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ULTRASTRUCTURAL FEATURES OF HUMAN REISSNER'S MEMBRANE

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Abstract

Ultrastructural features of human Reissner's membrane were investigated in two groups of similarly aged patients. Five patients had age-related normal hearing (ARNH) and four patients had acquired sensorineural hearing loss (SNHL) from causes other than age. The membrane consisted of a mesothelium facing the perilymph and an epithelium facing the endolymph. The two cell layers were separated by a basement membrane. The mesothelium was formed by wide spread thin cells with a smooth surface. The epithelial cells assumed two different shapes, flat and rounded. Both epithelial cell types were covered with many short microvilli. In all specimens, the rounded cells were arranged in bands, strands, whorls and clusters. The size of bands and whorls was larger in the lower half of the basal turn and decreased gradually towards the apex. Bands and whorls were both larger in specimens from patients with SNHL than in those with ARNH and expanded up to the middle turn. In patients with SNHL, some flat cells had relatively few long microvilli. The epithelium showed more pronounced cellular changes in patients with SNHL than in those with ARNH and these alterations are discussed in relation to sensorineural degeneration.

Key Words: Human, Reissner's membrane, inner ear, hearing loss, ultrastructure.

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Introduction

Reissner's membrane consists of two cell layers, an epithelium facing the endolymph of the scala media and a mesothelium facing the perilymph of the scala vestibuli. This membrane not only forms a barrier between the two fluid compartments but is also involved in maintaining ion and fluid transport (4, 5, 8, 11). An intact membrane is necessary for hearing.

Light microscopic studies have shown that in adults, the epithelial layer of the membrane differs markedly from that of infants and children. In adults, the epithelial cells have an irregular shape and form densely arranged bands and whorls, the latter with clusters of protruding cells. In infants and children, there is a regular pattern of polygonal cells (2). Only a few studies have pointed out morphological changes in the membrane associated with presbyacusis and other forms of sensorineural hearing loss, which includes Menière's disease (2, 10, 11). In guinea pigs and other laboratory animals, no differences in structural composition have been reported between young and adult animals Reissner's membrane (1, 6, 9). Their arrangement resembles that of human infants (2, 10).

The aim of this study was to investigate the ultrastructure of Reissner's membrane in temporal bones from patients both with age-related normal hearing (ARNH) and sensorineural hearing loss (SNHL) and to compare the findings between the two groups.

Material and Methods

Reissner's membrane from 16 temporal bones of 9 Caucasian patients were examined. The hearing of all patients was documented by pure tone audiometry within the six months which preceded death. Five patients had ARNH (Table 1), i.e., their pure-tone audiogram was better than the 75th percentile of the age and sex-related normal audiograms as defined by Robinson and Sutton (7). Four had SNHL (Table 2), that is they had pure tone thresholds worse than the 75th centile for their age and this additional hearing loss had been caused by factors such as noise. The precise degeneration patterns of some of these patients cochleas have been described

earlier (3). The inner ears were fixed within 2 hours of death by perilymphatic perfusion through the round and oval windows with 2.5% glutaraldehyde and 3% paraformaldehyde in 0.2 M phosphate buffer at pH 7.3. After removal at autopsy 1-2 days later, the temporal bones were kept in fixative until a complete microdissection was carried out after post-fixation with 1% phosphate-buffered osmium tetroxide. The specimens of Reissner's membrane were dehydrated in a graded series of ethanol, critical point dried in CO_2, mounted on stubs with colloidal silver, and sputter-coated with gold-palladium. The preparations were examined in a JEOL-JSM 25S scanning electron microscope. The number of rounded cells was estimated over the total width of the membrane. The percentage of rounded cells in relation to the total number of epithelial cells was determined for each cochlear turn.

Results

Patients with age-related normal hearing (ARNH)

The perilymphatic side of Reissner's membrane was lined by a thin layer of **mesothelium**. The adjacent cells were mostly lying side by side and in some instances overlapping their neighbours. The cells presented a smooth cell surface. In some cells, a single kinocilium was often found close to a protruding nucleus (Fig. 1). Some discontinuities in the layer were observed.

