Cell Tiss. Res. 193, 11–23 (1978)

Cell and Tissue Research

Ultrastructure of the Different Zones of the Tectorial Membrane*

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Summary. The tectorial membrane (t.m.) of mammals, which lies over the organ of Corti, is made up of an agglomerate of protofibrils of varying degrees of hydration. Two types of protofibrils are clearly distinguishable in the mouse t.m. While type-A protofibrils are straight and unbranched (thickness: 110 Å) demonstrating a periodic structure (period = 70 Å), type-B protofibrils are branched and coiled (thickness: 150–200 Å). These protofibrils could be systematically ordered according to the different t.m. zones. Type-A protofibrils predominate in the basal layer and in the entire middle zone, where they are interlaced with strongly hydrated type-B protofibrils. Weakly hydrated type-B protofibrils essentially make up the marginal zone (with the marginal net), the covering net, Hensen's stripe and the immediate contact layer with the limbus.

Key words: Tectorial membrane – Organ of Corti – Electron microscopy.

The tectorial membrane (t.m.) of the organ of Corti in mammals is composed of carbohydrates (Schätzle, 1971; Tachibana et al., 1973) secreted into the extracellular space of the scala media (Voldrich, 1968; Arnold and Vosteen, 1973). The t.m. is positioned directly above the accessory pole of the sensory hair cells of the organ of Corti. The role of the t.m. in the transformation of the stimulus is not understood, and is the subject of some controversy (Davis, 1957, 1968; Naphtalin, 1964, 1965; Lawrence, 1967, 1975; Lawrence et al., 1974; Ross, 1974; Flock, 1977).

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* This work was carried out in the Sonderforschungsbereich 50 "Kybernetik", München, which is supported by the Deutsche Forschungsgemeinschaft

** The author thanks Prof. Dr.-Ing. E. Zwicker for his promoting interest and valuable discussions and Prof. Dr. G.A. Manley for his help in improving the English version of the manuscript. Mrs. A. Eberth and Miss R. Scheeler assisted technically, Mrs. A. Schumann typed the manuscript. For these experiments the scanning electron microscope of the Sonderforschungsbereich 51, the transmission electron microscope of the Lehrstuhl für Metallurgie and Metallkunde der Technischen Universität München and the TEM-Laboratory of PD Dr. med. M. Theopold were used. This help is gratefully acknowledged

The different regions of the t.m., which can be readily identified by light microscopy (Held, 1926; Neubert, 1949), can also be distinguished by scanning electron microscopy (Lim and Lane, 1969; Lim, 1972; Ross, 1974; Hoshino, 1974, 1976, 1977; Tanaka et al., 1976). The correlation of light-microscopically identifiable structures in the scanning electron microscope is difficult, because of extensive shrinkage of the hydrated carbohydrates of the t.m. (Kronester-Frei, 1978a, b).

Lim (1972) has given a clear survey of current and old nomenclature and attempted to correlate observations made with light, electron and scanning electron microscopes. He proposed to distinguish in general limbal, middle and marginal zones.

Ultrastructural investigations of the t.m. have been carried out by Spoendlin (1957), 1966), Iurato (1960, 1962, 1967) and Kimura (1965). The limiting factor in these investigations was obviously the poor contrast of the protofibrils, an additional factor in the comparatively poor understanding of the t.m. This may also explain the lack until now of a systematic analysis of the ultrastructure and arrangement of individual zones. One such attempt to correlate zonation and ultrastructure is that of Lim (1972). In recent electron-microscopic investigations, the t.m. was found to consist of fine fibrils in an amorphous ground substance (Iurato, 1962, 1967; Engström und Wersäll, 1958; Wislocki and Ladman, 1954; Lim, 1972, 1977). Some investigators have shown that the diameters of the fine fibrils differ among species but are constant within species: guinea pig 40 Å (Wersäll and Engström, 1958), squirrel monkey 126 Å (Kimura, 1965) and rat 100 Å (Iurato, 1960). Other results indicate different diameters within the same species: rat: 40–50 Å, 160 Å; Ross (1974); cat: variable; Spoendlin (1957).

In this investigation the ultrastructure of carbohydrate protofibrils of the t.m. and their distribution in its different zones in the mouse was studied systematically by means of scanning and transmission electron microscopy. Using a staining technique specifically designed for the t.m., it was possible to obtain better contrast and discrimination of the protofibril structures. A newly developed technique (Kronester-Frei, 1976a, b) enables study of the ultrastructure with the transmission electron microscope after examination of the tissue in the scanning electron microscope, so that specific types of surface ultrastructure as seen in SEM could be identified with TEM.

