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Histological Study of the Thin Replacement Membrane of Human Tympanic Membrane Perforations

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A histological study was done on the thin, nearly transparent replacement membrane of tympanic membrane perforations. Human tympanic membranes that were rejected for transplantation, were studied by light and electron microscopy. The abrupt reduction in thickness at the margin of the covered perforation, is entirely due to the reduction of the lamina propria. Even in the thinnest parts of the replacement membrane, a lamina propria is present, separated by continuous basement membranes from the epithelium and mucosa, and measuring no more than some 2–3 μm in thickness. This lamina propria consists of fibrils and interfibrillar matrix, but fibroblasts appear to be lacking. The epithelial layer does not contain basal cells, confirming the thesis that the upper layers are not generated by in situ proliferation, but that they have migrated from the periphery. *Key words: middle ear, tympanic membrane, injury, pathology, ultrastructure.*

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When tympanic membranes are traumatically perforated, spontaneous healing almost always occurs. The replacement membrane is often a membrane of normal thickness or it can be even thicker than the original one. The healing mechanism and the structure of the replacement membrane were described by Reijnen (1), Clawson (2), Reeve (3), Stenfors (4, 5) and Yamashita (6). Yet, sometimes this replacement membrane has a very thin, nearly transparent aspect. In this study we describe the structure of this thin covering sheet by using light and electron microscopical techniques.

MATERIAL AND METHODS

Prelevation

In the context of transplantation projects, cone-shaped os petrosum specimens were prelevated from autopsies and fixed in a 4% formaldehyde solution, buffered with phosphate to pH 5.5. Many of these os petrosum specimens were rejected for transplantation because of all kinds of defects or pathologies. Some of them showed atrophic areas, resembling those that can sometimes be seen in clinical and experimental circumstances in the healing process of ear drum perforation, i.e. well defined, distinct areas with abrupt transition between normal and atrophic tissue. Therefore they were interpreted as covered tympanic membrane perforations and they were kept for this study. From these selected cones, os petrosum was maximally removed under an operation microscope (Zeiss OpMi-1) and the cavum tympani was opened to allow an approach to the tympanic membrane from the medial side. After careful removal of the tympanic membrane, it was cut into two pieces, dividing the atrophic area into a segment for light microscopical and another one for electron microscopical observation.

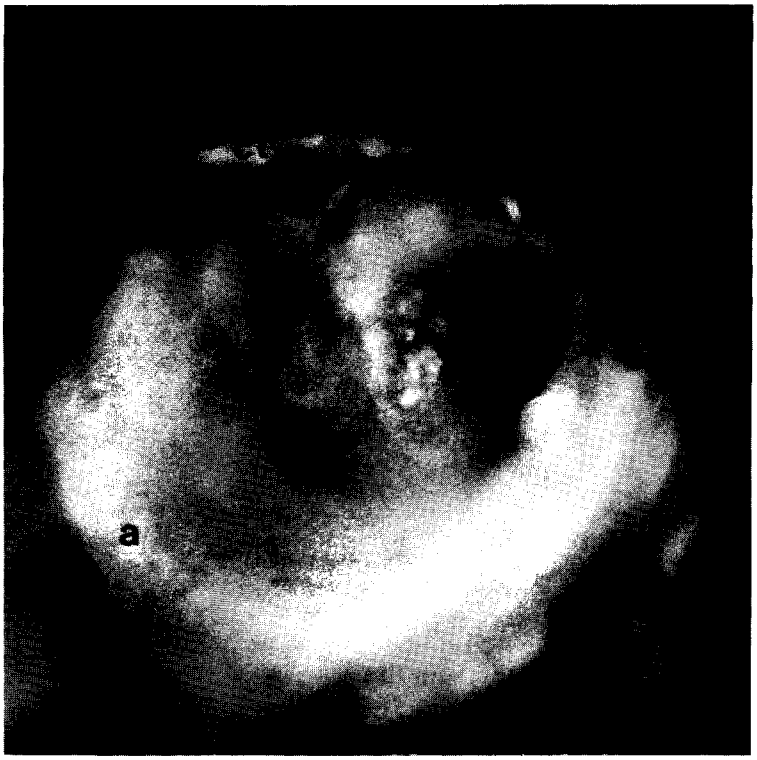

Fig. 1. Tympanic membrane, seen from the cavum tympani. (*m*) malleus, (*ct*) chorda tympani, (*a*) annulus, (*cp*) covered tympanic membrane perforation.

