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Section 3: Histology and Pathology:

Part 1: Ear

Chapter 20: Scanning Electron Microscopic Morphology of the Ear

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Since the introduction of the scanning electron microscope (SEM) to the field of biology in the mid 1960s, substantial progress has been made in its application to the study of the ear. Three-dimensional views of the ear on an ultrastructural level obtained with the SEM have added a new dimension to the understanding of this complex organ. These new views bridge the gap between the understanding of the ear provided by light microscopy and that provided by transmission electron microscopy (TEM). This chapter is intended to provide otolaryngologists with a better concept of the morphology of the ear through the use of scanning electron microscopy. It is not an exhaustive description of all the anatomic structures in the ear; some important structures are not discussed since the SEM has not provided new insight into them.

How the SEM Differs From the TEM

Unlike the conventional TEM, which uses thin sectioned tissue, the SEM allows the examination of most biologic tissue without sectioning, thereby providing a surface view of the cells. In the SEM, a narrow, constantly scanning electron beam, about 60 Å in diameter, is focused on cell surfaces that have been coated with a thin layer of conductive metal. Some new specimen preparation techniques require no heavy metal coating because of improved electron conductivity as a result of heavy impregnation of tissue with osmium or tannic acid, or both. As the electrons bombard the surface of the metal-coated tissue in a vacuum tube, they cause the emission of secondary electrons, which are collected, electronically amplified, and projected on a cathode ray tube. The SEM, therefore, consists of a column (vacuum tube), a vacuum system, and an electronic signal amplifier and displayer.

In contrast to the complex image processing in the SEM, the image in the TEM, as in the light microscope, is a result of heavy metal staining of the tissue, which provides electron contrast for a fluorescent screen. In the TEM, sectioned and stained specimens are placed in the pathway of the electron beam, but in the SEM, the bulky specimens are placed at terminals of the primary electron projection. This latter arrangement allows greater maneuverability of the specimen in the SEM than is possible in the TEM.

The x-ray emissions generated by electron bombardment of the tissue can be collected and analyzed using the energy-dispersive x-ray (EDX) analyzer. When the x-ray analyzer is coupled with the SEM system, localization of elements (elemental mapping) can also be done (see later section, Inner Ear Fluid Space). Using standard solutions, quantitative measurement of

electrolytes in the inner ear fluids can be obtained.

Middle Ear

Tympanic Membrane

The tympanic membrane is formed by the pars flaccida and the pars tens. The *pars flaccida,* also known as Shrapnell's membrane, consists of the epidermal middle lamina propria, and inner mucosal layers. The middle lamina propria is made up of loose connective tissues with elastic and collagen fibers. The abundance of elastic fibers accounts for the elasticity of this part of the membrane, although the pars flaccida is generally thicker than the pars tensa in humans (Lim, 1970). The pars flaccida is of particular clinical importance in relation to the development of cholesteatoma. The *pars tensa* also consists of three layers. However, the lamina propria in the pars tensa consists of outer radial and inner circular fibers. These fibers have been considered to be collagen, but recent electron microscopic and biochemical studies have shown that they are fine fibrils of an unidentified substance, possibly keratin or reticulin (Johnson et al, 1968). The outer radial fibers are generally well developed near the umbo, whereas the circular fibers are better developed in the periphery of the membrane. In addition to these two distinct fiber layers, there are a few parabolic, traverse, and crescentic fibers that are also a part of the total tympanic membrane fiber arrangement.

Lining Epithelium of the Eustachian Tube and the Middle Ear Mucosa

The mastoid, tympanic cavity, and eustachian tube are integral parts of the tubotympanic complex and are covered by mucosal epithelium. The lining epithelium of the eustachian tube is formed by ciliated cells (80 per cent in humans) and secretory cells (goblet cell) (Sade, 1966; Lim and Shimada, 1971). The side near the pharyngeal opening contains seromucous glands that open to the epithelial surface. On the other hand, the lining of the middle ear cavity is generally described as a simple mesothelial-like squamous epithelium. Recent electron microscopic studies have provided evidence that the lining of the middle ear cavity is formed by numerous ciliated, as well as secretory, cells (Lim et al, 1967; Kawabata and Paparella, 1969; Hentzer, 1970). About 10 per cent of the epithelial cells are ciliated on the promontory of the normal adult (Shimada and Lim, 1972). A typical mucous blanket can be seen on the promontory and in the antrum. This information suggests that the middle ear cavity and the eustachian tube are provided with a mucociliary transportation system that could be considered part of the defense system of the middle ear.

Experimental evidence has shown that a functioning mucociliary system is essential for fluid evacuation from the tympanic cavity (Bakaletz et al, 1987), and that the respiratory viruses, such as influenza A or respiratory syncytial virus, destroy the ciliated cell, resulting in tubal mucociliary dysfunction that leads to secondary bacterial infection (Lim et al, 1987). In addition to this mechanical defense system, the presence of a biochemical defense system, such as bactericidal lysozyme and lactoferrin, has been demonstrated (Lim et al, 1976; Hanamure and Lim, 1986).

Inner Ear

Inner Ear Fluid Space

The inner ear, or labyrinth, is composed of two parts, the *bony labyrinth* and the *membranous labyrinth.* The former is made of hard ivory-like bone and encases the liquid-filled (endolymph) membranous labyrinth, which is surrounded by another fluid (perilymph). The membranous labyrinth is divided into two parts: the *pars superior,* which includes the utricle and the semicircular canals, and the *pars inferior,* which includes the cochlea and the saccule. These two parts are connected to the endolymphatic sac by the endolymphatic duct.

The *perilymph* communicates with the cerebrospinal fluid (CSF) via the cochlear aqueduct, which is loosely filled with perilymphatic connective tissue. These connective tissue cells appear porous under the SEM and are considered to be involved in filtration and modification of perilymph (Duckert, 1974). The perilymphatic connective tissue is unique in that the fibrils are the same as those of the spiral ligament. The *perilymphatic connective tissue* is present only on the pars superior side, and in some species, the connective tissue and perilymph of the pars superior are separated by the *membrana limitans*.

When cochleae are fresh frozen in liquid nitrogen and freeze-dried, a large amount of organic substance can be preserved in the endolymph. As mentioned previously, the SEM equipped with an EDX analyzer makes it possible to study the elements (such as electrolytes) present in the inner ear fluids as well as in tissue such as the cupula, tectorial membrane, spiral limbus, and spiral ligament, which are presumably bathed in either endolymph or perilymph. The EDX analyzer is extremely sensitive and can measure elements in amounts as small as 10^{-18} gm and, using proper standards and computation, gives a quite reliable and repeatable quantitative (or semiquantitative) analysis of biologic fluid and tissue samples in the picoliter range (Anniko et al, 1985; Ryan, 1984). An EDX analyzer that is attached to the SEM can also display the localization of elements (element mapping).

The *endolymphatic duct system* encompasses the endolymphatic duct and the endolymphatic sac. The *endolymphatic duct* is formed by the saccular duct and utricular duct. The utricular duct opening at the utricular side is formed by an infolding of the utricular membrane and is called the *utriculoendolymphatic fold* or *valve* (Bast and Anson, 1949). The utricular duct is collapsed and is considerably narrower than the saccular duct. The function of the endolymphatic duct is to drain the endolymph toward the endolymphatic sac.