Materials and Methods

The inner ears of 15 mice, 4 guinea pigs and 2 squirrel monkeys were used in this investigation.

Mice were anaesthetized with ether and rapidly decapitated. Then the bullae were opened, immersed in 6.25% glutaraldehyde in phosphate buffer (pH 7.4) and opened at the round and oval windows. After 2 h, the stabilizing solution was replaced by a solution of 2% glutaraldehyde in phosphate buffer which was also convenient for storage. Before the material was processed for transmission and scanning electron microscopes, according to current methods of Luft (1961) and Cohen (1968), the tissue was fixed in 1% osmium tetroxide in phosphate buffer for 2 h. For transmission electron microscopy, the epon-embedded cochleae were cut up with a saw according to the method of Spoendlin (1974), under an operating microscope while observing them with a light-fibre illumination technique (Kronester-Frei,

1976b) and then mounted for semithin and ultrathin sectioning. In this way, it is possible to avoid decalcification. The semithin sections were stained with toluidine blue (Fig. 2a). After staining with uranyl acetate and lead citrate for 12 h, the ultrathin sections were studied in a Siemens Elmiskop I.

In anaesthetised guinea pigs and squirrel monkeys (0.3 ml/kg Narcoren) the middle ear was opened and the fixative injected into scalae vestibuli and tympani, before the inner ears were removed. After stabilization with glutaraldehyde, the tissue was (in some cases) dissected in buffer, i.e. the scala media was opened to stain the t.m. with Janus green and then embedded in Epon. In the other cases, the tissue was prepared after fixation in a way comparable to that of the mouse cochleae.

For scanning electron microscopy, the tissue was critical-point dried, sputtered and examined in a Cambridge Stereoscan Mark 2A. Thanks to the light-fibre illumination technique it was possible to dissect these dried cochleae and to process them for transmission electron microscopy (Kronester-Frei, 1976b).

Results

1. Ultrastructure of Protofibrils

In the mouse, two clearly distinguishable types of protofibrils were revealed by TEM:

Type A: straight, unbranched protofibrils, 110 Å in diameter, with a periodic structure (periodicity = 70 Å, Fig. 3a).

Type B: coiled, branched protofibrils, 150–200 Å in diameter, occuring in two interchangeable states of hydration (Fig. 5a).

These protofibrils can be made clearly visible with a conventional staining procedure for proteins when the staining time is prolonged from 1 to 12 h. This staining method takes advantage of the binding properties of the carbohydrates, which, according to Gottschalk (1972), consist mainly of carbohydrate-protein complexes.

2. Zones of the Tectorial Membrane and Their Protofibrils

The zones of the t.m. (Lim, 1972) are described here according to their fibrillar structure.

Limbal, middle and marginal zones are easily recognizable in the scanning electron microscope. Fig. 1a shows the upper surface of the t.m. in mouse. Fibres on the limbus run toward the apex at an angle of 64°, and in the middle zone at an angle of 52°. The fibres of the cover net (marginal zone) run obliquely toward the base of the cochlea; near the border of the marginal zone they run parallel. In Fig. 1b the t.m. has been turned over and shows on the underside (left to right) marginal zone, impressions from the hairs of the outer hair cells, Hensen's stripe and the edge of the spiral limbus. The fibres of the middle zone run towards the apex with an angle of 29° between marginal zone and Hensen's stripe and of 16° between Hensen's stripe and the limbus. The schematic representation of a radial section (Fig. 1c) indicates the areas of the membrane that are illustrated in Figs. 2–5.

a) Limbal Zone. According to Neubert (1949), the t.m. is cemented to the spiral limbus by a sticky, jelly-like substance. Using TEM it becomes obvious that the