Light microscopical preparation

The removed tympanic membranes were fixed in a 4% formaldehyde solution. Dehydration was carried out in 95% isopropylalcohol (3×1 h) followed by 100% ethylalcohol (2×1 h). After clearing with toluene (2×1 h), the specimens were embedded in paraffin wax. Sections of 6 μm were made and hematoxylin/phloxine/saffron stain was applied.

Electron microscopical preparation

The removed tympanic membranes were fixed in a 4% formaldehyde solution. After fixation, the specimens were rinsed with sodium-cacodylate buffered saline (CBS) + 7.5% saccharose, pH 7.4 for 30 min. Post-fixation was carried out with osmium tetroxide (1%) in phosphate-buffer (pH 7.4) for 2 h. After another wash with CBS, the specimen was dehydrated in increasing concentrations (70–90–100–100%) of ethanol. Propylene oxide was used as transitional solvent before embedding the specimens in epoxy resin (LX-112, Cat. 21210; Ladd Research Industries, Burlington, Vermont). Thin sections were stained with 5% aqueous uranyl-acetate and 0.3% aqeous lead-citrate.

RESULTS

Operation microscope

Views on the tympanic membranes, seen from the cavum tympani by means of an operation microscope, show malleus, chorda tympani and annulus (Fig. 1). The atrophic area is a very thin, nearly transparent membrane that is very well defined, with an abrupt transition between normal and atrophic tissue. This kind of lesion corresponds to the thin replacement

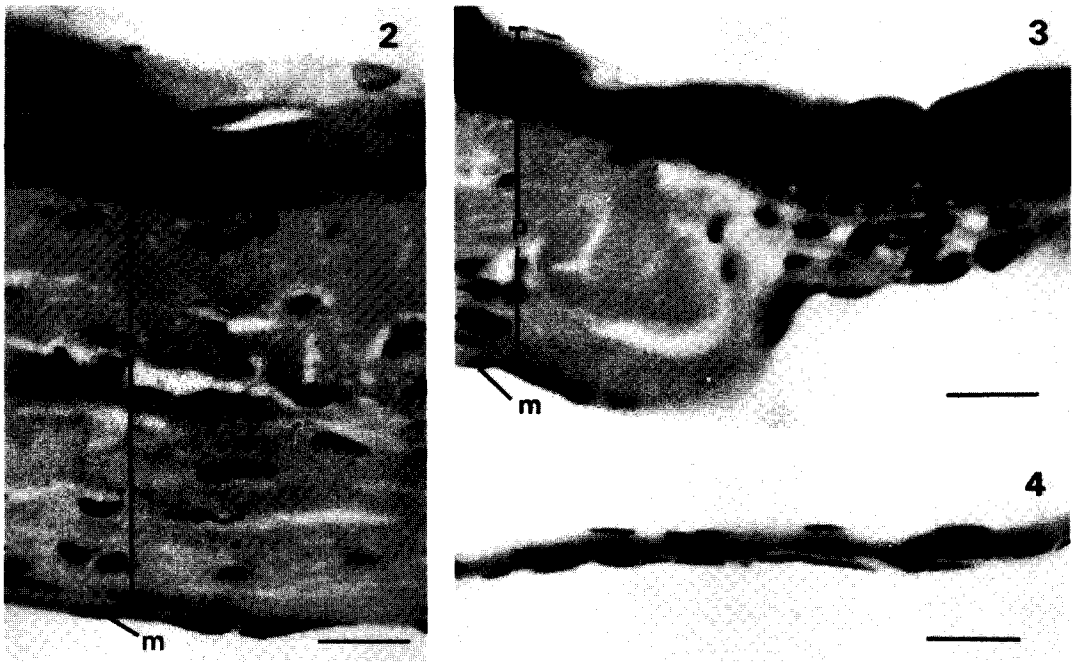

Fig. 2. Light micrograph of the original 'thick' part of the tympanic membrane. No abnormal morphology is seen. (*e*) epithelial layer, (*p*) lamina propria, (*m*) mucous layer. Bar = 20 μm.

