The Tectorial Membrane of the Rat [1]

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ABSTRACT Histochemical, x-ray analytical and scanning and transmission electron microscopical procedures have been utilized to determine the chemical nature, physical appearance and attachments of the tectorial membrane in normal rats and to correlate these results with biochemical data on protein-carbohydrate complexes. Additionally, pertinent histochemical and ultrastructural findings in chemically sympathectomized rats are considered. The results indicate that the tectorial membrane is a viscous, complex, colloid of glycoprotein(s) possessing some oriented molecules and an ionic composition different from either endolymph or perilymph. It is attached to the reticular laminar surface of the organ of Corti and to the tips of the outer hair cells; it is attached to and encloses the hairs of the inner hair cells. A fluid compartment may exist within the limbs of the "W" formed by the hairs on each outer hair cell surface. Present biochemical concepts of viscous glycoproteins suggest that they are polyelectrolytes interacting physically to form complex networks. They possess characteristics making them important in fluid and ion transport. Furthermore, the macromolecular configuration assumed by such polyelectrolytes is unstable and subject to change from stress or shifts in pH or ions. Thus, the attachments of the tectorial membrane to the hair cells may play an important role in the transduction process at the molecular level.

The present investigation is an outgrowth of a prior study of the effects of chemical sympathectomy upon the structures of the inner ear. None of the findings of this prior study were more interesting than those concerning the internal structure and the attachments of the tectorial membrane. In chemically sympathectomized rats, the filamentous organization of the membrane was often remarkably evident ultrastructurally, and the membrane showed a proclivity for remaining attached to the tips of the hairs of the outer hair cells. These results raised questions concerning the chemical nature and attachments of the tectorial membrane in untreated rats, and whether or not these properties had been altered by chemical sympathectomy.

A search of the literature showed that the answers to the questions posed were not to be found there for, although the membrane has been extensively studied from the time of Corti (1851) to the present, the origin, attachments, chemical nature, physical appearance and functions of the tectorial membrane remain matters of dispute. As will be made evident in the Review of the Literature which follows, one can find support for almost any interpretation of the structure of the tectorial membrane or its degree of attachment to the organ of Corti. Although the tectorial membrane is generally accepted to be rich in acid mucopolysaccharide, this point is also a matter of some contention.

It was decided, therefore, to undertake histochemical and scanning electron microscopical research which might provide new information on certain of the controversial aspects of the tectorial membrane (chemical nature, structure, and attachments) in rats with developed auditory systems (rats beyond 14 days of age). It was hoped that new insights into the functions of the membrane would result from elucidation of these particular points.

It became apparent, however, that a clearer understanding of the functional significance of the tectorial membrane depended not only upon such chemical or

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structural evidence as could be obtained by the methods chosen, but also upon correlation of this data with that obtained by biochemists working in the field of protein-carbohydrate complexes. Biochemical knowledge of such complexes has advanced rapidly in recent years and has reached a point where its application to structures such as the inner ear membranes could be most enlightening, not only with respect to understanding tectorial membrane functions in the normal auditory organ, but also in perceiving possible mechanisms underlying some degenerative diseases of the inner ear. The integration of histochemical, ultrastructural and biochemical data is, then, a further important aim of this report.

Review of the Literature

From the earliest descriptions of its microscopic appearance, the tectorial membrane has been considered to be a fibrillar structure (Boettcher, 1859; Kölliker, 1854, 1861; Waldeyer, 1873; Henle, 1880; Retzius, 1884). Many observers have considered the membrane to be comprised of fibrils embedded in a gelatinous or amorphous matrix (Retzius, 1884; Kolmer, '07; Hardesty, '08, '15; Keith, '18; Wislocki and Ladman, '55; Iurato, '60; Lim, '72); to be stratified, or composed of lamellae in toto or in part (Henle, 1880; Held, '02, '09; Shambaugh, '07; Prentiss, '13); or to be a reticulum (Coÿne and Cannieu, 1895; Prentiss, '13). Still others have thought that the fibrils were either products of coagulation of endolymph (Czinner and Hammerschlag, 1897) or were continuations of the hairs of the hair cells (Ayers, 1891; Borghesan, '49; '52; Mygind, '52). Recently, Borghesan ('71) suggested that the hairs are deeply embedded in the membrane, and that they are artifactitiously elongated as the shrinkage forces pull the membrane away from the hairs.

Research carried out by phase contrast or with polarized light has suggested that the tectorial membrane is a filamentous structure, with the filaments coursing at 30°–40° angles in an apical direction (A. Hilding, '52; Iurato, '60). Transmission electron microscopy demonstrates that the membrane appears to be comprised of slender filaments which vary somewhat in thickness from one species to another, (40 Å, guinea pig, Engström and Wersäll, '58; 100 Å, rat, Iurato, '60; variable, cat, Spoendlin, '57). However, Spoendlin ('57) pointed out that the filaments could be artifactitious and Engström and Wersäll ('58) indicated that, because of their size, they cannot be the same as those observed with the light microscope. The last-named authors suggested that the tectorial membrane might be jelly-like with micellar structures interspersed in some substance which dissolves away in preparation of the tissue.

Scanning electron microscopy has generally revealed the tectorial membrane to be smooth on much of its under side but to be grossly fibrillar on its upper side and in the interior (Kosaka et al., '71; Lim, '72). In his diagram, Lim ('72) showed the smooth regions as separate sheets, corresponding to Hensen's stripe and to Hardesty's membrane.

All of the early investigators thought that the membrane was attached to the epithelial cells of the entire organ developmentally, but differed in their opinion of its degree of attachment in the adult. Thus, many have stated that the membrane was free, or "floated" over the organ of Corti in the adult (Kölliker, 1861; Helmholtz, 1863; Waldeyer, 1873; Retzius, 1884; Rickenbacher, '01; Hardesty, '08; Held, '09; Kolmer, '07, '27). Waldeyer commented, however, that he occasionally found the hairs of the outer hair cells imbedded in the membrane and, after hardening in alcohol, sometimes found the lamina reticularis carried away with the membrane as it contracted. Boettcher (1859) indicated that the membrane did not float free but was connected to the surface of the organ of Corti, an opinion shared by Coÿne and Cannieu (1895), Kishi ('07), Shambaugh ('07) and Prentiss ('13). Prentiss was emphatic in stating that he meant that the attachments were to the entire surface of the organ of Corti, as occurred in the fetal state. Corti (1851), Boettcher (1859) and Löwenberg (1864) considered the tectorial membrane to extend beyond the organ of Corti, even to the spiral ligament; Henle (1880) found it attached just beyond the last row of hair cells. Keith ('18) mentioned an attachment

of the tectorial membrane to inner supporting cells as well as a peripheral connection to outermost Deiters cells. Among more recent investigators, Wittmack ('36) maintained that the tectorial membrane attached to the hairs of both inner and outer hair cells; such connections were also found by von Békésy ('60) and Hawkins and Johnsson ('68). According to Wittmack ('36), other attachments existed to border cells and to Hensen cells. These results were supported by A. Hilding ('52), who also observed attachments to outer, but not inner, hair cells. The peripheral attachment of the tectorial membrane to the reticular lamina (von Békésy, '60), or to Hensen cells (Tonndorf et al., '62), has been described as firm, effectively forming a seal between the organ of Corti and the endolymph.

Scanning electron microscopists have found attachments of the tectorial membrane to various parts of the organ of Corti. Kosaka et al. ('71) reported indentations of the tips of the hairs of outer hair cells in the tectorial membrane; Lim ('72) found connections of the membrane to the outer hair cells and to the outermost row of Deiters cells; Tanaka et al. ('73) observed attachments of the membrane to both the inner and the outer hair cells. According to Lim ('72), the outer margin of the tectorial membrane is attached only as far as the outermost row of hair cells in some cases; thus, endolymph would have free access to the surface of the organ of Corti.

Transmission electron microscopists have generally failed to find the tips of the hairs connected to the tectorial membrane in mammals (Smith and Dempsey, '57; Spoendlin, '57; Engström and Wersäll, '58; Iurato, '61). However, a few attachments were seen by Kimura ('66) in the case of outer hair cells, and Iurato ('67) found the tips of the hairs of the inner hair cells in close contact with the membrane. In birds, not only were hair cells connected to the tectorial membrane, but also there were veil-like attachments between the tectorial membrane and the microvilli of the supporting cells (Dohlman, '71; Takasaka and Smith, '71; Rosenhall, '71).

Investigations using biochemical and histochemical techniques have led to divergent results. Biochemical studies have indicated that the fibrils of the tectorial membrane are not comprised of collagen (Bairati and Iurato, '57; Iurato, '60; and Naftalin et al., '64) and are not related to keratin (Bélanger, '56a). Naftalin et al. ('64) found the tectorial membrane to be a gel which binds calcium and magnesium, with calcium the more firmly bound, and to contain potassium in higher concentration than in extracellular fluid but lower than in endolymph. They and others (Ayers, 1891; Shambaugh, '07; Wittmack, '36) noted the great sensitivity of the tectorial membrane to the state of hydration. Investigators have found the tectorial membrane to be PAS-positive (Wislocki and Ladman, '55; Bélanger, '56a; Friberg and Ringertz, '56; Igarashi and Alford, '69; Vinnekov and Titova, '64). Results with toluidine blue are in dispute. Wislocki and Ladman ('55) and Friberg and Ringertz ('56) found the membrane to lack metachromasia, but Bélanger ('53, '54, '56a,b), Plotz and Perlman ('55) and Tonndorf et al. ('62) reported it to be metachromatic. Additionally, Igarashi and Alford ('69) found the inner ear membranes to stain moderately in alcian blue, and concluded that the membranes contain neutral and acid mucopolysaccharides. This conclusion is commonly accepted today. However, Iurato ('60, '67) showed that the tectorial membrane consisted mostly of protein, contained little carbohydrate and no hexuronic acid. He concluded, on these grounds, that the tectorial membrane cannot be composed chiefly of acid mucopolysaccharides.

MATERIAL AND METHODS

The experiments reported upon here were carried out on Sprague-Dawley rats ranging in weight from 30–385 gm. Some of the animals were chemically sympathectomized by use of 6-hydroxydopamine (6-OH DA) (Angeletti and Levi-Montalcini, '70). Newborn animals were injected subperitoneally with 6-hydroxydopamine 50 μgm/gm body weight, in ascorbic acid or in physiological saline daily from day 1 to day 7. Controls were left untreated or were injected with the vehicle only. The experimental animals were then prepared in the following ways.

Histochemical procedures

Twenty-four rats ranging from 25–75 gm and 15–35 days in age were used as normal controls. Five rats of the same ranges of body weight and age were injected with 6-hydroxydopamine. After decapitation, the entire skull with inner ears in situ was quickly frozen on dry ice while being sprayed with Cryokwik (International Equipment Co.). The frozen skull was then mounted and sectioned in a cryostat at −22°C. The 16 μ sections were picked up on warm coverslips and placed in small covered coplin jars in the cryostat for drying. The sections were then exposed to paraformaldehyde vapor for fixation at either 37°C for two hours, or at 50°C for one hour. Tissues were well fixed by either procedure, but the 37°C fixation was routinely followed as it was considered less drastic. Alternate sections were then treated in the following ways:

Periodic acid-Schiff (PAS). Sections were stained according to the method of Lillie ('65). Controls were exposed to the Schiff reagent only.

Toluidine blue. The toluidine blue method of Kramer and Windrum ('55) for metachromasia was followed; pH of the staining solution was 8.2.

Enzyme studies. Some sections were pretreated with enzymes to determine whether or not specific substrates were present in the tectorial membrane and contributed to the staining reaction noted. The enzymes were chosen for their usefulness in distinguishing glycoproteins from other substances which have, or might have, similar histochemical reactivity. The procedures followed were based upon data given by Thompson and Hunt ('66) and in the Worthington Manual ('72): amylase (Sigma, Worthington), for hydrolysis of glycogen, 20 mg/10 ml in Millonig buffer, pH 6, at 37°C for one hour; neuraminidase (Worthington), for hydrolysis of sialic acid, 0.2 mg/10 ml in acetate buffer, pH 5, at 37° for from 30 minutes to three hours; hyaluronidase (Worthington), for hydrolysis of hyaluronic acid, 5 mg/10 ml in Sorenson buffer, pH 6, at 37°C for from 1–20 hours. Controls were exposed to buffers only for the same periods of time.