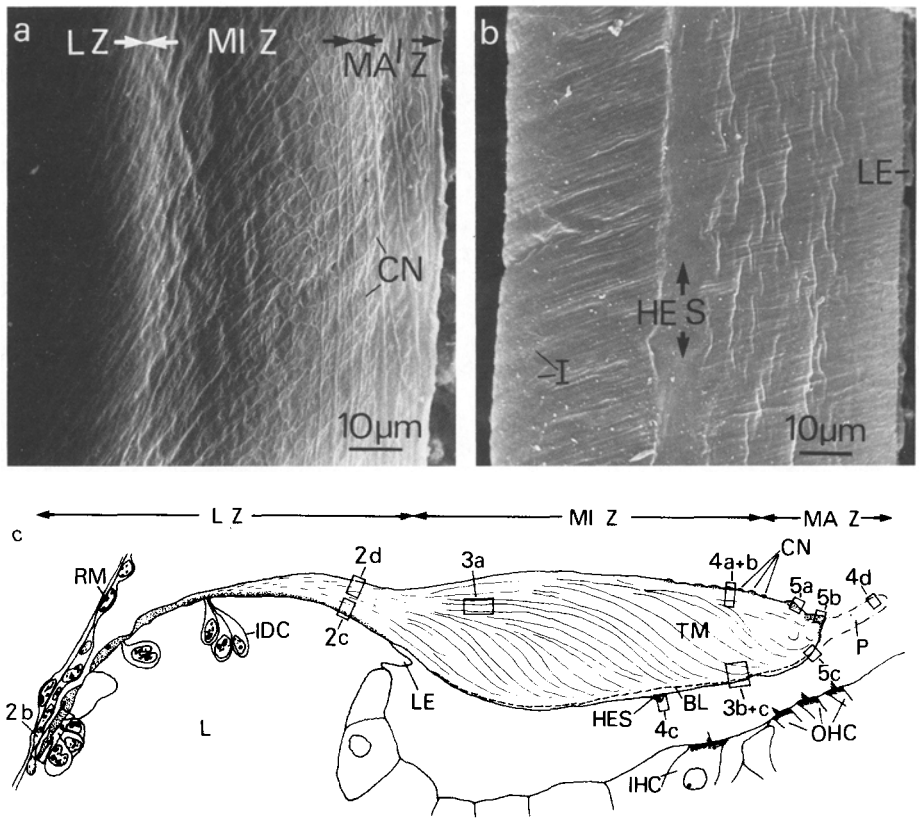

Fig. 1. a Upper surface of the tectorial membrane (TM), one turn from the oval window (mouse); **b** Underside of the TM, third turn (guinea pig); **c** Localization of Figs. 2–5 in schematic radial section of the TM. *BL* basal layer; *CN* cover net; *HES* Hensen's stripe; *IHC* inner hair cell; *IDC* interdental cells; *I* imprints; *L* spiral limbus; *LE* edge of the L; *L Z* limbal zone; *MA Z* marginal zone; *MI Z* middle zone; *OHC* outer hair cell; *P* processes of the MA Z; *RM* Reissner's membrane

contact surface of the t.m. with the interdental cells is made up of tightly packed type-B protofibrils (Fig. 2c). This layer is about 3 µm thick and covers the entire surface of the limbus. Axially, the t.m. consists of this layer only, but very close to Reissner's membrane it disappears (Fig. 2b). Axially, the t.m. contains more inclusions in fibrillar association, but these are lost laterally. These inclusions are apparently cell fragments, as pieces of cell membrane could be clearly identified (Fig. 2b). The significance of the invagination of the limbus epithelium in this region is not known (Fig. 2a). It is characteristic that the packed type-B protofibrils are frequently found here. In this region, microgranules (diameter 0.5–5 µm, Kronester-Frei, 1976a) are seen on the surface using SEM, as frequently observed in man by Lim (1972). Radially, the t.m. continuously increases in thickness. In the tympano-vestibular direction, the type-B protofibrils become more separated and are interlaced with radially—and obliquely—running (in the direction of the caecum cupulare) type-A protofibrils (Fig. 2c, d). The protofibril association is open to the endolymph and shows no clear edge at the t.m. surface (Fig. 2d).

b) Middle Zone. In this zone, the fibres which are obvious in the unfixed tissue (Lim, 1977; Kronester-Frei, 1978c) are clearly visible with light microscopy and are therefore not artifacts of fixation or preparation. Ultrastructurally, the surface that lies adjacent to the organ of Corti has a 1 μm thick layer of parallel, closely packed protofibrils of type A (Fig. 3b, c), which are not rigidly oriented radially, but run in the direction of the caecum cupulare (Fig. 1b). From this basal layer, the protofibril bundles (diameter of 0.3 μm) climb obliquely to the surface of the t.m., with an inter-bundle distance of 2.5 μm in the open protofibril association of type B (Fig. 3b). In the upper half of the membrane they run horizontally again (Fig. 3a), parallel to the basilar membrane, and end on the limbal slope in the limbal zone (see schematic drawing, Fig. 1c). These bundles of protofibrils appear as fibres in the light microscope.

The protofibril system in this zone is also open to the endolymph space. The "skin" on the t.m. surface described by Neubert (1949) was not observed.

The surface of the t.m. in the hair cell region has a cover net, which laterally merges into the marginal zone.

