaline cells in the bird cochlea as early as 1926. In general, however, hyaline cells and other supporting-cell types have been largely overlooked. Investigators have been primarily interested in anatomical and physiological properties of basilar papilla hair cells and their neural innervation. Therefore, little information regarding supporting-cell structure and function has emerged. In fact, confusion still exists in the literature over something as mundane as the naming of the various nonsensory cell types in the avian cochlea. A variety of different names have been used in the literature to identify the same cells in the avian cochlear duct. To

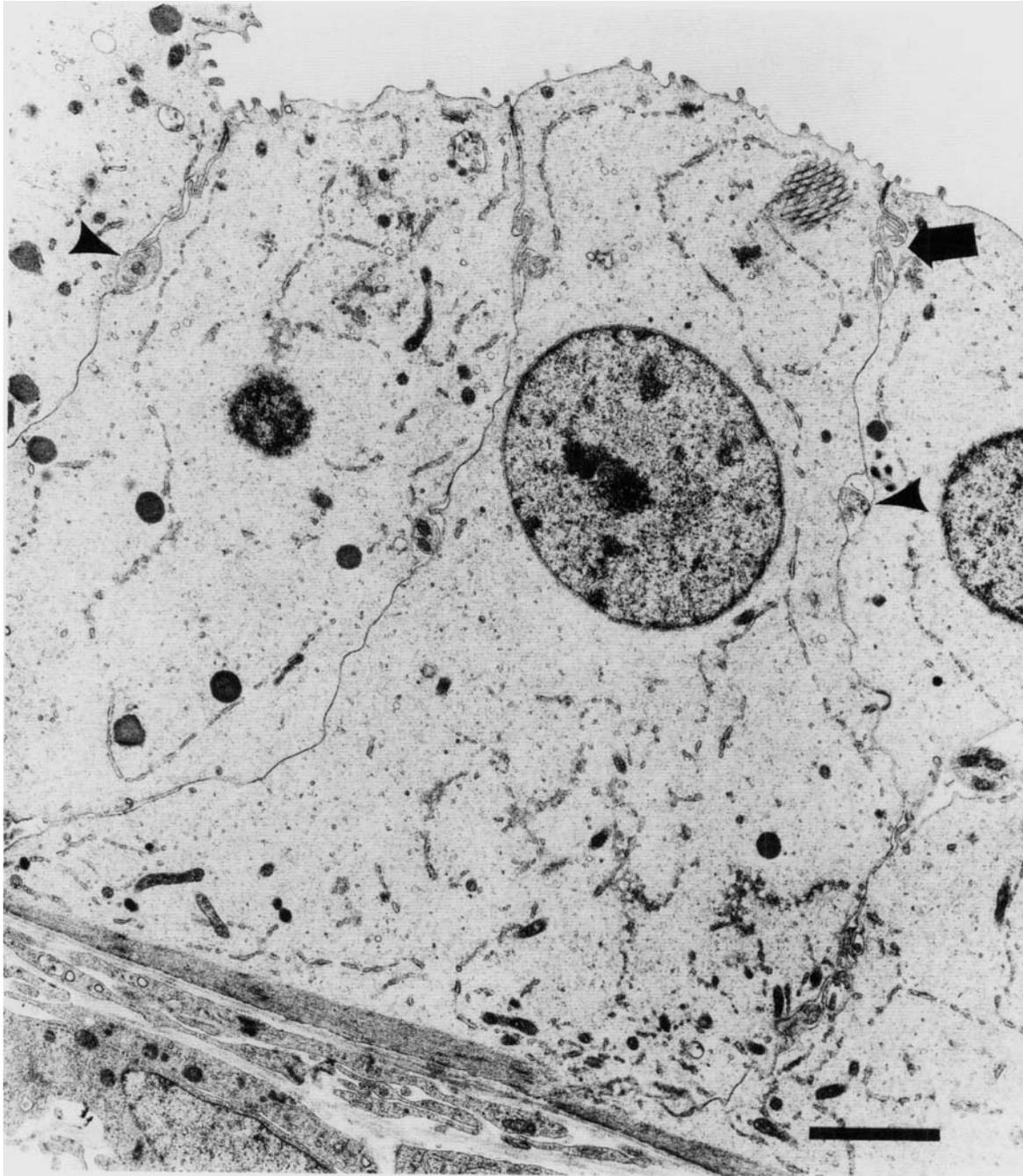


Fig. 8. Hyaline-cell interdigitations (arrow) and nerve fibers (arrowheads) are present in extracellular spaces adjacent to hyaline cells. Section from the high-frequency region of the cochlea from an 11 day old chick. Scale bar = 2 μ m.

illustrate, some of the names used previously for the cell type depicted in Figure 15b by "HM" are the following: 1) columnar cells (Fermin and Cohen, '84); 2) homogene cells (Jahnke et al., '69; Chandler, '84), and 3) hyaline cells (Retzius, 1884). Terms used by investigators for the cells labeled "CC" in Figure 15b are hyaline cells (Jahnke et al., '69) and clear cells (Chandler, '84). The cells labeled "H" in Figure 15c have been called epithelial cells (Retzius, 1884), epithelial cuboidal cells (Fermin and Cohen, '84), or hyaline

cells (Takasaka and Smith, '71; von Düring et al., '74). Names used previously for cells in Figure 15b,c labeled "C" and "V" include the following: 1) cuboidal cells (Takasaka and Smith, '71), 2) epithelial cells (Retzius, 1884), and 3) hyaline cells (Fermin and Cohen, '84).