The *endolymphatic sac* has two parts: the rugose portion and the smooth portion. Its function (particularly of the rugose portion) is known to be resorption of endolymph and digestion of foreign bodies (Lundquist, 1965). The rugose lumen is formed by a tall columnar epithelium, and this, in turn, is formed by two types of cells: dark cells and light cells. The dark cells have a smooth free cell surface, infolded nuclei, and osmiophilic cytoplasm and are thought to have the ability to phagocytize foreign particles. The light cells have abundant microvilli, apical pinocytic vesicles, and basal cell surface invagination and are capable of transporting

macromolecules (such as colloidal silver), suggesting that these cells are involved in fluid absorption. The endolymphatic sac also contains numerous free-floating macrophages, the function of which is known to be phagocytosis and antigen processing.

Based on this observation, together with the known presence of lymphocytes, it has been suggested that the endolymphatic sac is an immune organ (Lim and Silver, 1974; Rask-Andersen and Stahle, 1980). A study by Harris (1983) demonstrated that the inner ear (particular the perilymphatic compartment) can mount local immune response when challenged. This response is significantly lower when the endolymphatic duct and sac is obliterated, suggesting that the endolymphatic sac plays a significant role in mounting a local immune response.

Also, it has been shown that tracers introduced into the bullae of guinea pigs were observed in the endolymphatic sac, but when the endolymphatic ducts were obliterated, they were no longer found in the endolymphatic sac (Saijo and Kimura, 1984). This observation implies that the antigens can penetrate the round window membrane and enter the endolymphatic sac, where they can be processed by macrophages and presented to the immune competent cell.

The smooth portion of the endolymphatic sac is formed by smooth-surfaced epithelium and presumably is involved in pressure equalization between the CSF and the endolymph, since this part of the sac is located intradurally.

Blood Supply of the Inner Ear

The blood supply of the inner ear derives mainly from the *labyrinthine artery*, which is a branch of the anterior inferior cerebellar artery stemming from the basilar artery and divides into the *common cochlear artery* and *anterior vestibular artery*. The former divides into the *cochlear artery* and *vestibulocochlear artery*, which then divides into the terminal cochlear branch and the vestibular branch. The vestibular branch supplies the saccule and the greater portion of the semicircular canals and the basal end of the cochlea. The cochlear branch runs along the midmodiolus and anastomoses with the cochlear artery. The spiral ganglion, osseous spiral lamina, limbus, and spiral ligaments are supplied by cochlear and vestibular branches. The *anterior vestibular artery* supplies the vestibular nerves, all of the utricle, and a portion of the cristae and semicircular canals. In the internal auditory meatus, the *cochlear artery* runs a spiral course around the auditory nerve as the spiral modiolar artery. This artery divides into two main branches: (1) the radiating arteriole and (2) the branches that supply the limbus (limbal vessel), tympanic lip (vessels of the tympanic lip), and vessels of the spiral ganglion (Miodonski et al, 1978). Nutrients needed to support the organ of Corti are thought to be supplied by vessels of the tympanic lip and spiral tunnel vessel. The radiating arteriole runs over the scala vestibuli and supplies the stria vascularis, spiral prominence, and spiral ligament.

Venous drainage of the inner ear is a mirror image of the arterial supply and drains into the spiral midmodiolar vein. The apical coil of the cochlea is drained by the anterior spiral vein, and part of the middle turn and basal coil is drained by the posterior spiral vein. The spiral vein is then joined by the anterior and posterior vestibular veins to become the vein of the cochlear

aqueduct, which empties into the jugular bulb. The anterior vestibular vein becomes the labyrinthine vein and joins the superior petrosal sinus. The semicircular canals are drained by the vein of the vestibular aqueduct, which becomes the sigmoid sinus (Axelsson, 1968). In the scala tympani the vessels are mostly veins. SEM survey of resin-cast vessels of the cochlea clearly shows the complex arcade of blood supply of various parts of the inner ear, and the arteriovenous anastomosis is clearly demonstrated. The arteriole leaves distinct imprints of ovoid nuclei of the endothelial cells of the arteriolar wall, but the venule shows imprints of round nuclei of endothelial cells (Hodde et al, 1977).

Cochlea

The cochlea is encapsulated by hard bone and made up of fluid-filled scalae. The perilymphatic spaces (scalae vestibuli and tympani) are lined with mesothelial cells. The cochlea also contains specialized connective tissue, namely, the spiral ligament, limbus spiralis, and basilar membrane. The *cochlear duct,* coiled in the shape of a snail, is a tubular structure, lined by specialized epithelial cells and filled with endolymph. It is also called the scala media. The cochlear duct houses the *organ of Corti,* which is responsible for neural transduction, converting mechanical energy (sound) to neural impulses. Because of this important function, this sensory organ has been extensively studied. There are more than a dozen different cell types in the organ of Corti alone, which is formed by a mound of cells that are placed on the basilar membrane. These cells include one row of inner hair cells and three to four rows of outer hair cells, and all these cells are supported in most mammals by inner and outer pillar cells, Deiters' cell (outer phalangeal cells), inner phalangeal cells, border cells, Hensen's cells, Claudius' cells, Böttcher's cells, and inner sulcus cells. The top portion of the organ of Corti, which is called the reticular lamina, provides a tight seal with a mosaic arrangement of the surfaces of sensory and supporting cells. The sensory cilia project above the reticular lamina, which separates the endolymph and cortilymph, the latter of which fills Corti's tunnel and Nuel's space. Cortilymph presumably is similar to perilymph in chemical composition.

The unique features of the mammalian auditory organ, including the basilar membrane, tectorial membrane, and organ of Corti, are the differences in size and shape along its length that have a mechanical influence on the sensory transduction process. The mass is greater in the apex and becomes smaller toward the base. The angulation of the surface of the reticular lamina in respect to the basilar membrane in the apical cochlea of the chinchilla is about 35 degrees, whereas in the base, the angle is only about 3.5 degrees (Lim, 1986). The reason for this increased angulation in the apical coil is not fully understood; however, one can speculate that it modifies (bending by a shearing motion due to angulation) or adjusts the degree of stereociliary motion. Because of the location on the basilar membrane and ciliary height, the third row of the outer hair cells is likely to move to a greater degree than is the first row. However, the degree of ciliary motion of these cells may be modified by angulation of the reticular lamina in the organ of Corti.

Surface Morphology of the Organ of Corti

The reticular lamina is made of an intricate mosaic formation of sensory cells arranged in rows, which are held in position by the supporting cells. The phalanges of the third row of Deiters' cells are shield shaped, whereas those of the first and second rows are dumbbell shaped because they are sandwiched between the hair cells (Lim, 1980). The surface of the organ of Corti also changes along the length of the cochlea. The inner pillar cells that separate the inner hair cells from the first row of the outer hair cells are narrower in the base and wider in the apex.