Hensen's stripe, which lies on the tympanal side of the basal layer, has the ultrastructural appearance of dense protofibrils of type B (Fig. 4c).

c) Marginal Zone. The cover net of the marginal zone consists of coiled, irregular knots of type-B protofibrils. Fig. 4a shows a cross-sectioned fibre of the cover net with densely packed type-B protofibrils which are more strongly hydrated toward the center of the t.m. After staining with Janus green, the cover net shrinks considerably and the ultrastructure is drastically altered (Fig. 4b). Under these conditions, regions of electron-dense agglutinated bundles of protofibrils alternate with regions free of protofibrils.

The transition from the fibrillar basal layer (Fig. 5c) to the marginal border zone is clearly visible. The strong parallel orientation of the type-A protofibrils loosens (Fig. 3b) and the type-B protofibril portion lying between them becomes larger. In the marginal border zone the knot-like, densely packed protofibrils of type B predominate (Fig. 5b). The same situation can be found in the processes of the marginal net (Fig. 4d), as it can be seen in the apical coil of the squirrel monkey (Lim, 1977; Kronester-Frei, 1976a).

The ultrastructural similarity of the marginal border zone (Fig. 5b), the marginal net with processes (squirrel monkey, Fig. 5d) and Hensen's stripe (Fig. 5c) is obvious.

The ultrastructure of the most lateral extension of the cover net shows clearly (Fig. 5a) that the type B protofibrils, which are relatively widely separated in the interior of the membrane, are identical with the protofibril type of the knot-like structures of the marginal border zone.

3. Distribution of the Protofibrils in the Different Membrane Zones

As Table 1 shows, the type-A protofibrils are spread over the entire middle zone and partly over the limbal zone. Especially marked is the bundle pattern in the middle zone, and their tight packing in the basal layer over the hair cells.