Morphological features of nonsensory cells in and adjacent to the receptor epithelium are particularly interesting in light of recent findings from the sound-damaged basilar papilla which suggest that organ supporting cells and

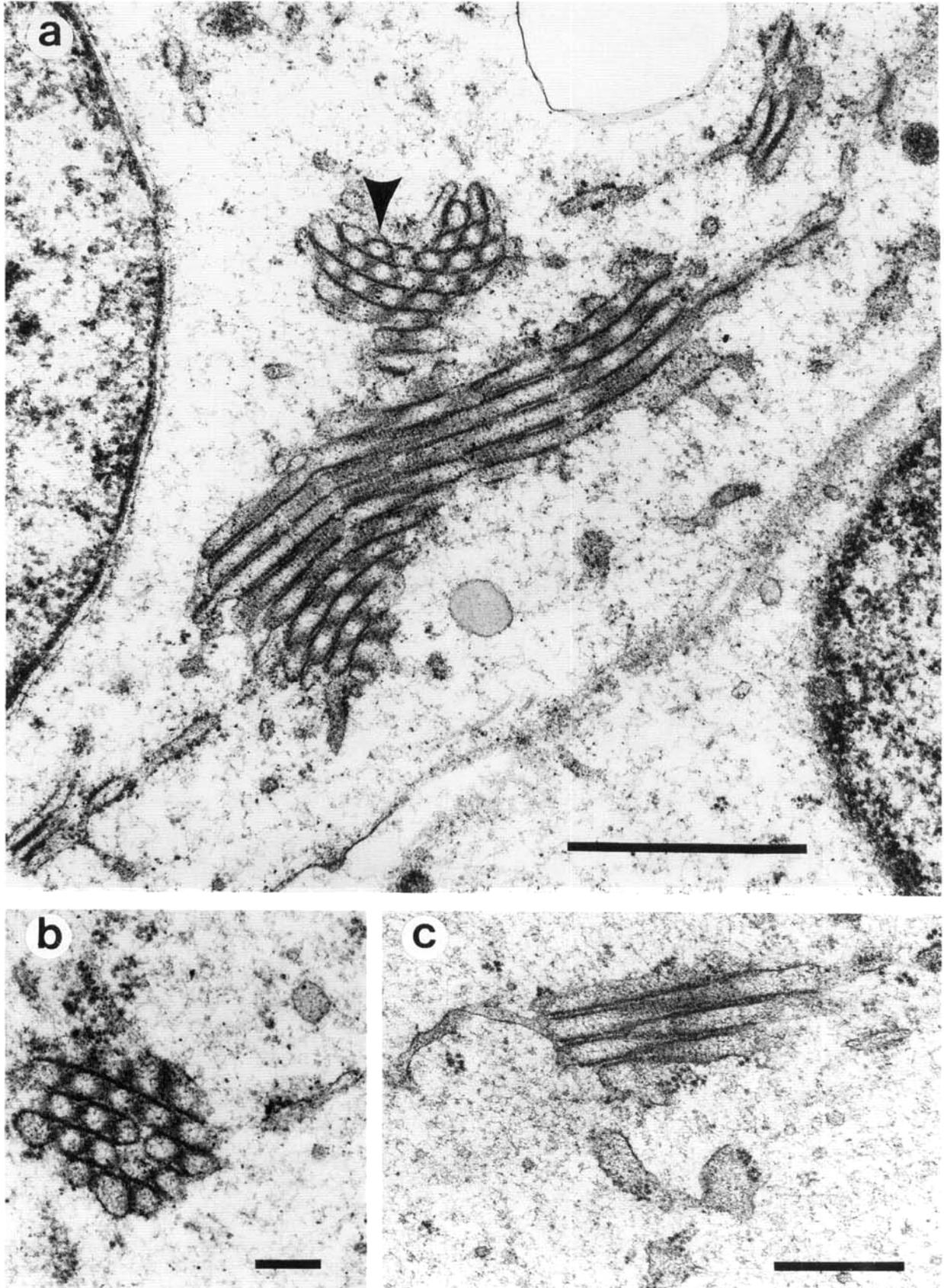


Figure 9 (see next page)

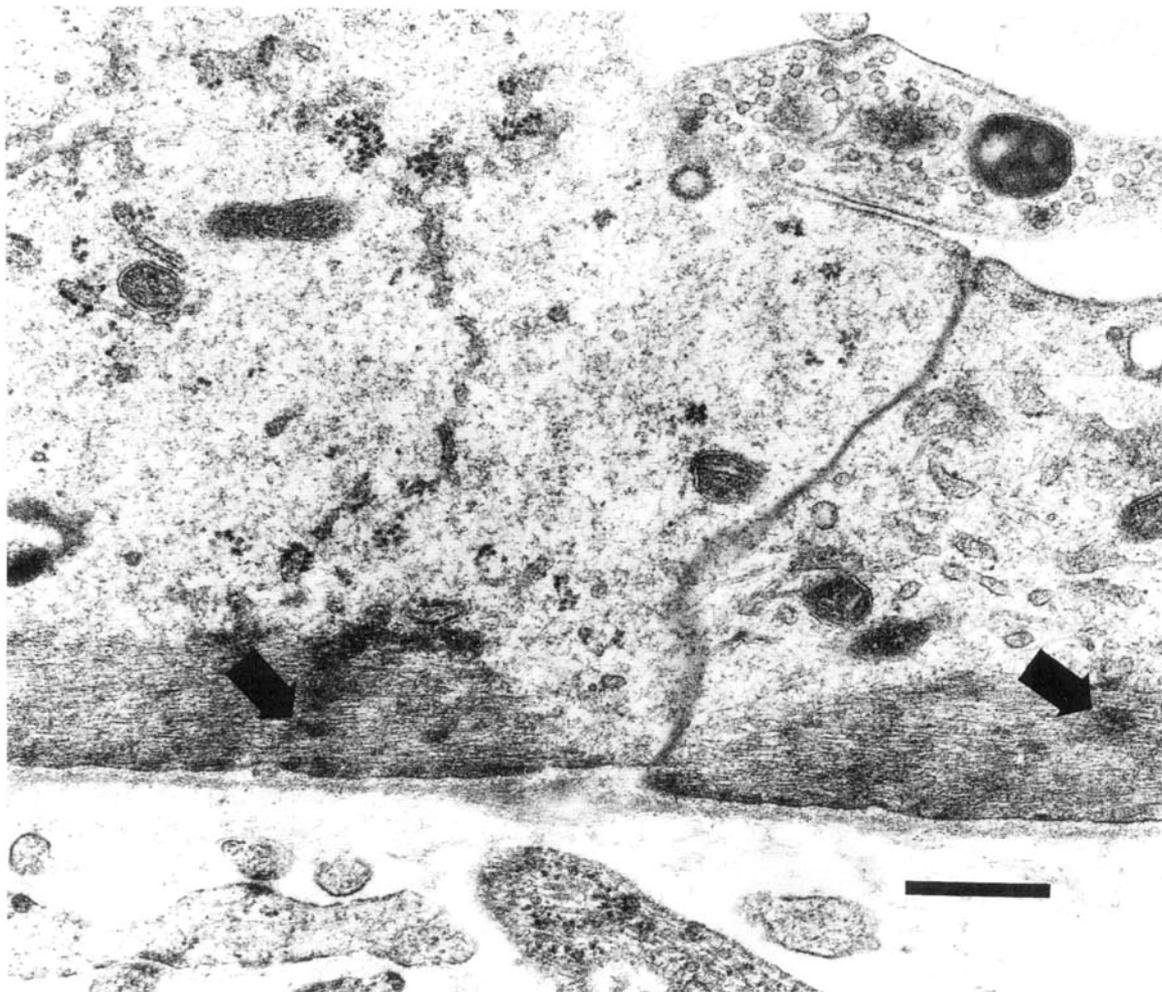


Fig. 10. Basal area of a hyaline cell located in the high-frequency region of the cochlea from an 11 day old chick. Numerous dense plaques (arrows) are interspersed among a network of dense filaments. Scale bar = 0.5 μm .

nonsensory cells inferior to the receptor epithelium participate in the repair of the sound-damaged end organ and are putative hair-cell precursor cells (Corwin and Cotanche, '88; Girod et al., '89).