Altogether, there are about 12,000 outer hair cells and 3500 inner hair cells in humans. The apical surfaces of the *outer hair cells* are ovoid at the basal turn but gradually become round (heart shaped) at the apical turn. The *stereociliary bundles* form a "W" or "V", on the tips of which are located basal bodies. Each ciliary bundle in a "W" formation consists of three or four rows of cilia of different heights, resembling steps. In the basal turn, the angle of the "W" formation is wider (about 120 degrees) and in the apical turn it is narrower (about 60 degrees). In the outer hair cells, particularly in the apical turn (and to a lesser degree in the middle turn), the "W" formation is slanted, the third row being the most pronounced and the first row being the least pronounced. This slanted arrangement of the "W" formation is negligible in the basal turn. The slanted arrangements exactly coincide with the slanted fiber arrangement of the overlying tectorial membrane (Lim, 1980).

The *apical surfaces* of the *inner hairs cells* are ovoid, with the long axis lying longitudinal to the cochlear spiral. The inner hair cells are partly surrounded by the inner phalangeal cells on the sides (along the longitudinal axis), the border cells on the medial side, and the inner pillar cells on the lateral side. The inner phalangeal cells are characterized by numerous long microvilli that separate the inner hair cell heads from the neighboring inner hair cells. The sensory cilia of the inner hair cells are also arranged in a "W" formation, but with much wider angles (almost straight lines) than those of the outer hair cell stereociliary formation.

Size and Dimension of Stereocilia. The inner hair cell stereocilia are larger in diameter than those of the outer hair cells (Lim, 1980). The diameters of the tallest stereocilia of the inner hair cells in chinchillas were measured at about 450 to 500 nm, and those of the first, second, and third rows of the outer hair cells were uniformly about 200 to 250 nm. There were no great differences in stereociliary diameters between the turns (apical and basal), but their heights were considerably different (Lim, 1980).

The number of stereocilia varies along the length of the cochlea as well as among the different rows of the outer hair cells. In humans, the number of outer hair cell stereocilia varies from 50 to 120 from the apical to the basal coil (Wright, 1984). In chinchillas, the number of stereocilia is also slightly different in the inner hair cells (77 in the basal turn and 65 in the apical turn). In the first and second rows of the outer hair cells, it is about 100 to 110 in the basal turn and 90 to 100 in the apical turn. However, the third rows have the greatest differences (80 in the basal turn and 18 to 40 in the apical turn). The stereociliar height shows a gradual increase along the length of the cochlea (Lim, 1980), and this height increase in the outer hair

cells is inversely related to the number of stereocilia along the length of the cochlea. In contrast, the number of inner hair cell stereocilia varies only slightly along the length of the cochlea (Lim, 1986).

Stereociliary Linkage System. The tallest cilia have tapered bodies with club-like blunt tips that are firmly embedded into the undersurface of the homogeneous layer (Hardesty's membrane) of the tectorial membrane (Kimura, 1966). The bodies of the cilia in the second and third rows are also tapered, but their heads are frequently pointed. These pointed tips are often connected to the bodies of the tall stereocilia by a tip linkage (see later). Osborne and associates (1984) and Pickles and colleagues (1984) described the *linkage* system of the stereociliary bundles. The linkages between neighboring cilia (side links) are amorphous materials that link (glue) together either in strands or in a broad area, whereas the tip linkages connecting the tips of the stereocilia to the neighboring stereocilia bodies are strands. The tip link after gold coating was measured to be about 15 to 30 nm in width and 15 to 100 nm in length; but in the absence of gold coating, the tip link measured only 3 to 6 nm in width (Furness and Hackney, 1985). The tip links are attached to the dark condensation of the stereocilia body (Furness and Hackney, 1985). It has been proposed that the side links may be needed to keep the stereocilia together as a bundle, while the tip links may be involved in the transduction process by mechanically affecting the ion channels (see later) (Pickles et al, 1984).

New Concepts of Auditory Sensory Transduction

The commonly accepted view of the mechanism of auditory sensory transduction is that the sound transmitted to the inner ear initiates a traveling wave in the inner ear fluid compartments. The traveling wave causes the maximum excursion of the basilar membrane at a specific location because of the varying width and thickness of this membrane along the cochlear partition. Therefore, the anatomic constraint of the basilar membrane dictates the tonotopicity of the auditory organ in mammals (von Békésy, 1960). However, in the alligator lizard, the basilar membrane does not have anatomic gradation, yet the nerve fibers innervating each hair cell are sharply tuned, and this tuning can be correlated with the gradation of heights of the stereociliary bundles (Weiss et al, 1976).

The important role the *sensory cilia* play in the sensory transduction process has been widely recognized (Flock et al, 1962; Hudspeth and Corey, 1977; Liberman and Kiang, 1978; Flock et al, 1985). Recent evidence indicates that the stereocilia from different hair cells are mechanically tuned to the frequencies to which those cells respond in the auditory organ of the alligator lizard (Frishkopf and DeRosier, 1983; Holton and Hudspeth, 1983). For example, a cell tuned to 1000 Hz has a stereociliary length of 30 microm, whereas a cell tuned to 400 Hz has a stereociliary height of 7 microm (Frishkopf and DeRosier, 1983). However, in cats, the ratio of bundle heights alone is insufficient to account for the actual tonotopic location, as measured by the neural characteristic frequency (Frishkopf and DeRosier, 1983). Other physical factors such as differing numbers and size of stereocilia, angulation of the "W" formulation, type of linkage system, and presence of a stereocilia-tectorial membrane coupling mechanism may also contribute to the tonotopicity (Lim, 1986).

In mammals and chicks, as in reptiles, stereociliary height is also tonotopically organized (Weiss et al, 1976; Lim, 1980; Tilney and Saunders, 1983; Wright, 1984; Saunders et al, 1985), and direct measurement of stereociliary stiffness has shown that the stiffness is inversely correlated with the lengths of the stereociliary bundles (Strelioff and Flock, 1984). Polymerized actin has been identified as the basic cytoskeleton of the stereocilia and accounts for their stiffness (Flock and Cheung, 1977). Recent experimental data suggest that the stereociliary stiffness may be modified, and its recovery from the reduction in stiffness requires intact cellular function (Saunders and Flock, 1985).

There is also good evidence to suggest that the morphologic changes involving stereocilia have a significant consequence on the single nerve neural tuning curve (Liberman and Beil, 1979; Liberman and Dodds, 1984). Therefore, it can be assumed that this unique stereociliary organization is the essential feature needed for auditory transduction and the tuning of the hair cells.

It is well known that the endolymph contains a high concentration of K+ and a high (+80 mV) electrical potential, in contrast to the high negative (-60 mV) intracellular potential of the hair cells. Recent evidence suggests that *calcium-sensitive potassium channels* exist on the apical portion of the stereociliary bundles in the frog saccule (Hudspeth, 1985). This finding implies that mechanical bending of the stereocilia would open or close the calcium-sensitive transduction channels present on the surface of the stereocilia. In fact, a linkage system connecting the tips of the stereocilia to the sides of the tall neighboring stereocilia has been suggested to be associated with the aforementioned ion channels (Osborne et al, 1984; Pickles et al, 1984). Modulated K+ ion passage through the stereociliary bundles would increase the intracellular K+ ions, requiring their elimination through the basolateral side of the hair cells by Ca-dependent K+ ion pumps. Such a concept, as proposed by Hudspeth (1985), is shown schematically.

