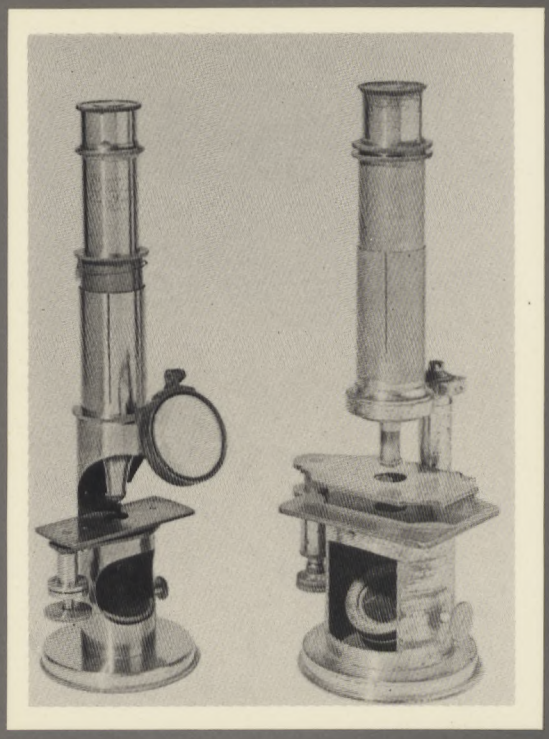
FUNCTIONAL STRUCTURE OF THE INNER EAR IN EXPERIMENTAL HYDROPS



F.W.J. ALBERS

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DE FUNCTIONELE STRUCTUUR VAN HET BINNENOOR BIJ EXPERIMENTELE HYDROPS
(MET EEN SAMENVATTING IN HET NEDERLANDS)

PROEFSCHRIFT

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Aan Ans en Ewout

Aan mijn ouders en schoonouders

The work presented in this thesis was performed in the Laboratory for Histophysiology and Experimental Pathology of the Department of Otorhinolaryngology, University Hospital Utrecht, The Netherlands.

CONTENTS

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CHAPTER 1

GENERAL INTRODUCTION

Introduction

In 1861 Prosper Menière[1] described the classical triadic symptomatology of hearing loss, vertigo and tinnitus, which he attributed for the first time to a labyrinthine disorder. Since Hallpike and Cairns[2] and also Yamakawa[3] in 1938 discovered hydrops of the endolymphatic system in the temporal bones of patients with Menière's disease, endolymphatic hydrops has been generally accepted as the basic histopathological substrate of Menière's disease. However, there is histological evidence that Menière's disease may exist without endolymphatic hydrops and that endolymphatic hydrops may be present without the classical symptoms of Menière's disease[4,5].
The production of an endolymphatic hydrops by obliteration of the endolymphatic sac and duct in animals has revealed considerable species differences in creating the hydrops[6]. The most consistent production of an experimental hydrops was reported in 1965 by Kimura and Schuknecht[7], who produced an endolymphatic hydrops with 100% accuracy in the guinea pig after surgical obliteration of the endolymphatic duct and sac. Despite the many discrepancies between experimentally induced hydrops and Menière's disease, such as the absence of episodic vestibular symptoms in the animal model, surgical obliteration of the endolymphatic duct and sac in the guinea pig is currently still the best available method for producing a persistent and progressive distension of Reissner's membrane.

Objectives of this study

Histological[8–11], electrophysiological[12–16] and biochemical[17–19] studies on experimental hydrops have revealed new interesting information with regard to the physiology and pathophysiology of the inner ear. In the light of this recent knowledge of inner ear physiology, the early pathophysiology in the cochlear duct after surgical obliteration of the endolymphatic duct and sac in the guinea pig has been the main focus of this study. Morphological changes of functional structures in the guinea pig cochlea have been analyzed in different time-sequence investigations.

In Chapter 2 the present knowledge of the light and submicroscopical morphology of the normal inner ear of the guinea pig is reviewed. The histological processing of the specimens used as illustrations in this chapter is described in Chapters 4-6.
In Chapter 3 a qualitative and quantitative investigation is presented of the early degenerative changes in the outer and inner hair cells one, two and four months after surgical obliteration of the endolymphatic duct and sac. The specimens were processed according to the block-surface technique and examined by interference differential (Nomarski) microscopy.

In Chapter 4 the initial ultrastructural changes in the stria vascularis and Reissner's membrane of the guinea pig after obliteration of the endolymphatic sac and duct are reported. The significance of these observations with regard to the pathophysiology of experimental hydrops is discussed.
In Chapter 5 the submicroscopical changes in the organ of Corti at one, two and three months after obliteration of the endolymphatic sac and duct are described. The possible consequences for the auditory transduction mechanism in experimentally induced hydrops are discussed.
In Chapter 6 changes in the selective contrast-enhancement of the cochlear duct glycocalyx after osmium tetroxide-potassium rutheniumcyanide post-fixation in this animal model are documented. The endolymphatic glycocalyx is considered to be of significant importance for the maintenance of the stereociliary cross-link systems of the inner ear sensory cells.
In Chapter 7 the regional and total cochlear blood flow after endolymphatic duct and sac obliteration is investigated using the non-radioactive microsphere method combined with serial sectioning of plastic embedded cochleae.
In Chapter 8 the results of this study are summarized and conclusions are presented.

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CHAPTER 2

MORPHOLOGY OF THE NORMAL GUINEA PIG COCHLEA

Introduction

In 1851 Alphonso Corti described for the first time in the Zeitschrift für wissenschaftliche Zoologie the sensory epithelium, spiral ganglion, tectorial membrane and stria vascularis of the mammalian inner ear[1]. In the second half of the 19th century many light microscopical investigations, among which are the classic studies of Gustav Retzius (1842-1919), revealed with surprising accuracy further information of the inner ear anatomy[2]. More detailed information on the morphology of the inner ear became available after the introduction of transmission electron microscopy (TEM) in the 1950s. Important contributions to the present understanding of the cochlear cytoarchitecture at submicroscopical level were provided by Engström and Wersäll [3,4], Smith[5,6], Spoendlin[7–11], Friedmann[12], Friedmann and Ballantyne[13], Iurato[14–16], Engström[17–19] and Duvall[20,21]. Quantitative and qualitative evaluation and documentation of cochlear pathology became possible after the development of the surface preparation technique[17,22–28]. A modification of this technique was introduced by Spoendlin[29,30] as the block-surface technique, which allows a combined investigation of the same specimen by light microscopy (bright field, phase contrast or interference differential (Nomarski) microscopy) and transmission electron microscopy.
At the end of the 1960s scanning electron microscopy (SEM) became available for inner ear research. In excellent studies by Lim[31], Bredberg[32–34] and Harada[35] the three-dimensional surface structures of the inner ear have been described in detail. In the last decade advances in tissue fixation and specimen processing for inner ear electron microscopy have considerably improved the preservation and contrast-enhancement of the cochlear ultrastructure[36–43]. A thorough knowledge of the light microscopical and ultrastructural morphology of the inner ear under non-pathological conditions is of the highest importance for the evaluation of the histopathology in experimental hydrops. Therefore a comprehensive overview of the basic cytoarchitecture of the functional structures in the cochlea at light microscopical and ultrastructural level is presented in this chapter. The specimens used as illustrations in this chapter have been processed as described in Chapters 4-6.

Micro-anatomy of the guinea pig cochlea

The inner ear (or labyrinth) of the guinea pig consists of the bony labyrinth and the membranous labyrinth. The cochlear part of the bony labyrinth forms an ascending spiral of 3.5 turns around the central modiolus. The cochlear canal is divided into three compartments: the scala vestibuli, the scala media (or cochlear duct) and the scala tympani. The scala vestibuli and scala tympani are perilymphatic spaces containing perilymph, which communicate with one

another through the helicotrema. The scala media contains the endolymph fluid.

As seen in midmodiolar sections, the cochlear duct is triangular in shape. (Fig. 1). The scala media is separated from the scala vestibuli by Reissner's membrane. The basilar membrane, which separates the scala media from the scala tympani, runs from the osseous spiral lamina to the spiral ligament. The organ of Corti, which is attached to the upper surface of the basilar membrane, consists of sensory cells (inner and outer hair cells) and supporting cells (inner and outer pillar cells, inner phalangeal cells and Deiters' cells). (Fig. 2). The surface of the organ of Corti is covered by the tectorial membrane, which extends from the spiral limbus to the outermost row of Deiters' cells or the Hensen's cells. The outer wall of the cochlear duct is formed by the stria vascularis and the spiral prominence, covering the spiral ligament.

The cochlea is innervated by both afferent and efferent nerve fibres. The afferent neurons with the bipolar ganglion cells form the spiral ganglion in Rosenthal's canal in the modiolus. The efferent olivocochlear neurons, known as the olivocochlear bundle (Rasmussen's bundle), originate in the homo- and contralateral superior olivary complex and reach the organ of Corti through Rosenthal's canal, forming the intraganglionic spiral bundle. The cochlea is also supplied with autonomic nerve fibres originating in the superior cervical ganglion. The most significant vascular areas supplying the cochlea are found in the modiolus, the osseous spiral lamina and the lateral wall, which includes the spiral ligament, the stria vascularis and the spiral prominence.

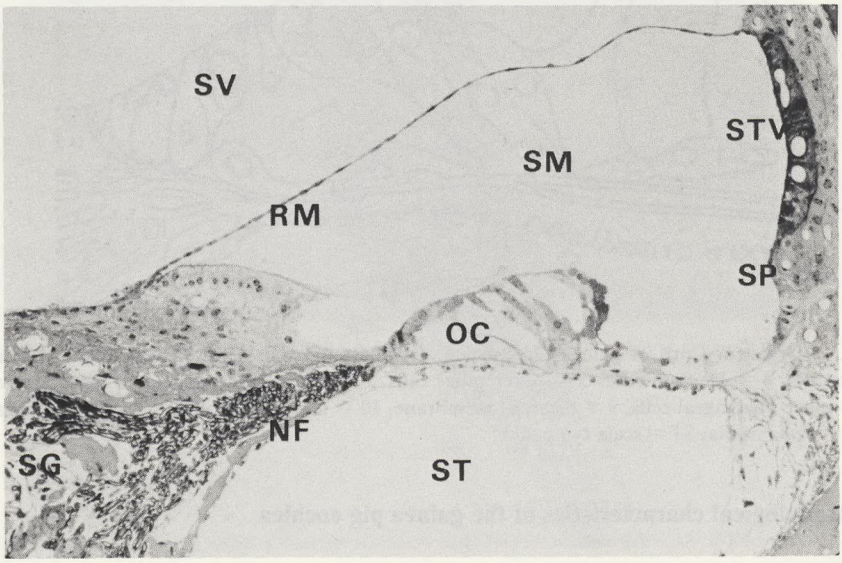

Fig. 1. Midmodiolar section of guinea pig cochlea. SV = scala vestibuli, SM = scala media, ST = scala tympani, RM = Reissner's membrane, STV = stria vascularis, SP = spiral prominence, OC = organ of Corti, NF = nerve fibres, SG = spiral ganglion.

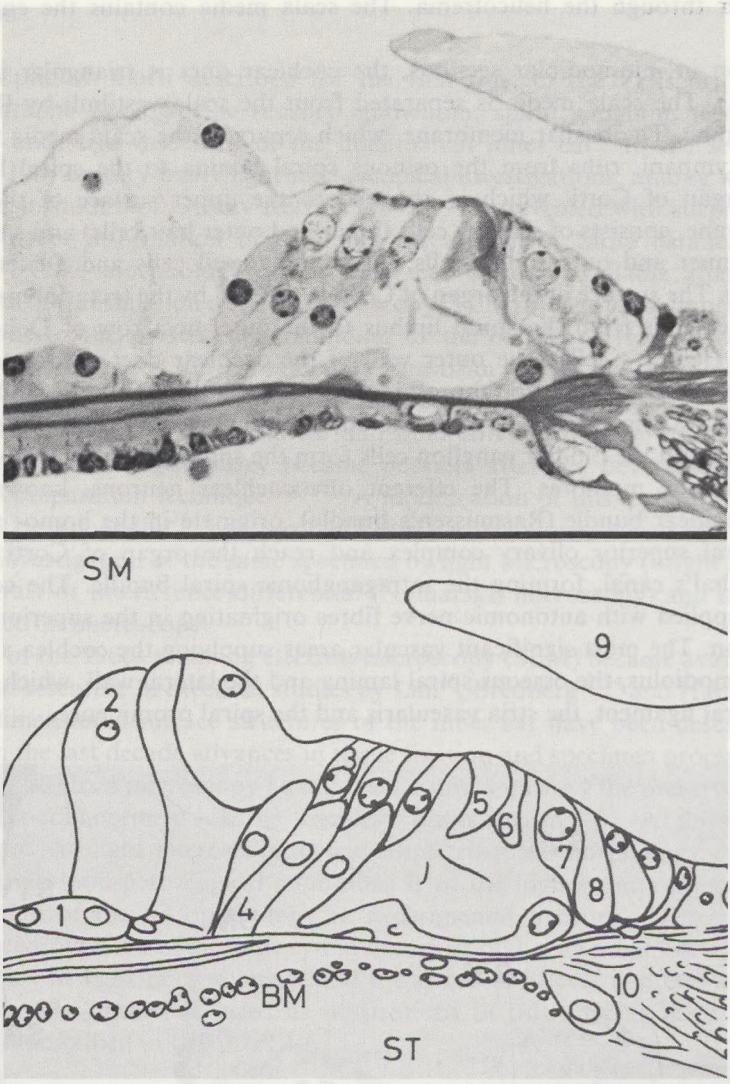

Fig. 2. Organ of Corti of guinea pig cochlea. 1 = Böttcher cells, 2 = Hensen cells, 3 = outer hair cells, 4 = Deiters' cells, 5 = outer pillar cells, 6 = inner pillar cells, 7 = inner hair cell, 8 = inner phalangeal cells, 9 = tectorial membrane, 10 = nerve fibres, BM = basilar membrane, SM = scala media, ST = scala tympani.

Morphological characteristics of the guinea pig cochlea

Reissner's membrane

Reissner's membrane (Fig. 3) consists of two different cell layers: the flat polygonal epithelial cells facing the scala media and the thin irregularly shaped

mesothelial cells facing the scala vestibuli. The epithelial cells and the mesothelial cells are separated by a basement membrane. Reissner's membrane is attached to the superior part of the spiral limbus and extends to the lateral cochlear wall, where the epithelial cell layer becomes continuous with the stria vascularis. The nucleus of the mesothelial cell has an ovoid shape. The cytoplasm contains a few mitochondria, glycogen particles, pinocytotic vesicles and sometimes a poorly developed Golgi apparatus. The mesothelial cells are loosely interconnected; desmosomes are infrequently observed between the interdigitating cell processes. Occasionally, adjacent mesothelial cells show disconnections or pores.

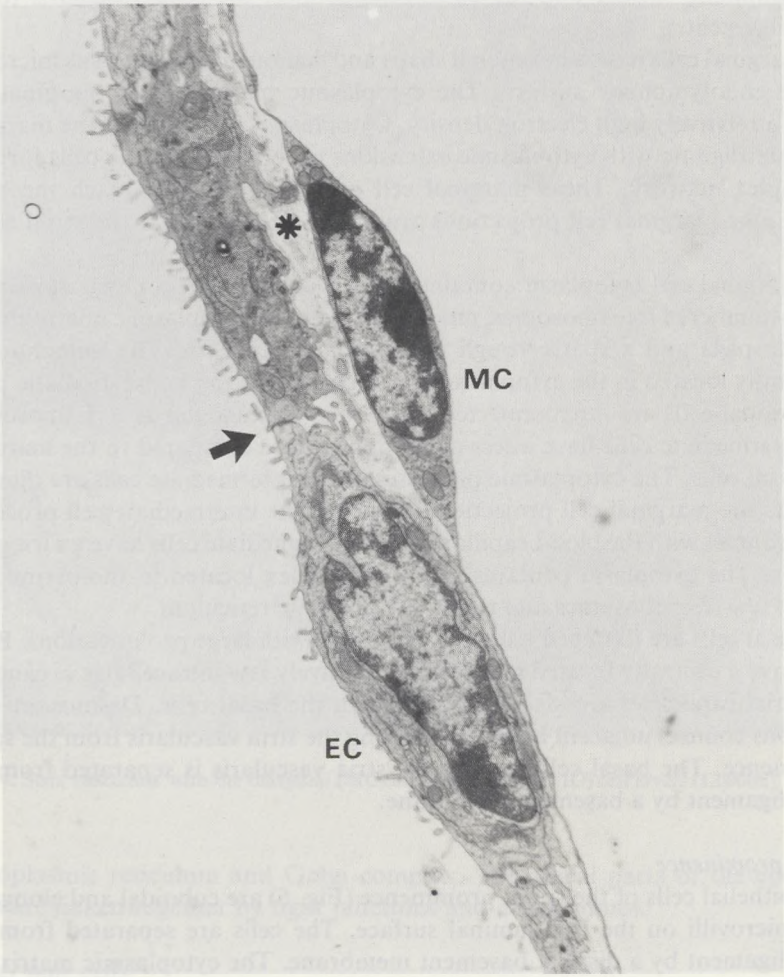

Fig. 3. Reissner's membrane with the mesothelial (MC) and epithelial (EC) cell layer, separated by a basement membrane (asterisk). The epithelial cells are interconnected by zonula occludens, zonula adhaerens and desmosomes. (arrow). (8,000x).

The epithelial cells demonstrate numerous microvilli on the endolymphatic surface. The cytoplasm contains an oval or multilobulated nucleus, a relatively well-developed rough endoplasmic reticulum and Golgi complex, lipid droplets, pinocytotic vesicles, lysosomes and mitochondria. Cell junctions between the epithelial cells consist of a zonula occludens at the endolymphatic surface, followed by a zonula adhaerens, desmosomes and interdigitating processes.

Stria vascularis

The stria vascularis is composed of three cell layers (marginal, intermediate and basal cells) and blood vessels. (Fig. 4, 5). The marginal cells border the endolymphatic surface and the basal cells connect the stria vascularis to the spiral ligament.

