THE ULTRASTRUCTURE OF THE SENSORY HAIRS AND ASSOCIATED ORGANELLES OF THE COCHLEAR INNER HAIR CELL, WITH REFERENCE TO DIRECTIONAL SENSITIVITY

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ABSTRACT

From the apical end of the inner hair cell of the organ of Corti in the guinea pig cochlea protrude four to five rows of stereocilia shaped in a pattern not unlike the wings of a bird. In the area devoid of cuticular substance facing toward the tunnel of Corti lies a consistently present centriole. The ultrastructure of this centriole is similar to that of the basal body of the kinocilium located in the periphery of the sensory hair bundles in the vestibular and lateral line organ sensory cells and to that of the centrioles of other cells. The physiological implications of the anatomical orientation of this centriole are discussed in terms of directional sensitivity.

INTRODUCTION

The sensory hairs of the receptor cells of the sensory epithelia of the inner ear and the lateral line organ constitute an important link in the transformation of mechanical stimuli into the electrophysiological responses of the sensory cells. With the aid of the electron microscope, the fine structure of the receptor cells has been investigated in detail (35, 7, 15, 31, 1, 18, 29, 11). In the crista ampullaris of the guinea pig, Wersäll (35) demonstrated a kinocilium located eccentrically in the bundle of sensory hairs arising from each receptor cell. The same configuration of the sensory hair bundle is seen in other vestibular epithelia, that is, in the utricle and the saccule (7), the lagena (26), and in the lateral line organs of fishes (33, 11) and of the toad (19). Held (16) and Kolmer (20) claimed the existence of kinocilia also in the cochlea, on the basis of work with the light microscope. However, the recent electron microscope studies of Flock, Kimura, Lundquist, and Wersäll (14) demonstrated the lack of a kinocilium on the hair cells of the cochlea, but the presence, in its place, of a centriole which is the structural equivalent of the basal body of the kinocilium. It has been shown by extensive correlation that the direction of this morphological polarization coincides with a functional directional sensitivity of the sensory cells. This has been demonstrated in many species along the phylogenetic scale (25, 13, 5, 14, 26, 10, 36, 11, 37). Békésy (2) has studied the directional characteristics and polarity of maximum cochlear microphonics produced by a vibrating needle placed on the upper surface of the tectorial membrane over the region of outer and inner hair cells, respectively. For the outer hair cells, his results coincide in the manner expected with the direction of