Crawford and Fettiplace (1981) reported that, in the auditory organ of the turtle, the sensory cells themselves have a *resonant membrane potential* to the injecting current, and each cell has a best resonant frequency. This membrane resonance is most likely mediated by the influx or efflux of K+ ions through the cell membrane. Furthermore, Crawford and Fettiplace (1985) demonstrated that the oscillation of the membrane potential is coupled with the oscillation of the stereociliary bundle. The different bundles vibrated with different amplitudes and frequencies. These frequencies coincided well with the frequency at which each hair cell was most sensitive to mechanically driven displacement. Recent studies show that the outer hair cell from the guinea pig demonstrated motile response by electrical stimulation (Flock, 1983; Brownell et al, 1985; Kachar et al, 1986; Ashmore, 1987; Zenner, 1988) as well as mechanical stimulation (Canlon et al, 1988). The long outer hair cell from the apical region of the cochlea was most sensitive to low-frequency mechanical stimuli, and the short outer hair cell from the basal region of the cochlea was most sensitive to high-frequency stimuli. These investigators concluded that the outer hair cell has individually tuned motile properties (Brundin et al, 1990). Although the exact role of this motile property in the auditory function is not clearly understood, it is postulated that this motile response may be involved in the active process of the organ of Corti. This active process may play an important role in the sharp tuning of the auditory sensory

cells and may also be responsible for the otoacoustic emission.

The Cellular Organization of the Sensory Cells

Outer Hair Cells. The *bodies* of the outer hair cells are cylindrical, and lengths of the cell bodies are graded along the length of the organ of Corti and also along the radial direction (Bohne and Carr, 1979). The hair cell is formed of cuticular plates (into which the stereocilia are inserted), basal body, Hensen's body, Retzius' body, and specialized endoplasmic reticulum, such as the subsurface cistern and subsynaptic cistern (Lim and Melnick, 1971).

The *cuticular plates* are known to be made up mainly of nonpolymerized actins (Flock et al, 1981) and also contains alpha-actinin (Drenckhahn et al, 1985), fibrin (Flock et al, 1981), and tubulin (Zenner, 1981). Flock and associates (1981) demonstrated that the actin of the cuticular plate in the frog vestibule shows a criss-cross arrangement, with an opposing directional arrangement of the actin filaments when decorated with myosin S1 fragments. Hirokawa and Tilney (1982) demonstrated that these actin filaments are linked by fine (3-nm) cross-linking filaments in vestibular sensory cells of the chick. These filaments attach to the inner leaflets of the plasmalemma and also to the rootlet filaments. A recent immunohistochemical study (Slepecky and Chamberlein, 1985) showed that the rootlets of the organ of Corti of the guinea pig contain tropomyosin.

Saunders and Flock (1985) recently observed the reduction of stereociliary stiffness following mechanical overstimulation of the dissected organ of Corti. Such stereociliary bundles of overstimulated cells showed recovery in about 15 minutes. Because of the frequent softening of the cuticular plates following acoustic trauma (Lim and Melnick, 1971; Hunter-Duvar, 1977), one can postulate that the anchoring mechanism of the rootlets with the cuticular plates has been disrupted (Saunders et al, 1985). When overstimulation occurs without the softening of the cuticular plates, then the stereociliary neck must blunt the exerted force because the stereocilia are thought to pivot at the neck portion. In fact, fracture of the necks of the stereocilia is often seen in acoustically traumatized cochlear hair cells (Engström et al, 1983).

It has been shown that the *subsurface cistern* is a stack of lamellar endoplasmic reticulum that completely surrounds the internal surface of the plasmalemma. The subsurface cisterns vary considerably among different outer hair cells of the same animals and among different species. The subsurface cistern connects with Hensen's (or the fenestrated lamellar) body, the nuclear membrane, and subsynaptic cistern. Kimura (1975) earlier established the existence of a connection between the subsynaptic cistern and subsurface cistern. Recently, we observed the consistent presence of an *apical cistern* in the inner and outer hair cells of the chinchilla (Lim, 1986). The apical cistern is connected to the subsurface cistern, Golgi's apparatus, and Hensen's body. It is postulated that it is responsible for the formation and maintenance of the glycocalyx, or it may also be involved in membrane recycling of the apical plasmalemma.

Because of the similarity between the sarcoplasmic reticulum of the skeletal muscle and the subsurface cistern of hair cells, and its close relationship to the subsynaptic cistern, it is

postulated that the subsurface cistern is involved in neural conduction or in mediating excitation-contraction coupling, as in muscle or in neurons in the central nervous system (Henkart et al, 1976). Lim and Melnick (1971) earlier postulated that neural conduction may be mediated by calcium binding and release to and from the subsurface cistern. Because the subsynaptic cistern, which is connected to the subsurface cistern, is under efferent influence and efferent stimulation modifies membrane electric resonance (Crawford and Fettiplace, 1981), and because acetylcholine (the presumed neurotransmitter of efferent nerve endings) can modulate contraction of outer hair cell motility (Brownell, 1983), it is tempting to suggest that the subsurface cistern mediates excitation-contraction coupling or decoupling by binding or releasing the calcium ions, as in the sarcoplasmic reticulum.

Hensen's body is a whorl formation of lamellar endoplasmic reticulum that is found in the subcuticular space, and all the cisternae are interconnected (Saito, 1983). The function of Hensen's body is not yet known, although it has been suggested to be a modified form of endoplasmic reticulum or Golgi's apparatus. The number of lamella of the subsurface cistern and Hensen's body increase in acoustically stimulated (physiologic) or pathologic ears (acoustic trauma (Enström and Ades, 1960) and experimental hydrops (Kimura, 1975)).

There are numerous round *cytoplasmic vesicles,* which are coated or uncoated, with diameters ranging from 3.5 nm to 100 nm in hair cells in the chinchilla. Some of these vesicles are considered to be *neurotransmitter vesicles* (Gulley and Reese, 1977; Saito, 1980), and some large vesicles are pinocytotic vesicles. The *coated vesicles* also are often fused with the plasmalemma, suggesting that they are engaged in either pinocytotic or exocytotic activity. Because the coated vesicles are commonly known to be involved in protein uptake (Luft, 1976), they are probably pinocytotic in nature. It is possible that they may also be involved in the membrane recycling process that maintains a constant total cell surface by countering an increase in membrane surface by constant fusion of neurotransmitter vesicles (Gershon et al, 1981). The membrane of the sensory cell would be enlarged as a result of sensory cell excitation because neurotransmitter vesicle membrane would add to the plasmalemma during exocytosis if the membrane were not compensated for with endocytosis. Because the coated vesicles are sometimes found fused with the tubulovesicular system (Dunn and Morest, 1975), it is postulated that the clear neurotransmitter vesicles can also derive from the tubulovesicular system. This recycling process is an integral part of the process of cell homeostasis that is required for cell function. It is interesting to note that these vesicular activities (endocytosis and exocytosis) are dramatically increased in the hair cells of ears that are exposed to noise (Lim and Melnick, 1971; Lim and Dunn, 1979) or in kanamycin-intoxicated hair cells (Lim, 1986).