The marginal cells have a hexagonal shape and demonstrate numerous microvilli on the endolymphatic surface. The cytoplasmic matrix of the marginal cell shows a relatively high electron density. Cytoplasmic processes of the marginal cells interdigitate with cytoplasmic extensions of the intermediate cells forming a complex network. These marginal cell processes can also reach the basal cells. Some marginal cell projections are in close contact with the strial blood vessels.

The marginal cell cytoplasm contains a spherical nucleus, a Golgi apparatus, a large number of free ribosomes, pinocytotic vesicles, cytoplasmic microtubules, lipid droplets and a sparse rough endoplasmic reticulum. The mitochondria are mainly located in the cytoplasmic processes. Adjacent endolymphatic parts of marginal cells are interconnected by zonulae occludentes and desmosomes.

The intermediate cells have a less dense appearance compared to the marginal and basal cells. The cytoplasmic processes of the intermediate cells are directed towards the marginal cell projections. Some of the intermediate cell processes make contact with the blood capillaries. The intermediate cells have an irregular nucleus. The cytoplasm contains a Golgi complex located in the perinuclear region, few free ribosomes and rough endoplasmic reticulum.

The basal cells are flattened and spindle shaped with large prolongations. Basal cells have a centrally located nucleus and relatively few intracellular organelles. The strial capillaries are also in contact with the basal cells. Desmosome-like junctions connect adjacent basal cells sealing the stria vascularis from the spiral prominence. The basal cell layer of the stria vascularis is separated from the spiral ligament by a basement membrane.

Spiral prominence

The epithelial cells of the spiral prominence (Fig. 6) are cuboidal and elongated with microvilli on the free luminal surface. The cells are separated from the spiral ligament by a distinct basement membrane. The cytoplasmic matrix has an electron dense appearance. The nucleus is large and usually lobulated. The cytoplasm contains only few mitochondria and a sparsely distributed rough

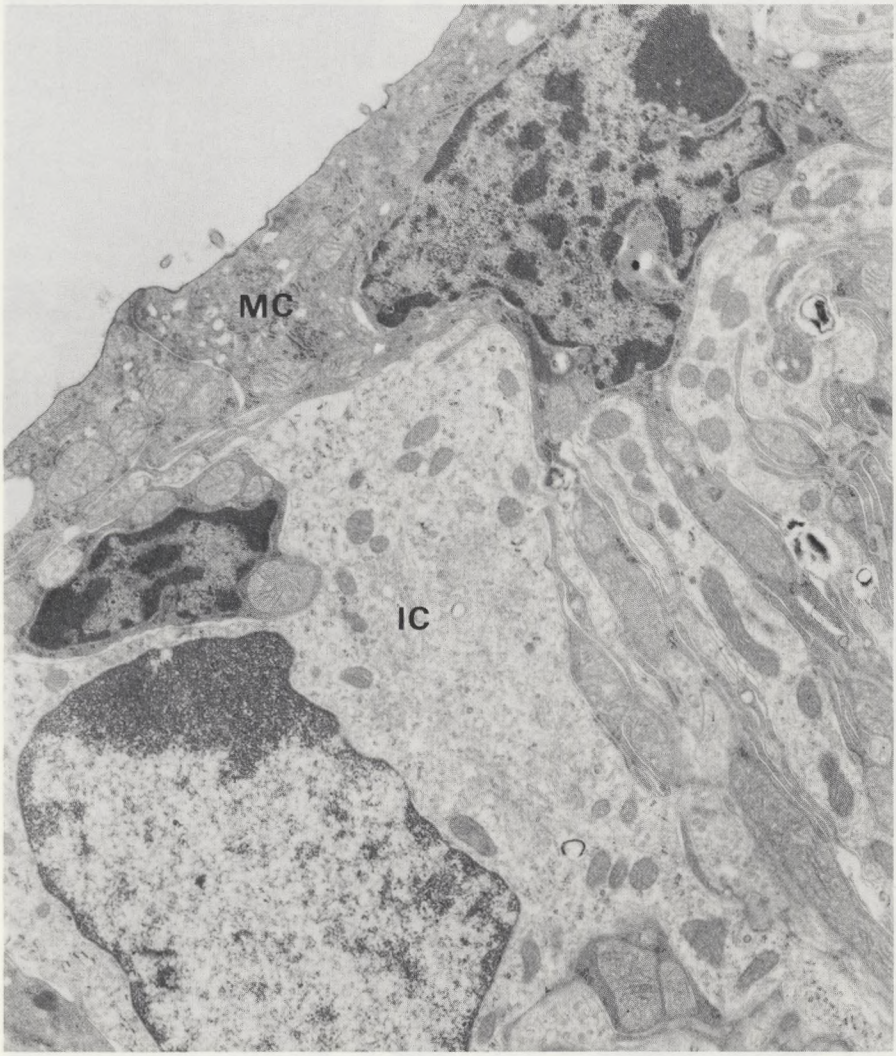

Fig. 4. Stria vascularis with the marginal (MC) and intermediate (IC) cell layer. (12,000x).

endoplasmic reticulum and Golgi complex. The apical parts of the epithelial cells are linked together by tight junctions and desmosomes.

Outer hair cells

The cylindrically shaped outer hair cells (Figs. 2, 7A-B, 8A-B) of the guinea pig are arranged in three parallel rows in the organ of Corti. The supporting framework for the outer hair cells is provided by the outer pillar cells and

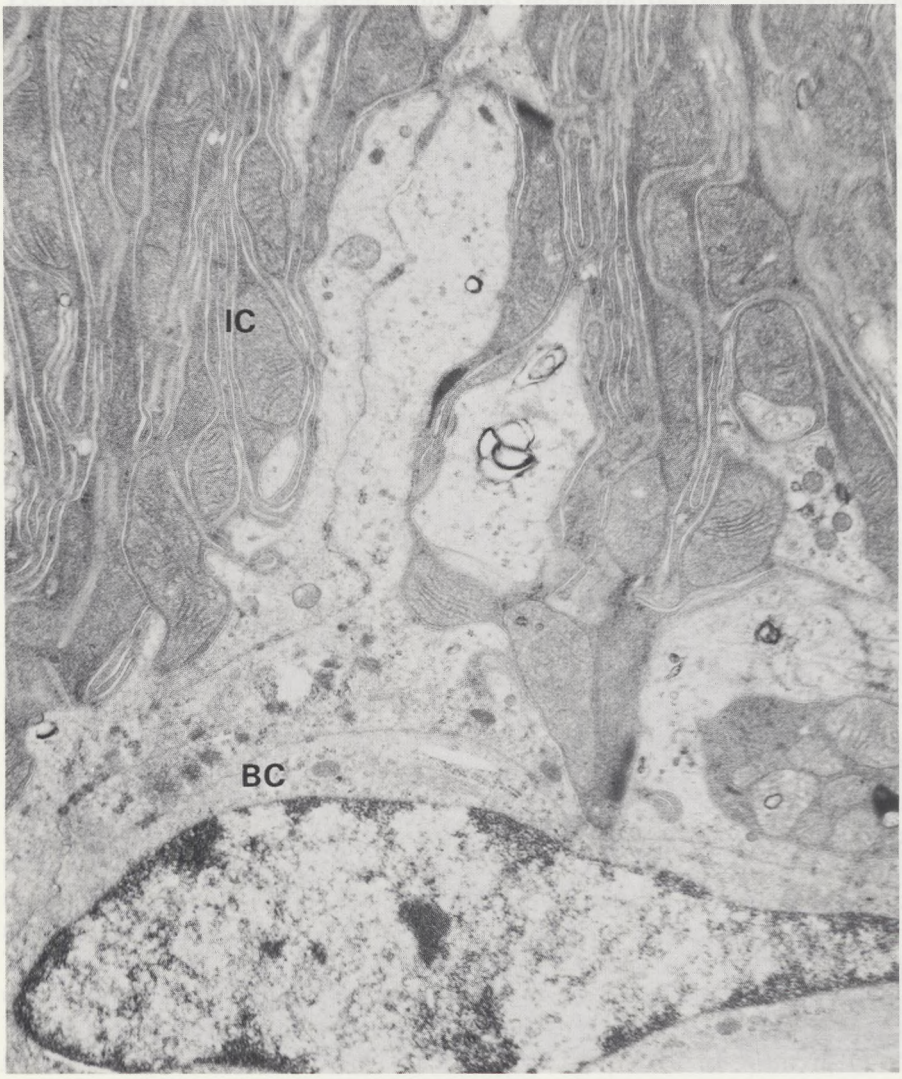

Fig. 5. Stria vascularis with the intermediate (IC) and basal (BC) cell layer. (12,000x).

the Deiters' cells (outer phalangeal cells), which contain large intracellular microfilaments. The outer pillar cells and the Deiters' cells participate in the formation of the reticular lamina at the apical surface of the organ of Corti. The apex of the outer hair cells is attached to the reticular lamina by tight junction complexes, preventing perilymphatic leakage. The cell bodies of the outer hair cells are surrounded by the Nuel's space, which is filled with Corti (peri)lymph.

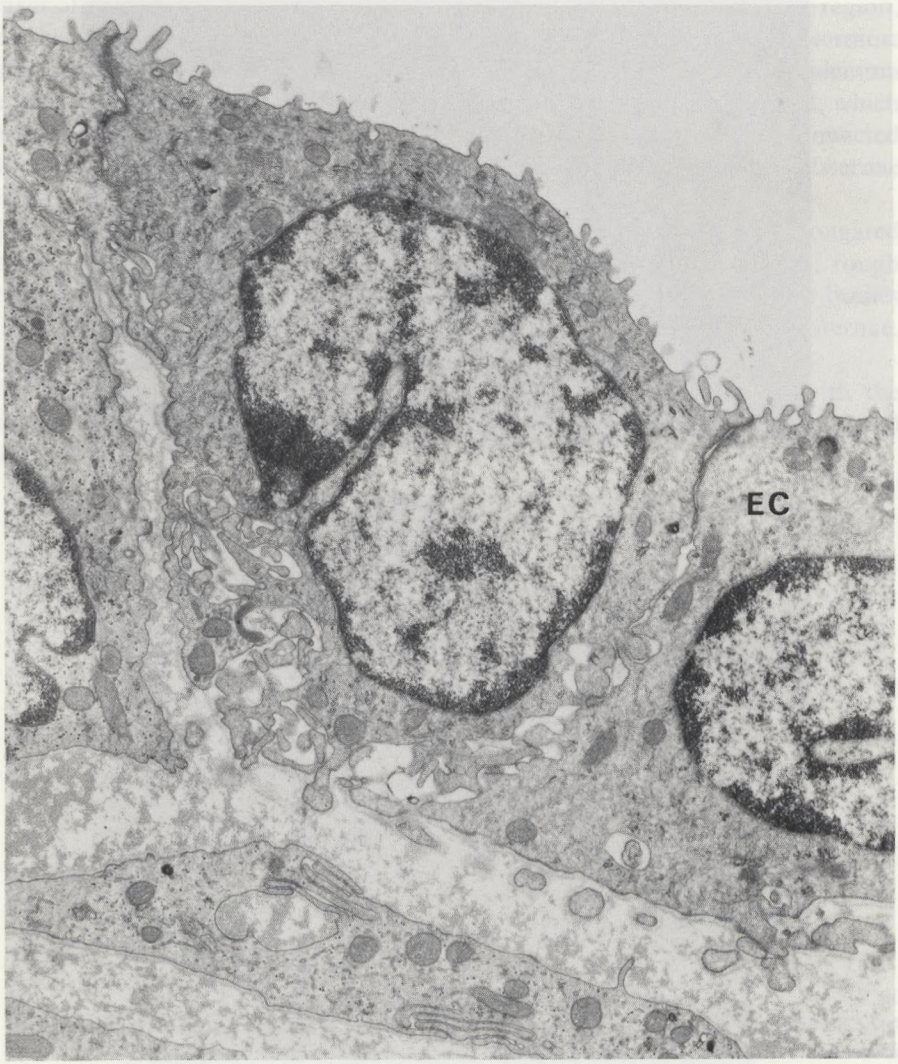

Fig. 6. Epithelial cells (EC) of the spiral prominence. (12,000x).

The stereociliary bundles on the surface of the outer hair cells show the characteristic W-pattern. (Fig. 7A). Each stereociliary bundle consists of three different rows of cilia with increasing heights. Interconnecting bridges between the individual stereocilia form an organized linkage system (Fig. 7B), which consists of three cross-link types: side-to-side links (between stereocilia of the same row), row-to-row links (between adjacent rows) and upward-pointing links (links from the tips of shorter stereocilia to the sides of the adjacent

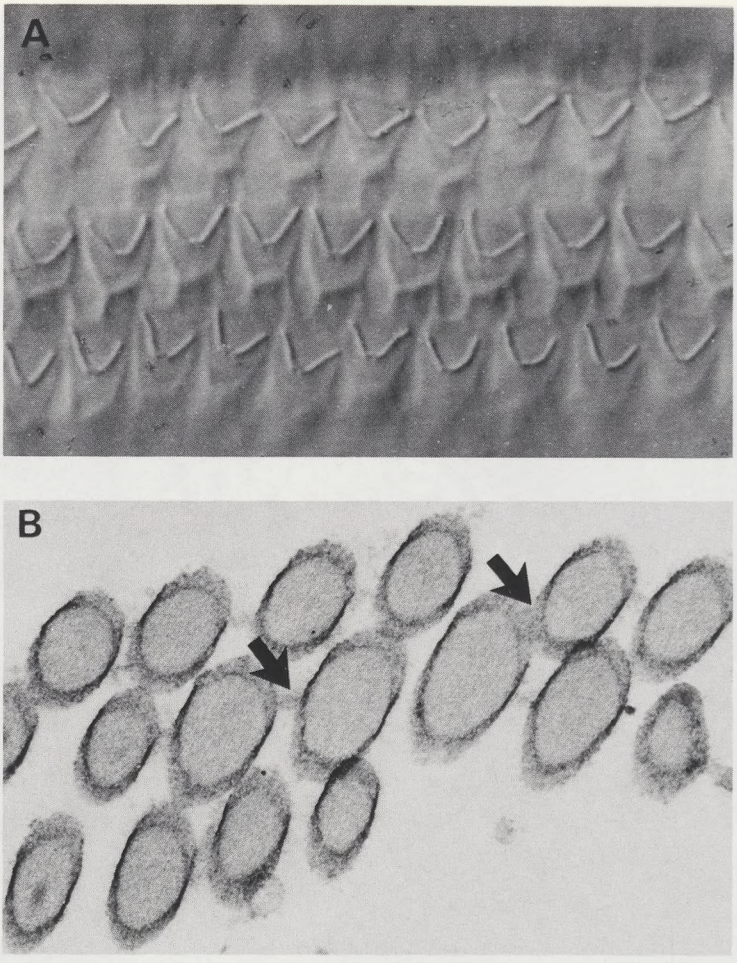

Fig. 7.
(A). Characteristic W-pattern of the stereociliary bundles of the outer hair cells. Interference differential (Nomarski) microscopy. (1,000x).
(B). Interconnecting bridges (cross-links) between the outer hair cell stereocilia. (arrows). (33,000x).

taller stereocilia). The stereocilia and the rootlets are composed of longitudinally arranged actin filaments. The stereociliary rootlets are anchored in the cuticular plate, which contains actin, myosin and other contractile cytoskeletal proteins. The rudimentary kinocilium with the basal body is located in the cuticle-free zone. (Fig. 8B).
Lamellar endoplasmic reticulum (subsurface cisternae) is situated along the entire internal surface of the plasmalemma. (Fig. 8A). The subsurface cisternae of the guinea pig outer hair cells demonstrate regional differences: the outermost

layer of the subsurface cisternae is a flattened cistern in the supranuclear region, but has a tubular arrangement in the subnuclear region. Between the outermost layer of the subsurface cisternae and the inner surface of the plasmalemma a layer of homogeneous substance with filamentous strands is observed, which is called the subplasma membrane. The subsurface cisternae are connected with the apical cisternae in the cuticle-free zone and the subsynaptic cisternae in the subnuclear region.

The subcuticular region of the outer hair cell contains round and elongated mitochondria, lysosomes, lipofuscin granules, a few Golgi complexes, rough endoplasmic reticulum with a small number of ribosomes and lamellar bodies (Hensen's bodies), which are in close contact with the subsurface cisternae. (Fig. 8A).

In the supranuclear zone the mitochondria are closely associated with the subsurface cisternae. The nucleus is round or ovoid and is mostly located in the basal part of the cell.

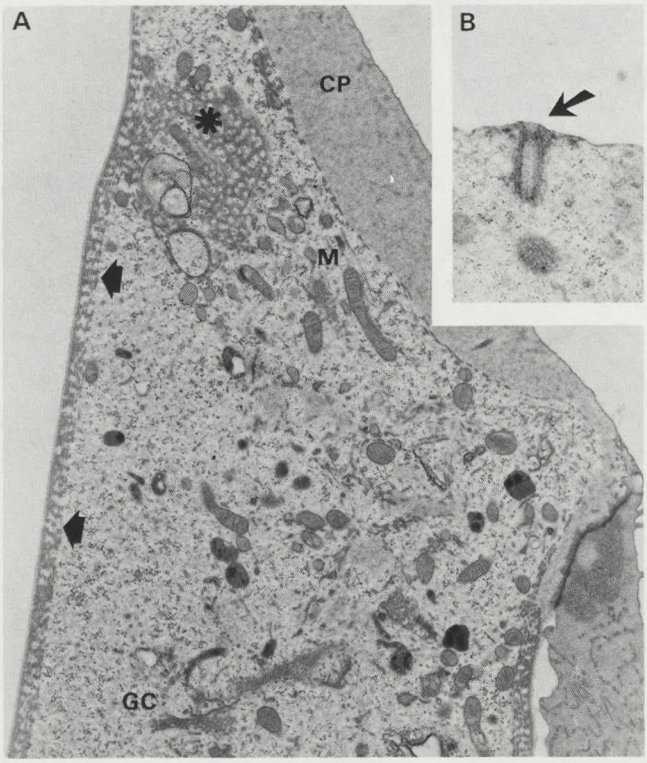

Fig. 8.