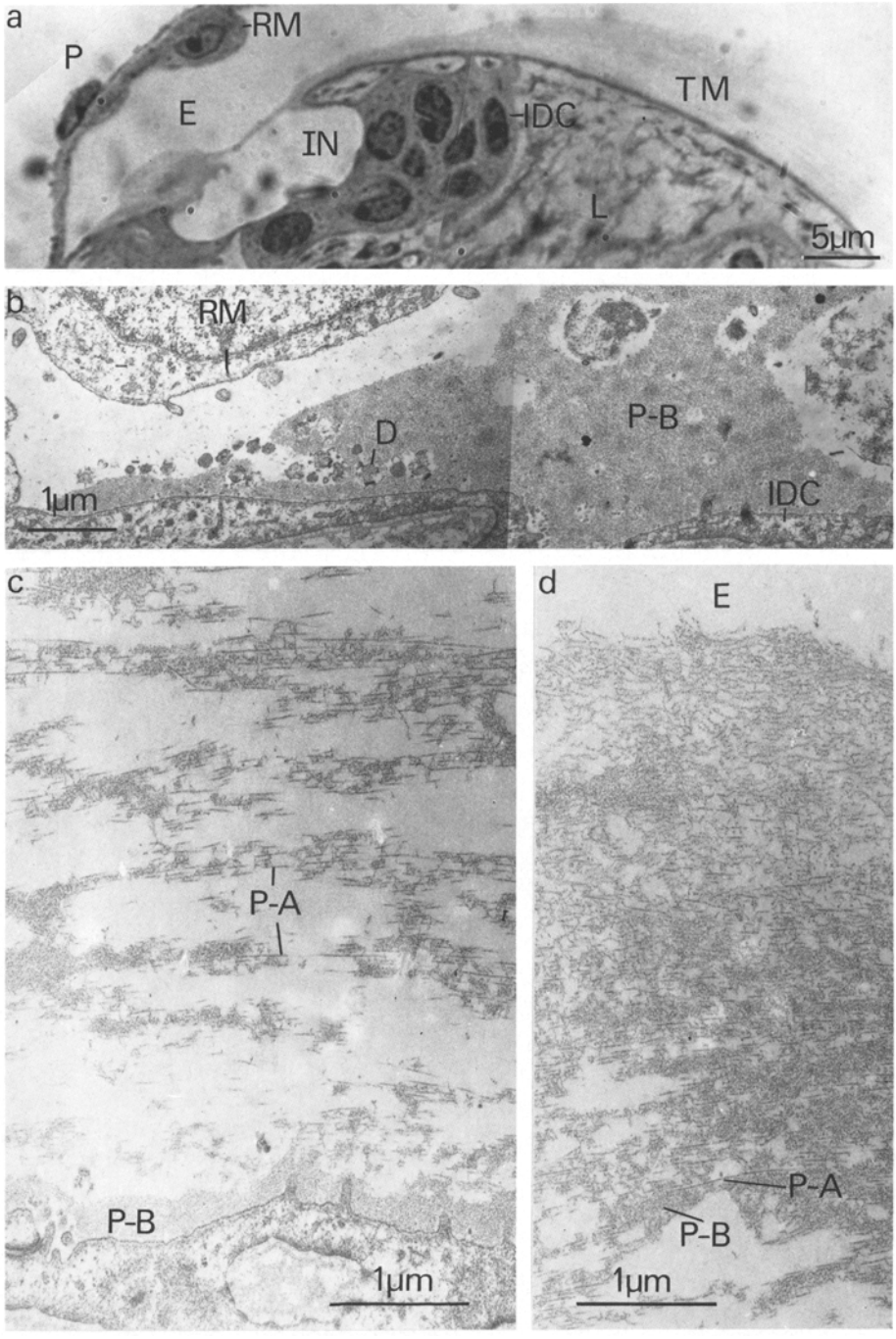

Fig. 2. a Radial section through spiral limbus (*L*), basal turn (guinea pig). Interdental cells (*IDC*) are more frequently found near Reissner's membrane (*RM*). **b** IDC and protofibrils type B (*P-B*) close to RM; **c** Lateral part of L; **d** vestibular surface of TM. *D* cell debris; *E* endolymph in scala media; *IN* invagination of limbus epithelium, *P* perilymph in scala vestibuli; *P-A* = protofibrils type A