Mitochondria are numerous in the outer hair cells and appear mainly in two groups: Supranuclear mitochondria are closely associated with the subsurface cistern, Hensen's body, and apical cistern; infranuclear mitochondria (or Retzius' bodies) are clustered in a small space between the nerve endings and nucleus in most mammals but not in gerbils (Smith, 1978) or bats (Kimura, 1975). When the mitochondria are numerous, they have a distinct pattern in size between the nucleus and nerve endings: those closer to the nucleus are larger than those near the nerve endings (Lim and Flock, 1985). The meaning of this pattern of mitochondrial distribution

is not known, but it may represent different metabolic states: the large ones are energetically charged and the smaller ones are energetically exhausted (Lim and Flock, 1985). However, such a pattern is not obvious in the supranuclear mitochondria.

Microtubules are often seen in the infranuclear region of hair cells treated with tannic acid. A small number of microtubules are also seen in the apical part of the hair cells. Because the microtubules are known to be involved in axoplasmic transport and in the formation of the coated pit (prevesicle) (Larp, 1984), the presence of microtubules in the basal part of the sensory cell may indicate their role in the formation and transport of neurotransmitters. Because the microtubules can also interact with actin filaments in the presence of microtubule-associated proteins (Pollard et al, 1984), it is possible that these structures can also be involved in the contraction of the cell.

Motile Activity of the Outer Hair Cells. Brownell (1983) and Brownell and co-workers (1985) observed that intracellularly and transcellularly injected currents evoke shortening of the dissociated outer hair cells by the depolarizing current and evoke lengthening of the cell by the hyperpolarizing current. Flock (1983) observed contraction and relaxation when the dissociated outer hair cells were bathed with contraction medium or relaxation medium for muscle. Zenner and his associates (1985) demonstrated the motile response of dissociated outer hair cells by increasing or decreasing the concentration of K+ and Cl- in the bathing medium. For relaxation of the contracted cell, Ca++ was required, but it was not required for the initial contraction. This author interpreted as evidence of the presence of an actin-dependent motile mechanism the fact that permeabilized outer hair cells contracted in the presence of ATP, which was inhibited by cytochalasin B.

Inner Hair Cells. The cell body is shaped like a wine bottle, and the nucleus is found in about the midportion of the cell. The structures of the cuticular plates are identical to those described for the outer hair cells, although the presence of striated bodies was recently reported to be associated exclusively with the inner hair cells in chinchillas (Slepecky and Chamberlain, 1982). The top of an inner hair cell contains a well-developed network of tubulovesicular system and numerous Golgi's apparatuses. There are also numerous vesicles in the infranuclear region. Although poorly developed, the *subsurface cistern* is present in the inner hair cell as a single layer, and the subsynaptic cisternae are not generally found in the inner hair cell (Kimura, 1975). The *synaptic bodies* are more frequently found in the inner than in the outer hair cells (Dunn and Morest, 1975; Kimura, 1975). Numerous *coated* and *uncoated vesicles,* like those found in the outer hair cells, are also observed (Saito, 1980). *Microtubules* are found in both the apical and the basal regions of the inner hair cells. There are two groups of *mitochondria:* supranuclear and infranuclear. The supranuclear mitochondria are scattered around the cytoplasm, and the infranuclear mitochondria, which are few in number, are clustered near the nucleus. No pattern of size distribution, such as that found in the outer hair cells, is seen. Numerous small afferent nerve endings but very few efferent nerves innervate the cells in contrast to the outer hair cells, which are largely innervated by the efferent nerve endings (Spoendlin, 1978).

Proposed Functions of Inner and Outer Hair Cells

The most important morphologic aspect of the organ of Corti is the dual organization of the hair cells - the inner and outer hair cells. The exact sensory transduction process on the cellular level and the roles the inner and outer hair cells play in this transduction process have not been well established. The meaning of the dual (motile versus nonmotile) sensory system in the organ of Corti has not been fully understood.

These two sensory cell groups have distinct differences in their location in respect to the motion of the basilar membrane. First, the inner hair cells are located on the edge of the osseous spiral lamina, placing them on the nonmoving portion of the basilar membrane; whereas the outer hair cells are located on the basilar membrane, which is expected to be moved freely by the traveling wave, making the outer hair cells the most likely candidates involved in motile activity (eg otoacoustic emission).

Both the inner and outer hair cells are mechanoreceptors with similar stereociliary organizations and synaptic apparatuses. However, the inner hair cells receive about 90 to 95 per cent of the afferent innervation, whereas the outer hair cells, which are three times greater in number, receive 5 to 10 per cent of the afferent innervation (Spoendlin, 1978). In contrast to this, the efferent nerves mainly innervate the outer hair cells, whereas only small fibers innervate the inner hair cells. Furthermore, the efferent fibers innervating the inner and outer hair cells are distinct in their origins. Small lateral olivocochlear neurons innervate the inner hair cell and large medial olivocochlear neurons innervate the outer hair cells. The medial efferents commonly innervate on the afferents, whereas the lateral efferents innervate directly on the outer hair cells (Warr, 1978).

As to the cellular organization, the outer hair cells possess a well-organized endoplasmic reticular system formed of subsurface cistern, subsynaptic cistern, Hensen's body, and apical cistern. The subsynaptic cistern apposes the efferent nerve endings (Lim et al, 1986); thus, the endoplasmic reticular system of the outer hair cells is under direct efferent modulation. In contrast to this specialized endoplasmic reticular system in the outer hair cells, the endoplasmic reticular system in the inner hair cells is poorly developed, although an incomplete single layer of the subsurface cistern has been observed occasionally. In the inner hair cells, the efferent system may modulate the afferent neural output rather than the sensory cells themselves, because of the numerous efferent synapses to the afferents.

As discussed earlier, Lim and Melnick (1971) suggested that this specialization of the endoplasmic reticular system in the outer hair cells strikingly resembles the sarcoplasmic reticulum of muscle cells, in which the contraction and relaxation of the muscle fibers is mediated by neural impulses via the release and uptake of calcium ions in the tubular sac. A recent hair cell model proposed by Hudspeth (1985) is compatible with this concept. It is also interesting that the large number of mitochondria are firmly attached to the surface of the subsurface cistern and Hensen's body. Because the mitochondria are the primary energy source of the cells, this morphologic arrangement suggests that the function of the endoplasmic reticular

system involved in sensory transduction requires high energy. Therefore, one can suggest that the outer hair cell is well equipped for the mechanically active transduction process.