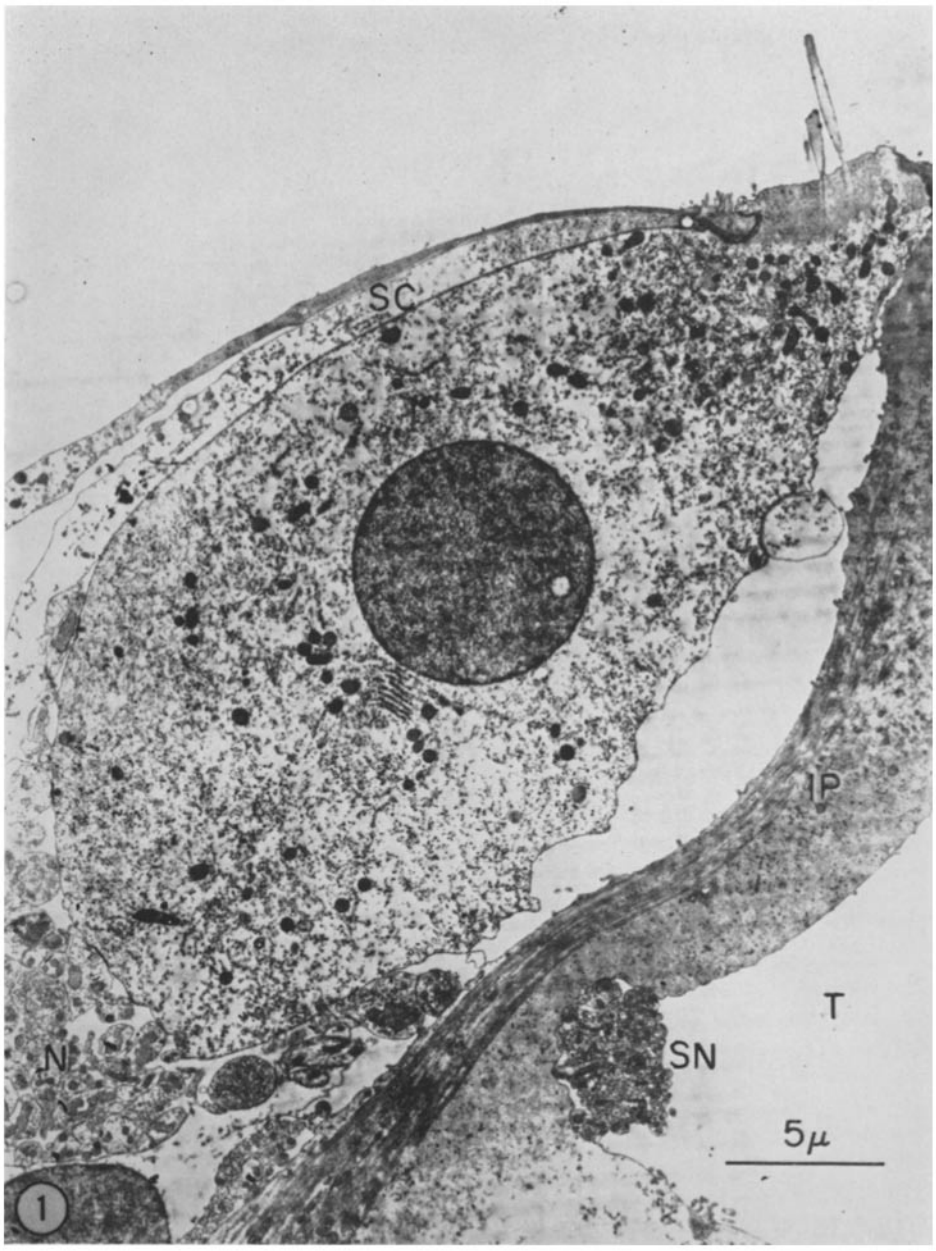


FIGURE 1 Inner hair cell of the guinea pig cochlea. The cell is eccentrically flask-shaped leaning toward the tunnel of Corti (*T*). It is enclosed by supporting cells (*SC*), its hair-bearing end protruding through the reticular lamina. Many nerve endings (*N*) are seen at the bottom of the cell. At the base of the inner pillar cell (*IP*) the inner spiral nerve bundle (*SN*) is seen. Osmium tetroxide fixation, uranyl acetate staining. × 4300.