(A). Apical part of outer hair cell. Subsurface cisternae (arrows) in multiple layers are found underneath the plasmalemma and are in close contact with the Hensen's body (asterisk). The subcuticular mitochondria (M) are round or elongated. GC = Golgi complex, CP = cuticular plate. (12,000x).

(B). Rudimentary kinocilium with basal body (arrow). (33,000x).

The infranuclear region (Fig. 9) is characterized by the presence of coated vesicles, microtubules, numerous tubular vesicles and mitochondria. Along the basal cytoplasmic membrane high endocytotic and/or exocytotic activity is observed. Dense synaptic bodies surrounded by synaptic vesicles are observed in the presynaptic cytoplasm adjacent to the afferent nerve endings. Flattened subsynaptic cisternae are located opposite to the efferent nerve endings.

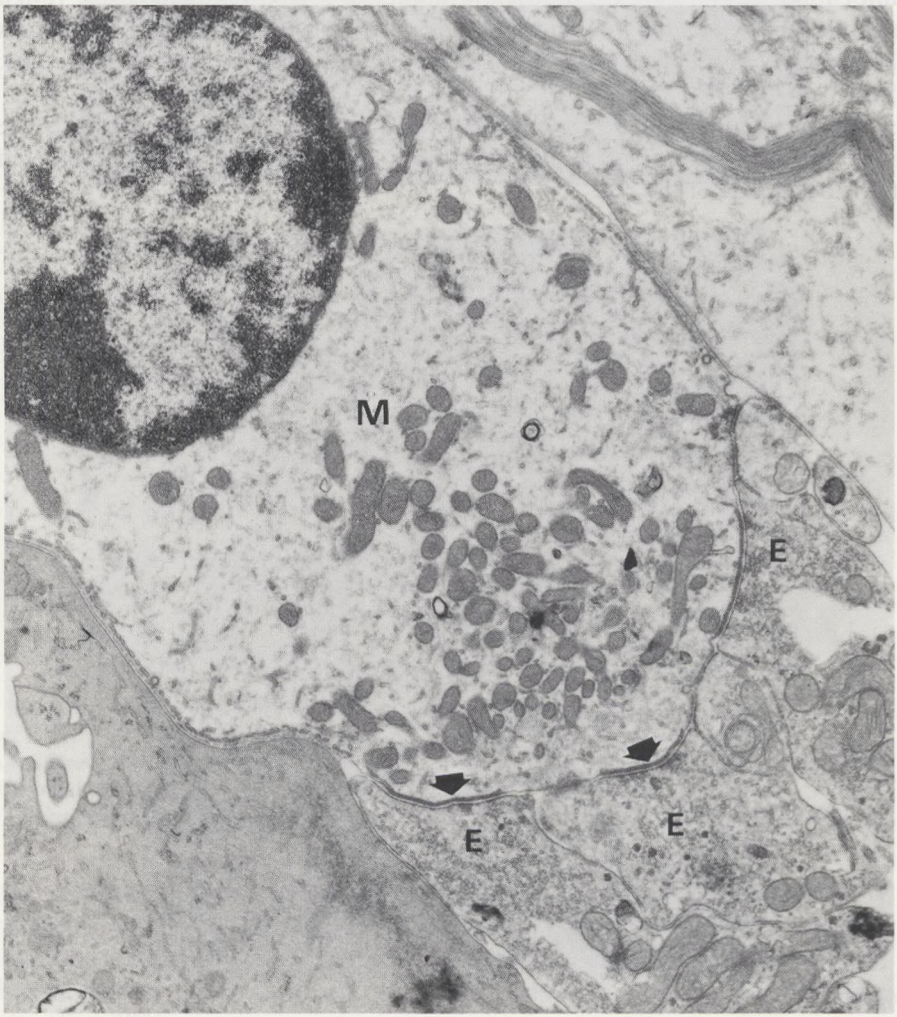

Fig. 9. Basal part of outer hair cell. Subsynaptic cisternae (arrows) are situated opposite to the efferent nerve endings (E). M = mitochondria. (12,000x).

Inner hair cells

The ovoid shaped inner hair cells form a single row along the longitudinal axis of the organ of Corti. The inner hair cells (Figs. 2, 10) are completely surrounded by the inner pillar cells and the inner phalangeal cells. The supporting cells are connected to the apical part of the inner hair cells with zonulae occludentes forming the reticular lamina.

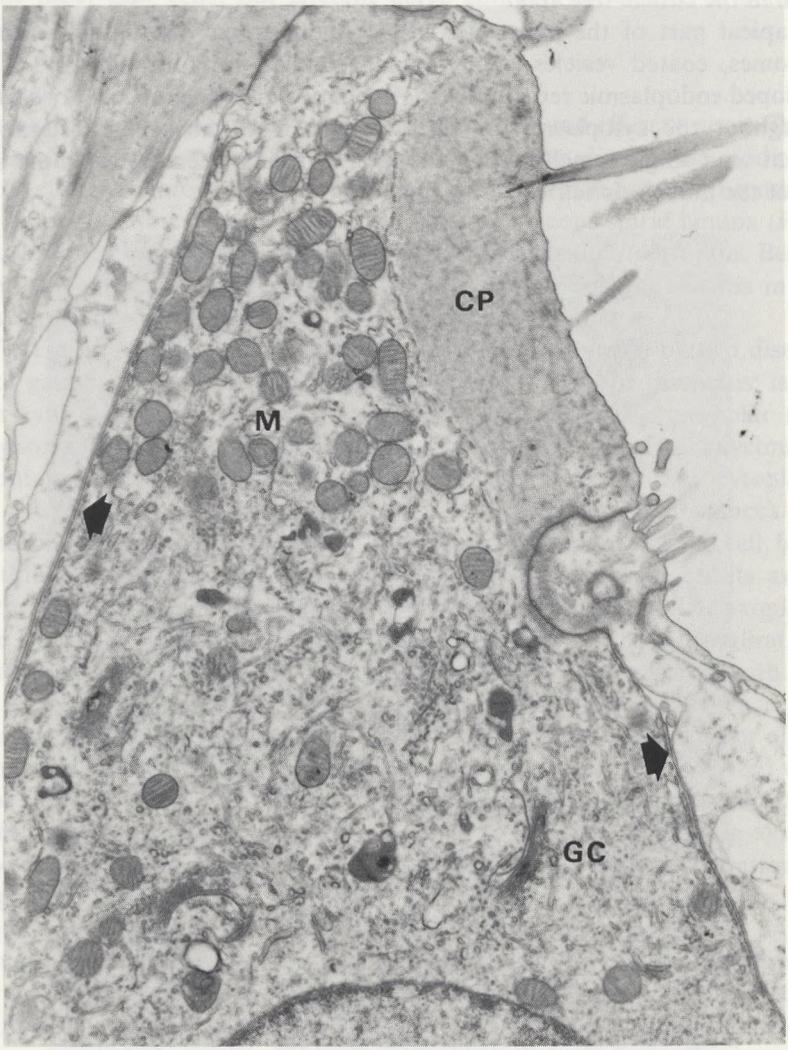

Fig. 10. Apical part of inner hair cell. Poorly developed subsurface cisternae (arrows) are located along the lateral plasma membrane. CP = cuticular plate, M = mitochondria, GC = Golgi complex. (12,000x).

The inner hair cell stereocilia are arranged in a few slightly curved rows running parallel to the longitudinal axis of the cochlear spiral. A difference in height exists between the stereocilia of the different rows. A network of interconnecting bridges between the stereocilia as described in the outer hair cells is also observed in the inner hair stereociliary bundle. The stereociliary rootlets of the inner hair cells show the same anchoring system in the cuticular plate as observed in the outer hair cells. A basal body as remnant of the kinocilium is frequently found in the cuticle-free area.

The apical part of the inner hair cell contains numerous Golgi complexes, lysosomes, coated vesicles, multivesicular bodies, microtubules and a well-developed endoplasmic reticulum. The subcuticular mitochondria are scattered throughout the cytoplasm. The poorly developed subsurface cisternae are present as a single lamella along the lateral plasma membrane of the apical part of the inner hair cell.

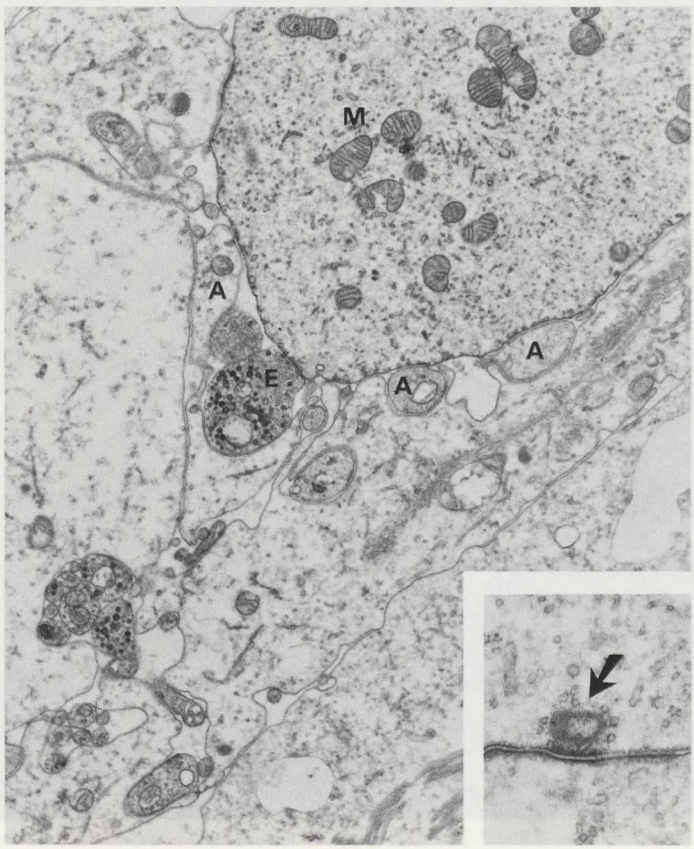

Fig. 11. Basal part of inner hair cell. Numerous afferent nerve contacts (A) are found, while efferent nerve endings (E) are sparse. M = mitochondria. (12,000x). The inset shows a synaptic body (arrow) surrounded by synaptic vesicles. (33,000x).

The round nucleus is located in the midportion of the cell and has an uniform chromatin contribution. The infranuclear mitochondria are grouped together around the nucleus. The infranuclear zone of the inner hair cell (Fig. 11) shows multiple microvesicles, coated vesicles, microtubules, large tubular vesicles and rough endoplasmic reticulum. Multiple synaptic bodies with presynaptic vesicles are located in the basal part of the inner hair cell, while subsynaptic cisternae are not commonly found. Numerous afferent nerve endings are in close contact with the inner hair cells, but efferent nerve contacts are sparse in contrast to the outer hair cells.

Neural elements

The guinea pig cochlea is innervated by both afferent and efferent nerve fibres. The afferent neurons are associated with the spiral ganglion in the modiolus, while the efferent neurons form the intraganglionic spiral bundle in Rosenthal's canal. The nerve fibres run in canals through the osseous spiral lamina (Figs. 2, 12A) and reach the organ of Corti through the habenula perforata. Before entering the organ of Corti all nerve fibres lose their myelin sheaths in the habenula perforata (Fig. 12B).

The afferent innervation of the guinea pig cochlea is provided by two distinct types of ganglion cells, which form the spiral ganglion in the modiolus: large, bipolar type I ganglion cells (90-95%) and smaller, pseudomonopolar type II ganglion cells (5-10%). The type I ganglion cell (Fig. 13A-B) is myelinated and contains a large nucleus with a pronounced nucleolus. The cytoplasm shows a well-developed rough endoplasmic reticulum (Nissl substance), mitochondria, Golgi complexes and lipofuscin granules. The type I cell body has a thin myelin sheath up to the first node of Ranvier, whereas its axons are surrounded by compact myelin layers of a uniform thickness. The axoplasm of type I nerve fibres contains mainly neurotubules. The type II ganglion cell is mostly unmyelinated and the cytoplasm has a pale appearance with less rough endoplasmic reticulum. In the type II axoplasm neurofibrils are predominantly found. The type I neurons provide the afferent innervation of the inner hair cells, while the outer hair cell innervation is directly associated with the type II neurons. The great majority of the afferent nerve fibres are associated with the inner hair cells, while only 5% of the total afferent neuron system provide the innervation of the outer hair cells. The afferent innervation of the organ of Corti is formed by the inner radial fibres, the basilar fibres and the outer spiral fibres. The inner hair cells are exclusively innervated by the unbranched inner radial fibres, which lead from the habenula perforata directly to the closest inner hair cell. The outer hair cells are innervated by the outer spiral fibres, which reach the area of the outer hair cells as basilar fibres. In contrast to the inner hair cell innervation the afferent nerves for the outer hair cells show considerable spiral distribution. The afferent nerve endings at the inner hair cells show multiple synaptic complexes with synaptic bodies of various sizes at the basal part of the inner hair cells. Synaptic complexes

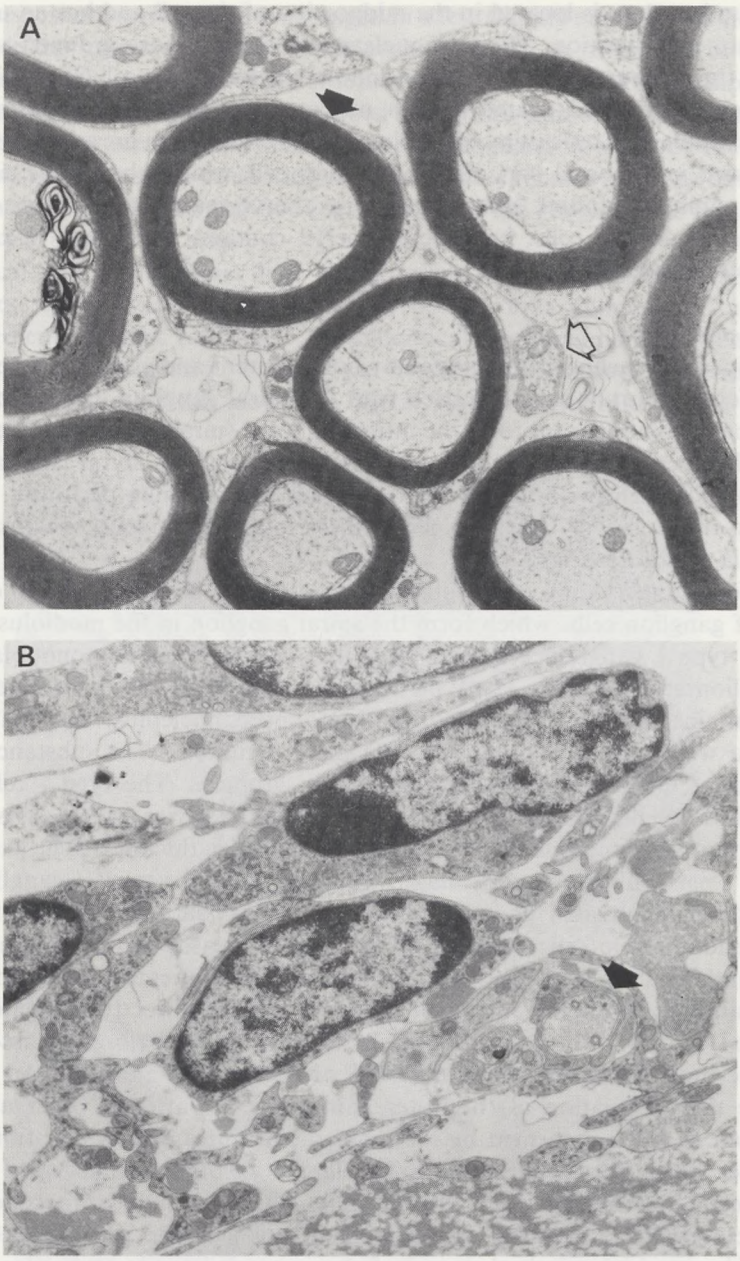

Fig. 12.
(A). Transverse section through the osseous spiral lamina demonstrating myelinated (arrow) and unmyelinated (unfilled arrow) nerve fibres. (12,000x).
(B). Unmyelinated nerve fibres (arrow) after entering the organ of Corti through the habenula perforata. (20,000x).

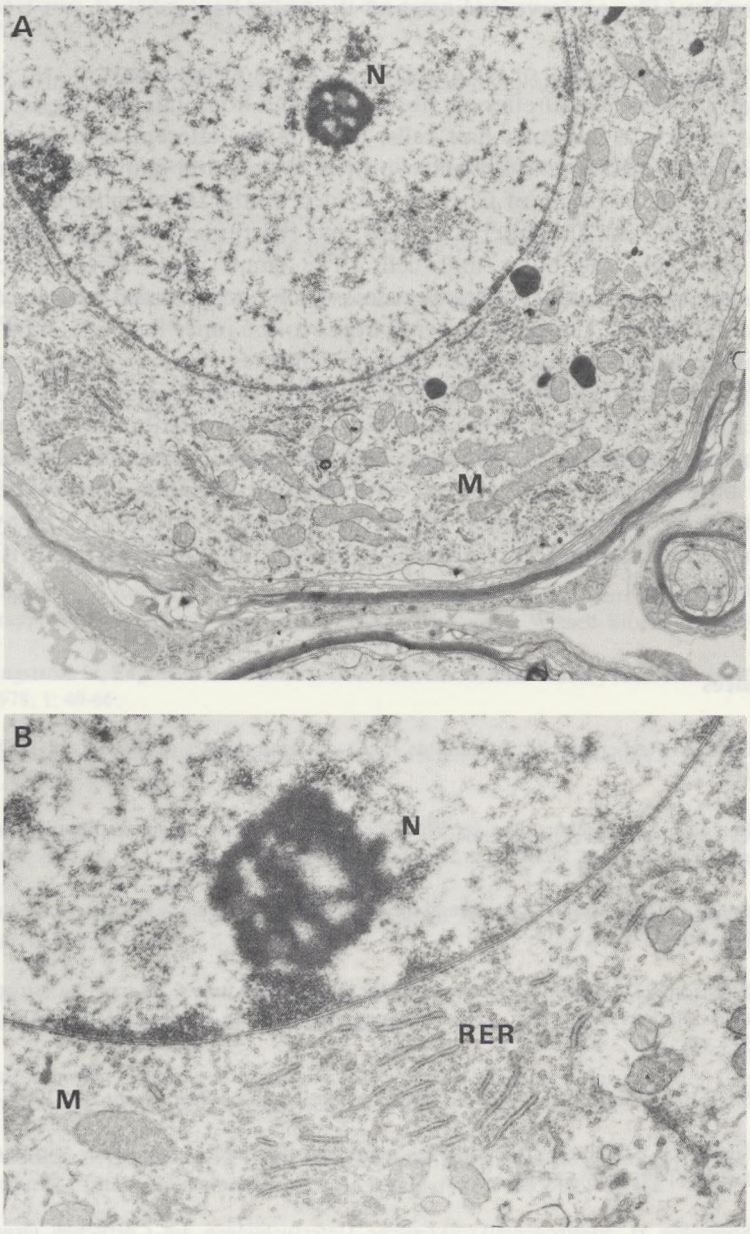

Fig. 13. (A-B). Type I ganglion cell with a pronounced nucleolus (N), mitochondria (M) and a well developed rough endoplasmic reticulum (RER) or Nissl substance. (A:12,000x and B:20,000x).

between the afferent nerve endings and the outer hair cells are not frequently found in the guinea pig.

