Paparella: Volume I: Basic Sciences and Related Disciplines

Section 3: Histology and Pathology:

Part 1: Ear

Chapter 19: Transmission Electron Microscopy of the Cochlea

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Microscopic examination of histologic specimens is one of the most valuable methods of obtaining biologic information. The development of phase microscopy attained the limit of resolution of the light microscope. This resolution approximates 0.25 microm, a limit imposed by the wavelength of light, precluding adequate resolution at magnifications greater than 1100 times. With increasing knowledge, it became evident that there were definitive structures smaller than those visualized by light microscopy. If better resolution and higher magnification could be obtained, the detailed form of these structures could be further studied. This has been achieved by the electron microscope, which utilizes a beam of electrons instead of light waves.

The first practical transmission electron microscope was invented more than 40 years ago. It soon came into use in metallurgy and related industries, but a lack of techniques for producing sufficiently thin tissue sections prevented its use as a practical tool in biology until the 1950s. The electron microscope allows resolution in the order of 1 to 2 Å (Å = 10^{-8} cm) and consequently magnifications of greater than a million times (Sjöstrand, 1967). Electrons pass through thin sections of a specimen and through systems of magnetic lenses and are finally made to impinge upon a fluorescent screen or a photographic plate. This electron beam is influenced to such an extent by its environment that it must be produced in a near vacuum. The sections must be extremely thin to allow "clean" penetration without scattering of the electrons. Producing sufficiently thin sections (250 Å) without chatter or folds is the chief technical stumbling block of biologic electron microscopy.

Electron microscopy has made new demands on fixation and preparation of specimens. At high magnification, postmortem changes are easily visualized, necessitating standards of specimen collection more rigorous than those of light microscopy. For ethical and anatomic reasons, biopsy of the normal human cochlea is not feasible, and postmortem material is rarely obtained within the necessary time limit. Therefore, experimental animals have provided the major material for study.

The final electron image projected onto the fluorescent screen or photographic plate is similar to a radiograph - it is one of lines, spaces, and shadows. There are few fixed parameters that will help to distinguish normal from abnormal morphology. Only by many hours of patient observation of normal and experimentally altered tissues with the electron microscope is this ability acquired.

Throughout this chapter, only an outline of electron microscopy of the cochlea is attempted. For the sake of brevity, descriptions are confined only to structures that can be delineated with the electron microscope and are of particular interest. Shown is a diagram showing a cross-section of the scala media and the location of cell types. The reader is referred to individual publications and to the texts on electron microscopy of the inner ear for more detailed descriptions (Enström et al, 1966; Spoendlin, 1966; Iurato, 1967).

General Considerations

Throughout the cochlea, the epithelium enclosing the endolymph is surrounded by a basal lamina (basement membrane) that is continuous except where the nerves penetrate the basilar membrane and behind the stria vascularis. The endolymphatic surface of this lining epithelium is a three-layered plasma membrane that is thicker than usual (130 Å as opposed to 90 Å) and is thrown into folds called microvilli. Each cell is attached to its neighbor by a so-called "tight junction complex" (*zonula occludens*, *zonula adherens,* and *macula adheres* or *desmosome*) (Farguhar and Palade, 1963). Neural elements do not extend into the scala media further than the hair cells of the organ of Corti.

Every cochlear cell has the usual cytoplasmic organelles commonly described by cell biologists. Only when their arrangements are significant will attention be drawn to them in the following text.

Limbus Spiralis

The spiral limbus is a platform composed mainly of connective tissue continuous with that of the basilar membrane. Its flat, superior surface is covered by T-shaped cells overlayed by the acellular tectorial membrane. The T or interdental cells are seen very clearly in embryologic development, as is the tectorial membrane. If one accepts the hypothesis that the tectorial membrane is secreted, then the T-cells are probably responsible for this function.

Inner sulcus cells line the limbus along its vertical surface, running from the auditory tooth of Huschke (the lateral border of the spiral limbus) along the basilar membrane to the inner supporting cells. The inner sulcus cells are similar in appearance to Claudius' cells.

Basilar Membrane

The basilar membrane is composed primarily of an extracellular amorphous substance and radial filaments. It is traditionally divided into an inner *pars tecta* and an outer *pars pectinata.* In the pars tecta, the filaments lie side by side, whereas in the pars pectinata, they are grouped into fibers ranging from 0.5 to 1.5 microm in diameter (Iurato, 1962). The filaments of the basilar membrane are continuous with those of the limbus and spiral ligament. Lining the tympanic surface of the basilar membrane is a layer of connective tissue that is continuous with the rest of scala tympani and contains capillaries beneath the organ of Corti.