The endolymphatic side of the membrane consisted of an **epithelium** which had more irregular features than the mesothelium (Fig. 2). The epithelium had two types of cells, **flat** and **rounded**. The latter assumed spindle and spherical shapes. The cells were slightly elongated in a radial direction and two or three rows of flat cells bordered Reissner's membrane on the side of the limbus. Each flat cell covered a larger area than the rounded cell and was more regular in shape than its rounded counterpart. The flat cells were polygonal with numerous short microvilli at the surface and margin (Fig. 2a). The rounded cells were arranged in four different patterns namely: whorls, clusters, bands, and strands. The surface of these cells was covered with numerous and short microvilli. Whorls were formed by spindle-shaped cells arranged circularly around an axis and were found in all cochlear turns (Fig. 2b). Clusters consisted of varying numbers of spherical cells protruding into the endolymphatic space and were often located in the center of the top of the whorls. Whorls and clusters covered a large area of Reissner's membrane in the lower half of the basal turn. Bands were formed by densely arranged spindle-shaped cells (Fig. 2c). In the upper half of the basal turn, and in the middle and apical turns the bands were broken up into strands alternating with flat cells (Fig. 2d). A schematic representation of the distribution of rounded epithelial cells throughout the cochlear turns is illustrated in Fig. 3. Rounded cells and their formations covered almost two third of the whole width of the membrane at the hook and lower basal turn and decreased gradually towards the apex.

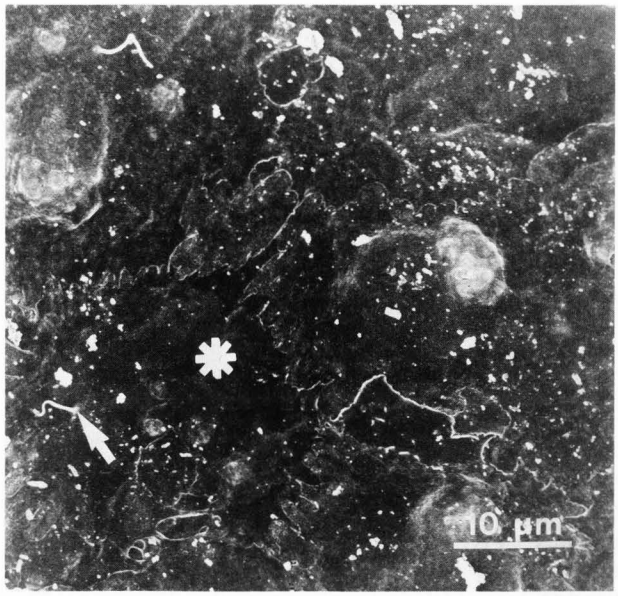

Figure 1. The smooth mesothelial surface from patients with ARNH. A mesothelial cell (asterisk) is seen overlapping the adjacent cells and a single kinocilium is present (arrow).

Table 1. Patients with age-related normal hearing (ARNH).

Case	Age (years)	Side	Audiogram
2	63	left & right	flat, 10-20 dB
4	72	left & right	sloping from 20-70 dB
5	61	left & right	flat, 10-20 dB
14	82	right	slightly sloping from 20-50 dB
16	90	right	sloping from 20-60 dB

Patients with sensorineural hearing loss (SNHL)

In comparison to patients with ARNH, the number of rounded cells was increased and covered a large area of the width up to the middle turn of the membrane. Similarly a gradual decrease towards the apex was observed (Fig. 3). Bands, whorls and clusters were larger in size with many cells protruding into the endolymphatic space. Spherical cells in clusters showed less microvilli and comparatively more degeneration than those in patients with ARNH (Fig.4).

The cell surface of some flat cells was covered by relatively few long microvilli. In the case of predominantly neural degeneration, flat cells with fewer microvilli or without any surface structures at all were more numerous and prominent than in other cases of SNHL. They were randomly distributed among the other cells (Fig. 4).