Recent data suggests that the outer hair cell is responsible for the sharply tuned tip and the inner hair cell is responsible for the threshold of the neural tuning curve from the single eight nerve fiber of cat's ears that have been traumatized with noise (Liberman and Kiang, 1978; Liberman and Dodds, 1984). These results strongly suggest that the outer hair cells are needed for frequency information. Because the total loss of all three rows of outer hair cells also reduces the auditory threshold by 40 dB (Dallos and Harris, 1978), it is also suggested that the outer hair cells may be contributing to the amplification of auditory sensitivity by interacting with the inner hair cells. For a number of reasons, the electrical interaction between the inner and outer hair cells is ruled out (Dallos, 1983). However, this interaction could be mechanical or neural, or both (perhaps via efferent). The stiffening of the stereocilia, or the motile activity of the outer hair cells may influence the stereocilia micromechanics of the inner hair cells. Because the tallest stereocilia of the outer hair cells are firmly attached to the tectorial membrane while those of the inner hair cells are free standing (Lim, 1980), the mechanical distortion of the tectorial membrane of the outer hair cells would influence the distance of the subtectorial space, thereby influencing the micromechanics of the stereocilia of the inner hair cells. Perhaps the motile activity of the outer hair cells is needed for amplification of the sensitivity of the inner hair cells as well as frequency resolution. Because the efferent input can modulate the motile activity of the outer hair cells, the efferent system may be involved in these processes. It is also possible that the efferent input could modify the mechanical configuration of the outer hair cells (Brownell et al, 1985) and that the efferent system can modulate the micromechanical environment of the inner hair cells. It is important to note that the auditory efferents have a dual system (lateral and medial) (Guinan et al, 1983) and may have differential neurochemical input to the inner and outer hair cells (Fex and Altschuler, 1985; Altschuler et al, 1986). This dual system may be needed to modulate the inner and outer hair cells separately to accomplish the amplification and spatial resolution of hearing.

Nerve Supply of the Organ of Corti

As mentioned earlier, the cochlea is innervated by two sets each of afferent and efferent fibers. The outer hair cell receives afferent innervation from type II cochlear ganglion neurons, whereas the inner hair cell receives type I cochlear ganglion neurons (Spoendlin, 1972). The outer hair cell receives large medial efferent fibers (myelinated) from the contralateral side of the medial superior olivary nucleus. The inner hair cell, on the other hand, receives lateral efferent fibers from the ipsilateral lateral superior olivary nucleus (Warr and Guinan, 1979). Therefore, the outer hair cell was innervated by two separate systems of afferent and efferent nerves. This pattern of dual innervation of afferents and efferents to inner and outer hair cells suggests that these two sensory cell groups have dual sensory functions and that the efferents have dual central regulatory functions.

The cochlear nerve, travels through thin bony plates (osseous spiral lamina) as bundles of myelinated nerve fibers. These bundles lose their myelin coating at the level of the basilar

membrane beneath the inner hair cells. The perforations in the basilar membrane are called the *habenulae perforatae.* These nerve bundles are formed by both afferent and efferent (olivocochlear) fibers.

As the nerve fibers enter the cochlea, they form the following bundles: (1) inner spiral bundles, (2) radial tunnel bundles, (3) spiral tunnel bundles, (4) outer spiral bundles, and (5) short radial fibers. There are about 20 different fibers innervating the inner hair cells, whereas one different fiber may innervate about 20 outer hair cells distributed along a certain length of the cochlea (Kim, 1984). It is believed that each of the nerve fibers supplies more than one outer hair cell and that they travel considerable distances either in the tunnel bundles or in the external spiral bundles. The population of the afferent and efferent nerve endings and their distribution in the guinea pig have been mapped out by Smith and Sjöstrand (1961). According to these researchers, the number of cochlear nerve terminals per cell seems to be greater in the apical part of the cochlea. In the chinchilla, 30 small endings (afferent) were counted on a single cell. It was further concluded that the afferent and efferent endings were approximately equal in number in the entire basal turn and on the first row of hair cells in all turns. But in the upper turns, the afferent nerve endings greatly outnumbered the efferent terminals.

Tectorial Membrane

The tectorial membrane is made up of gelatinous materials containing microfibrils and sulfated glycoconjugates in the ground substances (Iurato, 1967; Steel, 1985). Kronester-Frei (1978) classified two types of microfibriles: type A, which is banded; and type B, which is branched. The type A microfibrils appear square in cross-section (Kimura, 1984; Arima and Lim, 1988). Recent evidence suggests that the type A microfibrils may be type II collagen (Thalman et al, 1986).

The tectorial membrane can be roughly divided into three parts: (1) the limbal portion in contact with the interdental cell phalanx; (2) the midportion covering the inner sulcus, including the organ of Corti; and (3) the marginal network ("Randfasernetz"). The marginal network is believed to be attached to the third row of the Deiters' cell phalanges or Hensen's cells, or both, either by finger-like projections or by ladder-like structures. The outer surface of the membrane is loosely covered with a network of fibers similar to the material found in the marginal network (Lim, 1978). A linear band runs along the undersurface of the entire length of the tectorial membrane. This band, called Hensen's stripe, has trabeculae-like structures arranged in intervals that are the width of one inner hair cell. Whether or not the sensory hairs of the inner hair cells are firmly embedded in the membrane has been the subject of debate. Recent electron microscopic evidence suggests that the tall hairs of the outer hair cells are partially embedded in the undersurface of the tectorial membrane (Kimura, 1966). When seen by the SEM, it always shows three or four rows of imprints of the outer sensory cell hairs on the homogeneous layer (Kimura's membrane). In contrast to this, inner hair cell imprints are not commonly found, although a few sporadic imprints of inner hair cells in the basal turn have been reported in squirrel monkeys and kittens (Hoshino and Kodama, 1977).

Outer and Inner Pillar Cells

In cross-sectioned views, the outer and inner pillars can be seen to form a triangle and to support the sensory cells. The reticular laminae between the inner and outer hair cells are formed by the head plate of the inner pillar cells. The upper portion of the outer pillar cells gives firm support directly beneath the head plate of the inner pillar, but its phalanx forms a portions of the reticular lamina between the first and second rows of the outer hair cells. The pillars are arranged at regular intervals, allowing the cortilymph (Engström, 1960; Rauch, 1964) to flow freely in the tunnel and the radial tunnel fibers to pass freely between the pillars. The bases of the pillars are firmly attached to the surface of the basilar membrane by broad footings.

Inner Phalangeal Cells

The inner phalangeal cells support the inner hair cells. Their phalanges form a portion of the reticular lamina between the inner hair cells and border the pillars of the inner pillar cells and the border cells. Cytologically, inner phalangeal cells are similar to border cells. The presence of numerous long microvilli on the surface of the inner phalangeal cells suggests the possibility that these cells serve as anchoring point for the trabeculae of Hensen's stripe.

Deiters' Cell (Outer Phalangeal Cell)

The Deiters' cell consists of a tubular cell body, slender cell processes, and an umbrella-like phalanx. The upper part of the cell body embraces the lower part of the hair cell; therefore, this part of the cell is sometimes referred to as the Deiters' cell cup. The positions of the cell body and its cell phalanx are exactly one cell apart. In other words, as shown, a Deiters' cell embraces an outer hair cell (A), but its phalangeal process is attached between hair cells B and C, located toward the apex. The function of the Deiters' cell is to support the outer hair cells. In the event of outer hair cell degeneration due to acoustic trauma, the phalanx closes the gap left by degenerated outer hair cells. There is evidence that the phalanges of the third row of Deiters' cells might serve as anchoring points for the marginal network of the tectorial membrane (Kimura, 1966).