Our intentions were to describe the ultrastructural features of nonsensory cells bordering and inferior to the basilar papilla in the normal chicken. It is anticipated that these data will lead towards a better understanding of the

morphological features of potential hair-cell precursor cells. It is also hoped that our detailed study of the ultrastructural features of these cells will help clarify some of confusing nomenclature existing in the literature and assist in the adoption of a standard terminology for structures in the avian cochlea.

Border cells

The chicken's basilar papilla is composed of supporting and sensory cells. Several types of hair cells have been identified, namely tall, short, and intermediate hair cells (Takasaka and Smith, '71). In contrast, it has been assumed that the supporting-cell population is homogeneous; that, unlike the mammalian organ of Corti, only one type of supporting cell is present in the receptor epithelium. We propose that a heterogeneity of supporting-cell type exists in the chicken basilar papilla. Supporting cells at the inferior edge of the receptor epithelium are morphologically distinct from other basilar-papilla supporting cells and hence should be considered a separate cell type. We named the cells at the inferior edge of the papilla "border cells" and refer to the rest of the papilla supporting-cell popula-

Fig. 9. Unusual structure in hyaline cells. **a:** A striated rough tubular aggregate in the cytoplasmic matrix of a hyaline cell from the low-frequency region of the cochlea from a 22 week old chicken. The structure is composed of parallel arrays of striated tubules. Circular profiles (arrowhead) derived from transverse sections through the tubules are also present. Rough endoplasmic reticulum lies in the immediate vicinity of the structure and appears to be continuous with it. Scale bar = 1 μm . **b:** Cross section of the structure. Individual subunits are approximately 94 nm in diameter. Section from the high-frequency region of the cochlea from a 22 week old chicken. Scale bar = 0.2 μm . **c:** Rough tubular aggregate consisting of only a few tubular cisternae. Note the continuity between the tubular cisternae and the rough endoplasmic reticulum. Section from the high-frequency region of the cochlea from an 11 day old chick. Scale bar = 0.5 μm .

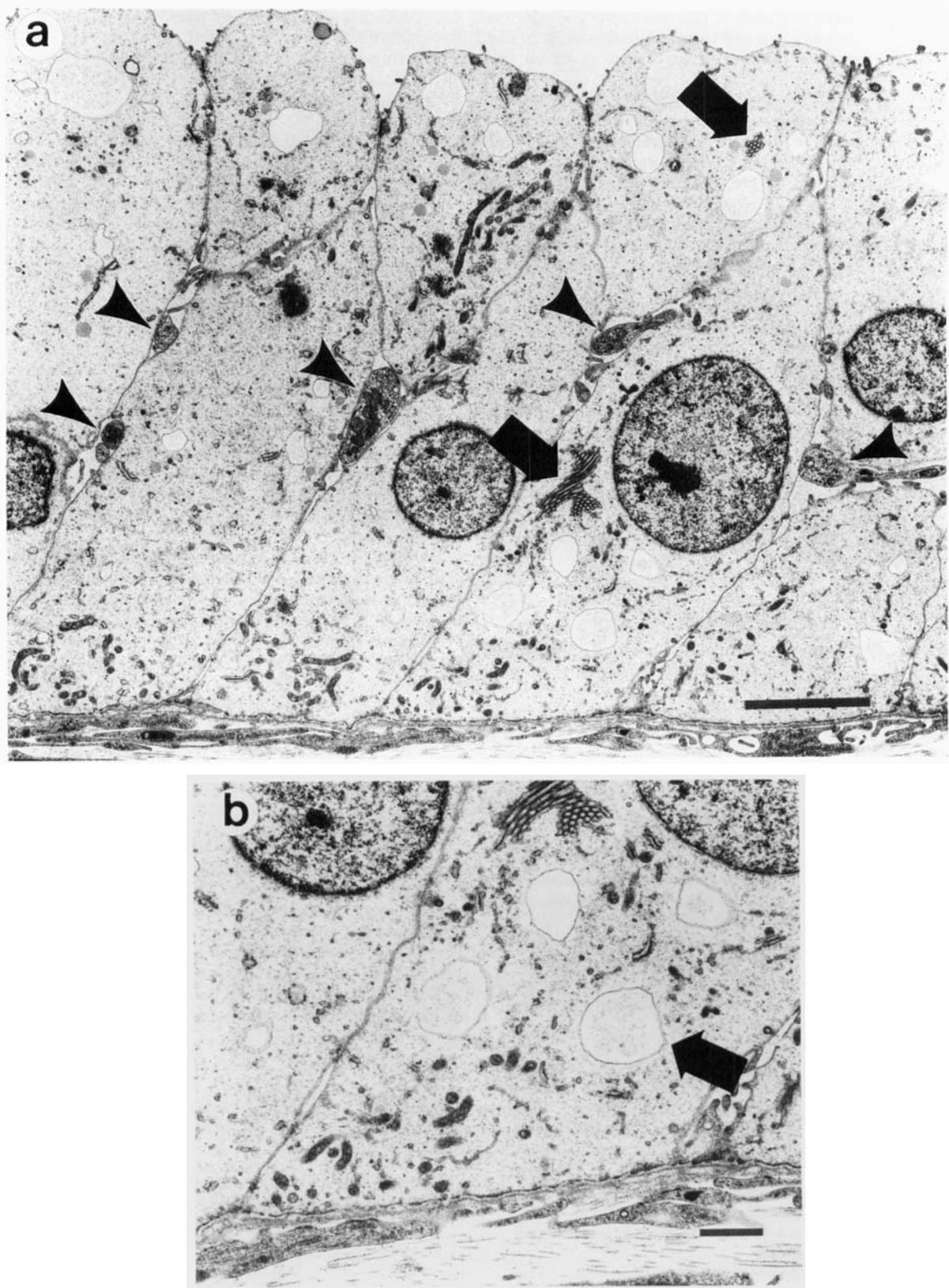


Fig. 11. Hyaline cells from the low-frequency region of the cochlea from a 22 week old chicken. **a:** Note the presence of the rough tubular aggregate (arrows) and observe the absence of filaments in the basal regions of these hyaline cells. Numerous neural profiles are present in

the extracellular spaces between hyaline cells (arrowheads). Scale bar = 5 μ m. **b:** A higher magnification of one of the hyaline cells shown in part a. Note the presence of the large vesicles filled with a fine filamentous substance (arrow). Scale bar = 1 μ m.