Ultrastructural procedures

Scanning electron microscopy. Cochleae from 34 normal, 13 6-hydroxydopamine treated, and five saline injected animals were prepared by a variety of procedures for scanning electron microscopy. Twenty rats were prepared by the following method, referred to subsequently as the "ordinary procedure" for scanning electron microscopy:

Animals were anesthetized with 3.5% chloral hydrate (1 ml/100 gm body weight), and then fixed by intracardiac perfusion with glutaraldehyde (3%)-paraformaldehyde (4%) in Millonig buffer, pH 7.4. Cochleae were removed and opened in fixative, and subsequently postfixed in 1% osmic acid in Millonig buffer. The tissues were dehydrated in alcohol up to 70%, and were stored in 70% alcohol until later dissection for freeze-drying. Cochleae were broken into large pieces, freeze-dried, mounted on stubs, coated with gold and observed in a JEOL JSM U3 scanning electron microscope.

Four rats were perfused and cochleae were removed and osmicated as outlined above. After osmication the cochleae were broken into segments and pieces of the tectorial membrane were removed from their attachments. These portions were then dehydrated through absolute alcohol, immersed in amyl acetate (2 changes, 15 minutes), and dried in a critical-point dryer (Denton).

The remaining 23 animals were not perfused, but were decapitated. Cochleae were removed and prepared according to the procedures outlined immediately below. These experiments were designed to reveal more about the physical appearance of the tectorial membrane, its attachments and ionic composition, under a variety of technical conditions.

First, cochleae from two untreated and from three 6-hydroxydopamine treated rats were opened directly in the glutaraldehyde-paraformaldehyde fixative. Except for this, the ordinary procedure for scanning electron microscopy was followed.

Second, in order to determine the consequences of poor fixation and of freezing-thawing-refreezing upon inner ear structures, two animals were prepared as follows: after decapitation, one cochlea was

allowed to remain unfixed for 15 minutes, whereupon it was opened in glutaraldehyde-paraformaldehyde fixative and subsequently prepared according to the ordinary method given above. The other cochlea was allowed to remain unfixed for 30 minutes, and then treated similarly. Cochleae from the remaining animals were opened rapidly, fixed immediately in glutaraldehyde-paraformaldehyde and prepared according to the usual procedure up to the quenching. At this point, segments of these cochleae were quenched, then thawed, then quenched again and freeze-dried.

Third, according to Hardesty ('15), fixation in Held's solution gives the best preservation of the tectorial membrane. Cochleae from two normal and from one 6-hydroxydopamine treated rats were opened in this fixative, left in it overnight, and then prepared according to the ordinary procedure for scanning electron microscopy.

Fourth, various combinations of glycerin in glutaraldehyde were tried in an attempt to prevent dehydration of the membrane during the process of fixation, by replacing the water with glycerin. One cochlea was opened in 10%, and the other in 20% glycerin in 2% glutaraldehyde. These were left in the solutions overnight, then dried briefly over silica gel, coated and viewed in the scanning electron microscope. Additionally, four cochleae were prepared by fixation in each of the above combinations of glycerin and glutaraldehyde, but were freeze-dried.

Finally, nine cochleae were removed, quickly opened to varying degrees, and quenched in liquid Freon cooled in liquid nitrogen. These cochleae were immediately freeze-dried. No fixation or dehydration was involved. Additionally, five cochleae were removed quickly from decapitated rats and immediately quenched in liquid Freon cooled in liquid nitrogen. These cochleae were opened in the liquid Freon, according to the technique of Flock ('73).

Analyses of fixed, dehydrated samples of tectorial membrane and of freeze-dried tissue untouched by fluid fixative or dehydrating agents were carried out in the scanning electron microscope, utilizing an x-ray analyzer and a Multichannel analyzer, Model 710 (Northern Scientific Company). Other samples of freeze-dried tissue were analyzed in an ARL EMX-SM electron microprobe, utilizing a Multichannel analyzer, Model 710 (Northern Scientific Company). For microprobe analyses, the samples were mounted on carbon stubs with carbon paint, and were carbon-coated.

Transmission electron microscopy. Tissues were obtained from seven untreated and 13 6-hydroxydopamine injected rats which had been prepared by intracardiac perfusion of glutaraldehyde-paraformaldehyde as given. After osmication, tissues for transmission electron microscopy were dehydrated and embedded in Epon. Thick sections (1–2 μ) were used for orientation purposes, then thin sections were cut and mounted on 200-mesh grids covered with Formvar membranes. Sections were stained with lead citrate (Reynolds, '63) and studied on an Hitachi Hu 11A electron microscope.

RESULTS

Histochemical findings

The tectorial membrane is sometimes torn away from the surface of the spiral limbus in cryostat sections, and may be folded upon itself to some extent. These are unavoidable artifacts in sections of undecalcified cochleae.

PAS stain. The tectorial membrane is highly PAS-positive in both normal and in 6-hydroxydopamine treated rats (figs. 1, 2). The dark, purplish-red color of the stained membrane is not uniform throughout, but this appears to be due to distortions (principally folding) of the membrane during cutting procedures rather than to the presence of fibrils.

The PAS reactivity of the membrane is unaffected by pretreatment with amylase, hyaluronidase, or their buffers. The fact that no detectable loss of staining capacity occurs after amylase treatment indicates that glycogen, which would stain with the PAS method, is not present in the tectorial membrane.

There appears to be a slight decline in the intensity of the staining reaction after exposure to neuraminidase. However, undue emphasis cannot be placed upon this change at the present time. The slight shift in color may be due not to the hydrolysis of sialic acid, but to the action of contaminant hexosidases, or to the blocking of

otherwise reactive sites by their binding with neuraminidase. Chemical analysis of the tectorial membrane should resolve this issue.

The tectorial membrane is an exceedingly pale pink color in control sections exposed to the Schiff reagent only. This result demonstrates that the reactions noted in membranes exposed to the entire staining procedure are not artifacts induced by the process of fixation in paraformaldehyde vapor.

Toluidine blue. The tectorial membrane is not metachromatic under any of the experimental conditions utilized here, indicating that it does not contain numerous free polyanions. The finding in the case of the tectorial membrane was checked against the staining reaction of mast cells, which were always metachromatic. The mast cells were present on every section in connective tissue remnants along blood vessels outside the skull.

The tectorial membrane stains dark blue in both normal and in 6-hydroxydopamine treated rats (figs. 3, 4). The membrane looks faintly striated, or even assumes a feathery appearance, in toluidine blue stain.

The entire section stains less intensely with toluidine blue after exposure to an enzyme-buffer solution or to a buffer alone. In the case of the tectorial membrane, the staining reaction remains orthochromatic, but the color assumed by the membrane shifts to a more pale shade of blue. The shift is slight in sections pretreated with amylase or hyaluronidase (fig. 5) or their buffers, but is pronounced in the case of pretreatment with neuraminidase (fig. 6). Tectorial membranes pretreated with the acetate buffer alone appear to be slightly less affected than when neuraminidase is included in the solution in the present series. These results suggest that the shifts in staining color noted in the tectorial membrane are at least partly related to the pH of the buffer solutions used (pH 6.0 in the case of the amylase or hyaluronidase; pH 5.0 for neuraminidase). However, as hyaluronidase had no remarkable effect upon the staining reactivity of the tectorial membrane, it can be concluded that hyaluronic acid is not present in significant amounts. Whether or not the shift in color noted after treatment with neuraminidase indicates that some sialic acid is present remains a question. This finding is subjective and must be considered tentative until definitive results are forthcoming from chemical analysis.

Ultrastructural findings

Scanning electron microscopy of the normal organ of Corti and its relationship to the tectorial membrane. The tectorial membrane ordinarily pulls away from its attachments to the organ of Corti during preparation of the tissue for scanning electron microscopy. If any part of it remains extended over the organ, it is usually purposely removed so that the entire surface of the organ of Corti can be seen. Under either of these circumstances, the true relationships of the membrane to the organ of Corti are generally obliterated. However, at times, shreds of the membrane remain attached accidentally, and adhering portions can be left intact on purpose so that some concept of the attachments can be gained.

When the membrane is completely removed from the cochlea, the hairs of the outer hair cells are generally erect and evenly spaced relative to one another (fig. 7). Sometimes the hairs slope slightly inward, toward the limbus, but this could be artifactitious and related to manipulation of the tissue during dissection.

The hairs are disposed in three rows of decreasing height on the surfaces of the outer hair cells, in a "W" configuration (fig. 7). The base of the "W" is broader in the first row of hair cells, with the "W" of the third row being most narrow. The tallest hairs are always outermost on any individual hair cell, but the hairs are of graded height from base to apex, being tallest at the apex.

The tips of the tallest hairs of any given set are smooth and bulbous when the tectorial membrane is completely removed from them (fig. 7). However, if the membrane is left as undisturbed as possible, these tips are covered over by the substance of the tectorial membrane (fig. 8). Moreover, the shafts of the hairs are interconnected by similar substance in such cases (fig. 8). Wherever the hairs are acciden-

tally slightly separated, the connections between them are evident.

In some cases, the attachments between the tectorial membrane and the hairs and between the hairs themselves are so strong that the hair cells are lifted from their basal connections (fig. 9). In other cases, the caps of the tips of the hairs strip away and hang from the underside of the membrane (fig. 10), or the hairs may pull the attaching filaments loose, so that imprints of their arrangement (also in the form of a "W") are visible in the tectorial membrane (fig. 9).

The hairs of the inner hair cells are thick, somewhat club-shaped structures forming what can sometimes be described as a shallow "W," but more often a crescent, on the cuticular surfaces (fig. 11). The hairs are distributed in three main rows on the surface of each cell, although extra hairs forming incomplete internal rows are common in the rat. The hairs of the outermost row on each hair cell are the tallest, with the inner rows formed of hairs of decreasing height. The tips of the tallest hairs are smooth and flat in ordinary scanning electron microscopical preparations, while the tips of the inner rows are smooth but angled in an upward and outward direction (fig. 11). The shafts of the hairs are rough in superficial appearance.

The tips of the tallest hairs of the inner hair cells are also attached to the tectorial membrane (figs. 12–14). In some preparations, when care is taken to leave portions of the tectorial membrane remaining adherent to the organ of Corti undisturbed, the shreds of the membrane attach to all the hairs of the inner hair cells, as though enshrouding them. At times, hairs of the inner hair cells have been found hanging from the tectorial membrane.

The hairs on each hair cell are interconnected, both between adjacent hairs of the same row and of neighboring rows (figs. 15, 16). Usually, the connections appear strand-like, but this is probably artifactitious and related to stretching of the substance as hairs are separated from one another either by accidental manipulation or during the drying process.

Finger-like shreds of the tectorial membrane have often been encountered attached to the microvilli of the entire surface of the organ of Corti, from inner border cells to the phalangeal processes of the outermost row of Deiters cells (figs. 17–19). That these attachments are to microvilli seems clear from electron micrographs taken at higher magnifications (fig. 20).

In the case of the outer hair cells, the presence of attachments to the tips of the tallest hairs, the roofing over of the hair region, and the attachments to the few microvilli present suggest that a fluid compartment exists within the limbs of the "W" formed by the hairs. It would seem that this small compartment may extend completely around the hairs on the basis of the distribution of the microvilli, although the present results can neither deny nor confirm this. The present findings concerning the attachments of the tectorial membrane to the organ of Corti are shown diagrammatically in figure 21.

Scanning electron microscopy of the tectorial membrane. Various techniques have been followed in an attempt to leave the tectorial membrane in as natural a condition as possible. This is a monumental task, for the membrane is extremely sensitive to any of the common fixatives as well as to alcohol, so that it always shrinks and pulls away from the organ of Corti during ordinary technical procedures. Nor does freeze-drying of the membrane in situ in a freshly sacrificed animal surmount these difficulties, for this procedure also results in shrinkage and detachment of the membrane as it is dehydrated. However, these and other studies dealt with here are useful, for they demonstrate unreservedly that the tectorial membrane can assume any one of several physical appearances, depending upon how it is treated.