The function of other *supporting cells,* such as *Hensen's cells,* the inner phalangeal cells, *border cells,* inner sulcus cells, and Claudius' cells, is not yet established. There is some good morphologic (Lim, 1987) and histochemical (Lim and Rueda, 1987) evidence suggesting that the inner phalangeal cells, Deiters' cells, and Hensen's cells may be involved in the production of certain components of the tectorial membrane during development.

Spiral Ligament

When examined with the SEM, the sponge-like spiral ligament appears as a meshwork of fibrils. According to Iurato (1967), these fibrils are chemically similar too keratin rather than to collagen. The spiral ligament extends to the scala vestibuli as well as to the scala tympani and is thereby soaked with the perilymph. Some areas of the spiral ligament facing the scala vestibuli

and the scala tympani are not completely covered with cells, thus allowing free flow of perilymph. The exact function of the spiral ligament is not known; however, it is believed that this ligament acts as an anchor for the basilar membrane and houses the stria vascularis.

Limbus Spiralis

The limbus spiralis is formed by loose connective tissue similar to the spiral ligament and contains large numbers of blood vessels. The basilar membrane also anchors to the limbus as it did in the spiral ligament. The surface of the limbus spiralis faces the scala media as well as the scala vestibuli. Borghesan (1957) postulated that the vascular zone of the limbus spiralis facing the scala vestibuli contributes to the formation of the perilymph.

The part that faces the scala media is covered mainly with Huschke's teeth cells (interdental cells). In cross-section, these cells appear to have the shape of the letter "T"; therefore, they are also referred to as the "T" cells. Iurato (1967) and other investigators believe that, in addition to their obvious function as anchors for the tectorial membrane to the limbus, the interdental cells also secrete tectorial membrane ground substances. Another less widely accepted function of the "T" cells is secretion of endolymph (Voldrich, 1967). Occasionally, indentation of the center portion of the phalanx of this cell is observed, but the functional meaning of this observation is unclear.

Reissner's Membrane

Reissner's membrane is formed by two layers of different types of cells. The epithelial cells facing the endolymph have junctions that tightly bridge the cell. When they are viewed from the surface, these epithelial cells reveal a regular hexagonal arrangement. The surface in contact with the endolymph is covered with numerous microvilli, whereas the cells facing the scala vestibuli resemble endothelial cells in that their cell margins loosely overlap, and they do not have a well-developed tight junction. According to the "radial flow" theory of endolymph production proposed by Naftalin and Harrison (1958), Reissner's membrane presumably serves as a filter, allowing certain ions to pass through while acting as a barrier to others. This concept is proved by the tracer study that showed that injected ferritin particles are transported through Reissner's membrane from the perilymph side toward the endolymph side but that when macromolecules are injected into the endolymph space, the particles did not pass through the membrane, suggesting that Reissner's membrane serves as an effective one-way barrier (Hinojosa, 1971).

Spiral Prominence and External Sulcus Cells

The epithelial cells covering the spiral prominence are elongated hexagons whose long axis parallels the plane of the basilar membrane, whereas the external sulcus cells are strictly hexagonal. When the external sulcus cells are covered by bordering Claudius' cells, their margins become indistinct. The function of these two cell groups is not clearly understood. Although Shambaugh (1908) hypothesized that the external sulcus cell has a secretory function,

morphologic evidence in support of this concept is weak. Recent electron microscopic studies have revealed that the external sulcus cells possess treelike cytoplasmic processes with numerous vessels wrapped around them. Saxen (1951) postulated that the external sulcus cells participate in phagocytosis and absorption of the inner ear fluid.

Stria Vascularis

When the stria vascularis is sectioned, it can be seen that it is formed by three different types of cells: outer marginal cells, middle intermediary cells, and basal cells. The outer marginal cells are hexagonal on the surface and are covered with a small number of microvilli and numerous pinocytic vesicles. The base of this cell interdigitates in a complex pattern with the intermediary cells. The outer marginal or epithelial cells possess large numbers of mitochondria. The stria vascularis is provided with a rich vascular network that forms a loop at the level of the spiral prominence. The function of the stria is still a source of controversy: some argue that it is absorptive, whereas others argue that it is secretory. In either case, the implication is that the stria contributes to the make-up of the endolymph.

Vestibule

The sensory organs of the vestibule or labyrinth in mammals are formed by three cristae ampullares and two maculae, a utricle and a saccule. They are the proprioceptors of rotatory and gravitational sensations. In addition to these organs, the vestibule has an endolymphatic sac that extends from the otoconial organs and terminates as a blind pouch intradurally. This sac is believed to perform absorptive and phagocytic functions.

Otoconial Organs

The *saccule* is the only vestibular organ that is directly connected to the cochlea by way of the *ductus reuniens* and embryologically belongs to the pars inferior, as does the cochlea. The saccule is in the shape of an inverted L, and its "knee" points toward the cochlea. The saccule is securely fixed in a bony depression, the spherical recess of the temporal bone. The *utricle* is in the shape of an open shell, with its plane approximately at right angles to the saccule and partially secured to a bony depression, the elliptic recess of the temporal bone.

Otoconial Membrane

Both the saccule and utricle are termed otoconial organs because of their otoconial membranes. This membrane is formed by two parts: a gelatin layer and statoconia. When the surface of the membrane is examined, the "snowdrift lines" of Engström can be seen. In the utricle, this snowdrift line is in the shape of a U and appears to be a depression. In the saccule, it is L-shaped and elevated like a snow-covered mountain ridge. These lines correspond to the "striola" of Werner, which is visible in a surface preparation as a light area on the sensory epithelium.

Only relatively recently, clusters of small holes in the otoconial membrane along the striola were observed with microradiography (Lindeman, 1969) and SEM (Lim, 1969). Why these holes are present is not clearly understood. However, the presence of holes along the snowdrift lines suggests the possibility that the endolymph can flow freely into the subcupular space in this region. In a test tube experiment with gel-grown calcite crystals, the smaller crystals are found, as a rule, along the gel interface. It is interesting to note that the smaller otoconia (statoconia) are found on the outer surface of the membrane and also along the snowdrift lines.

In mammals, the *statoconia* are believed to consist of calcium carbonate in calcite form. The statoconium has a smooth, barrel-shaped body with two pointed tips. The pointed end is formed by three surfaces. The size of a statoconium varies considerably, ranging between 0.5 and 10 microns.