The Organ of Corti

The organ of Corti consists of a row of inner hair cells and three rows of outer hair cells, associated neural elements, and different types of supporting cells. Occasionally there are "extra" outer hair cells, resulting in a fourth row.

The inner hair cell appears less differentiated than the outer hair cell. It resembles an obliquely placed type I vestibular hair cell. Interestingly, during embryonic development the outer hair cells also have this flasklike shape. The more elaborate ultrastructure of the adult outer hair cell may indicate a higher order of function. It has been suggested that the outer hair cell performs a greater degree of frequency analysis.

The cylindrical outer hair cells are longer in the apical turns than in the basal turns. Lining their vertical walls are layers of flattened cisternae. Mitochondria are collected in the subcuticular region and at the infranuclear pole and are dispersed along the vertical walls.

At the surface of the hair cell is a cuticular plate from which project stiff stereocilia which may be partially embedded into the overhanging tectorial membrane. These stereocilia are arranged in rows and form a characteristic pattern. Those of the outer hair cell are shaped like a W, with the limbs facing away from the stria vascularis. Some evidence (Flock and Cheung, 1977) indicates the presence of actin filaments. Toward the stria vascularis and at the apex of the formation, a basal body lies in a small afilamentous region of the cuticular plate (Engström et al, 1962; Flock et al, 1962). On the inner hair cell, the stereocilia form a flattened "M", like the wings of a bird in flight. The basal body or centriole is positioned in front of the pattern formed by the stereocilia, again toward the stria vascularis (Duvall et al, 1966). This arrangement is similar to that of the vestibular system (Flock, 1964), in which the basal bodies have associated kinocilia like those found in respiratory ciliated cells. Kinocilia extend from the basal bodies of the embryonic cochlea but are absent in the mature hair cell. The significance of this anatomic polarization in the cochlea is not understood, whereas the arrangement in the vestibular system coincides with the physiologic polarization. Serial sectioning has failed to identify more than one centriole, apart from the basal body, in any one hair cell (Duvall et al, 1966).

The major mechanical support of the organ of Corti is derived from the pillar cells and the reticular lamina (Engström and Wersäll, 1953). The latter is formed primarily by coalescence of tonofibrils of Deiters' cells.

Innervation of the Organ of Corti. Electron microscopy has expanded knowledge of the innervation of the organ of Corti (Spoendlin and Gacek, 1963; Smith and Rasmussen, 1965; Spoendlin, 1966, 1969). Ultramicroscopically, efferent nerve endings can be distinguished from afferent nerve endings. Unfortunately, the afferent and efferent nerve fibers cannot be easily distinguished morphologically. The following discussion outlines the innervation as it is precisely understood.

All nerves enter from the modiolus and are composed of afferent and efferent fibers. The spiral ganglion primarily consists of myelinated nerve fibers (afferent and efferent) and at least two types of bipolar neuron cell bodies. The type I cell bodies occur most frequently (at least in the cat) and are thought to innervate the inner hair cells. They have a large spherical-shaped nucleus and are wrapped in many layers of myelin. The type II cell bodies are fewer in number and smaller in size than the type I cell bodies. Type II nuclei are lobulated; their cytoplasm contains abundant microfilaments, smooth endoplasmic reticulum, and Golgi bodies, and they are unmyelinated. The type II cells appear to innervate the outer hair cells (Ruggero et al, 1982). Where these nerves lie in the osseous spiral lamina or beneath the basilar membrane, they are wrapped in myelin sheaths. Above the basilar membrane the myelin is absent, and nerves are covered by sarcolemmal sheaths only. Most of these fibers are collected into bundles that run spirally or radially.

Both the inner and outer hair cells have different and efferent innervation. Beneath the inner hair cells are inner spiral bundles composed mainly of efferent fibers that spiral in both directions along the cochlea. Some fibers cross between the inner pillar cells and form the tunnel spiral bundle. Transection of the olivocochlear bundle, which enters the cochlea from the brain stem via the vestibular nerve, results in degeneration of the tunnel spiral bundle, indicating its efferent nature. Intermittently, fibers of the tunnel spiral bundle turn at right angles, cross the tunnel, and innervate outer hair cells. Some of these fibers spiral a short distance in an outer spiral bundle before innervating outer hair cells.