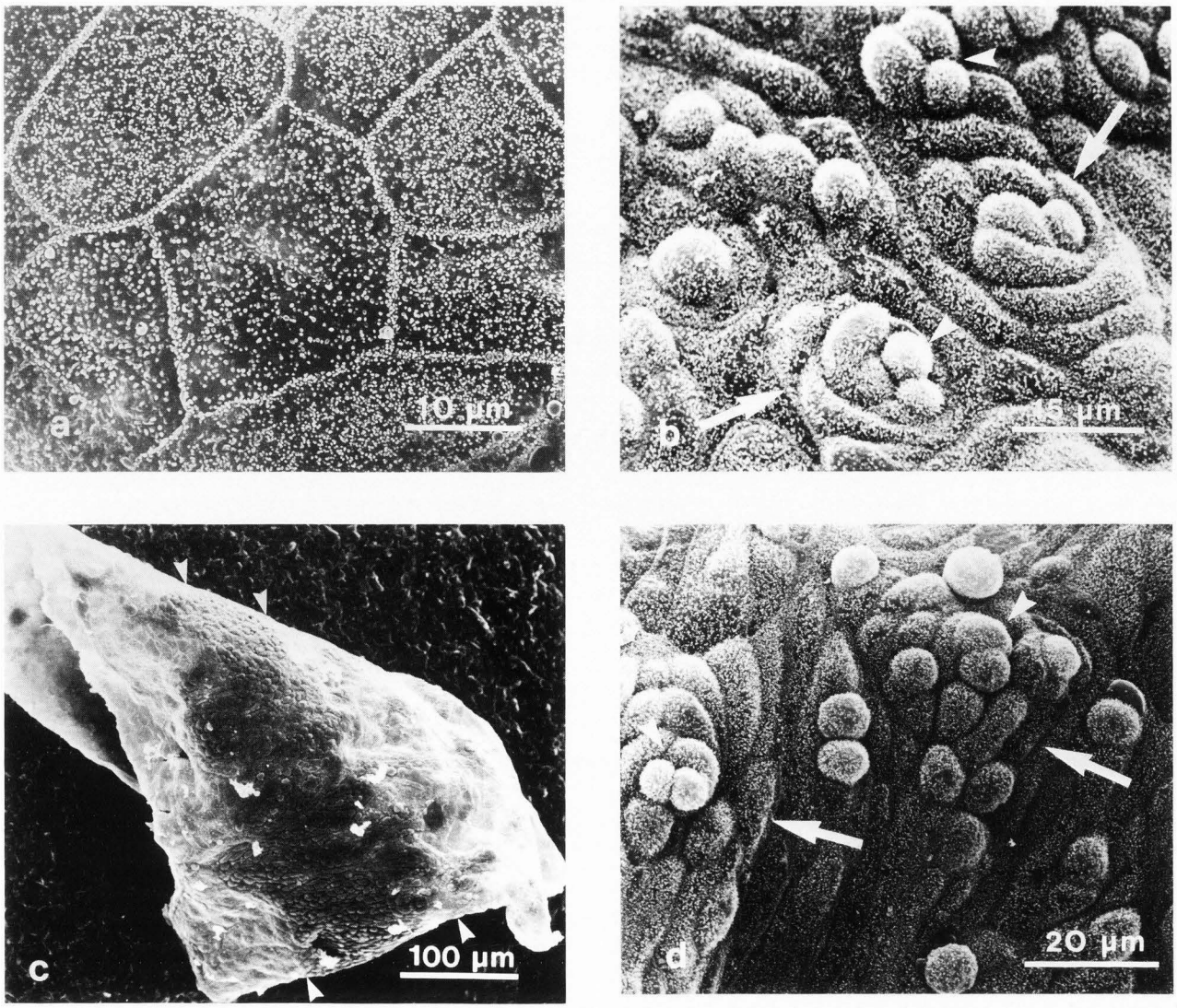

Figure 2. Epithelium from patients with ARNH. **a**. Flat cells with numerous and short microvilli. Accumulation of microvilli at the cell margin. **b**. Whorls (arrows) of spindle-shaped cells arranged around an axis with clusters (arrow-heads). **c**. A band (between arrow-heads) of densely arranged spindle-shaped cells. **d**. Strands (arrows) with clusters (arrow-heads) alternating with flat cells.