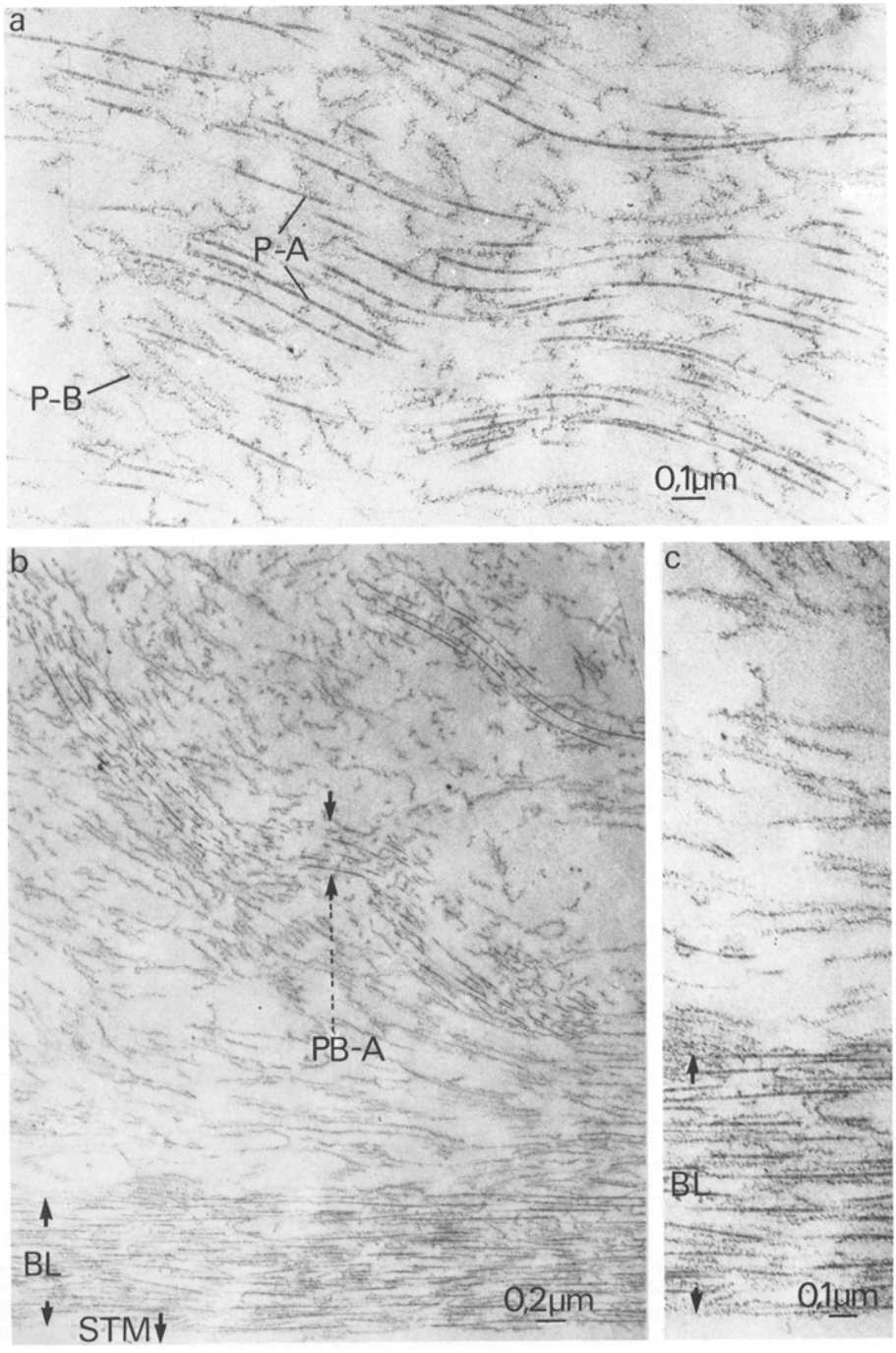

Fig. 3. a Protofibrils type A (*P-A*: $\varnothing = 110$ Å, periodic structuring) running parallel to the basilar membrane in loose association with the protofibrils of type B (*P-B*; $\varnothing = 150$–200 Å). **b** P-A in bundles (*PB-A*) and in the basal layer (*BL*), which limits the subtectorial space (*STM*) in vestibular direction; **c** magnified detail of b