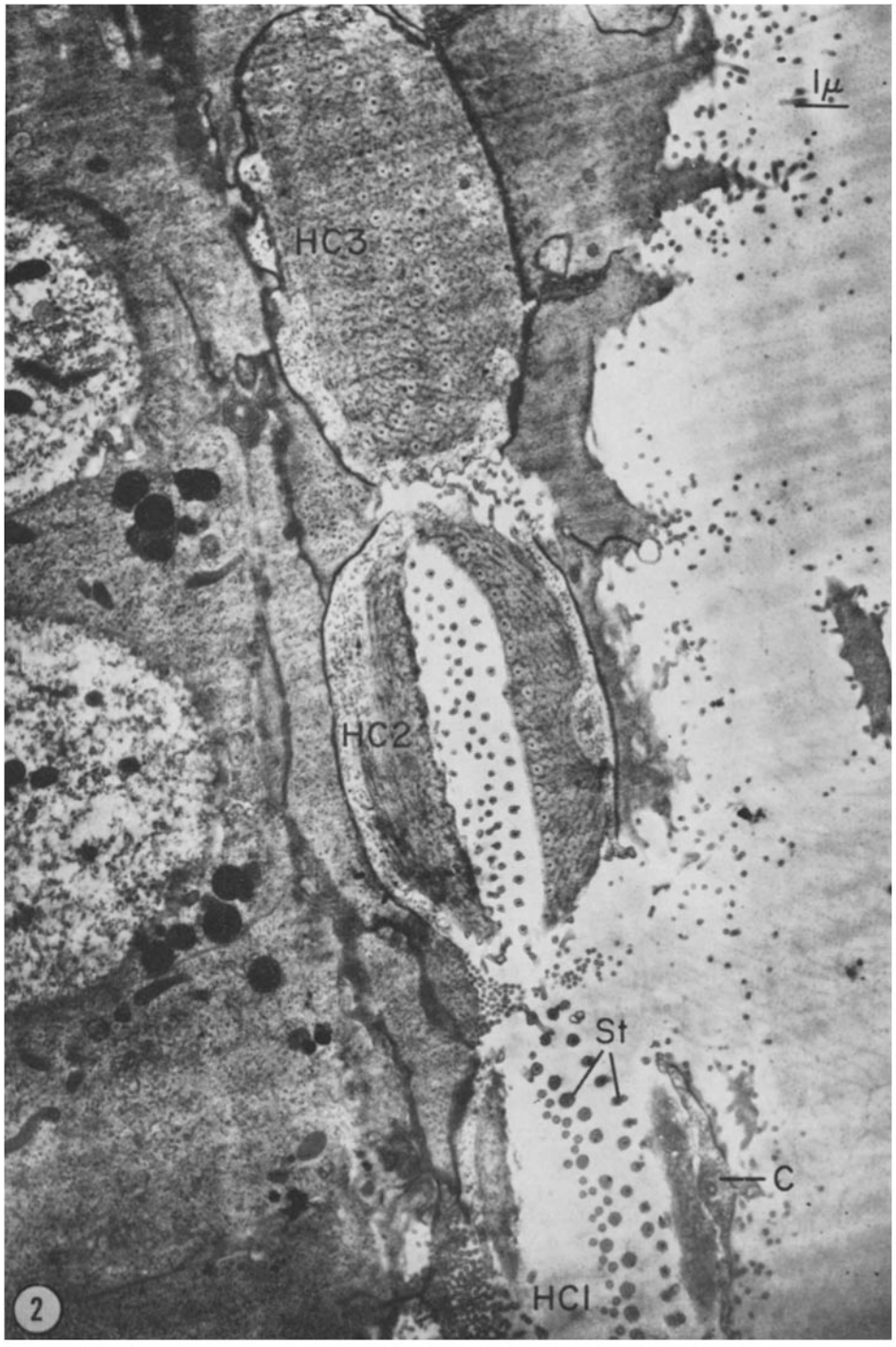


FIGURE 2 Section cut at slight angle to the reticular lamina through the hair-bearing ends of three inner hair cells (*HC 1*, *HC 2*, and *HC 3*). *HC 1* and *HC 2* are cut only partly below their surfaces, while *HC 3* is cut at the level of the cuticular plate. The sensory hairs or stereocilia (*St*) are arranged in wing-shaped rows. In *HC 3*, the rootlets passing from the sensory hairs down into the cuticle reveal this arrangement. In *HC 2*, four rows of stereocilia are seen but short stereocilia are also scattered in an irregular way, independent of these rows. In *HC 3*, at least 100 rootlets can be counted. In *HC 1* a centriole (*C*) is seen at a location corresponding to the cuticular defect appearing at a deeper level in *HC 2* and *3*. Osmium tetroxide fixation, uranyl acetate staining. × 7000.

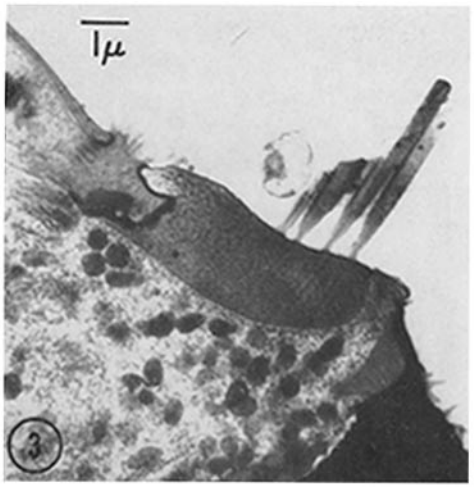


FIGURE 3 Top of an inner hair cell showing four rows of stereocilia protruding from the cell surface. Osmium tetroxide fixation, uranyl acetate staining. × 6000.

orientation of these cells (14). For the inner hair cells it appears, however, that Békésy's results do not correlate in the expected way with the direction of morphological polarization, a fact which will be demonstrated and discussed in the present article.