Most of the afferent fibers to the organ of Corti synapse directly with the inner hair cells, with 95 per cent of the fibers innervating the inner hair cells in the cat (Spoendlin, 1972). Some take a short spiral course in inner spiral bundles and then pass obliquely to the outer hair cells between the inner pillar cells below the tunnel spiral bundle. Cross-sections show that an average of one fiber passes between each pillar cell (Spoendlin, 1969) and then lies on the floor of the tunnel, often encased in tunnel cells. These afferent fibers join the three outer spiral bundles, which run basally. The small number of afferent fibers in the tunnel, compared with the large number of afferent nerve endings on the outer hair cells, indicates extensive nerve branching.

Afferent and efferent nerve endings are grouped at the lower pole of hair cells and differ ultrastructurally (Rodrigues-Echandia, 1967). The efferent endings are more prevalent on the outer hair cells in the basal turns, particularly the innermost rows. These endings are also more numerous than the number of efferent fibers crossing the tunnel, indicating extensive efferent fiber branching. Nerve fibers of afferent or efferent variety supply more than one outer hair cell, and any one hair cell is, therefore, supplied by more than one nerve fiber.

The efferent ending is large, and the flattened adjacent surfaces of the nerve ending and outer hair cell are called synaptic membranes. These synaptic membranes are separated by a synaptic gap of about 200 Å in the guinea pig. Above the synaptic membrane of the hair cell lies a flattened vesicle called the postsynaptic cisterna. The nerve ending cytoplasm is characterized by many large mitochondria and numerous synaptic vesicles, most of which are uniform in size. A few have dense cores and are larger than average.

The afferent ending is smaller than the efferent ending. It has a thickened postsynaptic membrane and contains smaller mitochondria. The synaptic vesicles are irregular in size. Attached to the thickened presynaptic membrane of the hair cell are small electrodense rods that protrude into the cytoplasm of the cell. These synaptic bars are surrounded by vesicles. The synaptic gap varies between 150 and 175 Å and may contain filamentous material that is often distinguishable as an electron-dense line.

The majority of endings innervating the inner hair cells are afferent. The endings are small and characterized by synaptic bars and thickened pre- and postsynaptic membranes. The efferents synapse on the afferent nerve fibers of the inner hair cells.

Hensen's Cells

At the lateral border of the organ of Corti, the cells of Hensen are collected together in a characteristic rosette, especially in the apical turns. On the endolymphatic surface, microvilli are more numerous and longer than those of any other cell type within the cochlear duct. The nucleus is centrally placed in the cell and lies along the radial axis of the rosette. Cytoplasmic lipid inclusions are found mainly in the cells of the apical turns. In the embryo, similar lipid inclusions are found in the inner supporting cells.

Claudius' Cells

The cuboidal Claudius' cells line the floor of the external sulcus from Hensen's cells to the external sulcus cells. Their function has long eluded investigators. The relatively empty appearance of the cell cytoplasm gives no clue to their function. It has been suggested (Duvall and Quick, 1969) that Claudius' cells may be one of the repositories of particulate matter, as is evidenced by the increase in lysosomes seen in these cells following experiments in which endogenous debris were created within, or exogenous tracers were introduced to, the *scala media*.

External Sulcus Cells

The external sulcus cells form a continuous band throughout the cochlear duct and are situated behind the spiral prominence epithelium and Claudius' cells (Duvall, 1969). In the apical turns, the external sulcus cells partly emerge from between these cell groups, exposing a surface to the endolymph. From the deep surface of the band, multicellular projections or pegs extend into the spiral ligament. The number and size of these pegs decrease toward the apex. Contrary to the popular belief, cells of the pegs are not arranged in a rosette and no extracellular lumen is present. A capillary network intertwines through the spiral ligament connective tissue around the pegs.

Microfilaments are a distinguishing cytoplasmic feature of the external sulcus cells. These are also found in the epithelium of the endolymphatic sac. The plasma membranes of external sulcus cells interdigitate extensively with each other.

Spiral Prominence

The spiral prominence forms a rounded bulge in the lateral cochlear wall below the stria vascularis near the basilar membrane. It is covered by a single layer of flattened cuboidal cells. The nuclei occupy a large portion of the cells and are usually bilobed. This cell layer resembles the light cells of the vestibular *planum semilunatum.* Subepithelial connective tissue composes the bulk of the prominence and is similar to that of the spiral ligament but has a greater cell density. One or more omega-shaped capillaries are always situated near the center of the spiral prominence.