Table 2. Patients with sensorineural hearing loss (SNHL).

Case	Age (years)	Side	Type of hearing loss	Audiogram
3	73	left & right	unspecified	sloping, 30-70 dB
6	54	left & right	neural presbyacusis	flat, 30-50 dB, dip at 4 kHz
7	71	left & right	noise induced	semiabrupt at 1 kHz from 25-30 dB
8	72	left & right	noise induced	abrupt at 1-2 kHz from 30 dB

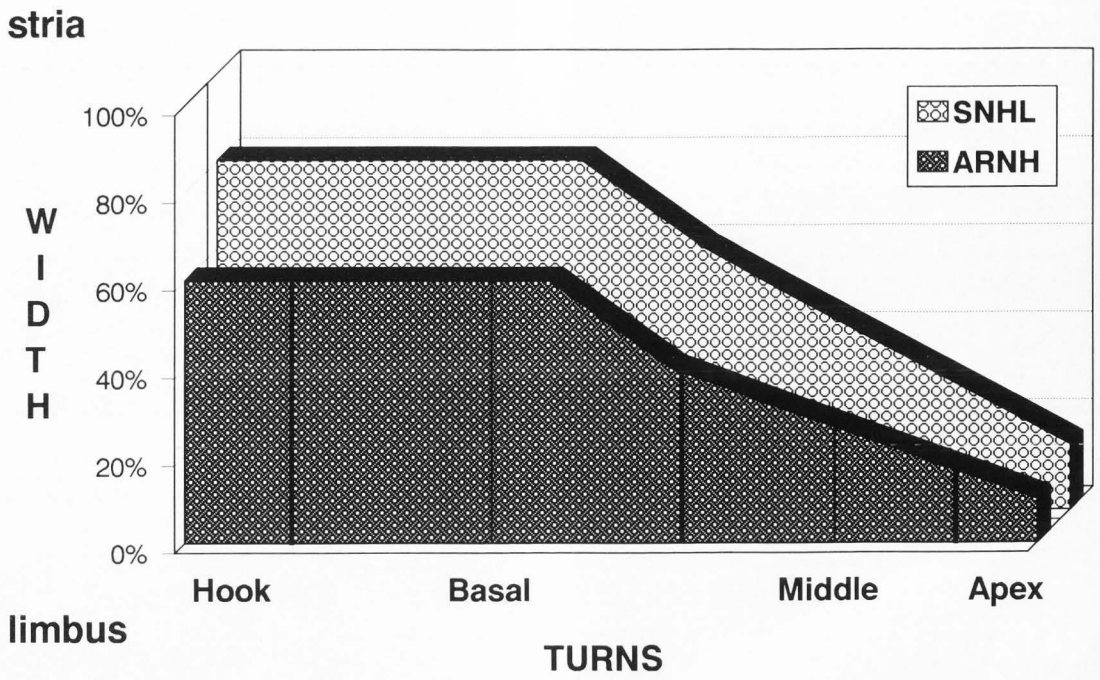

Figure 3. Analysis of the distribution pattern of rounded epithelial cells along the entire cochlea in patients with ARNH and SNHL.

Discussion

In this investigation, the surface features of Reissner's membrane were studied by scanning electron microscopy from patients with ARNH and compared with those from patients with SNHL. In patients with ARNH, some sensorineural degeneration and aging processes had also developed. Our two groups, however, were well matched with regard to age and were therefore directly comparable.