MATERIAL AND METHODS

Guinea pigs were used in this experiment. After decapitation, the cochleae were perfused with 1% osmium tetroxide buffered with veronal acetate (30), dehydrated in alcohol, and embedded in Epon (27). Serial sections were cut with glass knives on an LKB Ultrotome, collected from 5% alcohol on 100-mesh copper grids, and examined with a Siemens Elmiskop I, after staining in a 1.2% solution of uranyl acetate.

RESULTS

The guinea pig inner hair cell is eccentrically flask-shaped (Fig. 1), about 42 μ in length and 17 μ wide at its greatest diameter. Its long axis intersects the reticular lamina at an angle which makes it lean toward the tunnel of Corti, as well as toward the helicotrema (6). The hair cell is enclosed by supporting cells which snugly surround its oval hair-bearing end (Fig. 2). The supporting cells are firmly joined to the hair cells by desmosomes, thus forming the reticular lamina.

From the top of each sensory cell protrude rows of stereocilia, the long axes of which are perpendicular to the plane of the reticular lamina (Figs. 1, 3). The basic structure of the stereocilium has been described earlier by other authors (7, 18, 8). It is covered by a plasma membrane which is continuous with the three-layered cell membrane. It consists of a protoplasmic core, the center of which condenses towards the basal part of the cilium into a dense axial filament or rootlet. The

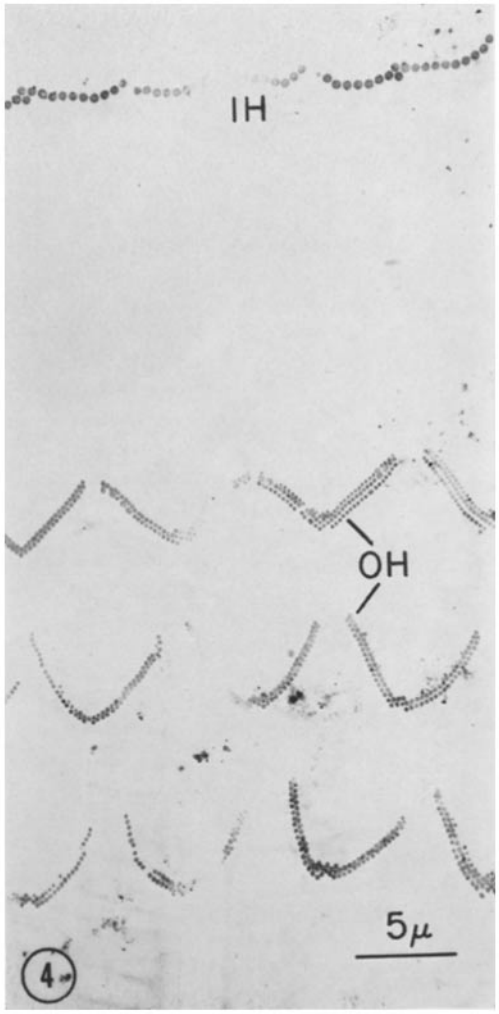
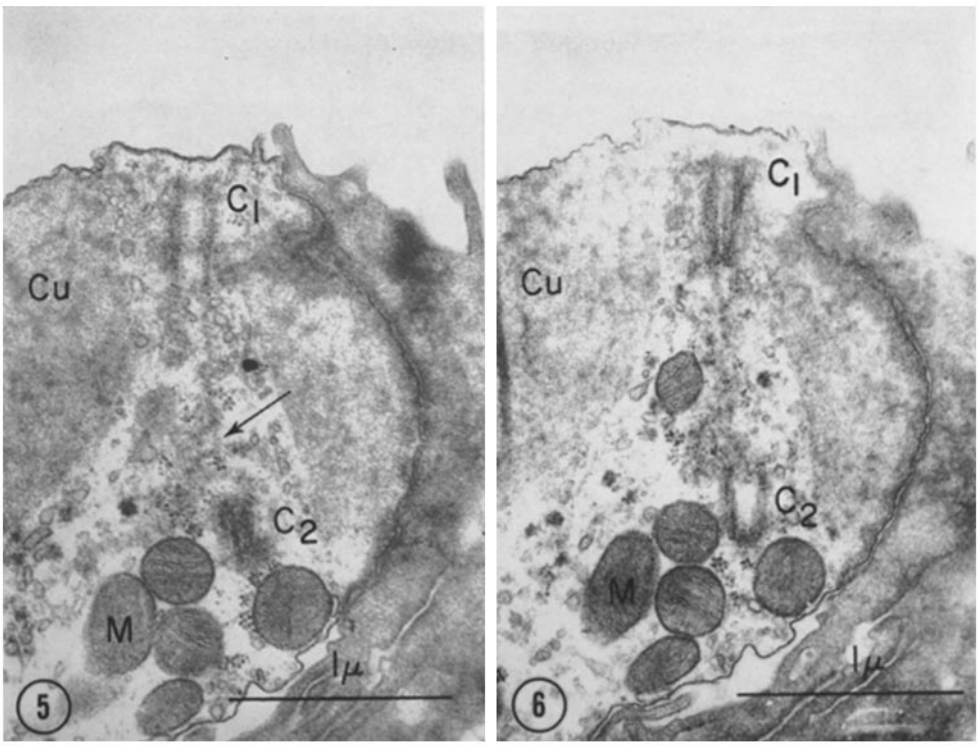