tion as "organ supporting cells." The question of whether a heterogeneity of supporting-cell types may also exist in the more superior regions of the papilla remains to be addressed.

Border cells can be differentiated from organ supporting cells on the basis of location and morphological characteristics. In a cross section of the chicken cochlear duct, several rows of border cells separate the sensory region from the hyaline cells and rest above the region of the basilar membrane where the transition occurs from the superior zone to the inferior zone. Border cells lie inferior to the hair cells and lie inferior to the inferiormost organ supporting cell. They abut the hyaline cells and are part of the basilar papilla proper.

Border cells have large luminal surfaces in contrast to organ supporting cells, and border-cell height varies in the radial direction, whereas the height of organ supporting cells is relatively constant. The fibrous part of the tectorial membrane extends to the microvilli of organ supporting cells but not to border-cell microvilli. Fine filaments or rootlets extend from the bases of microvilli atop the organ supporting cells but are absent in border-cell microvilli. A variety of cytoplasmic organelles are congregated in the apical regions of organ supporting cells in contrast to border cells, and organ supporting cells have a more extensive population of microtubules throughout the cytoplasm than border cells.

Hyaline cells

Dense filament networks. In the present study we have shown that dense networks of filaments appear in the basal portion of many hyaline cells. Our findings corroborate and extend those of Cotanche et al. ('90) and Drenckhahn et al. ('91) in the chicken and caiman inner ear, respectively. The presence of the basally located filament bundles in hyaline cells is dependent on cochlear location. A dense filament network is present in the basal regions of hyaline cells from high- and mid-frequency regions of the cochlea, but is absent in basal regions of hyaline cells located in the low-frequency region. In addition, we detected neuronal synapses on hyaline cells in all regions of the cochlea studied; on hyaline cells containing basally located filament bundles (high- and mid-frequency regions) as well as on hyaline cells not possessing a dense filament network (low-frequency regions). Thus, the presence of hyaline-neuronal synapses does not appear to correlate with the distribution of dense filament bundles in hyaline-cell basal regions.

Immunocytochemical studies of Cotanche et al. ('90) and Drenckhahn et al. ('91) demonstrated that the filament bundles in the basal regions of chick and caiman hyaline cells label heavily with antibodies to actin, myosin, and alpha-actinin, three main protein components constituting the contractile apparatus of muscle. They suggest that hyaline cells may be contractile cells capable of actively modifying the stiffness of or tension on the basilar membrane. The presence of efferent neural innervation to the hyaline cells may provide a means by which hyaline-cell contractibility can be regulated.

Striated rough tubular aggregate in hyaline cells. The discovery of an unusual structure, the striated rough tubular aggregate, in hyaline cells represents one of the more interesting findings. This study furnishes the first evidence for its existence in inner-ear cells. Its presence was

detected with all fixatives employed during the study, and features of the structure remain constant across the fixatives utilized, arguing that the presence and characteristics of the striated rough tubular array reported herein are not reflections of fixation artifacts.

At this time, we are unaware of other normal cells containing this cytoplasmic organelle. Other structures consisting of bundles of cisternae or tubular elements and closely related to the endoplasmic reticulum include Kolmer's crystalloid and tubular bundles observed in axons in the lateral vestibular nucleus of adult rats. Our structure bears some resemblance to Kolmer's crystalloid, a bundle of tubular elements observed in horizontal cells in normal human retina (Yoshida, '65; Uga and Ikui, '69) and endothelial cells of the human cornea (Jensen et al., '75), but differences in tubule diameter and appearance suggest that the structures are not equivalent. Specifically, the diameters of individual tubules constituting Kolmer's crystalloid (0.2–0.25 μm ; Uga and Ikui, '69; Jensen et al., '75) are several times larger than the tubular diameters observed herein (85–95 nm). Tubules in our structure show a cross-striated appearance whereas those constituting Kolmer's crystalloid do not, and empty and electron-dense spherical bodies are observed within the matrix of individual tubules in Kolmer's crystalloid but are not observed in our structure.

Bundles of closely packed tubules also have been described in preterminal segments of axons found in the lateral vestibular nucleus of the normal adult rat (Sotelo and Palay, '71). Differences in tubule diameter, intertubular distances, and associated endoplasmic reticulum elements suggest that this structure is not equivalent with our structure as well. Tubules in the lateral vestibular nucleus measure about 55 nm in diameter and are separated from one another by about 25 nm. Diameters of tubules in our structure are larger (85–95 nm), and intertubular distances are negligible. In addition, our structure is associated with rough endoplasmic reticulum whereas the tubular bundles in the lateral vestibular nucleus are reported to be continuous with smooth endoplasmic reticulum.

Structures resembling ours have been seen in tumor cells, and Ghadially ('88) coined the term "striated rough tubular aggregate" to describe them. Striated rough tubular aggregates have been seen in human neoplastic cells of: 1) a malignant stromal tumor (Tokue et al., '85); 2) an acinar cell carcinoma (Bockus et al., '85); 3) solid and cystic acinar cell tumors of the pancreas (Arai et al., '86); 4) a neuroendocrine carcinoma of pancreas and a neuroendocrine carcinoma of colon; 5) an undiagnosed tumor thought

Fig. 12. Hyaline-neuronal junctional complexes. **a:** Shown is a nerve fiber opposite a hyaline cell (H) from the mid-frequency region of the cochlea from a 12 week old chicken. Several small presynaptic densities surrounded by associated synaptic vesicles are present inside the nerve fiber (arrows). Numerous mitochondria are present in the neural element along with several dense-cored vesicles. A coated pit is present in the postsynaptic hyaline cell near the synaptic site (asterisk). A coated vesicle (arrowhead) is visible near another synaptic site on the same hyaline cell. Scale bar = 1 μm . **b:** Postsynaptic specializations observed in a hyaline cell. Shown is a neural element adjacent to a hyaline cell located in the high-frequency region of the cochlea from an 11 day old chick. A subsynaptic cisterna (arrowhead) is visible in the hyaline cell. Mitochondria are not observed in the neural element. Scale bar = 0.2 μm . **c:** Puncta adhaeren, an adhesive junction (arrowhead), between a hyaline cell (H) and a neural element from the mid-frequency region of the cochlea from a 12 week old chicken. Scale bar = 0.2 μm .