The efferent innervation of the guinea pig cochlea also consists of two different types of neurons. Small efferent fibres from the homolateral superior olivary nucleus innervate the inner hair cells, while larger efferent neurons from the contralateral accessory olivary nuclei project to the outer hair cells. The efferent innervation of the organ of Corti is provided by the inner spiral fibres, which are associated with the inner hair cells and the tunnel-crossing radial fibres, which innervate the outer hair cells. The small inner spiral fibres have enlargements filled with synaptic vesicles, which make contact with the afferent inner radial fibres. The efferent nerve fibres to the inner hair cells have almost exclusive synaptic contacts with afferent dendrites, while synaptic complexes with the inner hair cell are infrequently observed in the guinea pig. The efferent innervation of the inner hair cells shows a spiral distribution pattern. The efferent nerve endings of the outer hair cells have enormous contact areas at the base of the outer hair cell with multiple synaptic complexes. These efferent nerve endings contain numerous synaptic vesicles and many mitochondria. The distribution of efferent neurons to the outer hair cells is almost exclusively radial.

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CHAPTER 3

HAIR CELL COUNTS IN EXPERIMENTAL HYDROPS

Published in: Albers FWJ, Veldman JE, Huizing EH. Early hair cell loss in experimental hydrops. Ann Otol Rhinol Laryngol 1987; 96: 282-285.

Introduction

In spite of various histological and electrophysiological studies of the inner ear after surgical obliteration of the endolymphatic sac and duct little is understood about the mechanism involved in endolymphatic hydrops, which finally leads to the degeneration of the sensory cells in the organ of Corti. To elucidate the pathological mechanism involved in experimental hydrops, a systematic documentation of the early morphological changes in the organ of Corti after endolymphatic sac obliteration was made in the guinea pig. Routine light microscopy of histological sections has certain limitations for the investigation of early degenerative cellular phenomena. A quantitative assessment of outer and inner hair cell loss throughout the cochlea is unreliable with such routine histological techniques. Therefore, a qualitative and quantitative investigation of the early hair cell loss was carried out by means of interference differential (Nomarski) microscopy, according to the block-surface technique originally described by Spoendlin[1]. The submicroscopical studies described in Chapters 4-6 were based on the qualitative and quantitative data of this investigation.

Material and methods

Fifteen healthy, female albino guinea pigs (CPB-TNO, Zeist, The Netherlands; strain GpHi65 Himalayan, weight: 350-450 g) with a positive Preyer reflex were used for this experiment. Surgical obliteration of the endolymphatic duct and sac through an extradural posterior fossa approach[2,3] was performed under general Halothane® anaesthesia. After destruction of the intermediate part of the endolymphatic sac using a fine burr the remaining bony defect was filled with bone wax. The left ear was operated on, the right ear serving as a control. The animals were sacrified 1 month (N = 5), 2 months (N = 5) and 4 months (N = 5) after surgery.

Primary fixation was performed under sedation with sodium pentobarbital (60 mg/kg i.p.) by intracardiac perfusion with a tri-aldehyde fixative (3% glutaraldehyde, 2% formaldehyde, 1% acrolein and 2.5% DMSO in 0.08 mol/L sodium cacodylate buffer; pH 7.4). After temporal bone dissection additional immersion fixation with the same fixative was performed for 2 hours at room temperature.

Subsequently, the cochleae were decalcified in 10% EDTA-2Na (pH 7.4), post-fixated in 1% O_5O_4 at 4° C, dehydrated in graded alcohol and embedded in toto in Spurr's low viscosity resin.

After dividing the cochleae in a mid-modiolar plane, the distension of Reissner's membrane was examined by transillumination in the cochleae of the operated ears. Transverse sections were made and mounted on glass slides according to the block-surface technique[1]. (Fig. 1A-B).

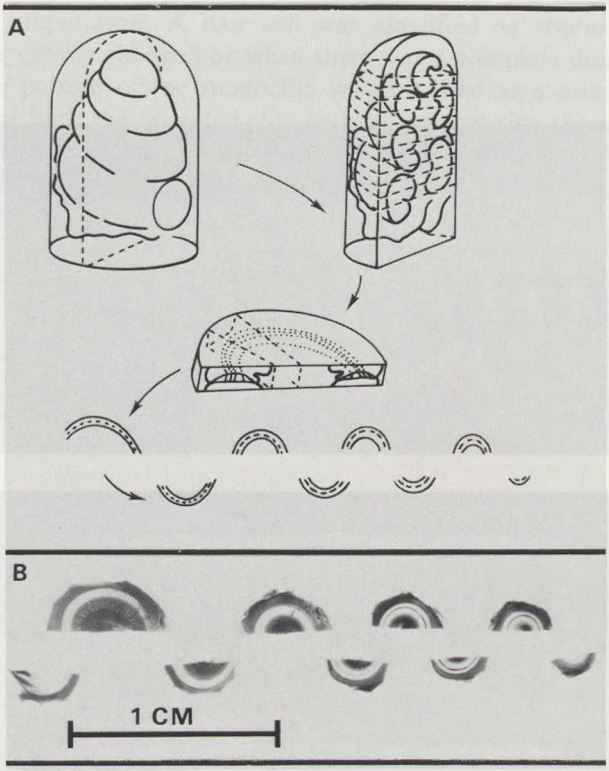

Fig.1.
(A). Schematic representation of the block-surface preparation.
(B). Original block-surface preparation of guinea pig cochlea.

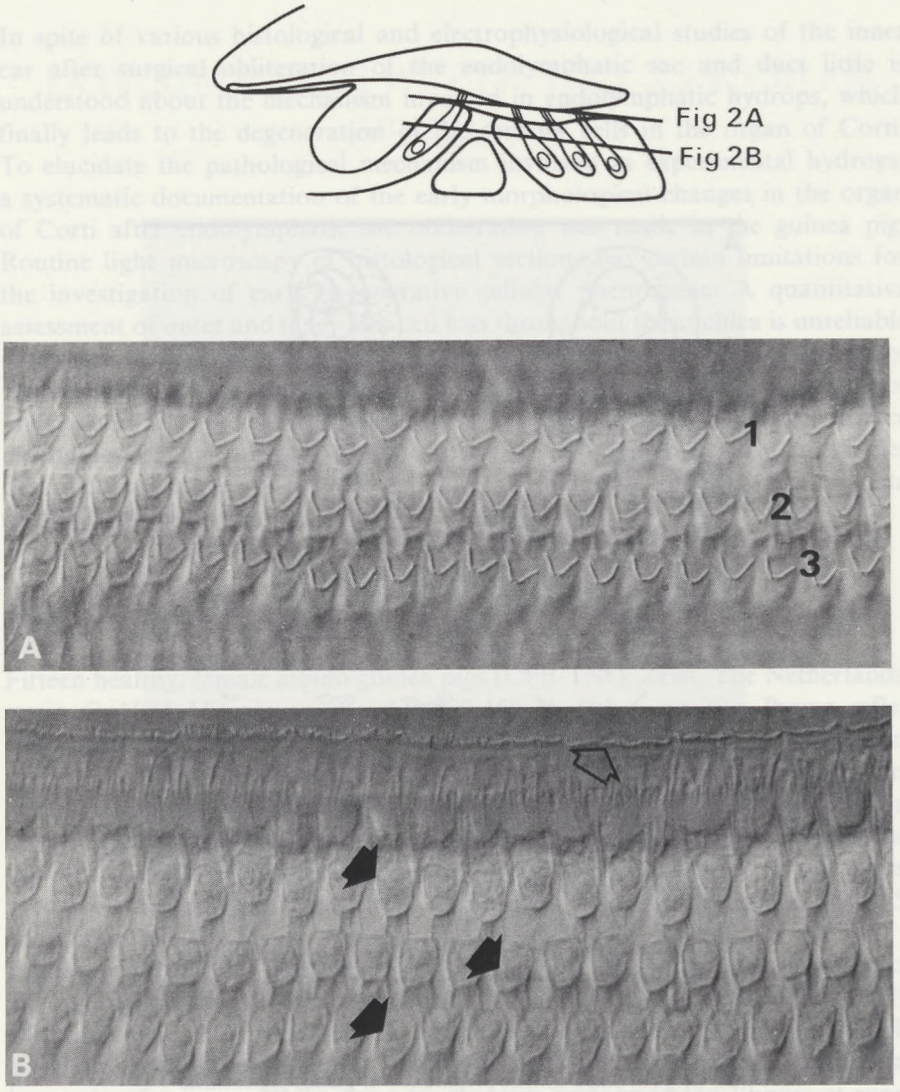

Fig.2.
(A). Stereocilia of the first (1), second (2) and third (3) rows of the outer cochlear hair cells. (400x).
(B). Stereocilia of the inner cochlear hair cells (unfilled arrow) and the cuticular plate of the first, second and third rows of the outer cochlear hair cells (arrows). (400x).

The organ of Corti of both the operated and control side was examined over the entire length of the basilar membrane using interference differential (Nomarski) microscopy. By means of this technique it is not only possible to study the general cellular pattern of the organ of Corti, both also to investigate stereocilia, cuticular plate and cell nuclei (Fig. 2A-B).
Data from the literature[4–7] on hair cell numbers in the healthy albino guinea pig were used for the quantitative evaluation. The presence or absence of the inner (IHC) and outer hair cells (OHC) was mapped in a cytocochleogram. A hair cell was classified as present when its appearance was normal as seen in the surface preparation. A hair cell was classified as absent when the appearance was grossly changed or when there was a complete disappearance of the cell. The pattern of the stereocilia was not used as a criterion, since the specimens of both the operated side and the control side showed distinct variations.
The preparation of the transverse sections unavoidably led to some hair cell loss. This preparation loss was calculated as the difference between the number of cells in the first row of the OHC's over the full length of the spiral organ[5] and the number of counted cells in the first row of the OHC's including the missing cells. The average preparation loss in this series of 30 cochleae was 9.1%.

Results

Cochlear transillumination
Examination by transillumination of the cochleae, divided in a mid-modiolar plane, showed a moderate to severe distension of Reissner's membrane in all operated ears, indicating an endolymphatic hydrops. All contralateral control ears had a normal appearance.

Qualitative cellular changes in the organ of Corti
Examination of the surface specimens showed a characteristic sequence of degenerative changes in the various elements of the organ of Corti.
Initial degeneration of the outer hair cells was marked by irregularities in the characteristic W-pattern of the stereocilia, followed by complete disappearance of the stereocilia.
At later stages of degeneration swelling of the nuclei and cell bodies became apparent in the outer hair cells. (Fig. 3).

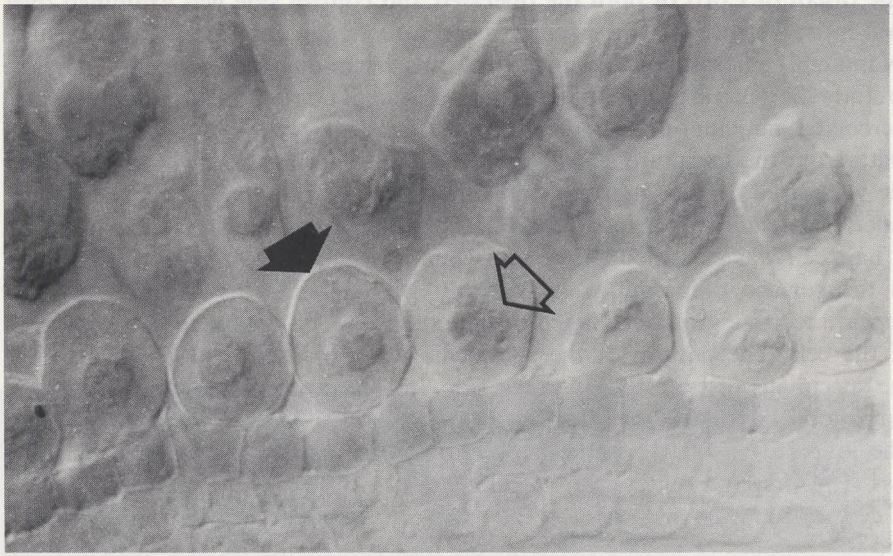

Fig. 3. Swollen nuclei (unfilled arrow) and cell bodies (arrow) in the first row of outer hair cells two months after endolymphatic sac obliteration. (400x).

The next phase was characterized by shrinkage of the cytoplasm and collapse of the cells, resulting in a phalangeal scar. These scars often showed a typical oval structure with an Y- or X-configuration, as frequently seen in hair cell degeneration. (Fig. 4).

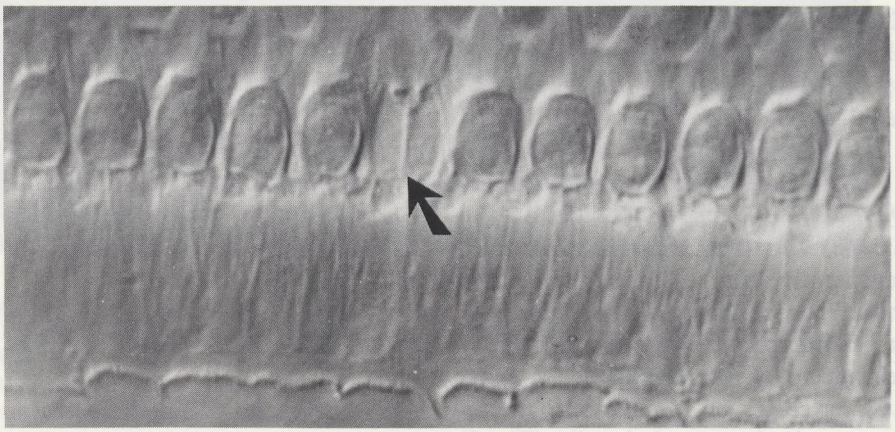

Fig. 4. Phalangeal scar (arrow) of outer hair cell two months after endolymphatic sac obliteration. (400x).

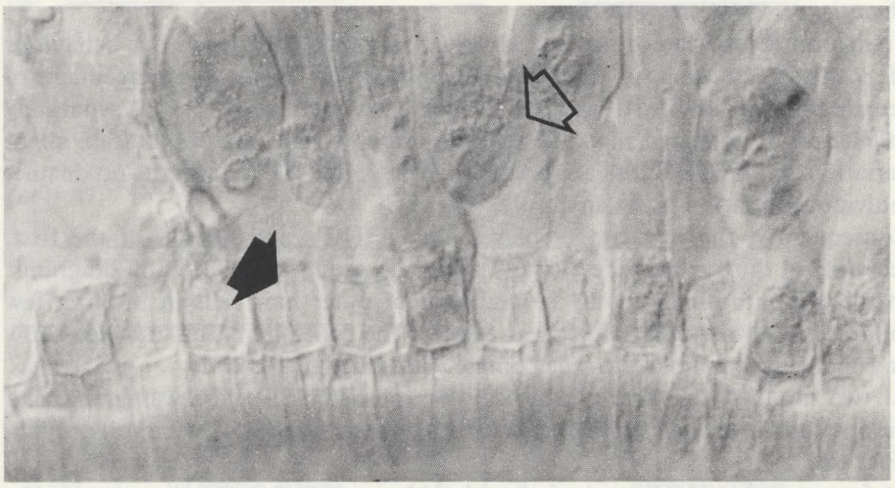

Fig. 5. Extensive loss of outer hair cells with phalangeal scars in the first row (arrow) and overgrowth of the phalangeal cells in the second and third rows (unfilled arrow) of the outer hair cells two months after sac obliteration. (400x).

Gradually the phalangeal scars concealed by overgrowth of the supporting cells resulting in an irregular cellular pattern on the surface of the organ of Corti. (Fig. 5)
Loss of outer and inner pillar cells was only seen in severely damaged specimens. (Fig. 6).

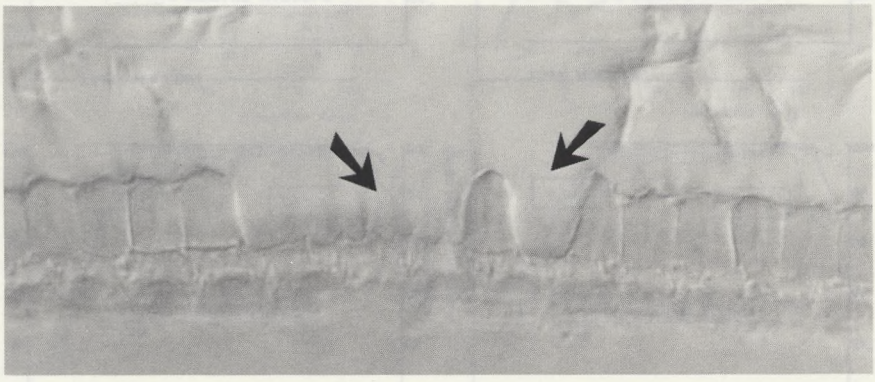

Fig. 6. Loss of outer pillar cells (arrows) in a severe damaged cochlea four months after endolymphatic sac obliteration. (400x).