The exact mechanism and origin of otoconia formation are not clearly understood; however, when isotope-labeled calcium was given to laboratory animals, some of the otoconia became labeled. This finding, according to Belanger (1956), indicates that new calcium is incorporated into the otoconia. Regarding the origin of otoconia, Vilstrup (1951) has suggested that these crystals are formed in the endolymphatic sac during the embryonic stage and transported to the otoconial organs (transportation theory). On the other hand, some believe that these crystals are formed in the gelatin membrane (in situ formation theory). In the latter theory the calcium carbonate crystallizes in the gelatin, using the gel molecules as "critical nuclei". The importance of gelatin (mucopolysaccharide) in otoconia formation has been given further emphasis by observations made by Erway and co-workers (1970). They were able to effect the absence or malformation of otoconia by eliminating manganese from the diet administered to pregnant mice in the early stages of pregnancy. These investigators reasoned that the manganese is essential for the normal biosynthesis of the mucopolysaccharide and that deficient mucoprotein is the underlying cause of otoconia malformation. Further, when the otoconia are decalcified, they leave protein residues.

The relationship between the *sensory epithelium* and the *gelatin membrane* has been the subject of considerable interest among morphologists and physiologists. Whether the gelatin substance completely covers the hairs remains a subject of dispute. Recent electron microscopic observations suggest that only the tall stereocilia and kinocilia are partially embedded in the gelatin layer, whereas the remainder of the stereocilia can float freely in the subcupular space (Igarashi and Kanda, 1969). Whole bundles of sensory hairs are surrounded by anchoring fibrils of the gelatinous substance (Dohlman, 1971). The supporting cells that surround the sensory cells contain numerous granules that are believed to be secretory in nature. Exactly what they secrete is still unknown; however, it is postulated that the veil-like anchoring fibrils are secreted by these supporting cells.

Sensory Epithelia

According to Wersäll (1954), the sensory epithelia of the vestibule are formed by two types of sensory cells. The type I sensory cell is goblet shaped and has a cup-like nerve ending

(nerve calyx) that is believed to be an afferent nerve ending. Occasionally, an efferent nerve ending is attached to the surface of the nerve calyx. At times, the nerve calyx embraces more than one sensory cell and forms double calyces. The type II sensory cell is cylindrical and is provided with button-like nerve endings, both afferent (agranular) and efferent (granular).

There are about 10,000 sensory hair cells in the utricle of the guinea pig and about 7500 sensory cells in the saccule (Lindeman, 1969). each hair of the sensory cells consists of one kinocilium and about 80 to 90 stereocilia. The kinocilium has 2+9 internal filaments similar to those of the motile cilia, but two central filaments are frequently interrupted in sensory cilia, whereas the stereocilia are formed by an amorphous matrix. This matrix is composed of actin and provides the stiffness required for sensory transduction (Flock and Cheung, 1977; Flock et al, 1977). The kinocilium is present near the largest stereocilia, which are arranged in church pipe organ patterns. The shearing motion toward the kinocilium from the side of the stereocilia presumably excites the sensory cells, and stimulation in the reverse direction inhibits the neural discharge.

This kinocilium-stereocilia arrangement is also referred to as the direction of sensory hairs, with the kinocilium at the head and the stereocilia behind. An arrow is generally used in a diagram to indicate this arrangement in a single sensory cell. This arrangement is polarized along the striola. In the saccule, the hairs on either side of the striola are turned away from one another, whereas in the utricle they face one another. In the ampulla of the semicircular canal, the direction of sensory hair arrangement is the same on each crista. In the horizontal crista, the direction of hair arrangement is toward the utricle, whereas it is opposite in the vertical cristae. The height of sensory cilia (particularly the stereocilia) in mammals varies considerably among sensory cells as well as between locations of the sensory epithelium. Stereocilia of the type I cells are, in general, thicker and taller than those of type II cells. Regardless of cell type, the cilia of the cells distributed in the striolar area (central) are shorter than those in the peripheral area (Lim, 1977).

Semicircular Canals and Cristae Ampullares

There are three semicircular canals connecting the three cristae ampullares and the utricle. The superior and posterior canals form the common duct. These three canals are arranged at right angles to one another. Because of this arrangement, these organs are considered to be sensing devices for angular motion in each respective plane. The walls of the ampulla and the semicircular canals are formed by two layers of cells: inner epithelial and outer mesothelial-like cells. The *crista ampullaris* forms a saddle-like mound of tissue that is richly supplied with myelinated nerve fibers and capillaries embedded in connective tissue. The large fibers predominantly innervate the central portion of the crista, whereas the small fibers predominantly innervate the peripheral portion. The surface of the crista is covered with sensory epithelium formed by two types of sensory cells and supporting cells identical to those of the macula. The only difference is that the sensory cells are much taller in the crista than in the macula, and their gradations in height are greater in the crista. The height of sensory cilia in the mammalian crista also varies considerably. The cilia in the central area are noticeably shorter than those of the cells

in the peripheral area (Lim, 1977). The long kinocilium is extremely wavy in appearance. The stereocilium takes the shape of a baseball bat. It has a narrow point inserted into the cuticular plate of the sensory cells and a dilated tip. The kinocilium, on the other hand, is usually uniform in thickness along its entire length. On top of these hairs is the cupula, which is formed by a mound of gelatin. The cupula has a definite texture in a fixed specimen. It contains numerous tubular structures and resembles a honeycomb. In the natural state and in freeze-dried specimens, the cupula is in contact with the dome of the ampulla (Steinhausen, 1933; Lim, 1975).

Whether the sensory hairs are fully embedded in the cupula has been the subject of the same kind of discussion that has focused upon the gelatin membrane of the otoconial organs. Electron microscopic findings seem to support the motion that there is a subcupular space. These findings suggest that the entire length of the sensory hair is not embedded in the cupula. The cupula is thought to be formed from a protein-mucopolysaccharide complex that is sulfated (Dohlman, 1960).

Planum Semilunatum

The half-moon-shaped areas on both sides of the lateral walls of the ampulla are designated as the plana semilunata. The function of the planum in mammals is not yet clearly understood. Dohlman (1960) demonstrated that when 35S is given to the pigeon, it is immediately incorporated into the planum cells and soon moved to the cupula. Therefore, in birds, the planum is considered to be secretory epithelium. Whether the secreta are endolymph components or part of the cupula is still open to question.

Dark Cells of the Vestibule

It is worth mentioning that the dark cells, so called because of their osmiophilic nature, have a distinct pattern of distribution in the vestibule (Kimura, 1969). The dark cells are present only in the slope of the crista. Among all the vestibular organs, the saccule is the only organ that does not possess these cells. Melanocytes have been observed lining the portion of the wall below the dark cells. The function of these cells is not yet clearly understood. Kimura and others have postulated that the dark cells might be responsible for contributing to the vestibular endolymph formation. These cells are morphologically similar to the marginal cells of the stria vascularis of the cochlea in that they have numerous vesicles and enormous interdigitations of the cytoplasmic membrane. Because of these morphologic characteristics, it has been suggested that the dark cells have an absorptive function (Dohlman, 1960). It is also interesting to note that the dark cells appear to produce a mass of debris on the cell surface, easily recognized with the TEM. When examined with the SEM, these debris were found to be otoconia dissolving as a result of contact with the dark cells.

The function of the melanocytes in the vestibule is not clearly understood. Erway and others (1970) have hypothesized that the melanocytes in the vestibule contribute to the normal metabolism of manganese, which is considered essential for the biosynthesis of mucopolysaccharides.