In patients with ARNH, more epithelial surface changes were observed than on the mesothelium. The mesothelial cells had some discontinuities which have also been observed in guinea pigs by some (1, 9) but not by other (6). The rounded cells of the epithelium dominated the hook and lower half part of the basal turn in the form of whorls, clusters and bands. These findings are in agreement with those reported in other human series (2, 10). The rounded cells might represent the "dense band of smaller cells" reported by Johnsson (2) as also the "dark area with a large number of small epithelial cells" observed by Watanuki *et al.* (10). These rounded cells are thought to represent the most active epithelial cell population for the purpose of remodeling the membrane throughout its length. Some spherical cells arranged in clusters seemed to be in the process of degeneration (as confirmed by transmission electron microscopy) and being shed into the endolymph. These epithelial cell arrangements may reflect the need for increased ion and fluid transport, possibly working against a greater hydrostatic gradient. If so, it corresponds to areas of sensorineural degeneration present in the hook and basal turn of patients with ARNH.

In patients with SNHL, the density of microvilli was decreased on some flat epithelial cells. The whorls and clusters were increased in number and size and expanded up to the middle turn of the membrane. The increase in size and number of the epithelial clusters was found more often near the basal end than in the other parts of the cochlea and correlated with advancing age (2, 10). In patients with SNHL, the increase of these epithelial changes from the hook to the apex might be related to sensorineural hearing loss; whether as a cause or consequence is a matter of speculation.

The present study is a part of an on-going long term research program which aims to elucidate the role of Reissner's membrane in the aging process and its alterations in acquired hearing loss.

Acknowledgements

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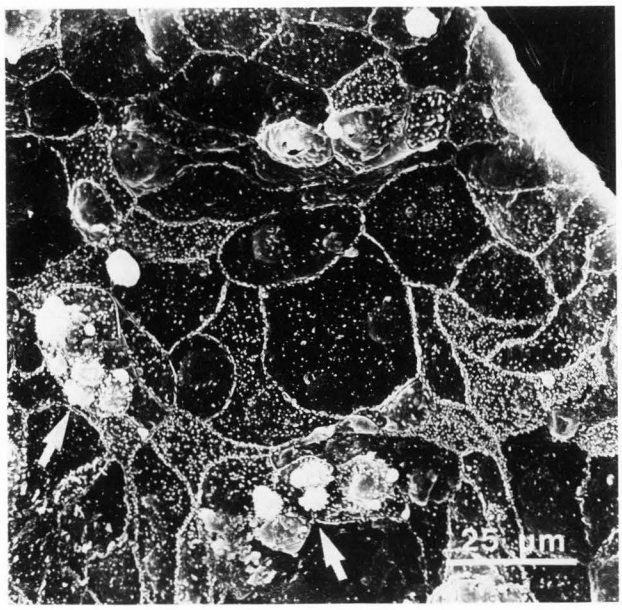

Figure 4. Case 6 with neural presbyacusis. Flat epithelial cells with absent or very few microvilli distributed randomly among the other cells. Clusters in process of degeneration (arrows).

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Discussion with Reviewers

R.S. Kimura: The description of the spherical cell does not provide any indication of the degenerative process. The electron micrographs show a distribution of microvilli similar to that of adjacent round cells. Why do you think that the spherical cells are in the process of degenerating?

Authors: The spherical cells are either single or arranged in clusters. In patients with ARNH, the great majority of spherical cells have a similar distribution of microvilli as the rounded cells. Only very few spherical cells have less microvilli than their neighbour cells. In patients with SNHL, the number of spherical cells showing fewer microvilli is increased. These cells seemed to be in the process of degeneration and being shed into the endolymph. Further studies by transmission electron microscopy have confirmed this hypothesis (Felix *et al.*, Acta Otolaryngol (Stockh) 1993; **113**; in press).

D.J. Lim: What is "age-related normal hearing"? Is it the same as presbycusis? If so, please state; if not, do these subjects have normal hearing (age adjusted)? Please clarify.

Authors: The audiograms of these patients with age-related normal hearing (ARNH) had to be better than the 75th percentile of the age- and sex-related normal audiometric curves as defined by Robinson and Sutton (text ref. 7). In addition, these patients had experienced only usual domestic, occupational and urban noise. None had been exposed to heavy industrial noise. They all had clinically normal hearing and had normal tympanic membranes and middle ears. To the best of the investigators' knowledge, none had received any ototoxic drugs. According to the above criteria, we consider that ARNH is not the same as presbycusis. These patients had "age adjusted" normal hearing.