The tectorial membrane is gel-like in appearance in sections cut in a cryostat, fixed over paraformaldehyde vapor, and kept dry over silica gel. Membranes fixed in situ, osmicated, then removed from their attachments prior to dehydration are not fibrillar although they may appear to be thrown into folds and wrinkles (figs. 22–24). The substance of the membrane is homogeneous and gel-like in appearance. Membranes which have been fixed, osmicated, dehydrated up to 70% alcohol and allowed

to enter the freeze-drying state in situ have their upper surfaces thrown into a series of ridges which lend them a fibrillar appearance (figs. 25–26). The ridges may be curved, straight, or form broad longitudinal bands. The ridges are not of similar orientation even in adjacent, small portions of the membrane. The under side is rough and sometimes has large superficial holes, as though the substance of the membrane had pulled apart (fig. 27).

If the tectorial membrane is quenched in liquid Freon cooled in liquid nitrogen and immediately freeze-dried, the membrane on the whole looks like a series of interconnected ribbons of gelatinous material (fig. 28), although it may appear to be a gelatinous sheet at other sites (fig. 29). In cross section, the tectorial membrane, freeze-dried and untouched by fluid fixatives or dehydrating agents, is gel-like in appearance (fig. 30). The interior of the membrane at first impression is striated. However, at higher magnifications the gelatinous nature of the interior is positively revealed, with the striations being simply thickenings of the constituent gelatinous material.

Tectorial membranes fixed in 25% glutaraldehyde and freeze-dried without alcohol dehydration resemble greatly in appearance the freeze-dried membranes just described (figs. 31, 32). In cross section the membrane is gelatinous, with thickenings of the gel-like substance radiating in a direction toward the under side of the tectorial membrane.

Membranes prepared by the Held fixative appear rough and granular, and fragments of them commonly remain attached to the surface of the organ of Corti. However, in those spots free of attaching tectorial membrane, the reticular lamina is vacuolated as though poorly fixed. The hairs of the hair cells, in contrast, are often nearly typical or entirely normal in overall appearance.

When glycerol is used with the glutaraldehyde in an attempt to replace the water of the membrane, the tectorial membrane is gelatinous in appearance. The membrane is also gelatinous-appearing if only dried over silica gel while left in situ or when entire cochleae are prepared by the Flock ('73) method. However, in all these cases, it is nearly impossible to define the external limits of the membrane.

Purposely induced artifact. After delayed fixation, the hairs of both inner and outer hair cells are often thread-like; adjacent hairs may fuse with one another, and the tips of the hairs of inner hair cells are sometimes flattened and expanded. The reticular lamina of the supporting cells is usually vacuolated and the microvilli are poorly preserved. The tectorial membrane unfixed for 30 minutes after death of the animal lacks organization and is vacuolated.

Freezing-thawing of presumably well-fixed cochleae results in artifacts which are different from those just described. The hairs of the outer hair cells have bizarre configurations. The hairs of all three rows on the hair cell frequently merge together in a solid line in a "V" shape. At other times, the limbs of the "V" are broken into sections, as though hairs are missing at intervals. On the other hand, the hairs of the inner hair cells often retain their individuality and form three rows on each hair cell surface. The reticular laminar surfaces of the supporting cells are sometimes intact, although large holes are of common occurrence. Tectorial membranes subjected to freezing-thawing generally have a reticulated or shredded appearance over the proximal one-half to two thirds of their extent. The portion over the organ of Corti resembles a vacuolated, glassy sheet. The under side is usually smooth and glassy.

X-ray analysis of the tectorial membrane. X-ray analyses of tectorial membranes prepared for scanning electron microscopy proved to be inadequate, because the use of aluminum stubs, of silver paint, and of gold coating interferes with the detection of ions which are present in small quantities. The analyses are useful, however, for a comparison of results obtained from freeze-dried membranes with fixed and dehydrated tissues shows that all ions are greatly reduced after the membranes are subjected to various fluids. This emphasizes the need for use of membranes untouched by fixatives or dehydrating agents when analyses are carried out.

The use of carbon paint and stubs in the procedure for microprobe analysis of

the tectorial membrane introduces a different artifact. Phosphorous and silicon are present in the paint, and silicon occurs in the stub; these ions must be discounted.

The analytical data given below are based upon information obtained from three samples of freeze-dried membrane with the upper side (toward the endolymph) and four samples with the lower side (toward the organ of Corti) exposed in the microprobe. The samples represent three different cochleae and include portions of the tectorial membrane from base to apex.

The x-ray analyses described here are not quantitative, but give information concerning the ions present and their relative concentration in scans of equal duration and of approximately equal counts of emitted rays per second (1000). These analyses show that the ions present in the tectorial membrane are not distributed in similar proportions on its two sides (figs. 33, 34). On the upper side (fig. 33), K^+ and Cl^- ions are present in almost equivalent amounts, although K^+ is slightly more plentiful. Na^+ is present in small and Mg^{++} in trace quantities, but Ca^{++} does not appear to exist in sufficient quantity to be detected. On the under side of the membrane (fig. 34), Cl^- is the most plentiful, followed by K^+ and then by Na^+. Na^+ is abundant on the under side of the membrane. Mg^{++} and Ca^{++} are present only in trace quantities. A small amount of sulfur is found on both the upper and the under sides and is likely incorporated into the protein of the membrane.

Transmission electron microscopy of the tectorial membrane. The portion of the tectorial membrane considered in detail here is the region of attachment to the outer hair cells. Thus far, no essential differences have been found between tectorial membranes obtained from untreated and from chemically sympathectomized rats.

The interior of the membrane is comprised of numerous filaments which are either fine (~30 Å wide), possessing numerous cross-hatchings so that they look feathered, and lacking apparent organization; or else the filaments are coarse (~160 Å wide), less branched and aggregated into groups having similar orientations (fig. 35) In these groups, the coarse and the fine filaments are cross-linked (fig. 35). There are small areas within the membrane which seem to have undergone lysis, and detritus is entrapped in these spaces (fig. 35).

The capping material from the tips of 15 hairs remains attached to the under side of the tectorial membrane shown in figure 35. The filaments of the tectorial membrane in the immediate vicinity of the tips of the hairs are more closely related to one another spatially than are those in the interior of the membrane, and are fine (~40–50 Å). In this part of the membrane the fine filaments emerge from the coarse ones directly nearest them, as though unwinding much in the manner of fraying rope. The fine filaments turn out almost at right angles from the coarser ones, lie close to one another, and finally cross the halo-like region around the tips of the hairs. As the filaments cross to the hairs, they become fine and are spatially evenly arranged (~30 Å apart). as though held in register. It is this even spacing of the filaments which lends the halo-like appearance to the zone of attachment. The filaments then course without break into the caps of the hairs, into material which would seem to correspond to a cell coat. In all cases, adjacent filaments appear to be cross-linked.

DISCUSSION

The chemical nature of the tectorial membrane. One of the more important issues to resolve is whether the tectorial membrane consists of acid mucopolysaccharide or of glycoprotein substances. Acid mucopolysaccharides (glycosaminoglycuronoglycans) are widely distributed in connective tissues and are large molecular weight protein-carbohydrate complexes with polysaccharide chains composed of repeating disaccharide units. These repeating units contain uronic acids (glucuronic or iduronic acid) and N-acetylhexosamine (N-acetylglucosamine or N-acetylgalactosamine). With the exception of hyaluronic acid most acid mucopolysaccharides contain ester-sulfate bonds. Glycoproteins on the other hand, are generally of lower molecular weight and branched in structure, lack a serially repeating unit and are high in protein content. In general, they lack

ester-sulfate bonds and contain a diversity of sugars or sugar derivatives, including N-acetylglucosamine, mannose, galactose, and/or fucose or sialic acid (Gottschalk, '66). Glycoproteins are currently of great interest for they are being shown to be important constituents of cell coats and to participate in the immune reaction; in surface charge, cell permeability and electrical properties of cells; in cell division; and in determining cell to cell reactions (Rambourg, '71).

The present histochemical results show that the tectorial membrane is highly PAS-positive and is not metachromatic, in agreement with the original findings of Wislocki and Ladman ('55). These observations must be interpreted in the light of recent histochemical and biochemical evidence which shows that only glycoproteins are highly PAS-positive, while mucopolysaccharides react slowly and little, if at all (Glegg et al., '52; Leblond et al., '57; Rambourg et al., '66; Rambourg, '71). On this basis alone, the tectorial membrane would appear to contain glycoprotein(s).

A metachromatic reaction on the part of a substance does not automatically categorize it as an acid mucopolysaccharide, but only indicates that anions with which the dye can react are present. The existing discrepancies in the metachromatic reaction of the tectorial membrane reported in the previous studies are at least partially explainable on the grounds that much of the work was carried out on inner ears after exposure to various fluids for fixation and/or dehydration, or even after decalcification, and often on embedded material. These manipulations could alter the ionic composition of the membrane. In the present study, use of cryostat sections from cochleae frozen in situ within the skull and fixed in vapor rather than fluid avoids the possibility that metachromasia has been artifactitiously conferred upon the membrane.

The lack of metachromasia in toluidine blue sections indicates that the tectorial membrane is not highly negatively charged. Based on their studies of interdental cells of the spiral limbus, Arnold and Vosteen ('73) concluded that the tectorial membrane consists mostly of neutral glycoproteins synthesized by these cells. Lawrence ('65) produced experimental evidence which indicates that the tectorial membrane is, in fact, electrically neutral.

Attachments of the membrane to the organ of Corti. The present scanning electron microscopic findings show that the tectorial membrane is attached to the tips of hairs of the outer hair cells, to the tips and shafts of hairs of inner hair cells, and to the microvilli of the surface of the organ of Corti from the inner border cells to the phalangeal processes of the outermost Deiters cells. Thus far, attachments to the Hensen cells have not been found.

Although the connections to the microvilli could not have been observed with the light microscope, my present findings are in general agreement with those of Prentiss ('13) and the other few, early investigators who held that the tectorial membrane was attached to the surface of the organ of Corti in adult mammals (Boettcher, 1859; Coÿne and Cannieu, 1895; Kishi, '07; Shambaugh, '07). My results in juvenile and adult rats are strikingly similar to those obtained by Held ('09) in fetal and newborn animals. Held previously described not only the attachments of the tectorial membrane to the organ of Corti shown here, but also the existence of a compartment around the hairs of the outer hair cells. He indicated, however, that these relationships did not persist beyond the neonatal state.

Alternate viewpoints that the shreds of material adhering to the microvilli are precipitated macromolecules formerly in solution in a subtectorial fluid, or are pieces of membrane which have accidentally fallen into position, have been considered and discounted. A subtectorial fluid would be small in quantity; its macromolecular content would have to be very high (and the fluid viscous) to account for the volume of material I have observed clinging to the microvilli. If endolymph were considered to be the subtectorial fluid, the chances of the shreds being precipitated macromolecules diminish remarkably. The viscosity and the protein content of endolymph are low in mammals (in the human: viscosity, 1.03–1.05 relative to water, 1.0; total protein content, 20–30 mg/100 ml; Rauch, '64). Moreover, the organization of the shreds and their resemblance to, and con-

tinuity with, the tectorial membrane speak for their being part of that membrane. Accidentally deposited debris is never organized and never specifically related to particular structures.

The physical nature of the tectorial membrane. My conclusion that the tectorial membrane is a glycoprotein must be considered together with the findings of Naftalin et al. ('64) that the membrane is a gel containing approximately 90% water in the adult guinea pig. This suggests that the tectorial membrane is a system of glycoprotein molecules in close association with fluid, forming a colloidal or semisolid structure, and that no further "amorphous" material is present. The amount of fluid entrained by the membrane appears to change between the fetal and the functional states, for embryologists have consistently found the membrane to be attached to the entire organ of Corti developmentally but not in the adult. The ease of preservation of the connections to the microvilli in birds implies that the membrane is less hydrated than in mammals. The extreme sensitivity of the membrane to states of hydration must reflect an important in vivo property which has been little explored.

The conclusion that the tectorial membrane is basically a gel has significant ramifications in considering the ultrastructural results which have been interpreted to indicate that the membrane is largely fibrillar or consists of fibrils in an amorphous matrix. As shown here, the grossly fibrillar appearance of the membrane described by many workers is artifactitious and related to the fixation and dehydration procedures followed.