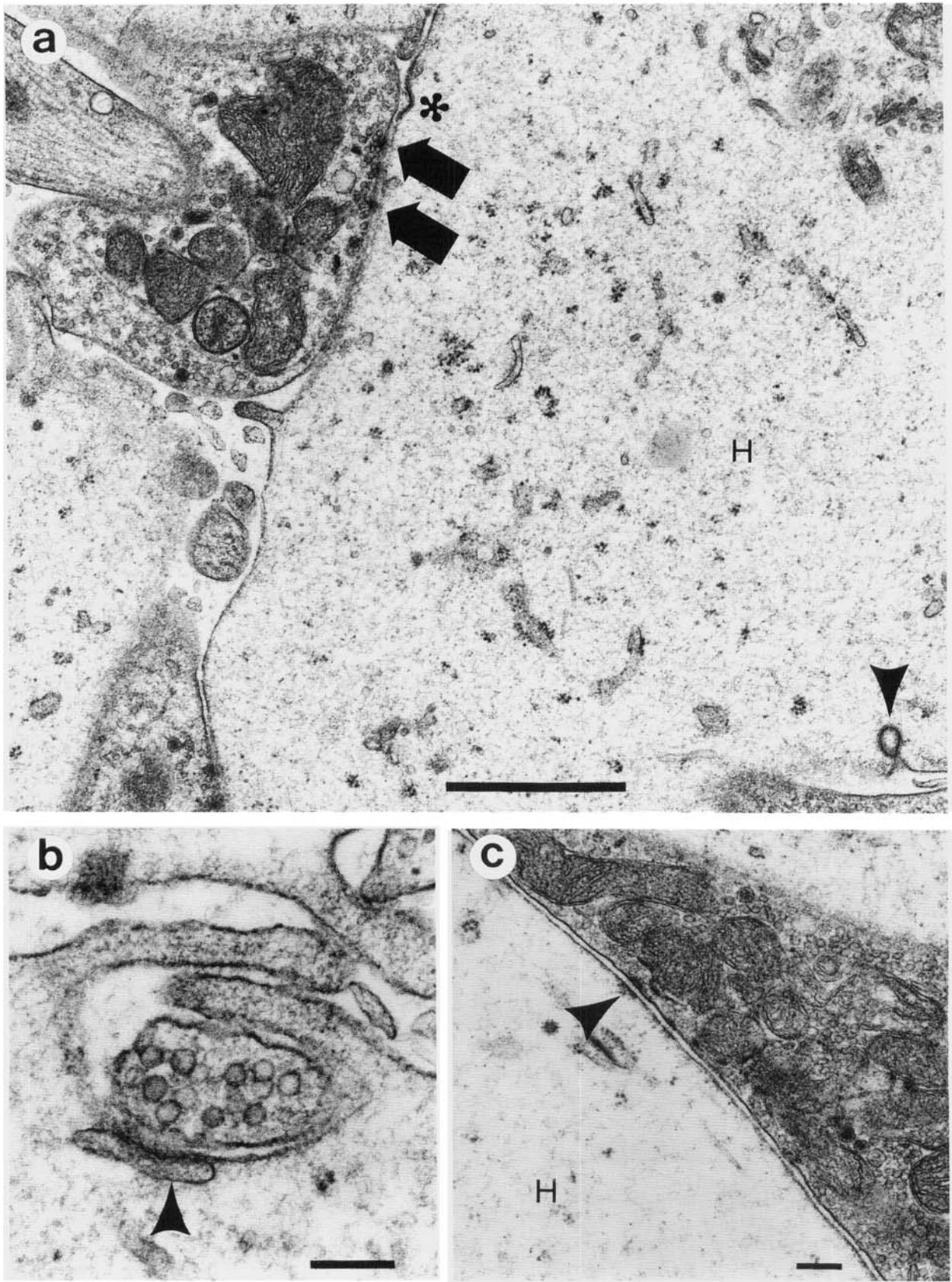


Figure 12

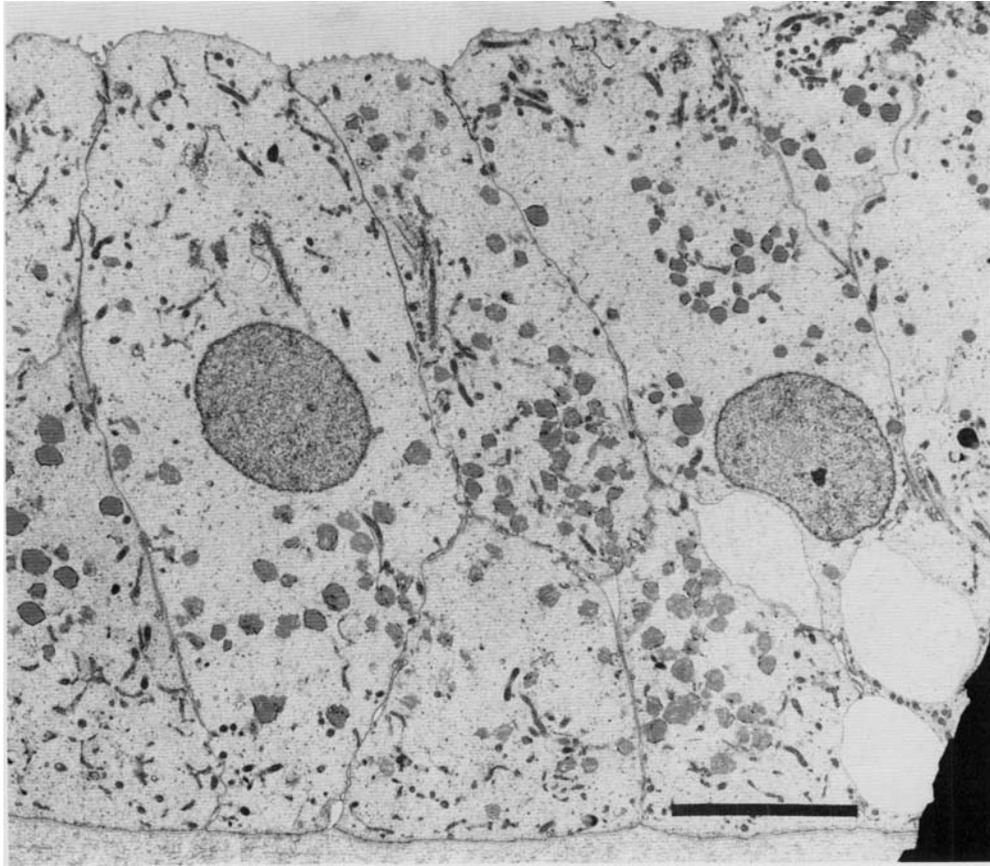


Fig. 13. Vacuole cells from the low-frequency region of the cochlea from an 11 day old chick. Scale bar = 5 μ m.

to be either a malignant haemangiopericytoma or a neuroendocrine carcinoma (Ghadially, '88); 6) a pheochromocytoma (Ghadially, unpublished observation); and 7) adenocarcinoma of the breast (Nordquist, personal communications). Like the structure we describe, striated rough tubular aggregates are composed of a group of ribosome-studded tubules lying in the cytoplasmic matrix. The tubules show a striated appearance. Tubular diameter can approach values reported herein (e.g., diameter of tubules in the stromal tumor is 85 nm: Tokue et al., '85).

The significance of striated rough tubular aggregates in the various human neoplastic cells is not known. It has been speculated that the rough tubular aggregate represents an abnormal proliferation of the rough endoplasmic reticulum engendered by the neoplastic state (Ghadially, '88). With regards to the hyaline cell, conclusions regarding the function(s) of the rough tubular aggregate cannot be drawn. It may be related to the genesis of rough-surfaced endoplasmic reticulum in hyaline cells. Continuity with rough endoplasmic reticulum suggests that the organelle may be involved in protein synthesis. The presence of rough tubular aggregates and large vacuoles in hyaline cells leads to the speculation that hyaline cells may play an important secretory role. Hyaline cells may secrete a substance into the endolymphatic spaces which in turn could potentially affect the hair-cell environment. Neural-hyaline synaptic contacts may regulate the secretory functions of hyaline cells.

Even though the functions of the tubular aggregate are unknown at present, its presence in hyaline cells and absence from other cell types in the chicken cochlea makes it a useful marker for the identification of hyaline cells.

Neural innervation to border cells and hyaline cells

This is the first report of neural-supporting cell synapses existing in the inner ear of birds. We have observed neural-border cell and neural-hyaline cell synapses in cochleae of juvenile and adult chickens. Neural-border cell and neural-hyaline cell specializations do not exhibit pre- and postsynaptic thickenings but do exhibit synaptic features such as accumulation of presynaptic vesicles at the contact site, small presynaptic densities, a synaptic cleft-like extracellular space occupied by a granular material of low electron density, and subsurface cisternae in the postsynaptic cell. All these morphologic features characterize normal synaptic contacts between neurons. Dimensions of the synaptic vesicles and cleft width for neural-border cell and neural-hyaline cell synapses fall within ranges reported for interneuronal synapses of the central nervous system (Peters et al., '91).

Although neural-border cell and neural-hyaline cell synapses do not exhibit several features characteristic of classical synapses (e.g., Pre- and postsynaptic membrane thickenings), we suggest that the presence of the specializations described above indicates that these sites are special-