FIGURE 4 Section parallel to the reticular lamina through the sensory hairs of inner and outer hair cells from the first turn of a guinea pig cochlea. The stereocilia of the outer hair cells (*OH*) are arranged in the shape of a "W" built up by three rows of stereocilia. Of the inner hair cell stereocilia (*IH*) only those of the tallest row are seen. They are arranged in a "bird-wing" pattern. Note the difference in diameter between inner and outer stereocilia. Osmium tetroxide fixation, uranyl acetate staining. × 2700.



FIGURES 5 and 6 Serial sections through the apical part of a guinea pig inner hair cell cutting longitudinally the centriole in the cuticular opening (C_1). This centriole shows two plates of osmiophilic material within its core, one at its apical end, one at its lower end (Fig. 5). The subjacent centriole (C_2) is seen more clearly in Fig. 6. The arrow indicates a strand of osmiophilic protoplasm running between two centrioles. Note the accumulation of mitochondria (M). Cu = cuticle. Osmium tetroxide fixation, uranyl acetate staining. × 30,000.

rootlet is anchored into a cuticle, an electron-opaque plate which is fairly homogeneous except for laminated condensations (Figs. 2, 5). In the guinea pig we find at least four rows of stereocilia (Figs. 2, 3). They form a pattern not unlike the wings of a bird (Figs. 2, 4). The first two rows (toward the tunnel of Corti) follow this pattern exactly, whereas the remaining rows are less organized, making it difficult to determine the exact number of rows (Fig. 2). The stereocilia decrease in length and diameter as they recede from the tunnel of Corti (Fig. 3). The cilia of the first two rows have a maximum diameter of about 0.35 μ, which is almost twice that of the outer hair cell stereocilia (0.2 μ) (Fig. 4). The diameter is relatively constant throughout the length of the cochlea in the guinea pig, whereas in the rat it was found by Iurato (18) that the diameter increases from base to apex. Each of the first two rows are composed of 22 to 25 stereocilia. Including the irregular other rows, we can count up to 120 stereocilia per cell. This is comparable to our outer hair cell count (100 to 120 stereocilia). The stereocilia of the third row are considerably shorter and slightly slimmer than those in the first two rows. The remaining stereocilia are short and thin (Fig. 3), but have the same basic structure as the larger ones. In the cuticular plate, an indentation is found facing towards the tunnel of Corti and corresponding to the indentation in the rows of stereocilia (Fig. 2). In this area a centriole is invariably present just beneath the surface membrane (Figs. 2, 5, 6). In many of our sections a second centriole was found beneath the superficial one. Both of these centrioles appeared to be joined by a strand of electron-opaque material (Figs. 5 and 6). The cytoplasm adjacent to the centrioles is always rich in mitochondria (Fig. 1). Serial sections of inner hair cells failed to demonstrate