Membranes dehydrated after being freed of their attachments are not fibrillar, but are gelatinous in appearance. These results demonstrate that great viscous forces exist in the glycoprotein of the tectorial membrane which keep it a cohesive mass when it is dried free of attachments. The surfaces of membranes simply dehydrated, or fixed and dehydrated, in situ show various patterns in the residual, still cohesive material. The particular pattern seen reflects the changing forces of stress placed upon the viscous membrane which is anchored at both ends as fixation and/or dehydration begins, but which shrinks and pulls away from its attachments to the organ of Corti as withdrawal of water continues. Consistent curling back of the outer margin of the membrane suggests a greater effect of dehydration upon the upper than on the under surface.

Thickenings of the gelatinous substance of the tectorial membrane radiate from the region of the limbus toward the organ of Corti in cross sections of membranes prepared by either freeze-drying or by fixation in 25% glutaraldehyde prior to freeze-drying. They may correspond to the "fibrils" seen with phase-contrast or polarized light microscopy (A. Hilding, '52; Iurato, '60) and to the fibrillar structures depicted by Held ('09) as anchoring the tectorial membrane to the reticular surfaces of the supporting cells of the organ of Corti in the fetus. These thickenings would appear to correspond to the groups of similarly oriented filaments observed by transmission electron microscopy (fig. 35). The aggregates of filaments do not correspond to "fibrils," but possibly represent orientations assumed by the glycoprotein macromolecules due to the tensions placed upon the membrane by its attachments.

Correlation of data obtained by transmission electron microscopy of the tectorial membrane with biochemical and physical data. The filaments observed in transmission electron microscopy of the tectorial membrane are the subunits of the cohesive material seen with the scanning electron microscope. They are presumed to be aggregates of macromolecules comprising the glycoprotein of the membrane. The arrangements of the macromolecules are dependent upon electrostatic interactions between or within the polyelectrolytes which act to attract or repel one another, upon the steric configurations of the molecules themselves, and upon the effects of tensions (stress) or of ions in the entrained fluid.

The electron microscopical observations taken together with the well-known physical visco-elastic property of the membrane lead to the inference that the polyelectrolyte glycoprotein molecular configuration is the extended flexible coil (Gibbons, '66). One consequence of this configuration is that the molecules can interact physically

with one another to form a complex network which will possess properties making it important in fluid and ion transport. When flexible polyelectrolyte molecules become very extended, hydrodynamic interaction ceases to a point where appreciable fluid can pass between and through the molecules. Such molecules are said to be partially or free draining, in contrast to more coiled or entwined molecules through which less water can pass (Gibbons, '66).

Both the more closely entwined and the more extended-appearing, thread-like filaments occur in the tectorial membrane, and in differing concentrations from one site to another (fig. 35). Thus, fluid transport capabilities are likely to differ from one site to another within the membrane. However, the amount of fluid entrained is possibly in constant flux, for the configuration of the molecules is unstable and can change (by further extending or recoiling) due to solvent-solute interactions, slight shifts in pH or ions, stress, or even Brownian movement (Gibbons, '66).

Ions can be moved through a complex, polyelectrolyte network by either of two methods: free flow with the fluid solvent, or by ion-exchange. In either case, the hydrated radius of the ions (ionic size) and the charge on the polyelectrolyte molecules must be considered pertinent to their movement.

The ionic composition of the tectorial membrane: its functional significance. The present findings show that the tectorial membrane has some pore spaces that are large (Ca^{++} has a relative hydration of 22 compared to 5.4 for K^+ and 8.4 for Na^+; Höber et al., '45), and that it will admit both cations and anions. The findings also demonstrate that although the tectorial membrane contains essentially the same ions as endolymph, it does not possess them in similar relative concentrations on both sides of the membrane. The relative concentrations found here for the ions on the upper surface correspond well with figures expressed in milligram-equivalents per liter for endolymph (in the human: Na^+, 6–30; K^+, 128–169; Cl^-, 100–123; Rauch, '64). However, the relative concentrations of the ions ($Cl^- > K^+ > Na^+$) present on the underside of the membrane are different; nor do they correspond to figures given for the ionic content of perilymph (in the human: Na^+, 118–169; K^+, 2.9–8.2; Cl^-, 102–135; Rauch, '64). Thus, the electrolyte composition of the fluid in the colloid (tectorial membrane) is different from either endolymph or perilymph, as Naftalin et al. ('64) have previously reported.

Naftalin et al. ('64) have shown that the membrane binds some of the K^+, and binds Ca^{++} more tightly than Mg^{++}. These findings taken together with the present results indicate that the tectorial membrane is not simply a sponge for endolymph. Rather, if the membrane is electrically neutral as the present histochemical and prior electrical evidence (Lawrence, '65) suggests, it stands as a barrier between regions of different electrolyte composition to retard diffusion of ions from the one region to the other. The cells around the endolymphatic space must be discharging or actively removing ions differently from those cells lying under the membrane. As the shifts noted are primarily in concentrations of Na^+ and K^+, there are the ions which are most concerned.

The fact that the ionic composition of the tectorial membrane differs from that of endolymph means that a potential difference will be produced, for potentials, which can be quite high, are created wherever charge separations exist. A series of finite charge separations probably occurs within the tectorial membrane itself. However, if the entire membrane is considered as one liquid phase and endolymph as another, then a charge separation exists at or near the interface between the two. That this is the case has already been shown by Lawrence ('65), who found a shift in potential to occur from about 6–7 mv negative (approximately base line) to about 70 mv positive as his recording electrode left the tectorial membrane to enter endolymph. Other charge separations possibly exist between the tectorial membrane and the fluid compartments around the hairs of the outer hair cells, although there is presently no electrical evidence for their existence. Additionally, the tectorial membrane, by its anatomical position, participates in the production of a charge separation between the organ of Corti and the

endolymph. Lawrence ('65) found a shift from 40 mv negative in the organ of Corti to 70 mv positive in the endolymph, with the shift toward zero taking place at the tectorial membrane. Thus, the tectorial membrane would appear to play a role in the production of certain inner ear potentials, although the specific functions of these potentials are currently unknown.

Functional significance of the attachments of the tectorial membrane to the hair cells. The concept that the extended, flexible coil is an unstable molecular configuration would appear to be of extreme significance when considering the function of the attachment between the tectorial membrane and the tips of the hairs of outer hair cells. The filaments by which the structures are attached to one another are thread-like, held in register with one another, and continuous with the cell coat of the tips of the hairs. They become intimately related to or even a part of the cell membranes of the hair cells on the one hand, and are continuous with the substance of the tectorial membrane on the other. Stimulation of these filaments by any of several means, (including stress or local shifts in pH or in ionic environment) could result in changes in configuration of the molecules, leading to alteration in the permeability of the hair cell membrane to ions, and to production of a receptor potential. This would bring the transduction of acoustical into electrical energy down to a molecular level as Naftalin ('65) and Lawrence ('65) have indicated is essential at lowermost thresholds of hearing.

In a sense, the tips of the outer hairs, the hairs of the inner hair cells, and the tectorial membrane are continuous, for they are physically bound together, and their attachments may correspond to a binding of one cell coat to another. This would suggest that the hairs and the tectorial membrane are also functionally united, and that the tectorial membrane and not the basilar is the important structure in delivering acoustical energy to the hair cells. While this concept is not new (von Ebner, '02; Shambaugh, '07, '08; Hardesty, '08; Prentiss, '13) it has all but been abandoned since the work of Békésy ('60). Most noted among recent proponents of this concept are Naftalin and his group ('64, '65), although Mygind ('52) and Borghesan ('71) have also supported it.

One of the arguments against bending of the hairs, as postulated by Békésy, has been the fact that the hairs are stiff (Waldeyer, 1873) and will break before bending (Engström et al., '62). In my preparations for scanning electron microscopy, hairs are sometimes broken at their bases and moved en masse from their positions due to the fact that they are strongly tied together by a binding material. The presence of this substance would preclude bending of the hairs, for the hairs would be restrained in movement by their coupling to one another. In my scanning preparations, both the hairs of outer and of inner hair cells show this binding; previously, Lindemann and Bredberg ('72) observed similar connections in the case of hairs of inner hair cells in the cat. The binding material seen by this method probably corresponds to the amorphous or granular substance found along the surface of the shafts of the hairs by all who have studied the hair cells by transmission electron microscopy. It may be a cell coat; some have postulated the material contains acid mucopolysaccharide (Küttner and Geyer, '71).

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PLATE 1

EXPLANATION OF FIGURES

1 The tectorial membrane (TM) is PAS-positive in the normal rat. SL, spiral limbus. Rat 21 days old. × 310.

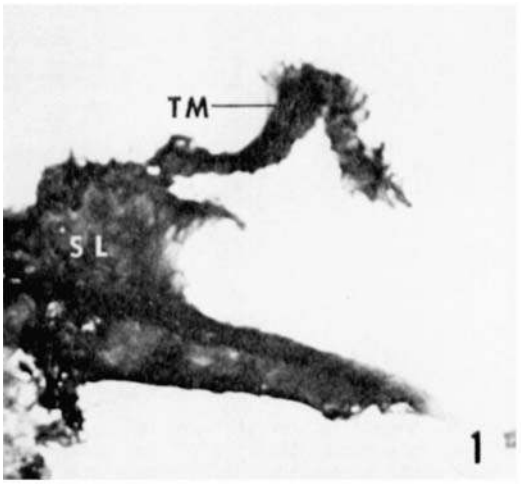
2 After chemical sympathectomy with 6-hydroxydopamine, the tectorial membrane (TM) remains PAS-positive. SL, spiral limbus. Rat 22 days old. × 300.

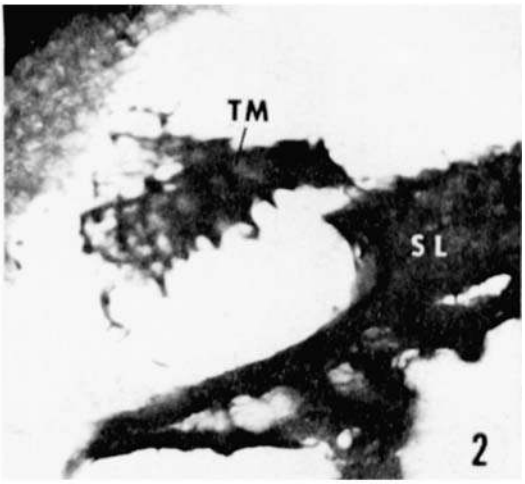
3 The tectorial membrane (TM) stains orthochromatically in toluidine blue. The membrane often has a feathery appearance with this stain. SL, spiral limbus. Rat 24 days old. × 350.

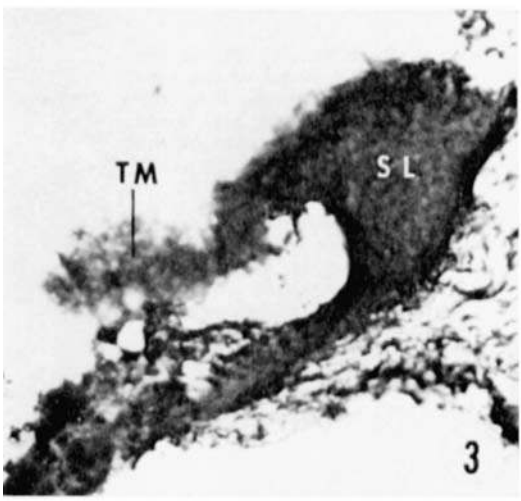
4 After chemical sympathectomy, the tectorial membrane (TM) remains orthchromatic in toluidine blue. The intensity of the staining reaction is approximately the same as in untreated rats. SL, spiral limbus. Rat 22 days old. × 345.

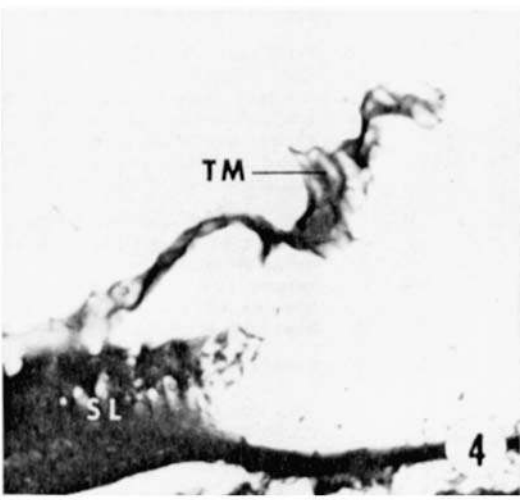
5 In this specimen, exposed to hyaluronidase for three hours prior to staining with toluidine blue, the tectorial membrane (TM), is orthochromatic and shows little change in staining reaction from the membrane shown in figure 3. SL, spiral limbus. Rat 25 days old. × 325.