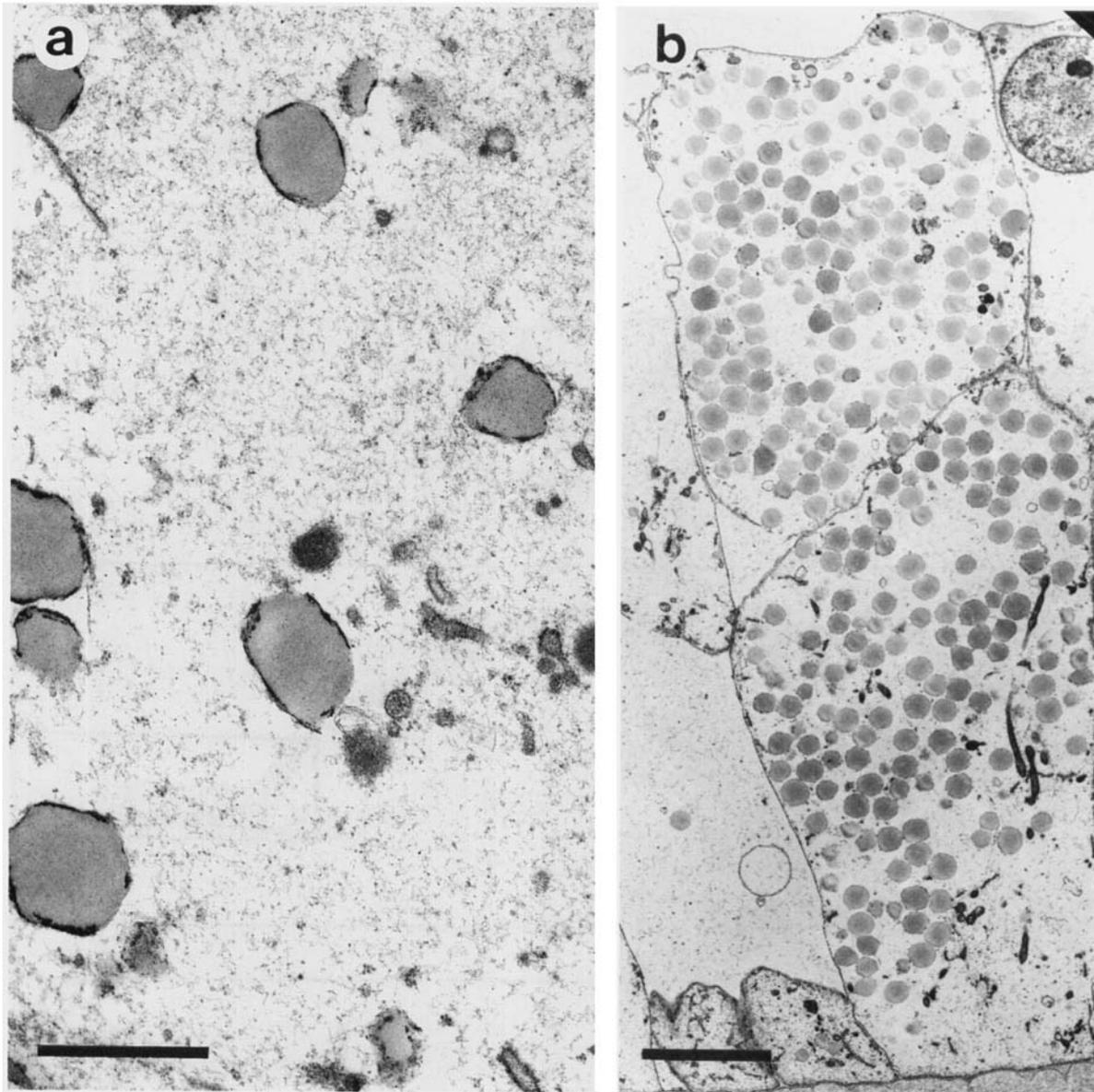


Fig. 14. Vacuole cells. **a:** High magnification of spherical, electron-dense vacuoles present in the cytoplasmic matrix of vacuole cells from the high-frequency region of the cochlea from a 12 week old chicken.

Scale bar = 1 μ m. **b:** Several vacuole cells from the low-frequency region of the cochlea from a 22 week old chicken with numerous vacuoles in their cytoplasmic matrix. Scale bar = 5 μ m.

ized neural-border cell and neural-hyaline cell contacts which allow for neural communication with these cell types. In general, efferent contacts in the inner ear do not exhibit much specialization. Contacts between vesicle-filled efferent swellings and afferent fibers in the inner hair cell area of the organ of Corti, as well as some contacts between efferent fibers and the inner hair cells themselves (e.g., Liberman et al., '90), look similar to those shown in figure 12a, i.e., no visible "postsynaptic" specialization and little "presynaptic" specialization beyond small punctate densities is observed. In the avian basilar papilla, neither of the apposed membranes is thickened in synaptic contacts between the hair cells and efferent fibers (e.g., Takasaka and Smith, '71; Hirokawa '78; Tanaka and Smith, '78).

Corroborating evidence for the existence of neural-nonsensory cell synapses in general comes from studies of normal nervous tissue development as well as studies of the normal central nervous system in situ and studies of central nervous system tissue cultures. Over the last quarter of a century there have been several descriptions of structures that appear to be axo-glial synapses. These structures meet the morphological criteria for synapses on the basis that there is a vesicle-containing profile forming a specialized junction with another structure, a neuroglial cell. Neural-glial synapses involving astrocytes were described in the cerebellum of the adult frog (Palacios-Prü, et al., '83), in the developing spinal cord (Hendrickson and Vaughn, '74; Vaughn, '89), and in the chick spinal cord in vitro (e.g.,