Quantitative OHC and IHC loss

To determine whether or not an individual variation in degenerative behaviour of cochlear hair cells in each experimental group exists, the percentage of remaining inner and first, second and third row outer hair cells was separately calculated. It appeared that one and two months after sac obliteration slight variations in the OHC loss are present. (Figs. 7, 8). At four months no distinct individual variation exists any more. (Fig. 9).

Consequently, the percentages of remaining IHC's and OHC's of the first, second and third rows were averaged for each group and plotted in diagrams. In the 1-month animals only a small loss of outer hair cells in the first few millimeters of the apical turn was found. The inner hair cells did not show any changes as compared to the control ears. (Fig. 10A-B).

At two months a distinct OHC loss was apparent. This loss was greatest at the apex and gradually decreased towards the base. The IHC's were still normal. (Fig. 10C-D).

At four months OHC loss had considerably increased in the direction of the basal turn. The IHC's now showed a initial degeneration in the most apical part of the cochlea. (Fig. 10E-F).

In the 1-, 2- and 4-month hydrops groups no difference in degeneration could be found between the three rows of the outer hair cells.

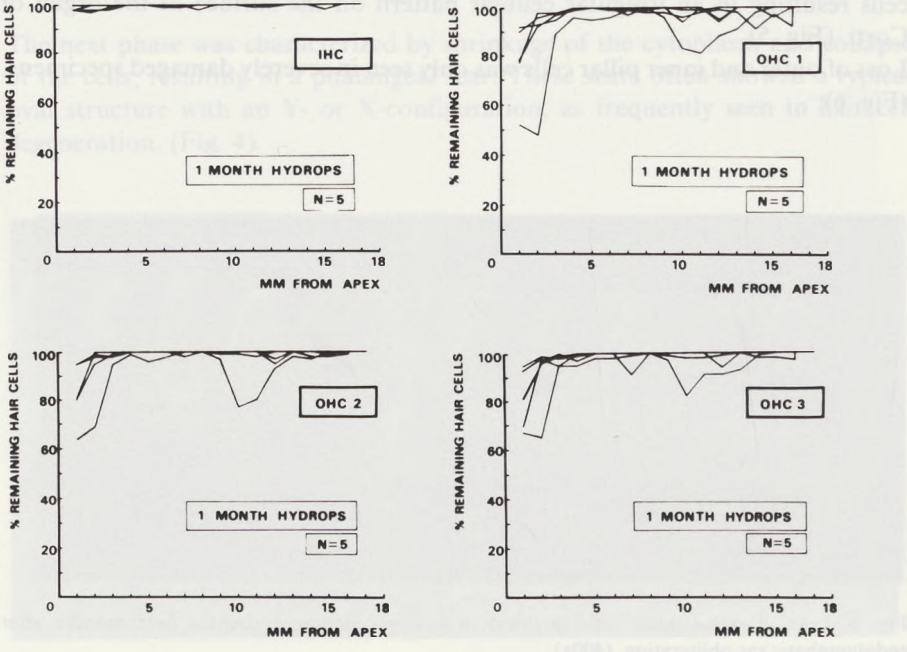

Fig. 7. Percentage remaining IHC and first, second and third row OHC one month after sac obliteration.

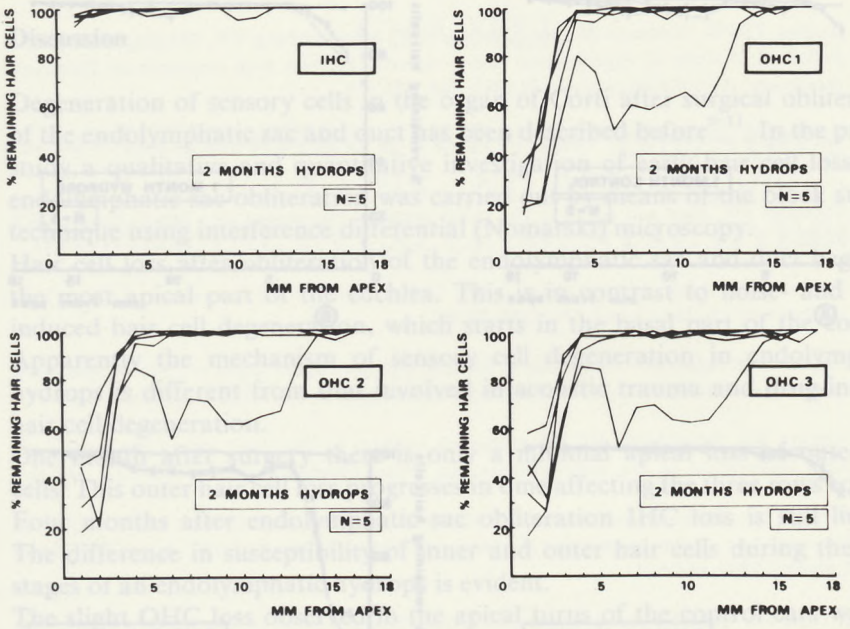

Fig. 8. Percentage remaining IHC and first, second and third row OHC two months after sac obliteration.

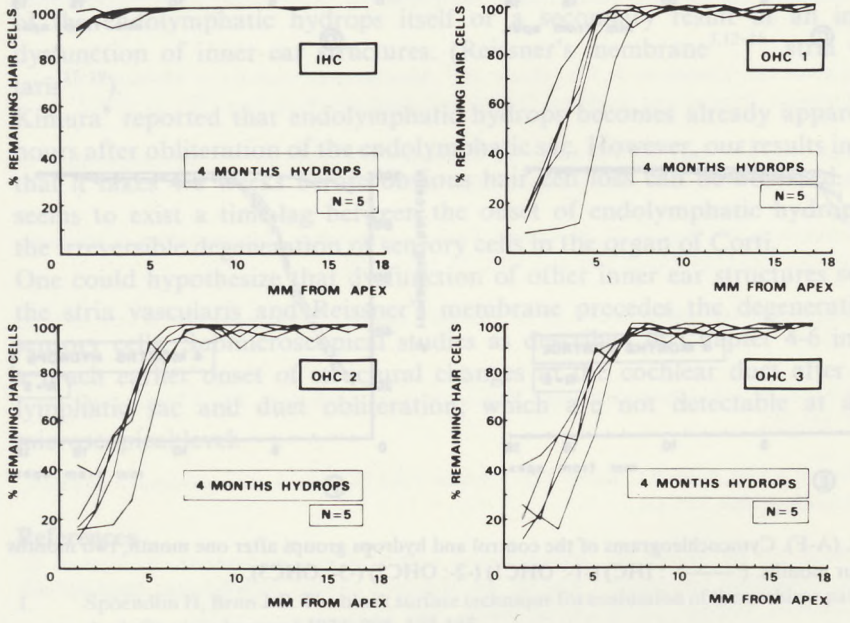

Fig. 9. Percentage remaining IHC and first, second and third row OHC four months after sac obliteration.

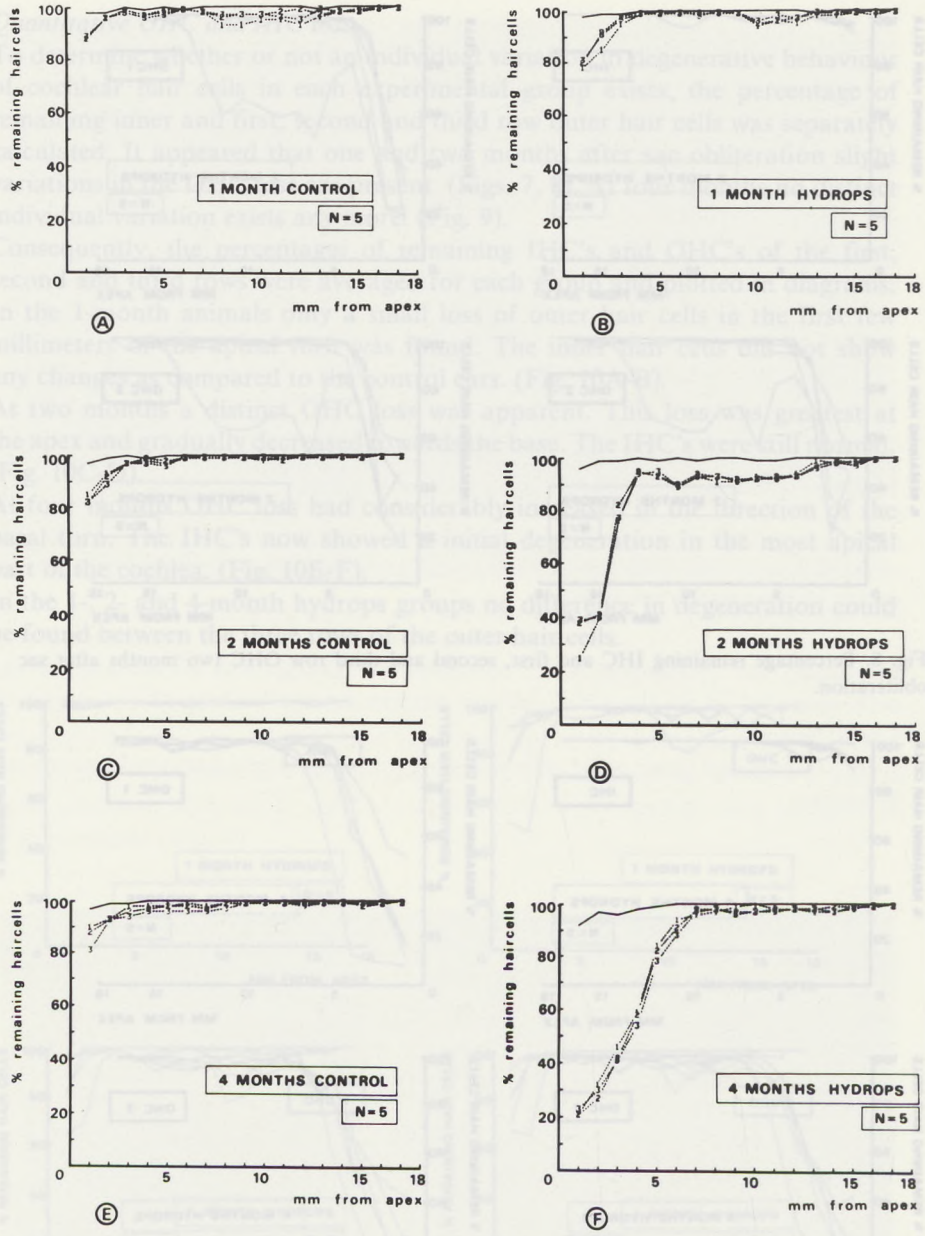

Fig. 10. (A-F). Cytocochleograms of the control and hydrops groups after one month, two months and four months. (——— : IHC) (-1-: OHC1) (-2-: OHC2) (-3-: OHC3).

Discussion

Degeneration of sensory cells in the organ of Corti after surgical obliteration of the endolymphatic sac and duct has been described before[8-11]. In the present study a qualitative and quantitative investigation of early hair cell loss after endolymphatic sac obliteration was carried out by means of the block surface technique using interference differential (Nomarski) microscopy.

Hair cell loss after obliteration of the endolymphatic sac and duct begins in the most apical part of the cochlea. This is in contrast to noise- and drug-induced hair cell degeneration, which starts in the basal part of the cochlea. Apparently the mechanism of sensory cell degeneration in endolymphatic hydrops is different from that involved in acoustic trauma and drug-induced hair cell degeneration.

One month after surgery there is only a minimal apical loss of outer hair cells. This outer hair cell loss progresses in time affecting the three rows equally. Four months after endolymphatic sac obliteration IHC loss is still limited. The difference in susceptibility of inner and outer hair cells during the early stages of an endolymphatic hydrops is evident.

The slight OHC loss observed in the apical turns of the control ears was not related to age. It is probably an artefact, caused by inadequate fixation of the most apical part of the cochlea.

It is still unclear whether the degeneration of sensory cells is a primary effect of the endolymphatic hydrops itself or a secondary result of an integral dysfunction of inner ear structures. (Reissner's membrane[3,12-16], stria vascularis[17-19]).

Kimura[8] reported that endolymphatic hydrops becomes already apparent 24 hours after obliteration of the endolymphatic sac. However, our results indicate that it takes 4-8 weeks before obvious hair cell loss can be observed. There seems to exist a time-lag between the onset of endolymphatic hydrops and the irreversible degeneration of sensory cells in the organ of Corti.

One could hypothesize that dysfunction of other inner ear structures such as the stria vascularis and Reissner's membrane precedes the degeneration of sensory cells. Submicroscopical studies as described in Chapter 4-6 indicate a much earlier onset of structural changes in the cochlear duct after endolymphatic sac and duct obliteration, which are not detectable at a light microscopical level.

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CHAPTER 4

ULTRASTRUCTURE OF THE STRIA VASCULARIS AND REISSNER'S MEMBRANE IN EXPERIMENTAL HYDROPS

Published in: Albers FWJ, De Groot JCMJ, Veldman JE, Huizing EH. Ultrastructure of the stria vascularis and Reissner's membrane in experimental hydrops. Acta Otolaryngol (Stockh) 1987; 104: 202-210.

Introduction

The histopathological changes in the cochlear duct after endolymphatic sac obliteration are characterized by an initial loss of outer hair cells followed by inner hair cell loss, both starting in the apical part of the cochlea[1-3]. Pathological changes in the stria vascularis and Reissner's membrane are also initiated in the apex of the cochlea[1,2].

The stria vascularis is considered to play a significant role in the osmoregulation and volume control of the inner ear fluids[4]. Reissner's membrane does not only form a barrier between the endolymphatic and perilymphatic compartment but is very likely also involved in ion and fluid transport between the two compartments[2,5]. Kimura et al.[6] demonstrated that obliteration of the ductus reuniens in the guinea pig produces a cochlear hydrops with a saccular collapse and a normal utricle. This experiment gives support to the theory of a longitudinal endolymph flow from the stria vascularis through the scala media and ductus reuniens to the saccule and endolymphatic sac and duct[7]. According to the radial flow theory endolymph is formed from perilymph by passing through Reissner's membrane and is locally absorbed by the stria vascularis[8]. Lawrence[9] introduced a unified concept of these theories in which the fluid flow is longitudinal with a radial exchange of solutes along the ducts.

In order to understand better the pathophysiology in experimental endolymphatic hydrops further investigation of inner ear structures involved in the control of the cochlear fluid balance is needed. In this experiment a time-sequence study is presented of the early ultrastructural changes in the stria vascularis and Reissner's membrane after surgical obliteration of the endolymphatic duct and sac in the guinea pig.

Material and methods

Nine healthy, female albino guinea pigs (CPB-TNO, Zeist, The Netherlands; strain GpHi65 Himalayan, weight: 350-450 g) with a positive Preyer reflex were used for this experiment. Surgical obliteration of the endolymphatic sac and duct was performed on the left ear, whereas the right ear served as control. The animals were subsequently sacrified one month (N = 3), two months (N = 3) and three months (N = 3) after sac obliteration.

Primary fixation was performed by intracardiac perfusion with tri-aldehyde fixative (3% glutaraldehyde, 2% formaldehyde, 1% acrolein and 2.5% DMSO in 0.08 mol/L sodium cacodylate buffer; pH 7.4). After temporal bone dissection, the cochleae were immersed in the same fixative for an additional two hours at room temperature. The cochleae were decalcified in 10% EDTA 2-Na (pH 7.4) and postfixed in 1% OsO_4 1% $K_4Ru(CN)_6$ (ICN Pharmaceuticals Inc./New York) in 0.1 M sodium cacodylate-HCl buffer; pH 7.4 for 2 h at 4°C. Dehydration was performed in a graded ethanol, 2,2-dimethoxypropane,

propylene oxide series and the cochleae were embedded in toto in Spurr's low-viscosity resin. The cochleae of both the operated and the control sides were divided along a midmodiolar plane and re-embedded in Spurr's low-viscosity resin.
From one-half of each cochlea semithin one-micron sections were obtained, stained with methylene blue and azure B and examined by light microscopy. From the other half of each cochlea ultrathin sections were made at three different levels in the cochlea using an LKB Ultrotome V and mounted on Pioloform-coated, single slot copper grids. The ultrathin sections were stained with methanolic uranyl acetate and Reynold's lead citrate and examined by transmission electron microscopy (Zeiss EM 109; 50kV).

Results

All cochleae of the operated side showed a distension of Reissner's membrane, indicating an endolymphatic hydrops. (Fig. 1). The most extensive dilatation of Reissner's membrane was found in the animals with the longest postoperative period (three months).

Stria vascularis
One month after endolymphatic sac obliteration a mild intercellular edema was observed in the marginal and intermediate cell layers of the stria vascularis in the apical turn. (Fig. 2). After the same period an increase in the number of coated and non-coated vesicles in the apical (endolymphatic) side of the marginal cells was found in the middle turns and to some extent also in the basal turns of the operated ears. (Fig. 3). A slight swelling of mitochondria was present in the marginal cells of the middle and basal turns.
After two months vacuolization by fusion of non-coated vesicles had developed at the basolateral side of the marginal cells in the apical part of the cochlea (Fig. 4). Severe local edema with decrease of cytoplasmic processes in the marginal and intermediate cells was observed. (Fig. 5). The most damaged specimen showed protusion of marginal cells into the endolymphatic space. In the three months animals atrophy of the intermediate cells and to a lesser extent of the marginal cells was observed in the most apical part of the cochlea. In one animal the basal cells were in direct contact with the marginal cells (Fig. 6).
The tight junctions between the marginal cells seemed always intact. No pathology of the basal cell layer was found. A slight edema between the marginal and intermediate cell layer started to develop in the lower turns of the cochlea.