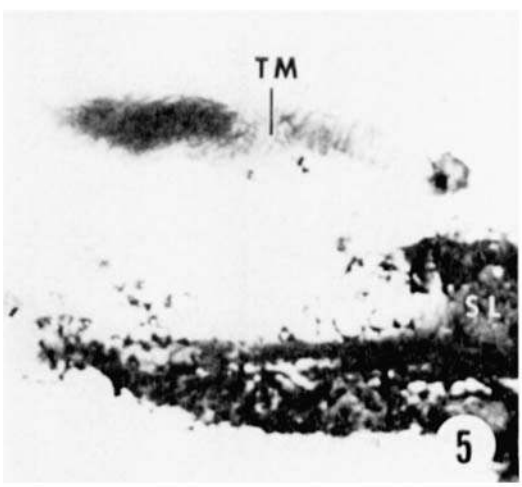
6 The tectorial membrane (TM) incubated in neuraminidase for 30 minutes prior to staining with toluidine blue stains a faint blue. SL, spiral limbus. Rat 25 days old. × 360.


TM
SL
1


TM
SL
2


TM
SL
3


TM
SL
4


TM
SL
5

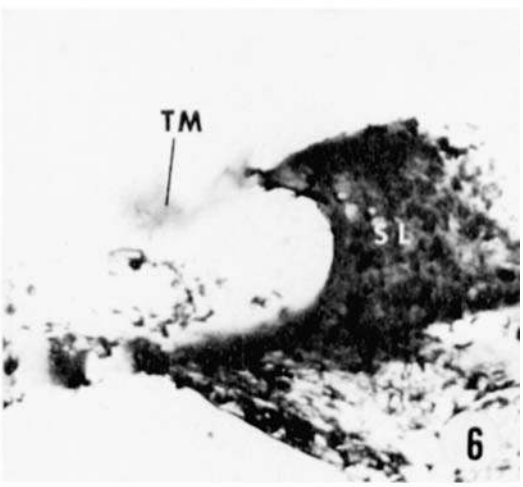

TM
SL
6

PLATE 2

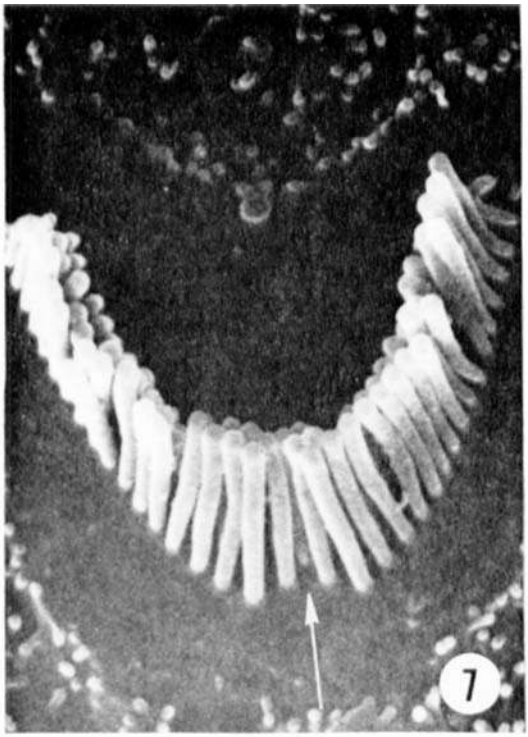
EXPLANATION OF FIGURES

7 The hairs of the outer hair cells of the normal rat are arranged in a "W" configuration (indentation between limbs of the "W" is marked with an arrow). The tips of the hairs are smooth and bulbous when entirely freed of tectorial membrane. Middle third of the cochlea. Rat 39 days old. × 18,000.

8 With care, the attachments of the tectorial membrane (TM) to the tips of the hairs of outer hair cells are preserved. Some of the connections between adjacent hairs are indicated by arrows. Note the few microvilli (M) on the cuticular surface of the hair cell. Some of the microvilli at upper right have bits of the substance of the tectorial membrane still adherent to them. Apical third of the cochlea. Rat 32 days old. × 20,000.

9 The hairs of the outer hair cells are so firmly attached to the tectorial membrane (TM) that they are sometimes torn loose from their basal relationships to the Deiters cells. Indentations, denoting the former positions of hairs of outer hair cells now pulled away, are obvious on the underside of the tectorial membrane (arrows). B, base of hair cell. Basal third of the cochlea. Adult rat, 320 gm. × 3800.

10 Shreds sometimes hang down from the tectorial membrane (TM), denoting positions of attachments (arrows) between the membrane and the tips of the hairs of outer hair cells. Middle third of the cochlea. Adult rat, 320 gm. × 3600.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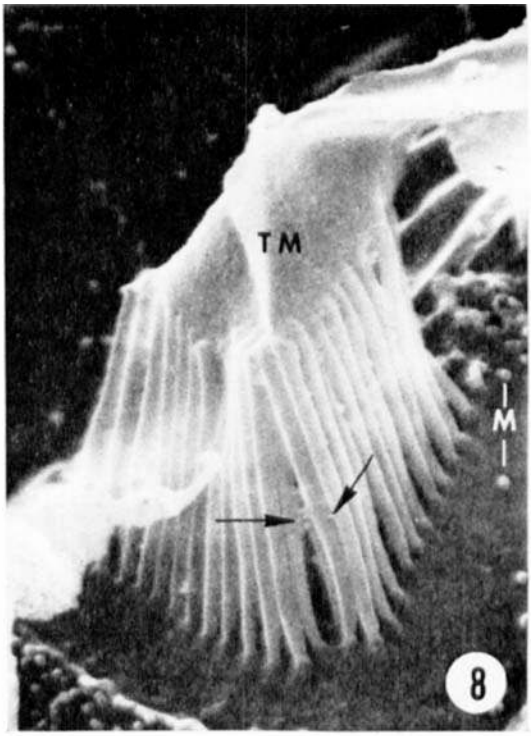
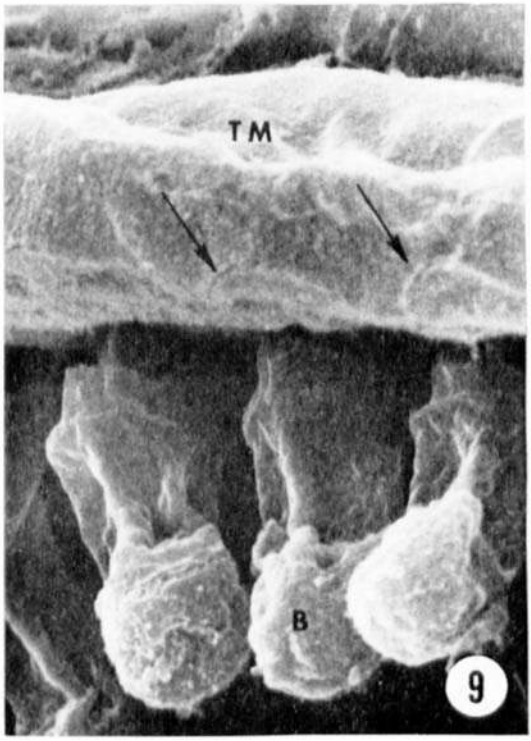

TM
M
8


TM
B
9

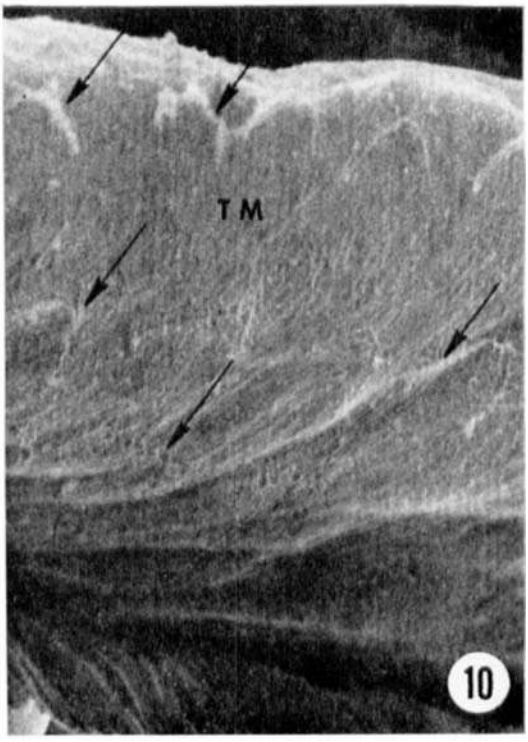

TM
10

PLATE 3

EXPLANATION OF FIGURES

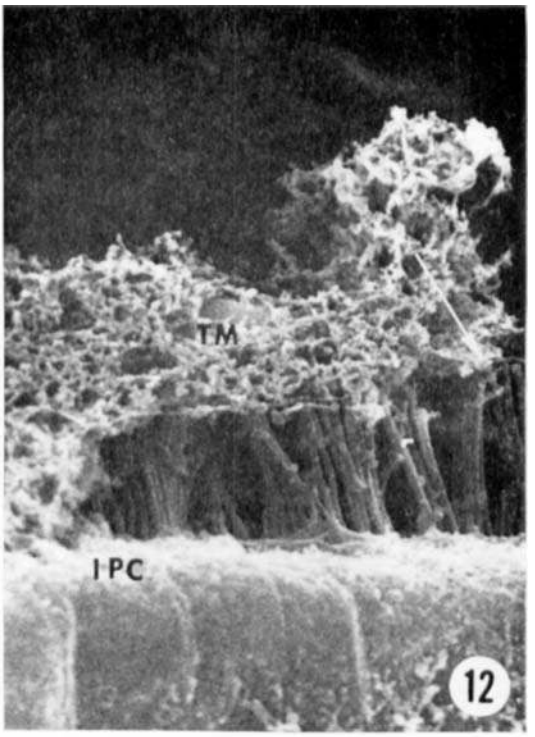
11 The hairs of the inner hair cells are arranged in three rows, forming a shallow crescent, with the tallest hairs outermost. When the tectorial membrane is completely removed from them, the hairs of the outermost row have blunt tips while those of the inner rows have tips which are flattened and sharply angled upward. IPC, inner pillar cell; IPhC, inner phalangeal cell. Middle third of the cochlea. Rat 39 days old. × 11,000.

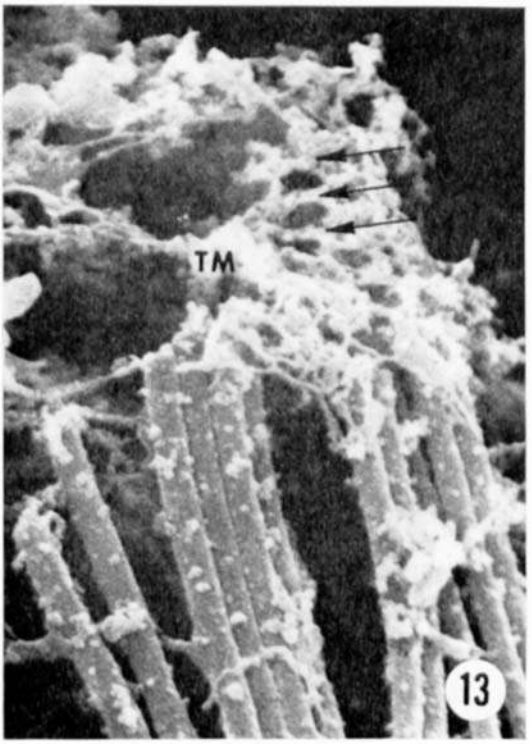
12 The hairs of the inner hair cells appear to be enshrouded by the tectorial membrane where it is left as intact as possible. At left, the tectorial membrane remains attached to the heads of the inner pillar cells (IPC). These attachments have been disrupted toward the center of the field. Toward the upper right, the attachments to the tips of the hairs of the inner hair cells are evident (arrow). Apical third of the cochlea. Rat 40 days old. × 3000.