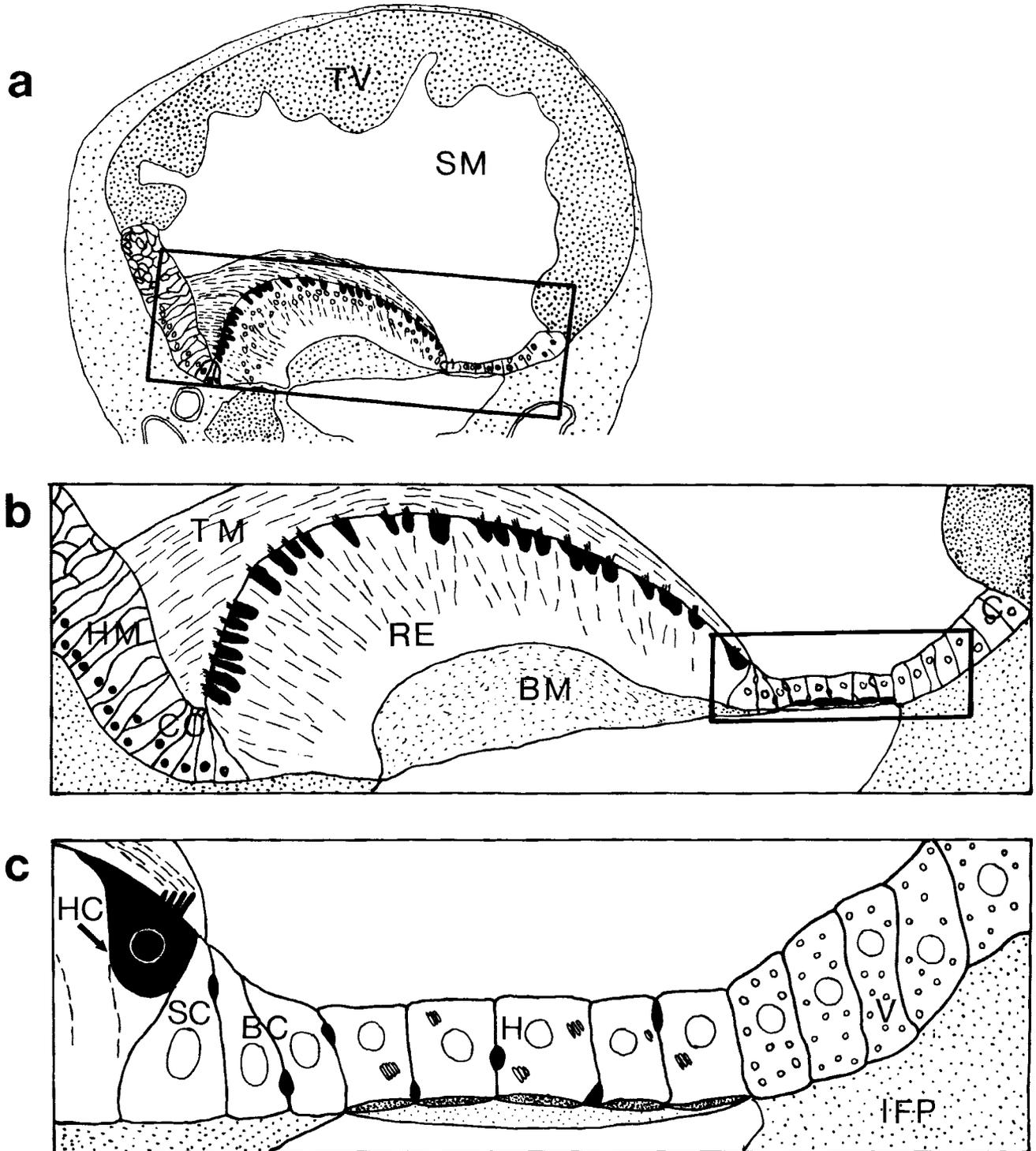


Fig. 15. Schematic reconstructions of chicken inner-ear tissue. **a:** Schematic reconstruction of a cross section of the cochlear duct from the high-frequency region of the chicken cochlea. SM, scala media; TV, tegmentum vasculosum. **b:** Schematic of the sensory epithelium and adjacent tissue. This is an enlargement of the boxed region in part a. HM, homogeneous cell; CC, clear cell; TM, tectorial membrane; RE, receptor epithelium; BM, basilar membrane; C, cuboidal cells. **c:** Schematic of the inferior region of the chick basilar papilla and the

neighboring supporting cells. This is an enlargement of the boxed region in part b. Striated rough tubular aggregates and dense filament bundles are depicted in the hyaline cells (H). Neural elements are depicted in intercellular spaces in the border cell and hyaline-cell region. Vacuoles are depicted in vacuole cells. HC, short hair cell; SC, organ supporting cell; BC, border cell; V, vacuole cell; IFP, inferior fibrocartilaginous plate.

James and Tresman, '69). Synaptic junctions with ependymal cells as the postsynaptic elements were encountered in the cerebral cortex of the adult turtle (Ebner and Colonnier, '75), in the rabbit brain (Leonhardt and Backhus-Roth, '69), and in the guinea pig hypophysis (Wittkowski, '73). Synaptic junctions involving tanyocytes have been observed in the median eminence (e.g., Wittkowski, '69; Rafols, '86). Transitory neural-glia synapses have been detected in tissue cultures (e.g., Palacios-Prü et al., '83) and in the demyelinating spinal cord (Soffer and Raine, '80).

Regarding the inner ear, neural-supporting cell synapses have been described in the auditory receptor organ of the reptile inner ear (in *Caiman crocodilus*: von Düring et al., '74; Drenckhahn et al., '91). In young and adult caimans, efferent nerve fibers form small en passant synapses with supporting cells in the region of the outer hair cells and with hyaline cells (von Düring et al., '74; Drenckhahn et al., '91). With respect to the avian inner ear, efferent innervation to the hyaline-cell region was reported in adult pigeons (Takasaka and Smith, '71) but synaptic contacts were not observed. In pigeons, the nerve plexus in the hyaline-cell region was shown by histochemical procedures to be positive for acetylcholinesterase (Takasaka and Smith, '71) suggesting that the fibers belong to the efferent system.

Features of neural-hyaline cell synaptic contacts in the chicken resemble those seen in the caiman. Specifically, synaptic vesicles are spherical, 30–50 nm in diameter, and have clear centers in neuronal-synaptic junctions in the chick. In the caiman the vesicles are spherical, have clear centers, and are 45–50 nm in diameter (von Düring et al., '74). Synaptic clefts in neuronal-hyaline junctions in the chicken are roughly 11–30 nm wide and in the caiman they are 50 nm wide (von Düring et al., '74). Synaptic contacts in the caiman are characterized by the accumulation of clear synaptic vesicles in the vicinity of presynaptic densities (Drenckhahn et al., '91), and postsynaptic thickenings are not apparent. Small presynaptic densities ringed by associated synaptic vesicles are present inside the nerve fiber in many chicken neuronal-hyaline synapses and postsynaptic thickenings are absent.