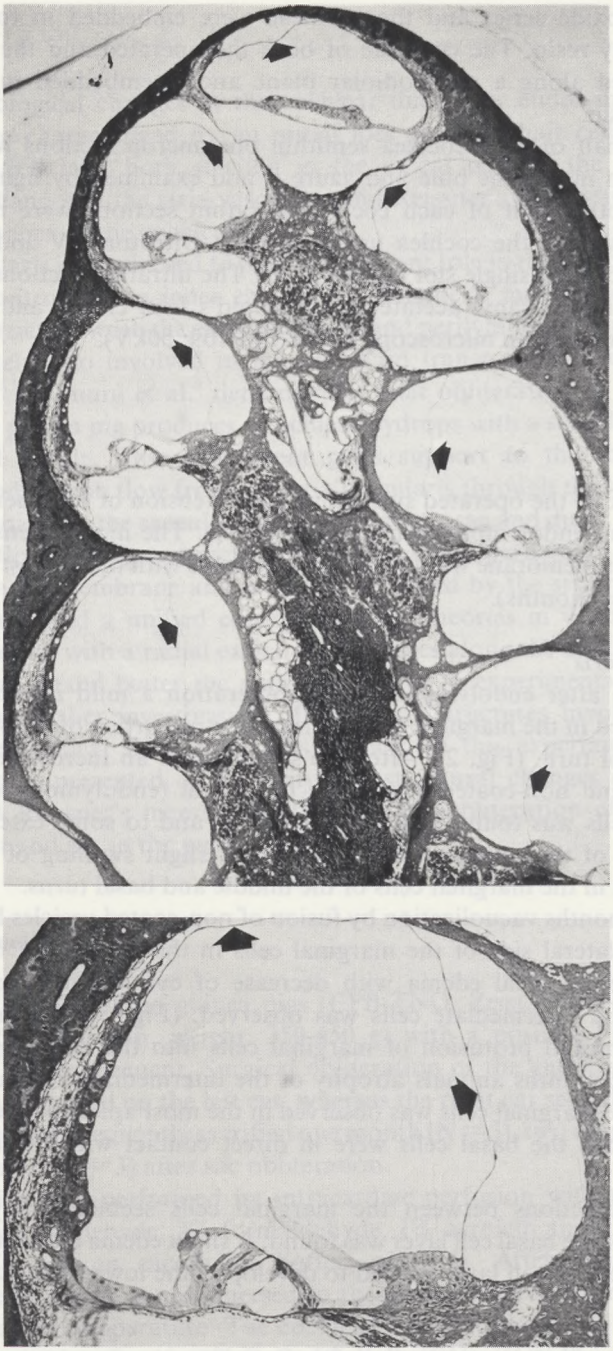


Fig. 1. Distension of Reissner's membrane (arrows) three months after surgical obliteration of the endolymphatic sac and duct.

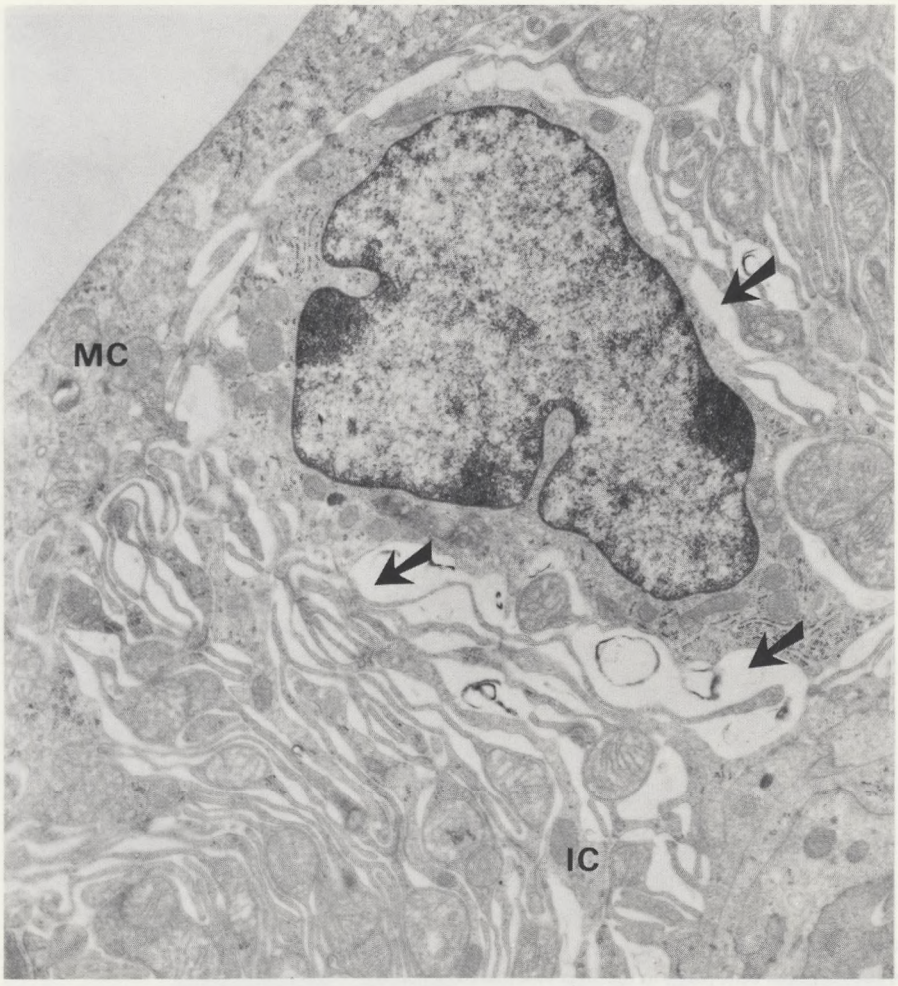


Fig. 2. Mild intercellular edema (arrows) between the marginal (MC) and intermediate (IC) cells of the stria vascularis. (20,000x).

Reissner's membrane

One month after obliteration extensive gaps in the mesothelial cell layer were found in the apical turns (Fig. 7). The epithelial cells did not show intracellular pathology.

After two months the mesothelial cell layer was missing over large areas not only in the apical part of the cochlea, but also in the middle turns. Slight vacuolization was found in the epithelial cells of the apical turns of the cochlea.

Three months postoperatively the defects in the mesothelial cell layer had

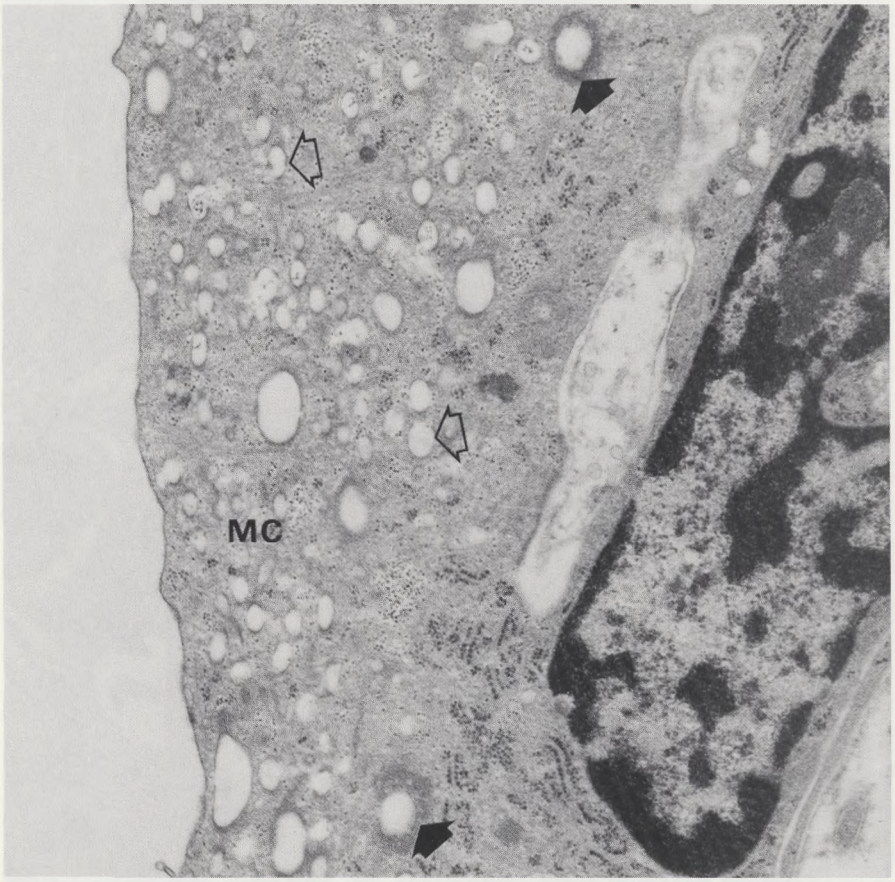


Fig. 3. Increase of coated (arrows) and non-coated (unfilled arrows) vesicles in the apical part of the marginal cells (MC) in the stria vascularis. (33,000x).

progressed towards the basal turn. Severe vacuolization with local disruption of mitochondria was observed in the epithelial cells of the apical turns (Fig. 8). The cell junctions of the epithelial cells had a normal appearance in all animals without widening of the intercellular clefts. The basement membrane seemed to be intact, but had locally a fuzzy appearance in the apical region. None of the animals showed a rupture of Reissner's membrane.

Certain epithelial cells in Reissner's membrane showed a condensation of cytoplasm with an increased electron density of the mitochondria. These electron dense epithelial cells were found more frequently in the most distended parts of Reissner's membrane.

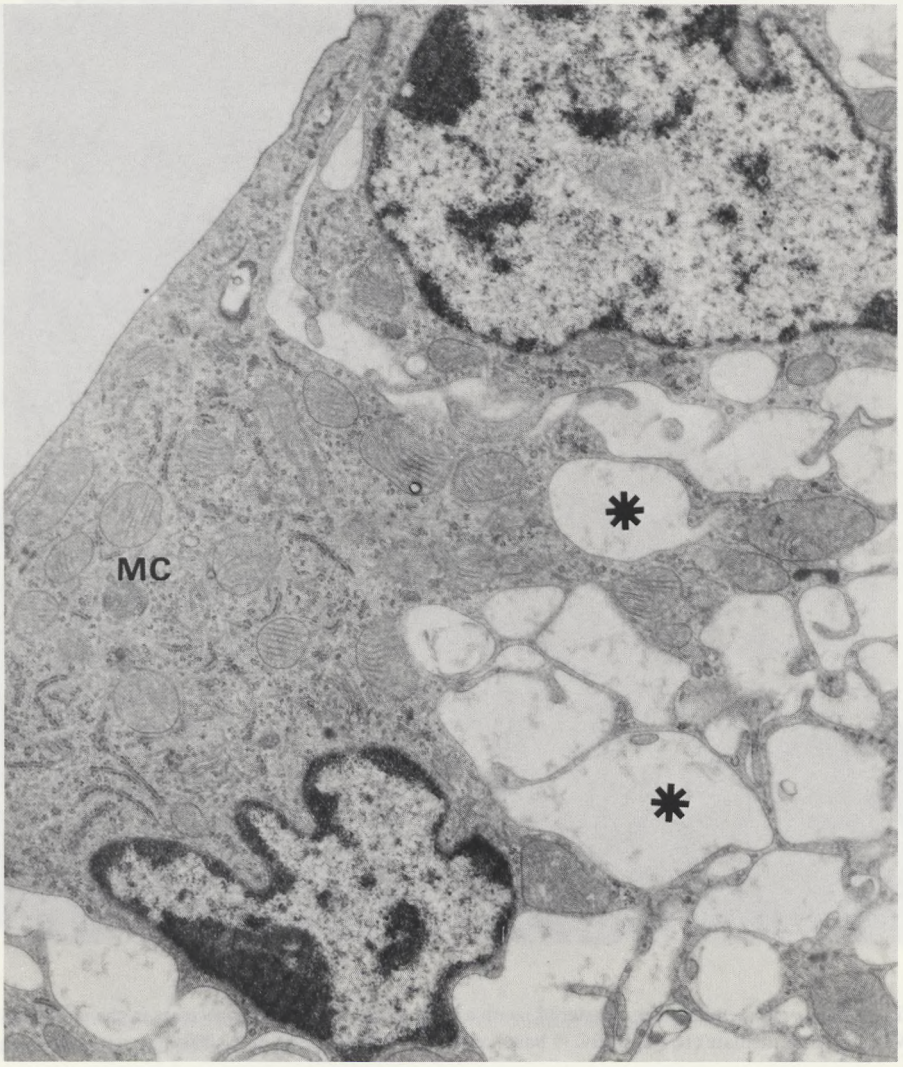


Fig. 4. Vacuolization (asterisk) at the basolateral side of the marginal cells (MC) of the stria vascularis. (20,000x).

Flattening of both the epithelial and the mesothelial cell layer was a common finding in the distended Reissner's membrane.
Connections between the lining cells in the wall of the scala vestibuli and the mesothelial cells of Reissner's membrane were found in severely hydropic animals. (Fig. 9).

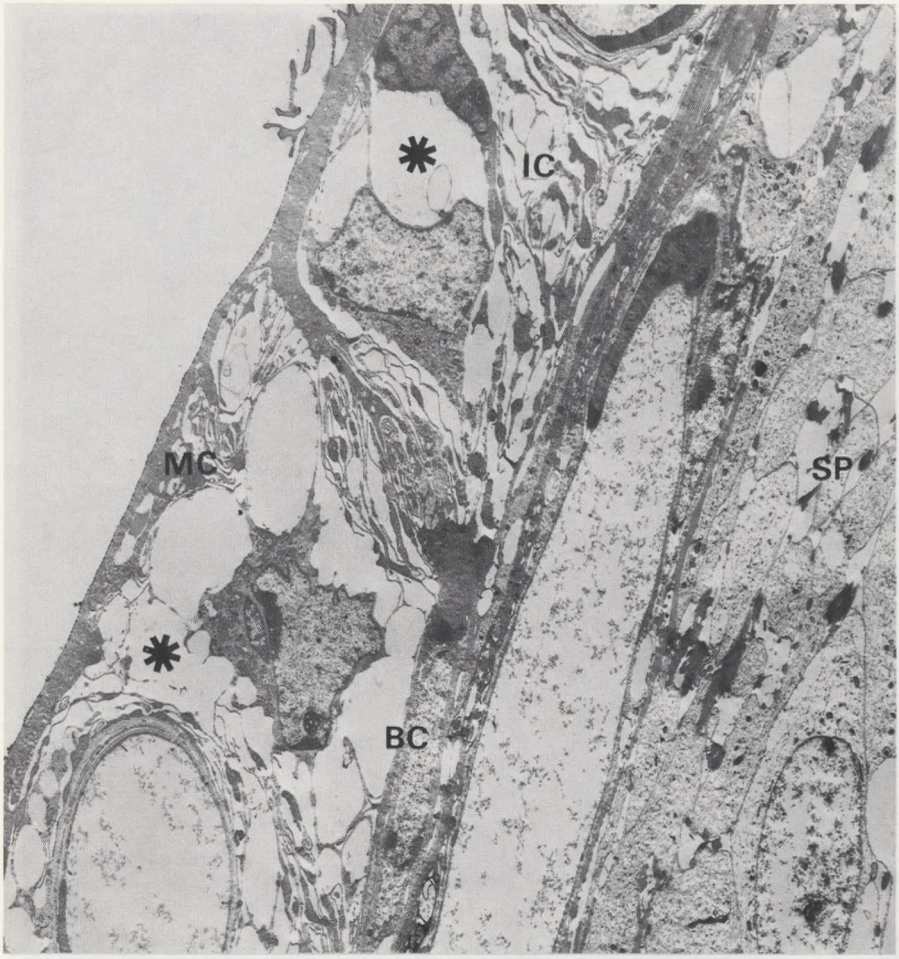


Fig. 5. Severe intercellular edema (asterisk) with a decrease of cytoplasmic processes in the marginal (MC) and intermediate (IC) cells. BC = basal cells, SP = spiral ligament. (3,000x).

In one three months animal a folding of Reissner's membrane with extension into the scala media was observed in the most apical part of the cochlea. (Fig. 10A-B). The mesothelial cell layer on both sides of the folding were fused to each other, while the epithelial cells showed a pronounced increase in electron density. The cell junctions between the epithelial cells were intact. The basement membrane had an irregular and fuzzy appearance along the entire folding.

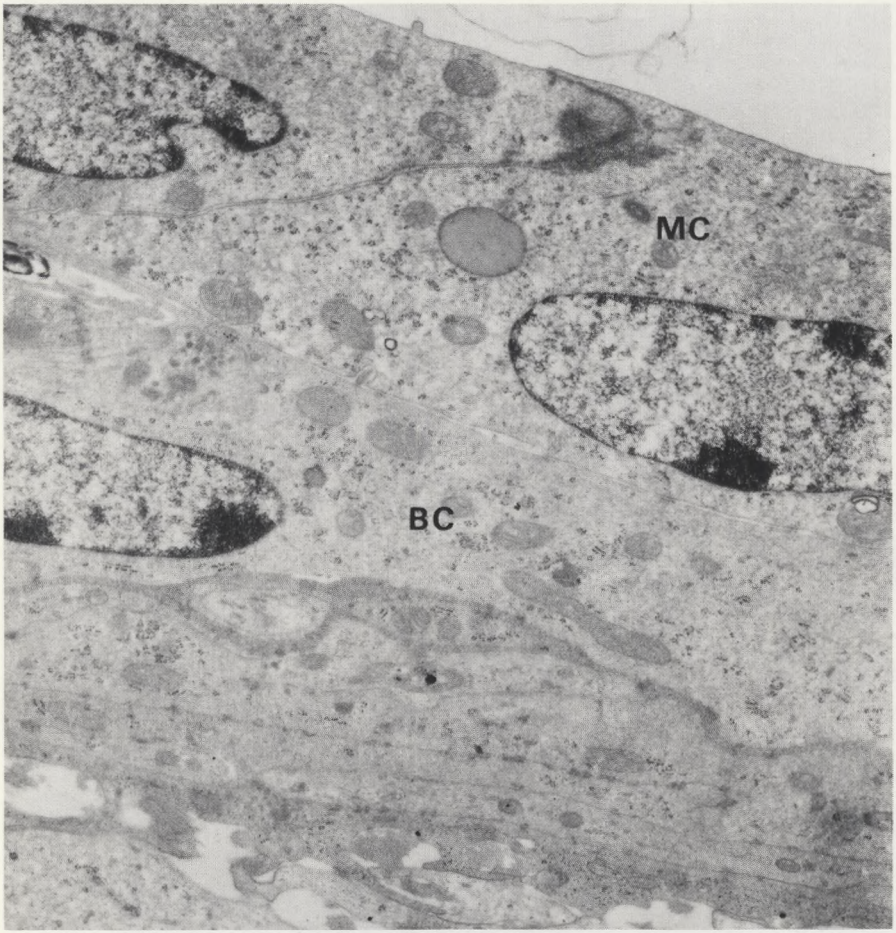


Fig. 6. Atrophy of the intermediate cells with direct contact between the marginal (MC) and basal (BC) cells. (20,000x).