Fig. 3. Light micrograph of the transitional zone between normal 'thick' and covering 'thin' membrane. (*e*) epithelial layer, (*p*) lamina properia, (*m*) mucous layer. Bar = 20 μm.

Fig. 4. Light micrograph of the thin replacement membrane, showing that a lamina propria appears to be lacking between the epithelial and mucous layer. Bar = 20 μm.

membrane that can sometimes be seen as the result of the healing process of ear drum perforations, and therefore it is interpreted as being a covered perforation.

Light microscopy

Light microscopical sections of the membranes demonstrate that the thickness abruptly diminishes at the edge of the covered perforation. In the 'thick' part of the tympanic membrane, the structure appears quite normal, showing an outer keratinizing stratified squamous epithelium, a lamina propria with outer radiate and inner circular fibre arrangement, and a mucosal monolayer (Fig. 2). The total thickness of the membrane is about 130 μm, with an outer epithelial layer of 30 μm, a lamina propria of 100 μm and a very thin mucous layer. Details of these structures do not reveal any abnormal morphological structure.

At the transitional zone between normal and thin membrane, the outer epithelium keeps its normal structure (Fig. 3). There are a basal cell-layer and some 4–5 superficial layers. The mucosal cells are predominantly of the flattened type. Almost no cuboidal cells can be recognized. The lamina propria abruptly becomes much thinner. The outer epithelial layer is 20 μm thick, the propria some 15 μm!

When proceeding to the centre of the replacement membrane, the membrane becomes still thinner, until it measures no more than 10 μm (Fig. 4). The outer epithelium consists of about three layers of flattened epithelial cells, resembling the very superficial cells of normal epithelium. Cornification is a constant finding. Basal cells are apparently lacking!

It is very difficult to discern a lamina propria. Yet, some flattened, fusiform nuclei are vis-

Fig. 5. Electron micrograph of the thin replacement membrane. The epithelial layer does not contain basal cells. A lamina propria is present, but fibroblasts cannot be visualized. Mucosal cells project microvilli into the cavum tympani. (*e*) cornified epithelium, (*p*) lamina propria, (*M*) mucosa. Arrow-heads: continuous basement membranes between propria and epithelium or mucosa. Bar = 2 μm.

ible just beneath the mucosa. The mucosal cells become very flattened and very long. At the periphery of the replacement membrane, some cuboidal mucosal cells can still be seen, but they are totally absent in the centre of the scar.

Electron microscopy

Electron microscopical sections of the membranes do not reveal any abnormal structure in the 'thick' part of the membrane.

The outer epithelium is of the stratified squamous type. About seven layers of cells can be distinguished. A basement membrane separates the outer epithelium from the lamina propria, in which fibroblasts and fibres are seen, embedded in a collagenous matrix. The fibres show the 64 nm banding, typical of collagen fibres. Small blood vessels can be recognized. A mast cell, filled with excretory granules, is a rare finding. The lamina propria is separated from the mucosa by a basement membrane. The mucosal monolayer consists of cuboidal cells, forming a tight sheet by junctions and complex interdigitations. Most of the cells project microvilli into the cavum tympani. Some cells have multiple cilia, whose structure shows the typical axonemal configuration of two central microtubuli and nine peripheral pairs of microtubuli.

The thin sheet covering the perforation consists equally of three layers (Fig. 5).

The outer epithelial layer is stratified and squamous. Proceeding to the centre of the replacement membrane, the number of cell-layers remains constantly about six. At the periphery of the replacement membrane, the epithelial cells are large and they are organized in the usual way, i.e. stratum basale, spinosum, granulosum and corneum. The epithelium is

some 20–30 μm thick. More to the centre, the epithelial cells become more flattened and much thinner. Some basal cell-layers are present, but they do not have the typical appearance of small roundish basal cells. They are flattened and very elongated, suggesting that they are merely compressed stratum spinosum cells. Multiple desmosomes confirm this finding. The very typical proliferative basal cells are lacking. This means that no proliferative capacity is present, so that the epithelial cells must have migrated from other sites on the membrane, confirming the findings of other authors (1). A basement membrane, separating the outer epithelium from the lamina propria is a constant finding, as well as the lamina propria itself, even in the thinnest part of the membrane. This is in contrast to the findings of Schuknecht (7) and Yamashita (6), who stated that a propria is lacking in these thin membranes. The propria is very thin in all parts of the covering membrane, merely 2–4 μm. This enormous reduction in thickness is the cause of the abrupt transition between normal 'thick' and covering 'thin' membrane. Fibroblasts could not be visualized! The propria appears to consist only of fibres and collagenous matrix. The fibres are banded and seem to be normal collagen fibres. No organized orientation of the fibres could be seen.