13 The attachments of the tectorial membrane (TM) to the tips of the hairs of the inner hair cells are shown here at higher magnification than in figure 12. Most of the tectorial membrane appears fibrous, but this is artifactitious and related to fixation and dehydration of the tissue. However, the regular arrangement of elements of the membrane attaching to the hairs can be observed at upper right (arrows). Note that bits of the tectorial membrane cling to the shafts of the hairs. Apical third of the cochlea. Rat 40 days old. × 12,000.

14 Connections of the tectorial membrane (TM) to the tips of the hairs of inner hair cells are also demonstrated in this scanning electron micrograph. Connecting filaments (F) remain attached to the second hair from the left, although the hair was loosened from the membrane. Apical third of the cochlea. Rat 40 days old. × 21,000.


IPC
IPhC
11


TM
IPC
12


TM
13

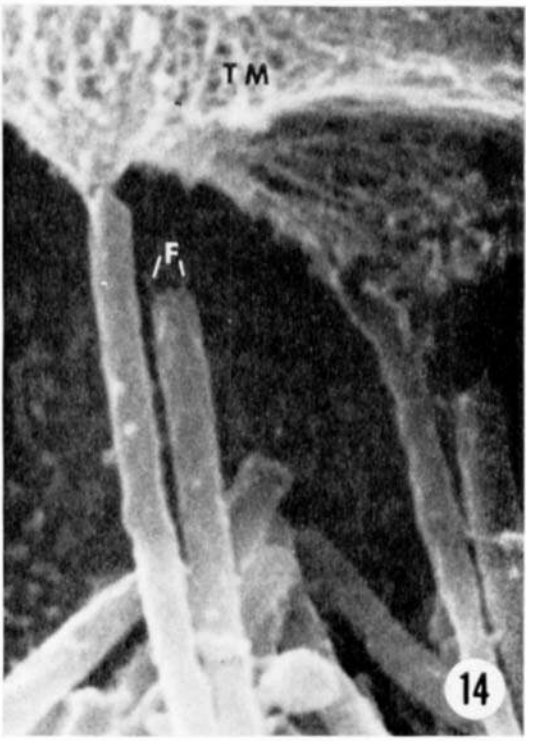

TM
F
14

PLATE 4

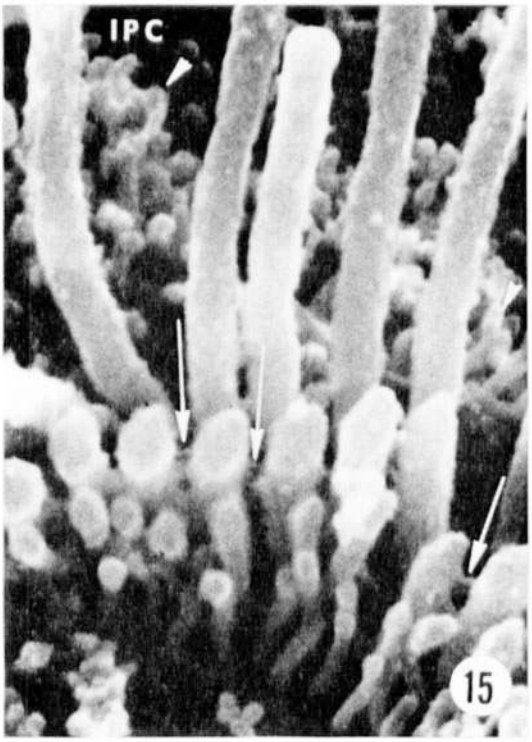
EXPLANATION OF FIGURES

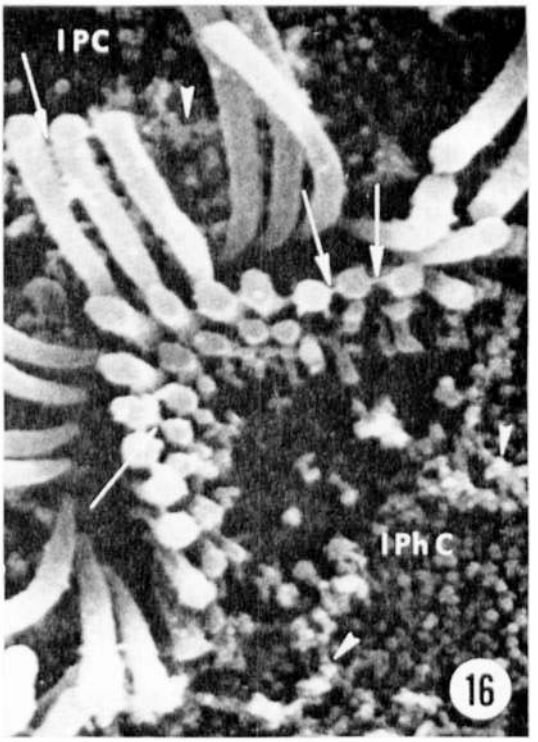
15 Filamentous strands connect adjacent hairs of the inner hair cells (arrows). Note that bits of the tectorial membrane (arrow heads) still cling to microvilli of the inner pillar cell (IPC) and also to hairs of the inner hair cell. Middle third of the cochlea. Rat 39 days old. × 24,000.

16 Connections between adjacent hairs of the same row and of the neighboring row on an inner hair cell are evident here (arrows). A few of the sites where remnants of the tectorial membrane remain attached to microvilli of the inner phalangeal cell (IPhC) and the inner pillar cell (IPC) are indicated with arrow heads. Middle third of the cochlea. Rat 39 days old. × 12,700.

17 Shreds of the tectorial membrane (TM) attach to microvilli of the inner phalangeal cells (IPhC) and border cells (BC) in this specimen. A few of the more obvious connections are indicated by arrows. Middle third of the cochlea. Rat 39 days old. × 8000.

18 Shreds of the tectorial membrane (TM) commonly attach to the microvilli of the inner pillar cells (IPC), outer pillar cells (OPC), and the Deiters cells (DC). Some shreds also adhere to microvilli of the cuticular plates of outer hair cells. A few of these attachments are indicated by arrows. Middle third of the cochlea. Rat 39 days old. × 8000.


IPC
15


IPC
IPhC
16


BC
TM
IPhC
17

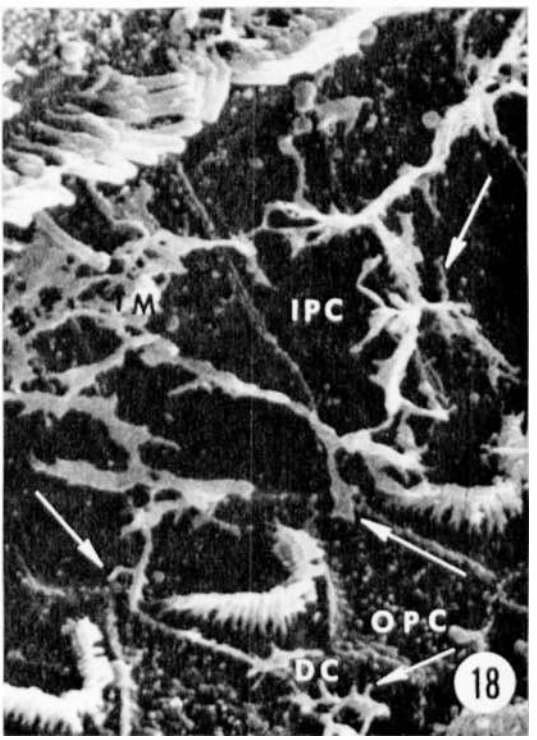

TM
IPC
OPC
DC
18

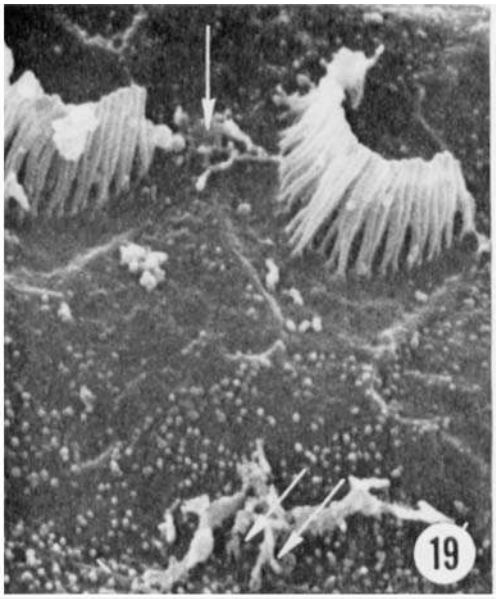
PLATE 5

EXPLANATION OF FIGURES

19 Connections of the tectorial membrane to the microvilli on the phalangeal processes of the second (single arrow) and third row (double arrows) of Deiters cells are indicated here. Rat 34 days old. Middle third of the cochlea. × 8000.

20 The shreds of tectorial membrane remaining adherent to the reticular lamina of the organ of Corti attach to the microvilli (arrows), as demonstrated at high magnification here. The connections shown are to microvilli of inner phalangeal cells. HIHC, hairs of an inner hair cell. Rat 34 days old. × 22,000.

21 This drawing illustrates the anatomical position of the tectorial membrane according to the present findings. The membrane is closely applied to the reticular laminar surface of the organ of Corti. It encloses the hairs of the inner hair cells, but is attached only to the tips of the hairs of the outer hair cells. The relationship of the membrane to the hairs (H) of the outer hair cells is shown in the insert; a fluid compartment (C) may exist around and within the limbs of the "W" formed by the hairs on the cuticular surface of each outer hair cell. The tectorial membrane is depicted here as a gel; internal organization of the membrane has been omitted.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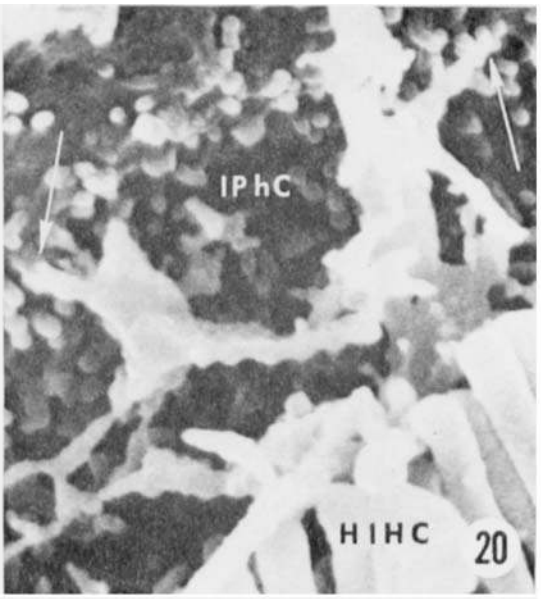
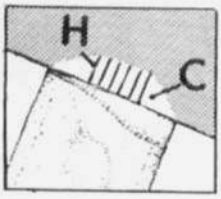