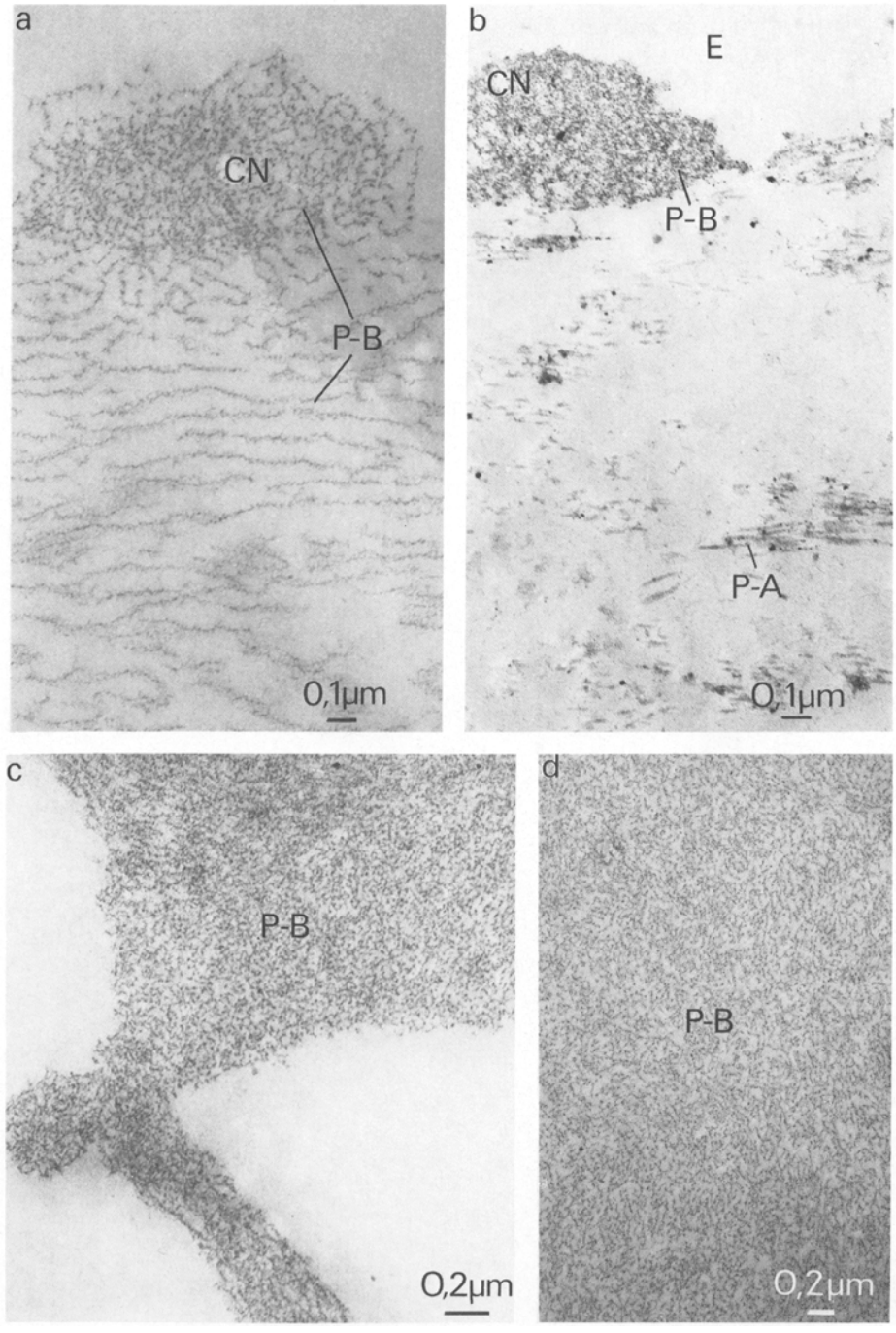

Fig. 4. a Cross section through a fibre of the cover net (*CN*) with protofibrils of type B (*P-B*; mouse), see Fig. 1 a; **b** CN after staining with Janus green (b-c = squirrel monkey); **c** Hensen's stripe; **d** processes of marginal net

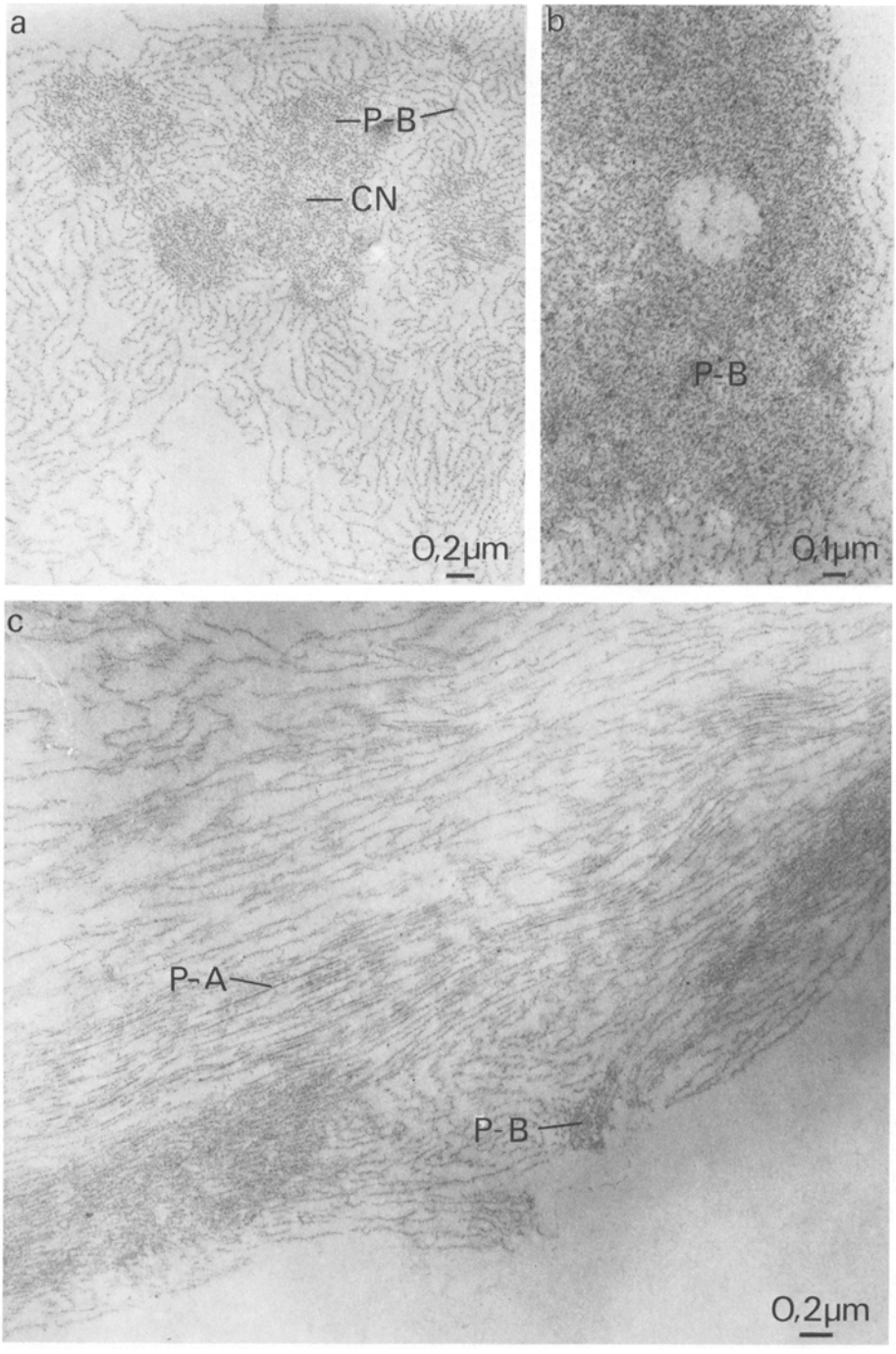

Fig. 5. a Lateral-most portion of the cover net (*CN*). The two different states of the type-B protofibrils are clearly visible (a-c = mouse); **b** lateral border zone of the tectorial membrane (*TM*); **c** basal layer (*BL*) of the underside of the TM near the transition to the marginal border zone