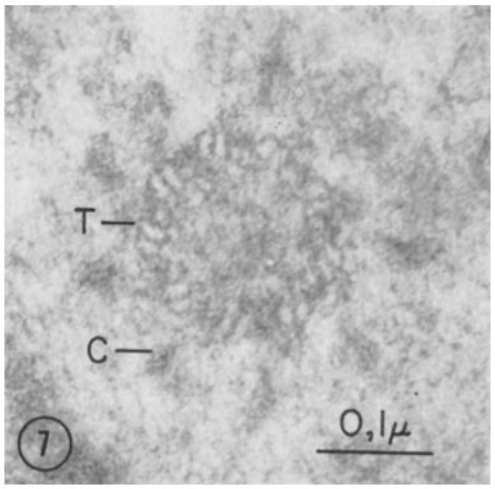


FIGURE 7 Cross-section through the apical end of an inner hair cell centriole at the level of its apical plate. The wall of the centriole is composed of nine triplicate tubules (*T*). At this level, spokes ending with a globular condensation (*C*) extend obliquely outward from each tubule. The lumen of the centriole is occupied by the apical plate. Osmium tetroxide fixation, uranyl acetate staining. × 150,000.

more than two centriolelike structures in any one cell.

The centriole is a cylindrical structure 0.5 μ long and 0.2 μ in diameter. Its axis is perpendicular to the apical cell surface, to which its upper end is closely apposed (Figs. 5 and 6). The wall of the centriole is composed of nine triplicate tubules arranged parallel to its axis. Its lumen contains two plates of electron-opaque material (Fig. 5). At the level of these plates, spokes radiate outward from the triplicate tubules. Those radiating from the superficial plate bend in a clockwise direction and possess a globular condensation at their distal end (Fig. 7). The tubules are joined to one another by osmiophilic strands on both their inner and outer margins (Figs. 8 and 9). Our concept of the ultrastructure of the inner hair cell centriole is presented in the schematic drawing in Fig. 10. For comparison, we note that the outer hair cell (7, 18, 14, 8, 22, 37) possesses three rows of stereocilia arranged in a W-shaped pattern (Fig. 4) and of decreasing length away from a similar centriole placed at the apex of the W facing toward the stria vascularis.

DISCUSSION

The basic structure of organization of the stereocilia is consistent for each cell type within an organ and among species. In the inner hair cell, the stereocilia are arranged in rows parallel to the tunnel of Corti. We find four to five rows of stereocilia, as did Held in 1926 (16). Other authors, and also one of us, have described two rows (7, 18, 8). The stereocilia of the two rows closest to the tunnel of Corti are definitely longer than those of the other rows, a fact which may easily cause an erroneous counting. Thus, the stereocilia of the inner hair cell become progressively taller toward the centriole, a feature also present in the outer hair cells (8, 14, 20), in the vestibular (8, 10, 36) and lateral line hair cells (13, 11).

The most striking dissimilarity between the cochlear and the other hair cells is the presence of a kinocilium in the sensory hair bundle of the vestibular and lateral line organ receptor cells, and its absence in the cochlear hair cells. Yet the cochlear inner and outer hair cells invariably possess a centriole which is the structural equivalent to the basal body of the kinocilium in the vestibular and lateral line organs. The presence of such a centriole has recently been shown also in the human cochlea by Kimura, Schuknecht, and Sando (22). It has recently been shown by Kikuchi and Hilding (21) that the hair cells in the developing

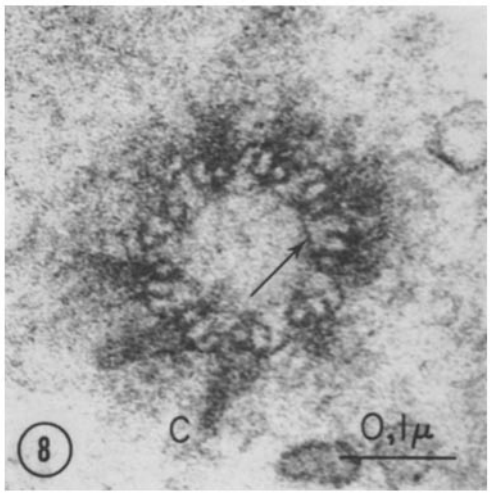


FIGURE 8 Section cut below the apical plate of an inner hair cell centriole. The triplicate structure of the tubules is evident, as are lines connecting the inner margins of the tubules (arrow). Outside each tubule a dense condensation (*C*) is seen. Three of these condensations have an elongated triangular shape and extend obliquely outward. Osmium tetroxide fixation, uranyl acetate staining. × 150,000.