IPhC
HIHC
20


H
C

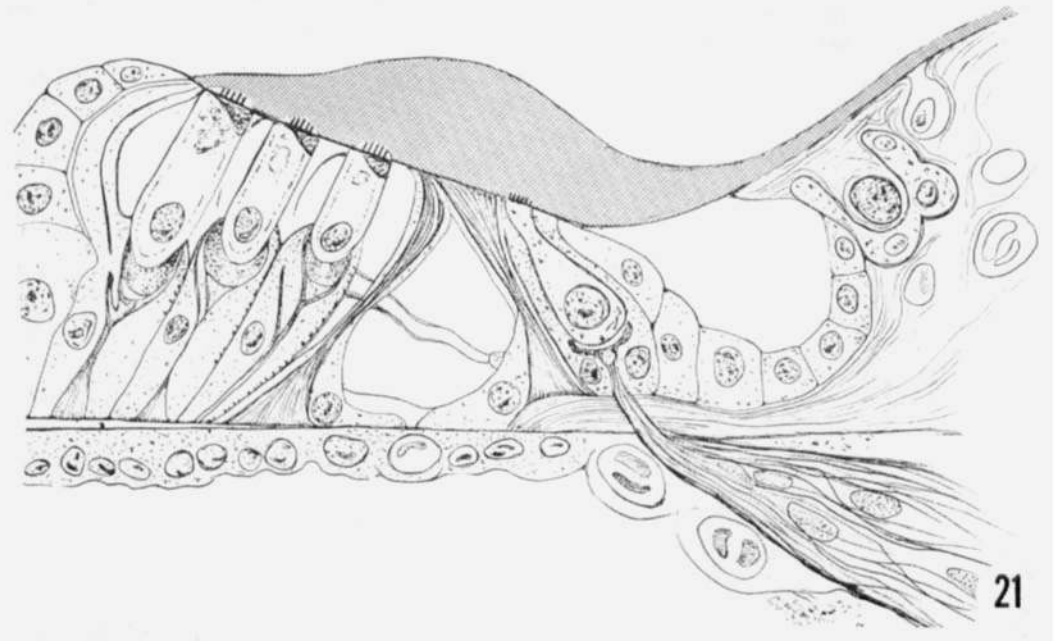

21

PLATE 6

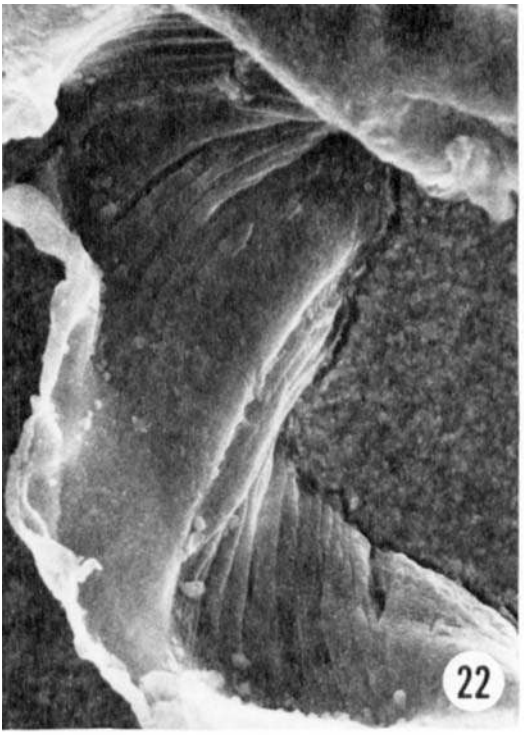
EXPLANATION OF FIGURES

22 Tectorial membranes freed of their attachments prior to dehydration appear gelatinous. Upper surface of the tectorial membrane, apical third of the cochlea. Critical point dried. Rat 39 days old. × 750.

23 A portion of the upper surface of the tectorial membrane from the same specimen depicted in figure 22 is shown here at higher magnification. Note the band-like thickenings of the gelatinous substance near the outer margin of the membrane (arrows). × 5400.

24 This scanning electron micrograph shows the under side of the tectorial membrane, as it appears when the membrane is dried free of its attachments. Critical point dried membrane. Rat 39 days old. × 6000.

25 Tectorial membranes fixed and dehydrated in situ commonly have their upper surfaces thrown into ridges, coursing in all directions. Near the peripheral margin of the membrane, at times, there are longitudinally running bands. The bands (arrows) shown here correspond in position to those shown in figure 23. Middle third of the cochlea. Rat 40 days old. × 2400.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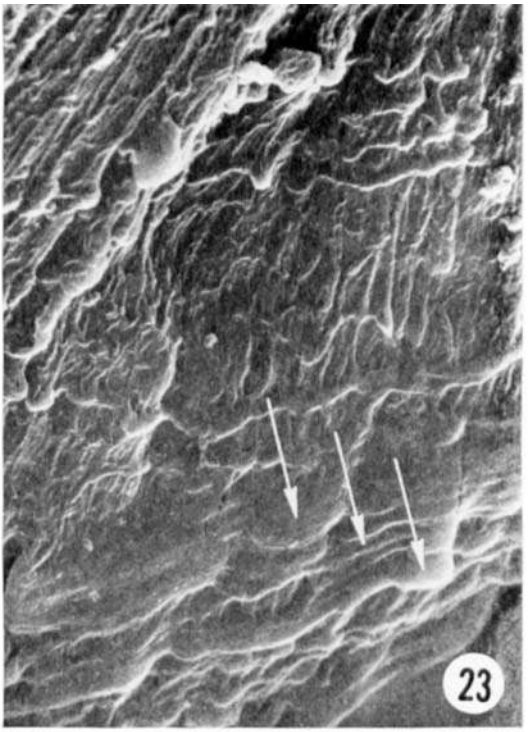
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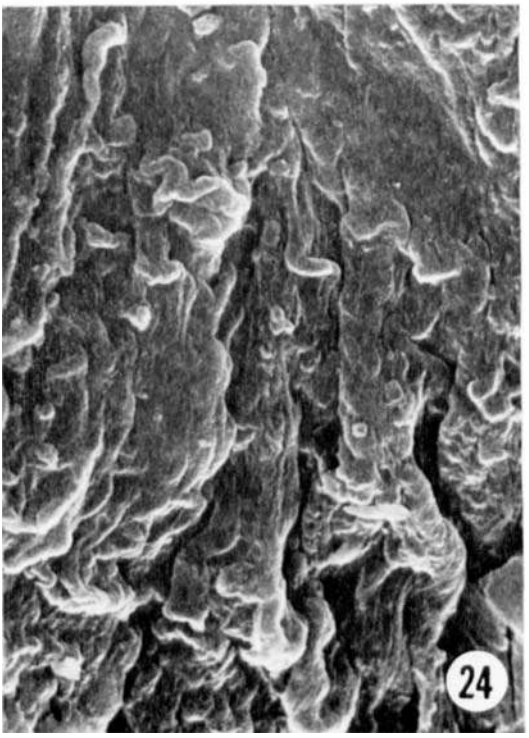
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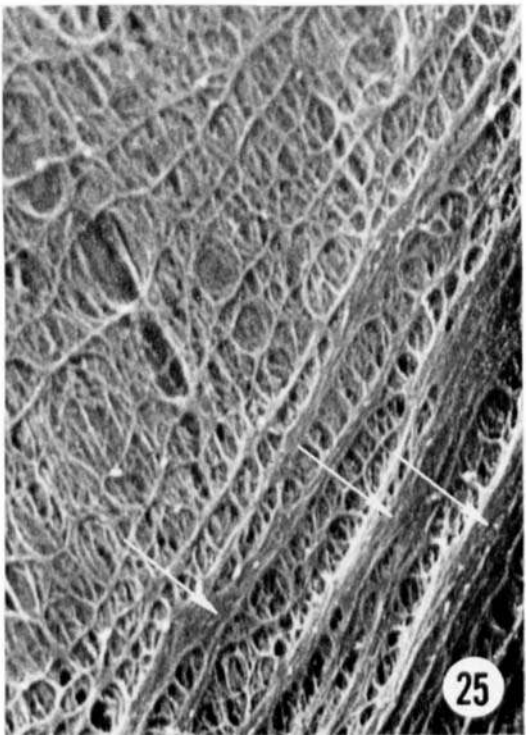

25

PLATE 7

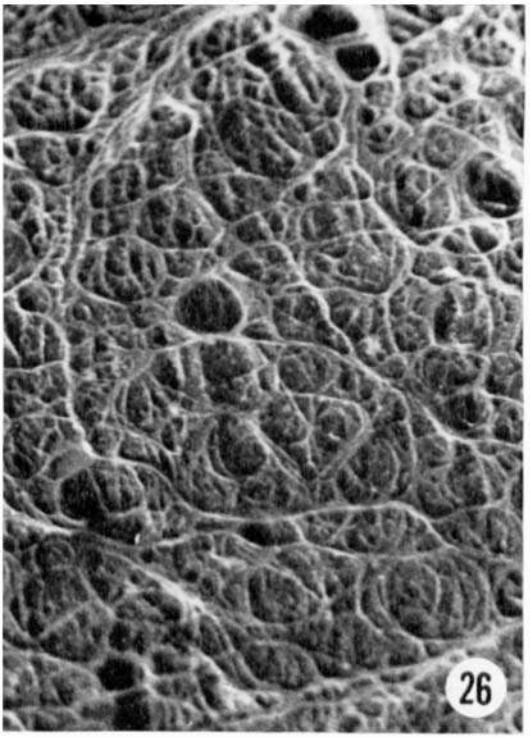
EXPLANATION OF FIGURES

26 The fine ridges, disposed in all directions, which are found on the upper surface of the tectorial membrane fixed and dried in situ are shown here at higher magnification than in figure 25. This portion of the membrane lies proximal (toward the spiral limbus) to the part shown in figure 25. Middle third of the cochlea. Rat 40 days old. × 4800.

27 A small portion of the under surface of the tectorial membrane from the same specimen shown in figures 25 and 26 is shown here. The under side (UTM) is curled back over the upper surface (UpTM) at the peripheral margin of the tectorial membrane. The under surface is rough and irregular. Where tears occur, the interior of the membrane appears fibrous. Middle third of the cochlea. Rat 40 days old. × 3750.

28 In some places, the upper surface of tectorial membrane (UpTM) freeze-dried without prior fixation or dehydration exhibits a series of ribbon-like, gelatinous structures (arrows). Here, at the peripheral border of the membrane, the under side (UTM) is gelatinous and curled back upon the upper surface. Middle third of the cochlea. Rat 41 days old. × 3750.

29 The gelatinous substance of the upper side of the freeze-dried tectorial membrane may be smooth, or show stubby protrusions. Middle third of the cochlea. Rat 41 days old. × 10,000.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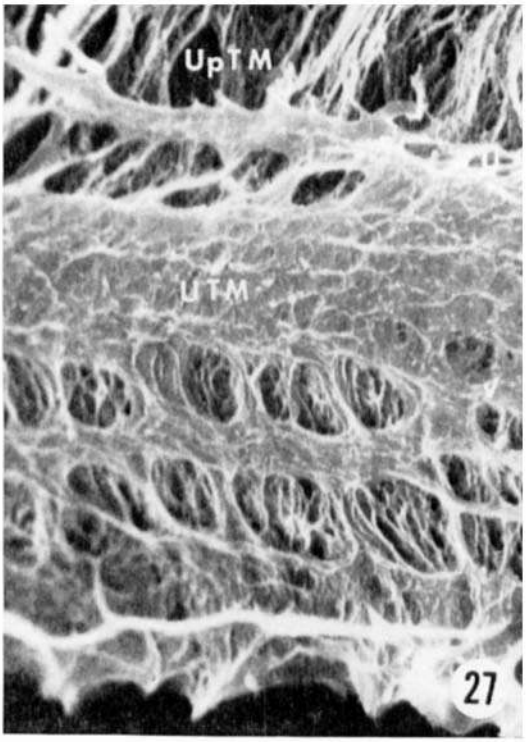
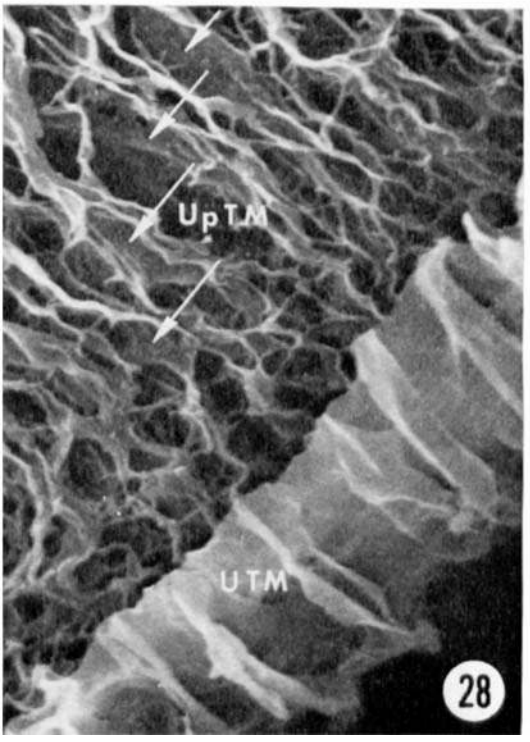