A continuous basement membrane between propria and mucosa is again a constant finding.

Mucosal cells are not as cuboidal as in the normal drum-membrane. Proceeding to the centre of the replacement membrane, they become flattened. Complex interdigitations and tight junctions are a rare finding in this part of the membrane. Microvilli are still present, while cilia have not been seen.

DISCUSSION

Small tympanic membrane perforations tend to heal spontaneously. About one day after traumatic membrane perforation, extensive oedema of the lamina propria occurs, and outer epithelial and inner mucosal layers are retracted. After 2 days, epithelial cells begin to proliferate, mainly at definite proliferative centres, i.e. the annulus, the handle of the malleus and the plicae mallei. After 3 days, the epithelial hyperplasia has increased, and also the connective tissue and the mucosal cells appear to be proliferating. In the following days the outer epithelium migrates over the perforation, followed by mucosal and young mesenchymal cells. As soon as the perforation is covered, mitotic activity decreases rapidly (1). The healing process thus seems to be initiated and guided by the outer epithelial capacity to proliferate. Mucosa and propria both follow the epithelial migration.

When a perforation does not close, it is likely that the reason for this failure has to be sought in a failure of the outer epithelium to cover the defect. Several hypothetical reasons can be mentioned: 1) the defect is too large, so that the epithelial sheet cannot bridge it; 2) large parts of the proliferative centres are damaged by the trauma, so that proliferation does not occur; 3) the etiological agent persists, making it impossible for the proliferating epithelium to close the defect, as would be the case when a ventilation tube is placed (in this latter case, epithelial hyperplasia lasts for at least 7 weeks (5)).

The difference between a 'restitutio ad integrum', in which a drum membrane of normal thickness and otological appearance is found, and a 'covered perforation', in which a very thin, nearly transparent sheet covers the original perforation, seems to be caused mainly by the different thickness of the lamina propria. In contrast to Schuknecht (7) and Yamashita (6), we could clearly demonstrate that even in the thinnest membrane, lamina propria is not lacking. Yet, whereas the lamina propria normally measures some 100 μm in thickness, it is no more than 2–3 μm thick in the covering sheet! Although light microscopy suggests that there would be some fibroblasts in the propria, we could not visualize them by electron microscopy. Collagen fibres are present. These data can be interpreted as if the matrix and the

fibres of the collagen tissue were formed by fibroblasts which are lying in the normal ('thick') part of the membrane. Apparently in a 'restitutio ad integrum' situation, the lamina propria contains normal fibroblasts, which are lacking in a 'covered membrane' situation. This might suggest that the normal reactive proliferation of fibroblasts after tympanic membrane perforation, is inhibited in some circumstances. The migration of epithelium is then followed by a passive 'diffusion' of collagen matrix, just enough to ensure the vascular needs of the epithelium, instead of being followed by an active proliferation and migration of fibroblasts. No evident explanation whatsoever can be given for the initial inhibition of the fibroblasts in this hypothesis.

Finally, a parenthesis has to be made. In experimental studies in which tympanosclerosis is evoked, one of the early changes in the tympanic membrane is the appearance of areas with reduced lamina propria. These areas resemble those we described as covered perforations, and they evolve further into tympanosclerotic plaques (Kuijpers W., personal communication). It might therefore not be impossible that the areas we described would also have evolved into tympanosclerotic plaques in vivo. However, we don not have any clinical evidence for this theoretical correlation.

Anyhow, the existence of these well defined areas with reduced lamina propria cannot be denied. A detailed morphological description has been given. The exact role of this phenomenon in the pathology of human tympanic membrane remains to be studied. It is very likely that clinical follow-up studies could provide much interesting information.

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