organ of Corti of the mouse do possess a kinocilium which is shed shortly after partus, leaving behind its intracellular portion which constitutes the centriole of the mature cell. Also, in sections from a 24-wk human embryo, a cilium has been found to protrude from the centriole of the external hair cells (34, 37). The centriole of the guinea pig cochlear inner and outer hair cells, when taken as a self-contained unit, appears to be symmetrical, i.e., it has no asymmetrical appendages. This is not true of the basal bodies of the kinocilia of vestibular sensory cells which possess an appendage projecting away from the stereocilia (26, 12).

To introduce the reader to the mechanism of hair cell function, one current hypothesis will be briefly described. In the acoustico-lateralis system the receptor cells are mechanically stimulated, through the sensory hairs, by the shearing displacement of the tectorial membrane, the cupula or the otolithic membrane, in relation to the surface of the sensory epithelium (17, 3). By the displacement of the sensory hairs the receptor mechanism is activated. A receptor potential (microphonic) is generated by the hair cell which ultimately regulates the flow of impulses in the innervating nerve fibers. It is well known that the cochlear microphonic is produced in the cochlea, and evidence points toward the hair cells as being responsible (3, 4). A basic principle of hair cell function is the directional sensitivity of the sensory units; that is, the response is dependent on the direction in which the hair bundle is displaced (9, 28, 23, 24, 3, 32). Displacement in a certain direction causes a negative receptor potential, which corresponds to the negative phase of the microphonics, and an increase in action potential frequency (excitation). Displacement in the opposite direction is followed by a positive potential and a decrease of impulse frequency (inhibition). For the vestibular and lateral line hair cells (25, 13, 5, 26, 10, 36, 11) and for the cochlear outer hair cells (14, 8) it has been found that this directional sensitivity coincides with the orientation of the morphologically polarized hair cells, as indicated by the position of the kinocilium or the centriole, and the increasing length of the stereocilia towards these structures. For the individual hair cell a displacement of the sensory hair bundle in a direction from the stereocilia toward the kinocilium or centriole causes a negative potential and excitation, while opposite displacement causes a positive potential and inhibition (cf. reference 36).

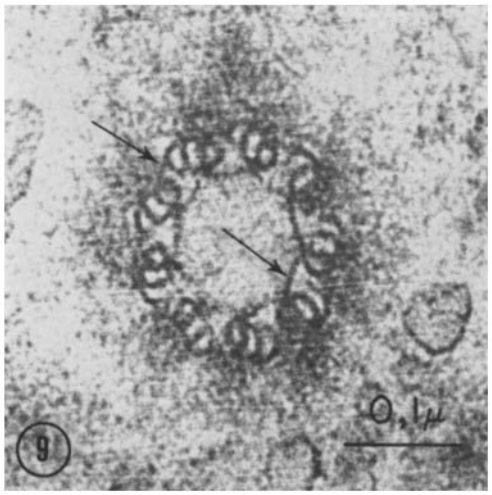


FIGURE 9 Cross-section through the lower end of an inner hair cell centriole showing clearly the architecture of its subunits. The tubules are joined to one another by osmiophilic strands at both their inner and outer margins (arrows). Inside, a line runs from the junction of the second and third part of one triplicate (counting clockwise) to the like position of the next. Outside the tubules osmiophilic condensations are seen. Osmium tetroxide fixation, uranyl acetate staining. × 150,000.