UpTM
UTM
27


UpTM
UTM
28

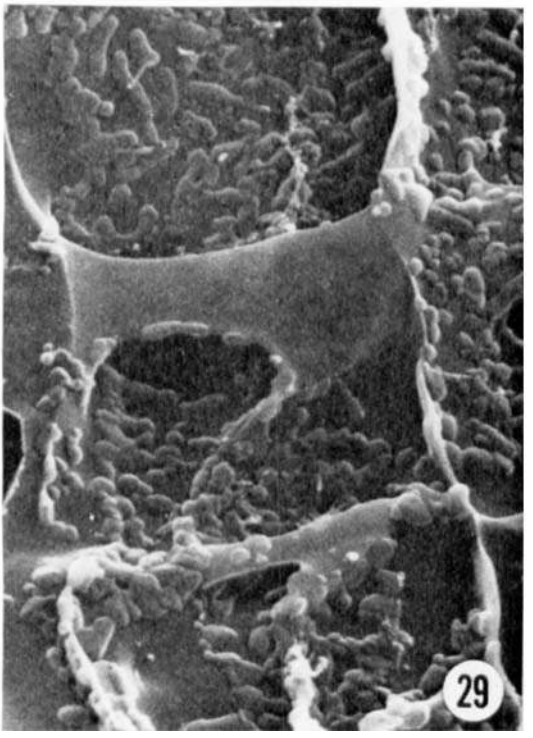

29

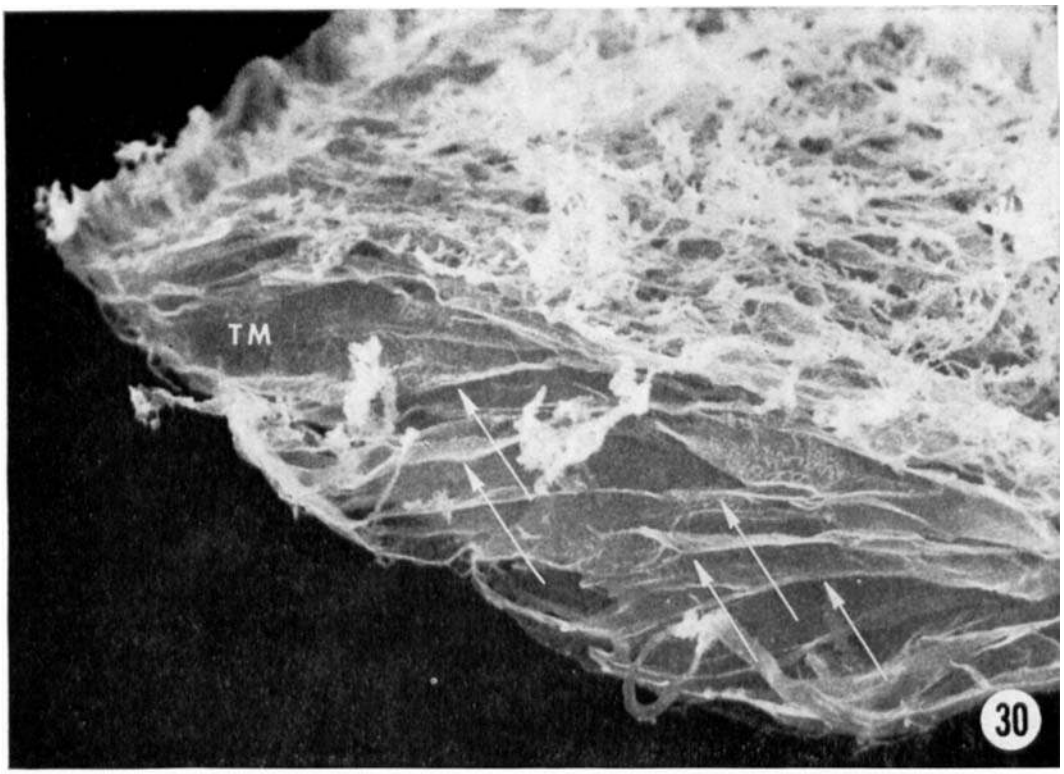
PLATE 8

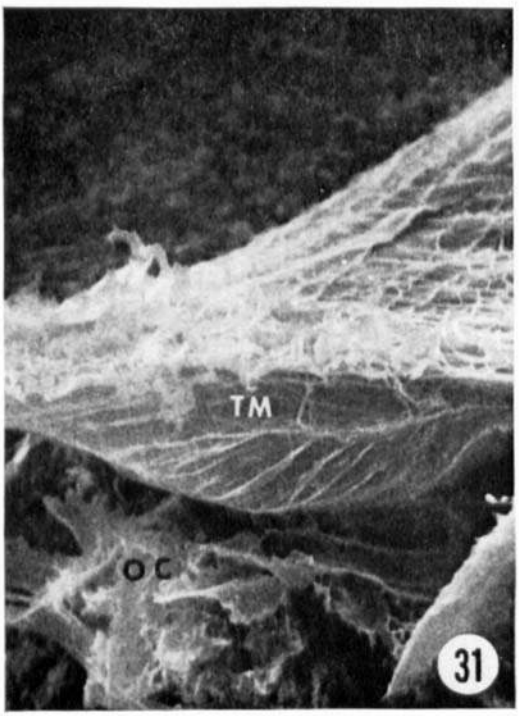
EXPLANATION OF FIGURES

30 A cross section of freeze-dried tectorial membrane (TM) is shown here. The interior of the membrane is gelatinous, not fibrillar. Thickenings of the gelatinous material are indicated by arrows. Basal third on the cochlea. Rat 41 days old. × 2300.

31 Compare this micrograph with figure 30. In cross section, the tectorial membrane (TM) fixed in 25% glutaraldehyde prior to freeze-drying resembles greatly the tectorial membrane subjected to freeze-drying only. The organ of Corti (OC) has been disrupted during preparation of the tissue. Basal third of the cochlea. Rat 71 days old. × 1270.

32 A portion of the interior of the tectorial membrane shown in figure 31 is presented here at higher magnification. The gelatinous nature of the membrane and the presence of thickenings (arrows) of the gelatinous material are evident. Rat 71 days old. × 32,000.


TM
30


TM
OC
31

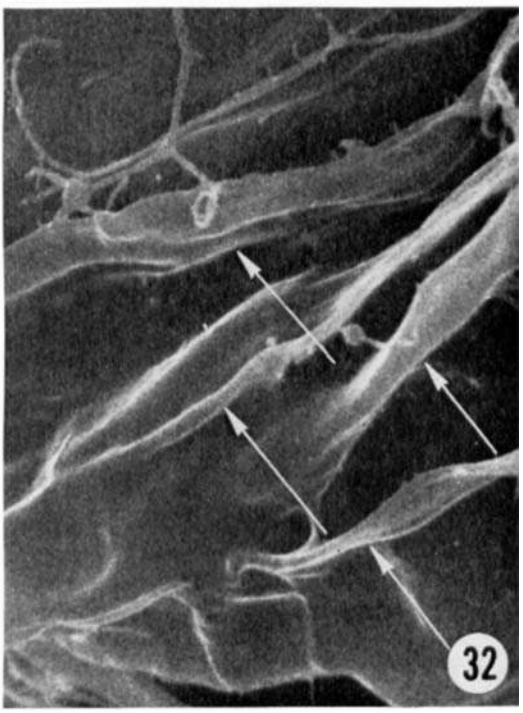

32

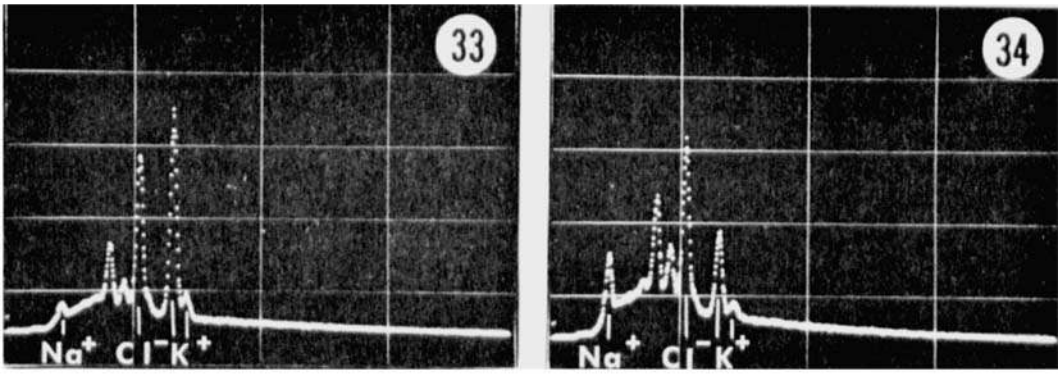
PLATE 9

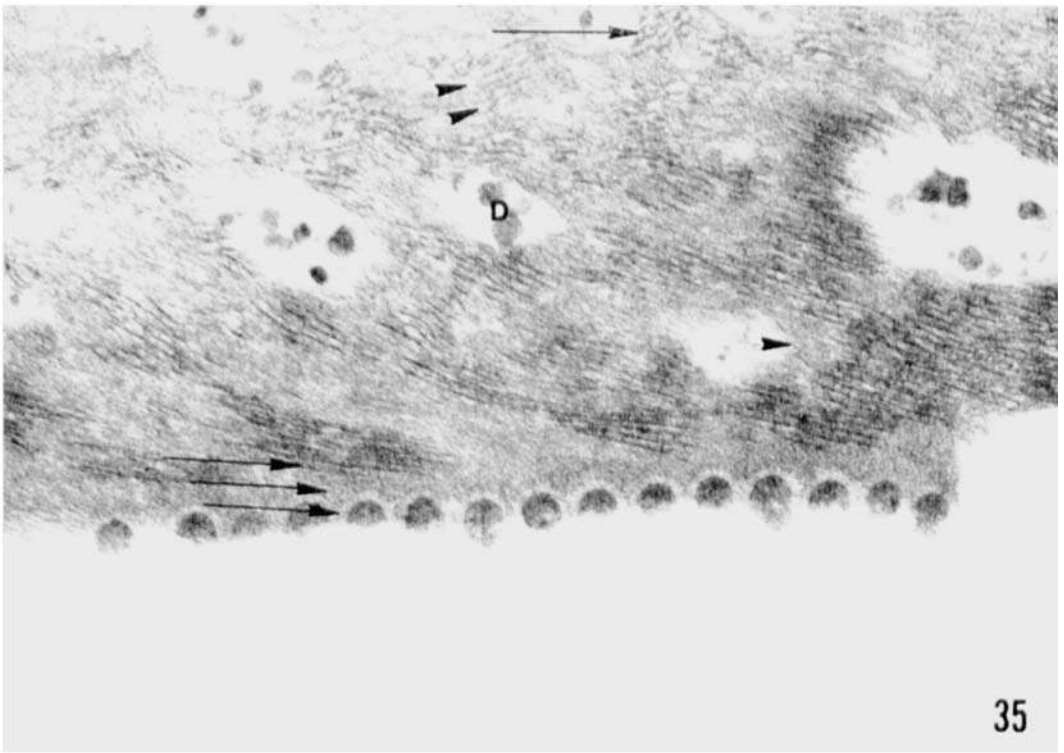
EXPLANATION OF FIGURES

33 This is an x-ray spectrum obtained upon the analysis of the upper surface of a segment of tectorial membrane from the basal third of the cochlea. The peaks given by Na^+, Cl^- and K^+ are indicated. The two major peaks between Na^+ and Cl^- are those given by phosphorus and sulfur, from left to right. Freeze-dried membrane. Adult rat, 385 gm.

34 This is an x-ray spectrum obtained upon analysis of the under side of a segment of tectorial membrane from the basal third of the cochlea. Compare with figure 33. Freeze-dried membrane. Adult rat, 350 gm.

35 A portion of the tectorial membrane, with capping material from the tips of 15 hairs still attached, is shown in this transmission electron micrograph. Some of the filaments on the interior are thick (⌣160 Å) and are aggregated into bundles (single arrow). Many of the remaining interior filaments are finer (⌣30 Å) and lack regular arrangement. Near the under side of the membrane, thicker filaments are continuous with finer ones which are more closely spaced; fine filaments then cross the halo-like region to the caps of the hairs in regular array (see the series of 3 arrows, lower center). The filaments appear to be cross linked (arrow heads). Detritus (D) appears entrapped in the clear spaces in the membrane. Rat 22 days old, 6-hydroxydopamine treated. Basal third of the cochlea. × 28,500.


33
Na+ Cl- K+
34
Na+ Cl- K+


D
35