Spiral prominence

Submicroscopical examination of the spiral prominence did not show any pathological alterations compared to the control cochleae.

Discussion

A striking similarity exists between the histopathological alterations of the stria vascularis in endolymphatic hydrops and the histopathology as observed

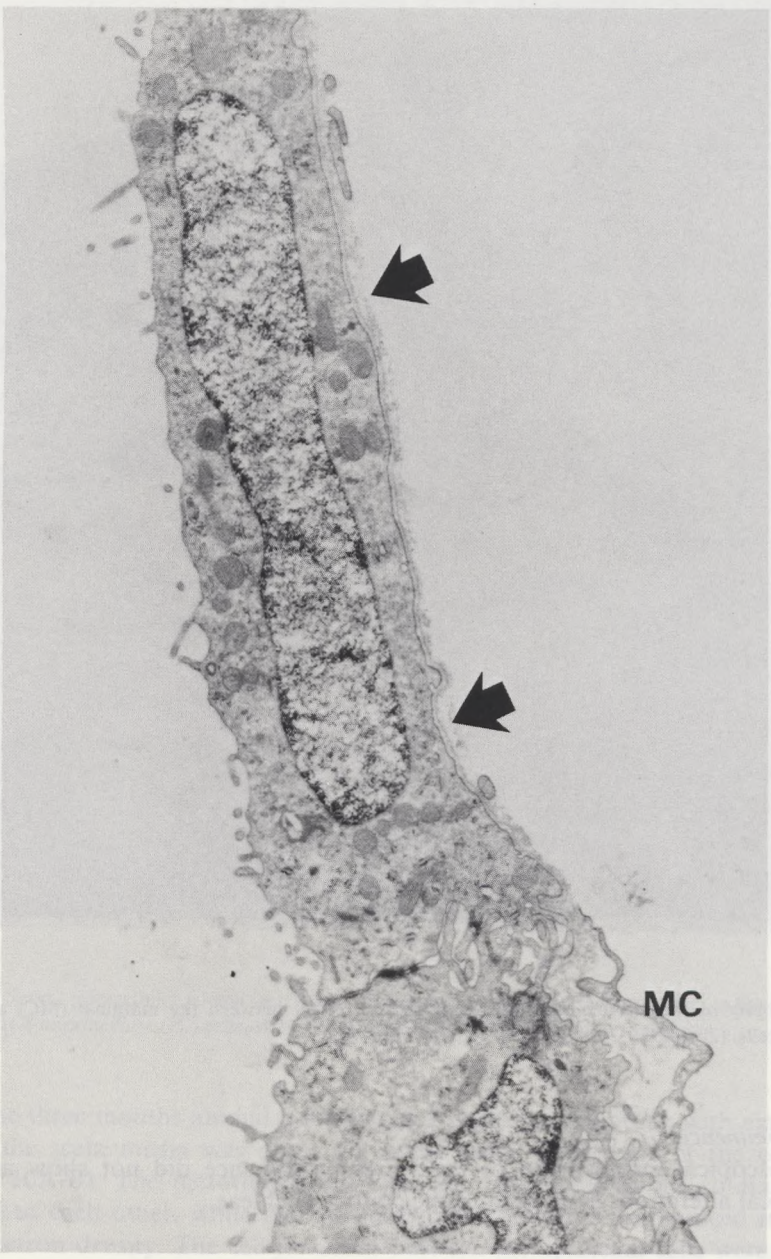


Fig. 7. Extensive gaps (arrows) in the mesothelial cell layer (MC) of Reissner's membrane. (12,000x).

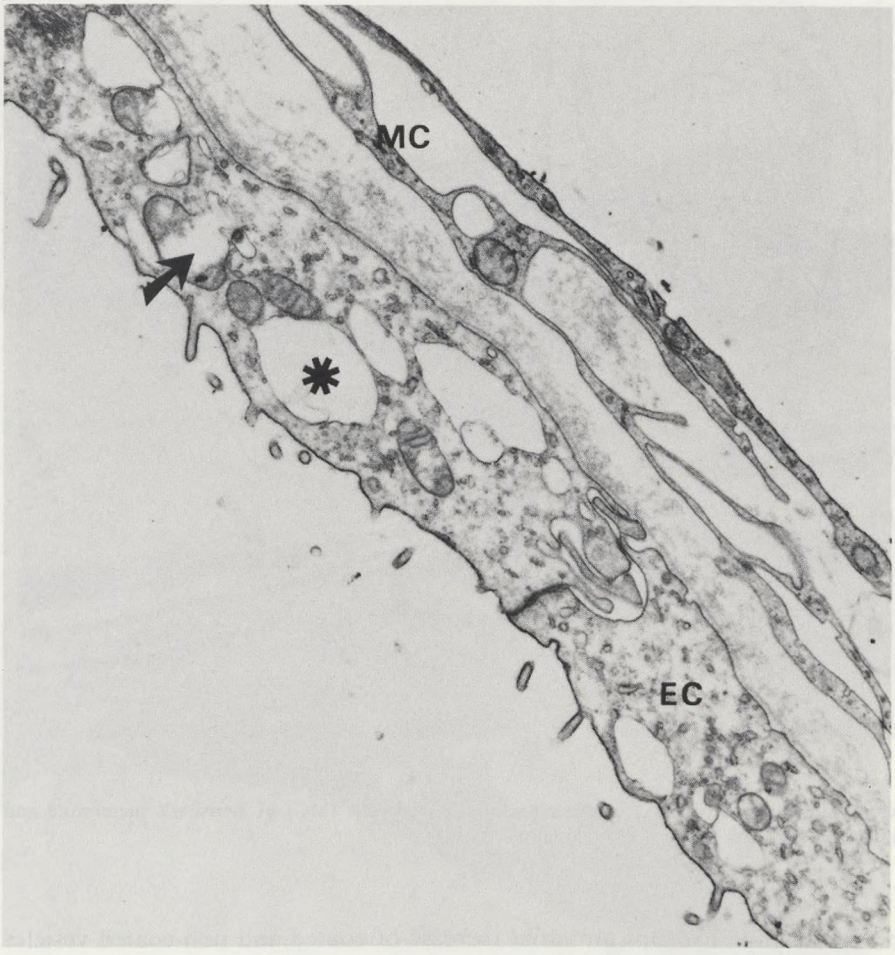


Fig. 8. Severe vacuolization (asterisk) with disruption of mitochondria (arrow) in the epithelial cells (EC) of Reissner's membrane. MC = mesothelial cell. (20,000x).

under other conditions in which the fluid balance is disturbed, as in loop diuretic ototoxicity[10,11] and after mechanical rupture of Reissner's membrane[12]. However, pathology of the stria vascularis in experimental hydrops[1,2] starts in the apex of the cochlea, whereas loop diuretics[10,11] have their initial effect in the basal coil. The stria vascularis is not only considered to be the site of endolymph production in the cochlea, but it may also have the capacity to resorb endolymph[8,9]. A great diversity of pathological conditions (metabolic, enzymatic or osmotic disturbances) probably interfere with the secretion/reabsorption capacity of the stria vascularis, which may ultimately lead to the histopathological changes mentioned earlier.

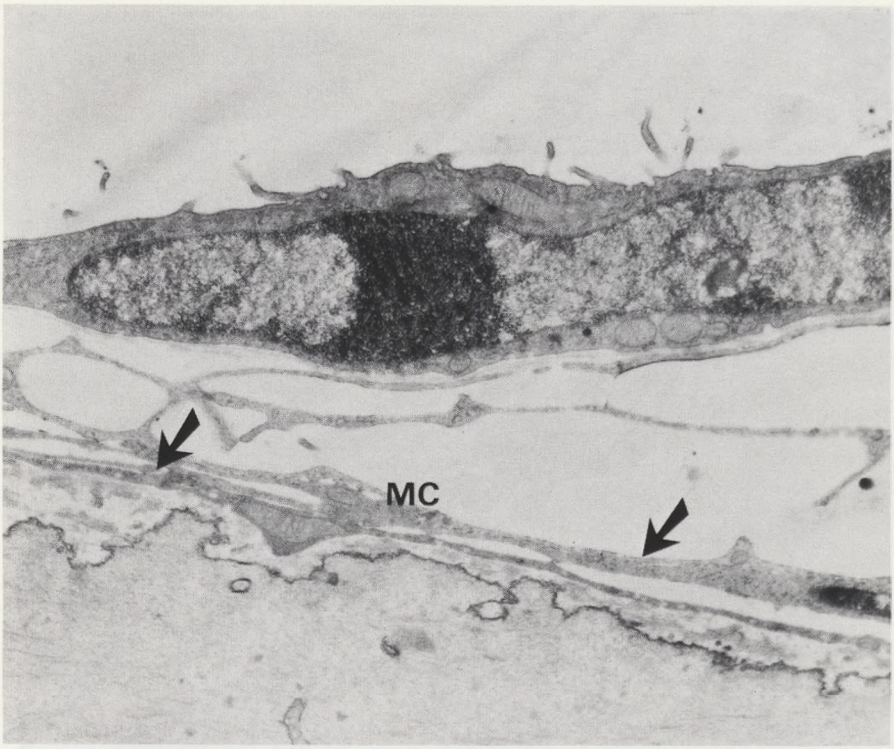


Fig. 9. Connections (arrows) between the mesothelial cells (MC) of Reissner's membrane and the wall of the scala vestibuli. (12,000x).

In experimental hydrops an initial increase of coated and non-coated vesicles in the apical region of the strial marginal cells is followed by intercellular edema between the marginal and intermediate cell layers. The next phase shows vacuolization formed by fusion of uncoated vesicles at the basolateral side of the marginal cells.

Voute et al.[13] demonstrated an increased sodium transport through frog skin in association with vacuolization of the lining epithelial cells and an increase of intercellular volume. A similar mechanism may well be operational in experimental hydrops, as earlier suggested for diuretic ototoxicity[11]. Obliteration of the endolymphatic duct and sac in the guinea pig causes an accumulation of endolymph resulting in an endolymphatic hydrops. Accumulation of endolymph could lead to an increase in reabsorptive activity of the stria vascularis in order to restore the disturbed fluid balance. This may result in an increased ion and/or fluid transport through the marginal cells with exocytosis at the basolateral side of the marginal cells. Expanding intercellular edema between

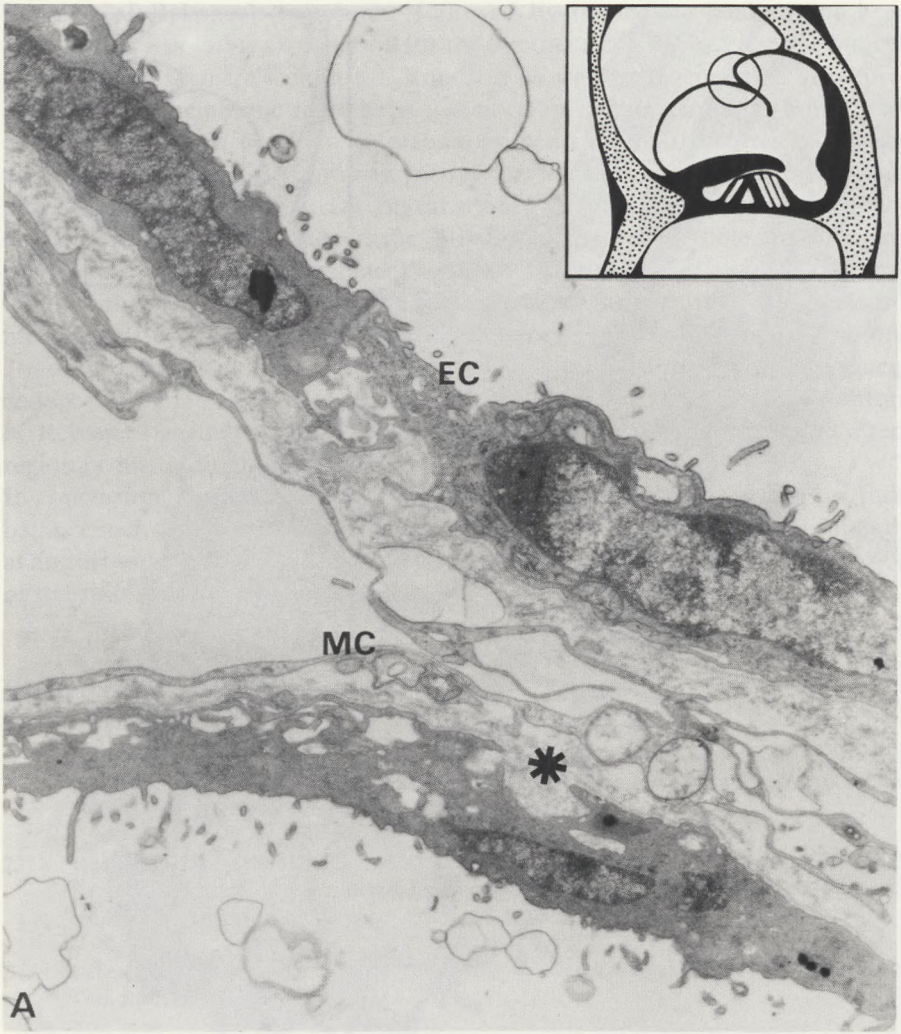
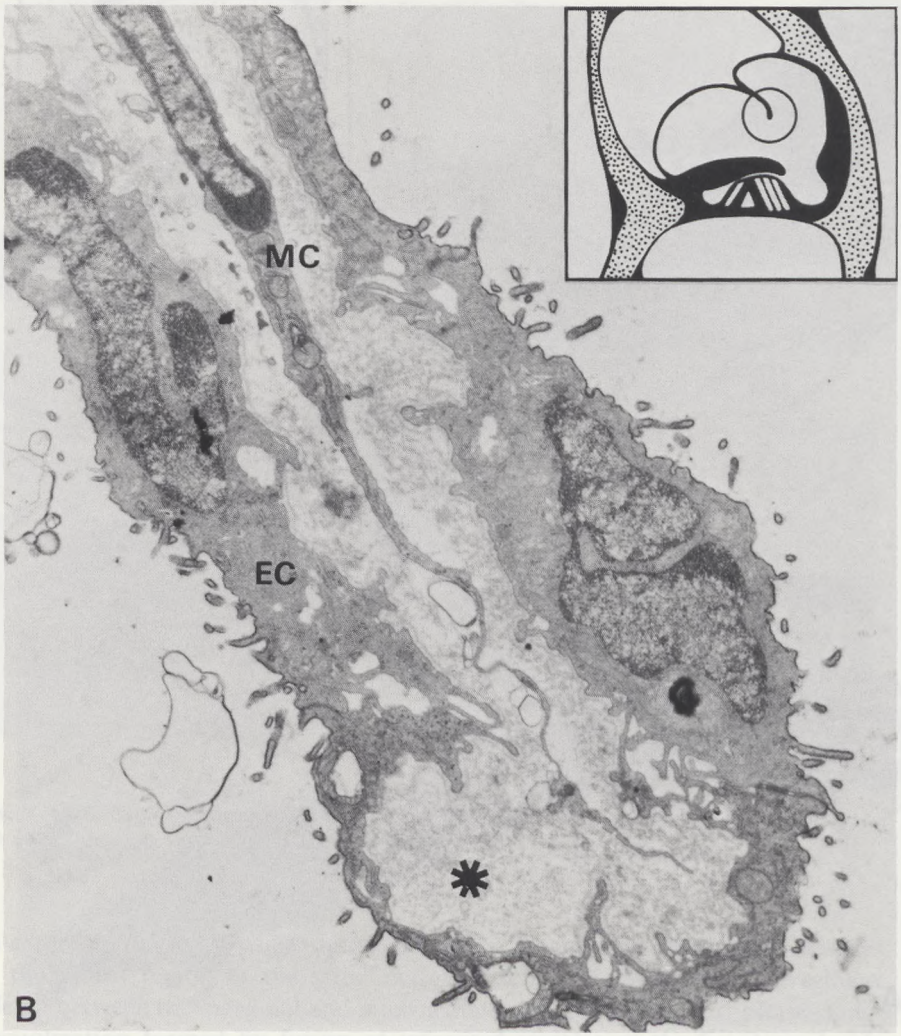


Fig 10 A-B. Folding of Reissner's membrane with fusion of the mesothelial cell layer (MC), increased density of the epithelial cells (EC) and a fuzzy appearance of the basement membrane (asterisk). (12,000x).

the intermediate and marginal cell layers could ultimately cause irreversible atrophy of the intermediate and marginal cells.

The wide gaps in the mesothelial cell layer of Reissner's membrane found in experimental hydrops are in accordance with earlier reports[1,2]. Disconnections between adjacent mesothelial cells have also been described in the normal guinea pig cochlea[14]. Indications of an increased permeabity of Reissner's membrane such as a widening of the intercellular junctions between the epithelial cells



were not found, although the basement membrane showed locally a fuzzy appearance.

An increased electron density in certain epithelial cells of Reissner's membrane was found in the most distended parts of Reissner's membrane. This phenomenon has also been reported in human endolymphatic hydrops[15], after kanamycin administration[16], and in acute atoxyl intoxication[17] possibly indicating a degenerative process. The disturbance in cochlear fluid balance in experimental hydrops might directly affect the transport activity of Reissner's membrane resulting in a dysfunction of the normal fluid transport, leading successively to degeneration of Reissner's membrane.