Ultrastructurally, both the inner and outer hair cell centrioles are oriented directionally toward the stria vascularis. Also, in both cells the stereocilia are of increasing length in this direction. Physiologically, Békésy (2) found that a vibrating needle placed on the surface of the tectorial membrane over the region of outer hair cells produced a maximum negative cochlear microphonic when the vibration was in a radial direction toward the stria vascularis. For the outer hair cell, the findings of Békésy, therefore, correlate with the morphological polarization of these cells in the same way as is found for the vestibular and lateral line organs. If the same correlation between functional and structural directionality holds for the inner hair cells, one would expect these cells also to generate maximal negative microphonics at radial displacement of the sensory hairs, since the centriole is oriented in that direction. Yet, Békésy found that when the vibrating needle was placed over the region of inner hair cells a maximal negative microphonic occurred when the vibration was almost longitudinal, towards the helicotrema. These experimental data accordingly disagree by 90° with the direction of maximal sensitivity expected on the basis of cell orientation. It should

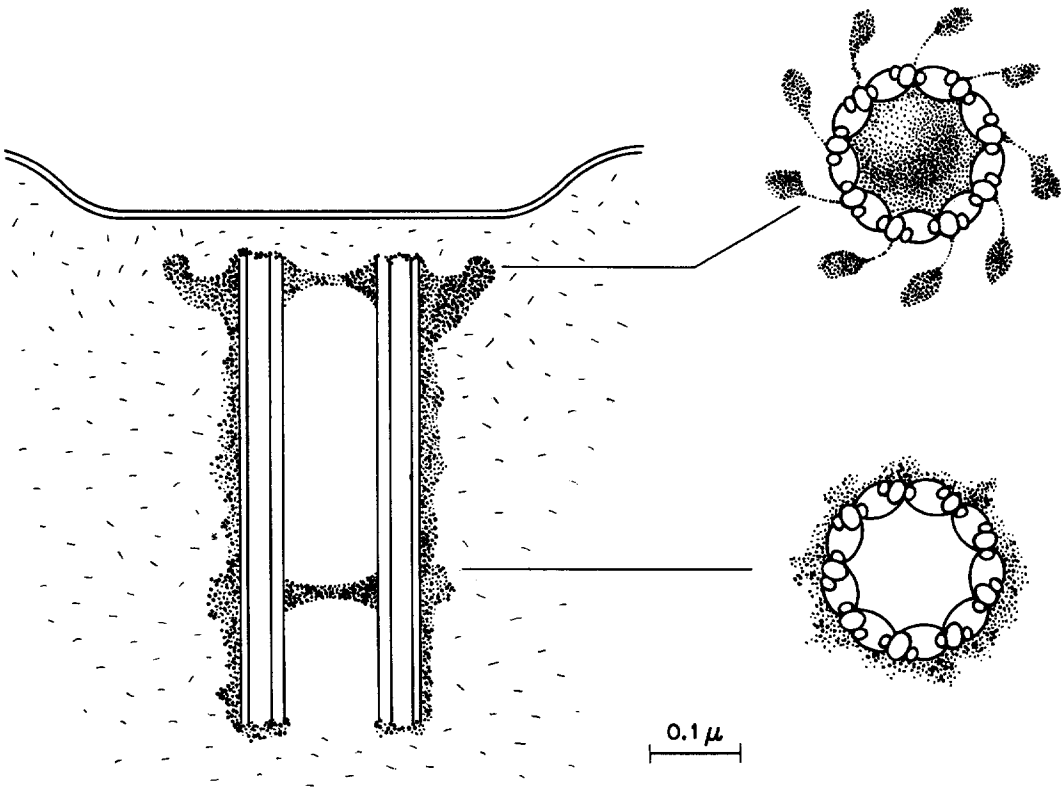


FIGURE 10 Schematic drawing of a cochlear hair cell centriole.

be noted that the vibrating needle in Békésy's experiment was applied to the vestibular surface of the tectorial membrane while the angular response of the hair cell depends on the direction of shearing displacement occurring between the lower surface of the tectorial membrane and the apical end of the hair cell. The possibility exists that when the vibrating needle was applied over the inner hair cells, longitudinal motion of the needle caused a radial displacement of the lower surface of the tectorial membrane. This disagreement between experimental results and inner hair cell orientation urges the reinvestigation of the fine movements of the cochlear partitions and associated electric potentials, hitherto mastered only by Békésy.

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