Stria Vascularis

The *stria vascularis* is considered a vascular epithelium (Rodrigues-Echandia and Burgos, 1965). It extends from the spiral prominence to the attachment of Reissner's membrane, thus forming the major portion of the lateral cochlear wall. It is composed of three types of cells - the marginal or chromophile (dark) cells, the intermediate or chromophobe (light) cells, and the basal cells. The capillary network within the stria vascularis is separated from the stria by a double basal lamina.

The marginal cells line the endolymphatic surface and are thought to be the only cell type derived from the otocyst (Kikuchi and Hilding, 1966). In a state of partial atrophy, they can resemble the dark cells of the vestibular labyrinth (Kimura, 1969). Their scala media surface has a few short microvilli. The basal portions of the cells are thrown into long, thin, tentacle-like projections, giving the cell a large surface area in relation to its volume. They interdigitate with similar, but somewhat thicker, processes of the intermediate cells. Processes from both types of cells frequently lie adjacent to the basement membrane of the capillaries. The marginal cell cytoplasm contains dense ground substance and numerous large mitochondria in their processes. Near the endolymphatic surface are a large number of vesicles with coated inner surfaces.

The intermediate cells lie behind the marginal cells. They have a pale cytoplasm with fewer mitochondria, and they are irregularly stellate in form. Projections of the intermediate cells extend toward the endoplasmatic surface to interdigitate with those of the marginal cells.

The basal cells are long, flat cells connected by frequent desmosomes and extensive tight junctions that apparently seal the stria vascularis from the underlying perilymphatic tissue of the spiral ligament. Their cytoplasm is pale and may contain lipid inclusions. They may lie adjacent to capillaries and often completely surround them. Groups of basal cells intermittently project toward the endolymphatic surface but reach that surface only at the upper and lower ends of the stria vascularis. There is not basement membrane separating the stria from the spiral ligament. In guinea pigs the intermediate and basal cell layers contain melanin granules. Melanocytes may be dispersed within the stria. The cytoplasm of all three cell types contains glycogen granules, but the granules are most numerous in marginal cells.

Certain morphologic features suggest possible functions: The anatomic appearance of the stria vascularis is consistent with secretion or absorption. The rich endowment of mitochondria indicates high metabolic activity within the stria. Elaboration of the cell margins, greatly increasing surface in relation to volume, suggests ionic transport (Fawcett, 1962).

After diuretic administration (Quick and Duvall, 1970; Brummett et al, 1977), venous occlusion (Kimura and Perlman, 1958), and intense sound exposure (Ward and Duvall, 1971; Duvall et al, 1974; Santi and Duvall, 1978), the most consistent morphologic change in the stria is intercellular fluid accumulation, or edema. Edema appears to be a reversible process that reflects a change in vessel permeability, as evidenced by increased vascular transport of the protein tracer horse-radish peroxidase. Nevertheless, the origin of this fluid and the mechanisms of its production are unclear.

Reissner's Membrane

In the guinea pig, Reissner's membrane has two cell layers. The *scala vestibuli* layer is thin and composes of elongated, flattened cells that are continuous with identical cells that line the entire scala vestibuli. Frequent pores are seen between adjacent cells (Duvall and Rhodes, 1967).

The flattened cuboidal cells of the *scala media* layer appear to have no formed connections with the scala vestibuli layer. However, between the layers, but always adjacent to the scala media layer, is a basal lamina.

From its structure, the scala vestibuli layer would seem to offer little resistance to the passage of fluid, electrolytes, and particulate matter. Farquhar and Palade (1963) feel that the basal lamina might play a significant role in selectivity. The microvesicles of the scala media layer suggest proteinaceous transfer; and, in fact, micropinocytotic transfer has been observed within these cells experimentally. Passage between cells connected by "tight junctions" is a matter of heated debate (Luft, 1966; Karnovsky, 1967; Revel and Karnovsky, 1967). Tracer experiments performed by the authors (1969) have shown passage through the scala media layer by micropinocytosis and not transfer through their "tight junctions". Electron-dense debris created within the scala media fail to pass further than lysosomes within the scala medial layer. It has been postulated, therefore, that Reissner's membrane offers a one-way barrier to particulate matter. More work is needed both to substantiate this hypothesis and to explore the movement of electrolytes and fluids in this region.

A brief review of the ultrastructure of the scala media has been presented. Electron microscopy has contributed significantly to our knowledge of this important organ. However, the electron microscope may have raised as many questions as it has answered. Morphologic studies alone cannot delineate function but can only suggest it. The combination of electron microscopy and the tools of histochemistry, biochemistry, and electrophysiology will further advance our knowledge of the cochlea. The future offers rich opportunities to inquiring minds willing to utilize all these tools.