Table 1

protofibrils	type A	type B	
TEM-structure	straight, unbranched $\varnothing \approx 110$ Å, period = 70 Å	coiled, branched $\varnothing = 150$–200 Å	
classification of carbohydrates (see discussion)	acid glycosaminoglycans	glycoprotein	
hydration		strong	weak
limbal zone	+	++	near Reissner's membrane, contact surface with the limbus
middle zone		+	
– run in bundles	++		
– basal layer	++		
– Hensen's stripe			++
– cover net			++
marginal zone		+	++
– processes			++

Type-B protofibrils occur throughout the entire t.m. Their strong hydration in the middle and limbal zones is striking. At the points of contact with the limbus, Hensen's stripe, in the marginal zone and its processes (marginal net) and in the cover net, these fibrils are poorly hydrated, i.e., they are closely packed.

Discussion

The concept that the tectorial membrane consists of fibrils in an amorphous matrix is incorrect and has been proposed without clear definition at both the light- and electron-microscopical levels. This investigation shows that the t.m. of the mouse consists only of protofibrils bound together in a complex network. Two protofibril types are clearly distinguishable, and for the first time could be assigned systematically to the various zones of the t.m. The protofibrils exhibit different degrees of hydration, which could be easily altered by changes in the ionic milieu (Kronester-Frei, 1978a, b). This explains the apparent structural variability of protofibrils as documented in the literature. The observations described herein make possible a simple summarization and bring some order to those reported in the literature: the protofibrils of type-B are identical with the amorphous matrix and bundles of type-A protofibrils are the fibres seen in light microscopy. The size and distribution of the protofibrils is very constant in both the mouse and the squirrel monkey (Fig. 4b–d). Observations on other species are not yet available. Evidence has been given that the t.m. consists of biochemically different components. While

Arnold and Vosteen (1973) and Ross (1974) mention the presence of neutral glycoproteins and Tachibana et al. (1973) of polarizable acid glycosaminoglycane (e.g. chondroitin sulphate), Schätzle (1971) found, histochemically, both components. The work of Kleine (1972) supports the idea that the type-A protofibrils, which are unbranched, straight and with a periodic structure, may consist of chondroitin-sulphate, for he found a periodic structure typical thereof. The branched, coiled structure of the type-B protofibrils is characteristic of glycoproteins (Gottschalk, 1972).

The evidence from TEM for the presence of two structural types of protofibrils is in agreement with biochemical and histochemical evidence for two carbohydrates. An unambiguous biochemical and morphological identification, as, e.g. for collagen, will only be possible when both types are isolated and the fractions are studied separately with TEM. It can be shown that the basal layer consists of densely packed, polarisable type-A protofibrils (acid glycosaminoglycanes) and that the subtectorial hair-cell space over the outer hair cells and pillar cells is closed off in the vestibular direction.

Hensen's stripe, which is located above the inner hair cells, consists of type-B protofibrils. The basal layer is identical with the homogeneous ground substance (Held, 1926), the homogeneous layer (Kimura, 1965), the basal homogeneous layer (Lim, 1972), the accessory membrane (Hardesty, 1908) and the granular layer (Tanaka et al., 1976). In the characterisations of this basal layer, the variability in the descriptions of the protofibrils is apparent. The problem lies probably in the planes of ultrathin sections; in a purely radial section the oblique protofibrils are cut obliquely or in cross section. Under poor conditions of discriminability, insufficient contrast gives the impression of a granular basal layer over the organ of Corti so that the structures described in TEM as granular or homogeneous depend on magnification and resolution.

The type-B protofibrils spread through the entire t.m. They have a low state of hydration wherever they come into contact with the organ of Corti, as in the limbal zone and in the marginal zone and, possibly, in the region of Hensen's stripe. These impressions are supported by investigations on unfixed, in-situ preparations having in-vivo-like ionic conditions (Kronester-Frei, 1978a–c), which indicate that in the future, dependence of the structure of the t.m. on ionic conditions must be taken into account.

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Accepted March 23, 1978