Preliminary findings from the auditory receptor organ of mammals, the organ of Corti, raise the possibility of the existence of neural-supporting cell synapses in the receptor epithelium of the mammalian inner ear. Efferent innervation to the intracellular spaces between Deiters' and Hensen's cells (Smith and Haglan, '73) or possibly the Hensen's cells themselves (Wright and Preston, '76; Stopp and Comis, '79) was reported in the guinea pig, but synaptic specializations have not yet been described. In the cat, recent light-microscopic observations of anti-synaptophysin immunostaining suggest that efferent fibers may synapse on Deiters' cells in apical regions of the cochlea (Lieberman et al., '90). These findings also remain to be confirmed by electron microscopic studies.

The central origins of the efferent fibers to the border and hyaline-cell region in the chick cochlea remain to be determined, and conclusions cannot be drawn at the present time about the function(s) of these neural-supporting cell junctions. A reasonable supposition is that the junctions allow some form of neuronal control over border-cell and hyaline-cell function, and that gap junctions between adjacent border cells, between adjacent hyaline cells, and between these cells and the neighboring supporting-cell types

might provide a means for widening or synchronizing the neuronal control over the region.

Interestingly, in the chicken, border cells may be potential precursors for regenerated hair cells (Oesterle, unpublished observations). It is plausible that border-cell innervation may be involved in the initiation and/or regulation of the regenerative process in the avian basilar papilla. Hyaline cells are involved in the regeneration and repair of sound-damaged basilar papillas and are also potential hair-cell precursors (Girod et al., '89). Recent electron microscopic and autoradiographic studies of sound-damaged ears demonstrate that hyaline cells are among the earliest cells to proliferate after extensive damage to the chick basilar papilla (Oesterle, unpublished observations). Possible roles of neuronal elements and neuronal-hyaline cell contacts in the regeneration process remain to be elucidated.

A second possible role for neuronal-hyaline junctions was put forth earlier; that the junctions may be important in neural regulation of the contractibility of hyaline cells and thus affect basilar membrane tensile characteristics. Lastly, the neural contacts may regulate as yet unknown secretory functions of hyaline cells.

Vacuole cells

Ultrastructural characteristics of vacuole cells were examined superficially in light of our ongoing studies of sound-damaged papillas. Preliminary observations of tissue from sound-damaged papillas where DNA labeling was used to indicate the presence of newly formed cells suggest that vacuole cells are probably not putative hair-cell precursor cells (Oesterle, unpublished observations). Consequently, ultrastructural characteristics of these cells were not examined in detail.

The appearance of the electron-dense vacuoles which are prominent in the cytoplasmic matrix of the vacuole cells suggests that the vacuoles may be lipid filled. Possible functions of these putative lipid-filled vacuoles or reasons for their marked accumulation in certain cells are unknown.

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