Rupture of Reissner's membrane followed by immediate healing has been proposed as a possible explanation for the fluctuant hearing loss and the attacks of vertigo in Menière's disease[18]. Ruptures as well as regenerative structures in Reissner's membrane have been described in human temporal bones with endolymphatic hydrops[19]. In animal experiments repair structures have been observed after surgical rupturing of Reissner's membrane and after acoustic overstimulation[20]. Light microscopical studies after surgical obliteration of the endolymphatic sac and duct in the guinea pig suggest possible ruptures and repair structures in Reissner's membrane[2,21]. The evidence at an ultrastructural level is still controversial. In the present study no ruptures of Reissner's membrane were found. The folding of Reissner's membrane observed in one 3-month animal could be a repair structure after a rupture and subsequent collapse. In contrast to the observations obtained after mechanical rupture of Reissner's membrane[12] no extensive degeneration was found in the direct region of the folding.

In conclusion, structures significant for the maintenance of the inner ear fluid balance such as the stria vascularis and Reissner's membrane participate already at an early stage in the sequence of events which occur after surgical obliteration of the endolymphatic sac and duct.

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CHAPTER 5

ULTRASTRUCTURE OF THE ORGAN OF CORTI IN EXPERIMENTAL HYDROPS

Published in: Albers FWJ, De Groot JCMJ, Veldman JE, Huizing EH. Ultrastructure of the organ of Corti in experimental hydrops. Acta Otolaryngol (Stockh) 1988; 105: 281-291.

Introduction

Recent advances in submicroscopical techniques have brought forward different new aspects of inner ear histophysiology and pathophysiology. Improved methods of tissue fixation and specimen processing in electron microscopy have led to a better preservation and an increased contrast-enhancement of cochlear tissues[1]. An organized linkage system of the stereociliary bundles has recently been demonstrated using special fixation techniques[2,3]. Special cytochemical staining methods have revealed the presence of cell coat material on the apical surfaces of the sensory cells, which has been proposed to play an active role in the auditory transduction process[4–8]. Furthermore, detailed submicroscopical studies of the outer hair cells indicate the existence of an interconnecting tubular system consisting of subsurface cisternae and lamellar bodies, which may be involved in neurotransmitter transport or in mediating excitation-contraction coupling[7,9,10].

The recent available information has led to new and interesting concepts with regard to the pathophysiology of acoustic trauma and aminoglycoside ototoxicity[11]. In this study, a detailed submicroscopical investigation of the initial changes in the organ of Corti after obliteration of the endolymphatic sac and duct is performed with special emphasis on the above-mentioned features to provide a basis for new concepts regarding the pathophysiology of experimental hydrops.

Material and methods

Nine healthy, female albino guinea pigs (CPB-TNO, Zeist, The Netherlands; strain GpHi65 Himalayan, weight: 350-450 g) with a positive Preyer reflex were used for this experiment. Surgical obliteration of the endolymphatic sac and duct through the extradural posterior fossa approach was performed on the left ear, whereas the right ear served as control. The animals were sacrified one month (N = 3), two months (N = 3) and three months (N = 3) after sac obliteration.

Tissue fixation and specimen processing for light and electron microscopy was performed as described in Chapter 4[12]. The cochleae of both the operated side and the contralateral control side were divided along a midmodiolar plane and re-embedded in Spurr's low-viscosity resin.

From one-half of each cochlea semithin one-micron sections were cut with glass knives on a Jung 1140 autocut microtome, stained with methylene blue and azure B in borax and examined by light microscopy. From the other half of each cochlea ultrathin sections were cut with a diamant knife at three different levels in the cochlea using an LKB Ultrotome V. The ultrathin sections were stained with methanolic uranyl acetate and Reynold's lead citrate and examined by transmission electron microscopy (Zeiss EM 109; 50kV).

Results

All cochleae of the operated side showed a distension of Reissner's membrane, indicating an endolymphatic hydrops. The most extensive dilatation of Reissner's membrane was found in the animals with the longest postoperative period (three months).

Glycocalyx

An extensive contrast-staining of the cochlear duct glycocalyx was observed in the 1-month hydrops cochleae and in all control cochleae. The apical surfaces of the sensory cells including the cross-links of the stereocilia were covered with an electron-dense layer. (Fig.1). There were no significant differences in

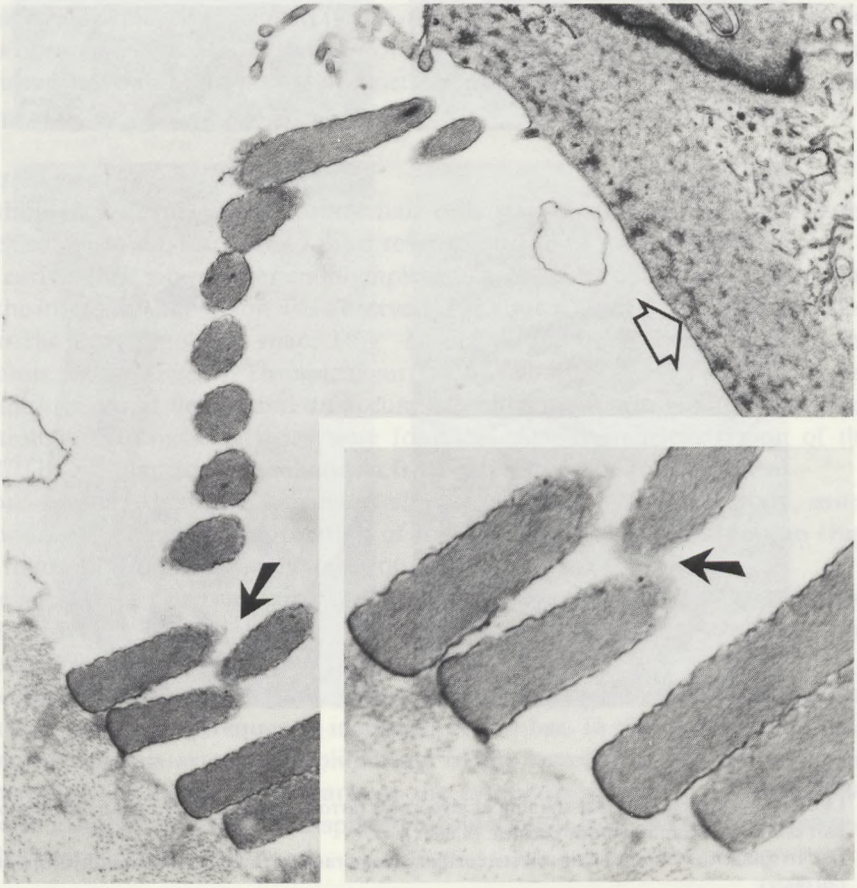

Fig. 1. Glycocalyx (unfilled arrow) of the endolymphatic surface of outer hair cell with cross-links (arrow) between the stereocilia in a 1-month hydrops cochlea. (20,000x). The inset shows a detail of the stereociliary cross-links (arrow). (33,000x).

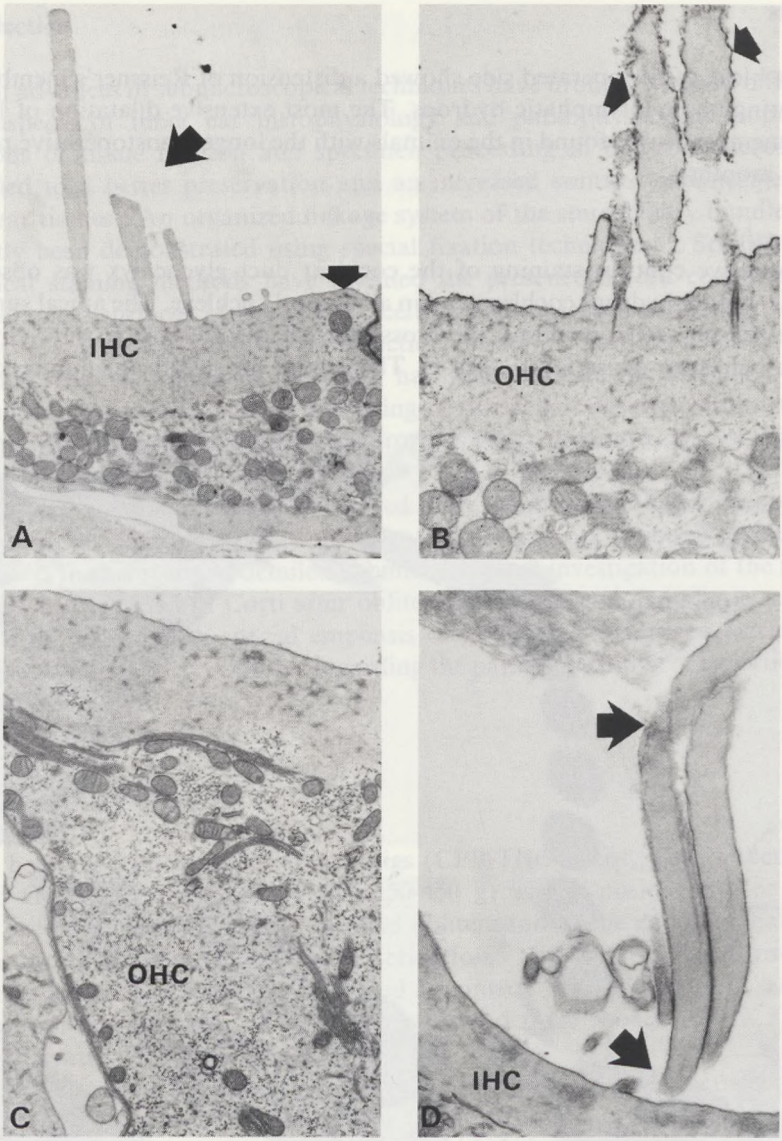

Fig. 2.
(A). Less prominent contrast-staining of glycocalyx (arrow) on the endolymphatic surface of inner hair cell in a 2-month hydrops cochlea. (8,000x).
(B). Irregularities of outer hair cell stereociliary membrane (arrow) in a 1-month hydrops cochlea. (20,000x).
(C). Loss of outer hair cell stereocilia in a 2-months hydrops cochlea. (10,000x).
(D). Bending at the neck and near the top of inner hair cell stereocilium in a 1-month hydrops cochlea (arrow). (33,000x).

glycocalyx distribution between the sensory cells and the supporting cells. Only a weak contrast-staining was observed on the basolateral surfaces of both the sensory cells and supporting cells.
A less prominent contrast-staining of the glycocalyx was found on the apical surfaces of the sensory cells and the supporting cells of the 2- and 3-month hydrops cochleae. (Fig. 2A). No differences in glycocalyx contrast-staining were observed between the apical part and the basal part of the hydrops cochleae.

Stereocilia
In all three groups a variety of pathological changes in the stereocilia was observed. These changes always started at the apex of the cochlea. The outer hair cell stereocilia were affected most frequently. In early hydrops, irregularity of the stereociliary membranes was a relatively common finding. (Fig. 2B). In severely damaged specimens fusion, disruption and ultimate loss of stereocilia was observed. (Fig. 2C). A number of inner hair cell stereocilia showed extreme bending, not only at the neck but sometimes also near the top of the stereocilium. (Fig. 2D).

Outer hair cells
Pathological changes in the outer hair cells started at the apical part of the cochlea. The first, second and third rows seemed to be affected equally.
As early as one month after endolymphatic sac and duct obliteration, distortion of the infracuticular region was observed. The cuticular plate showed protrusion into the endolymphatic space (Fig. 3) and displacement downwards to the nuclear region. (Fig. 4). The apical part of the cell often showed a large number of multivesicular bodies and an accumulation of lipofuscin granules. Concentrations of glycogen particles were found in the supranuclear region of the cell. The supranuclear mitochondria frequently appeared to be swollen.
More severely damaged specimens showed swelling of the cell body, mitochondrial condensation, formation of myeloid bodies and vacuolization (Fig. 5), followed by chromatin aggregation and nuclear pyknosis.
The subsurface cisternae initially showed an increase in the number of layers, followed by a dilatation of the cisternae and vacuolization starting in the supranuclear region.
The concentric layers of the lamellar bodies (Hensen's bodies) in the supranuclear region were frequently increased in number. In the cochleae of 2- and 3-month animals extensive proliferation of the lamellar bodies was observed sometimes occupying major parts of the cytoplasm (Fig. 6). Ultimately, the atrophic outer hair cells were displaced by outer pillar cells and outer phalangeal cells (Deiter's cells).

Inner hair cells
Morphological changes of the inner hair cells were only observed in the most apical part of the 3-month hydrops cochleae. The earliest changes were limited

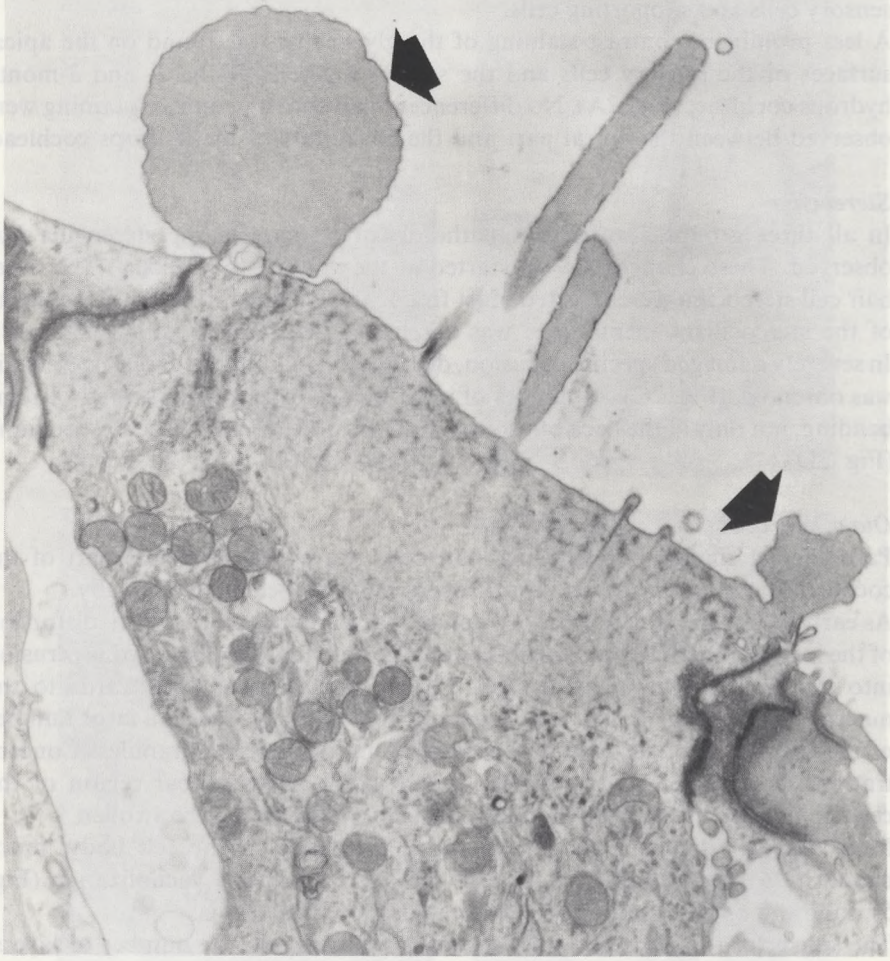

Fig. 3. Protrusion of the outer hair cell cuticular plate (arrow) into the endolymphatic space of a 1-month hydrops cochlea. (20,000x).

to the apical part of the cell. Distortion of the cuticular plate demonstrated similar characteristics to those observed in the outer hair cells. Condensation of the supranuclear mitochondria was observed together with loss of mitochondrial cristae. The subcuticular region showed abundant accumulation of lysosomes, coated and uncoated vesicles and lipofuscin granules.

No evident signs of degeneration were found in the poorly developed subsurface cisternae of the inner hair cells in contrast to the outer hair cells. In the basal

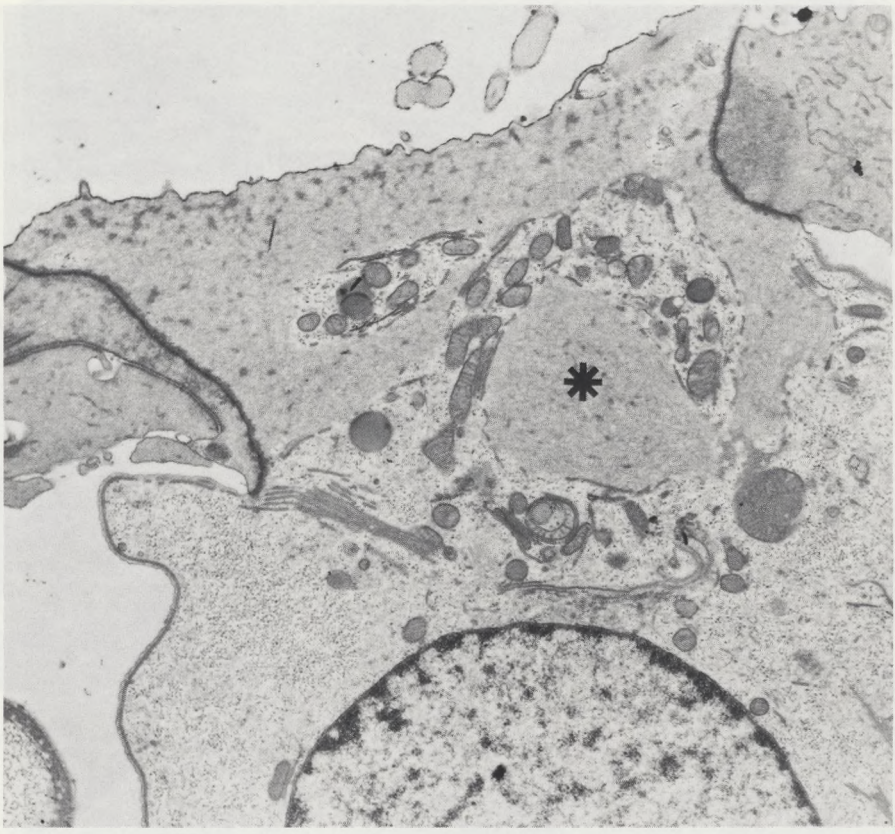

Fig. 4. Extension of the cuticular plate into the nuclear region (asterisk) of a 1-month hydrops outer hair cell. (20,000x).

part of the cell a slight increase of the coated and uncoated vesicles was found. The synaptic bodies seemed to be normal in appearance and number.
Further degeneration was marked by an irregular shape of the cell body and cytoplasmic vacuolization, again more pronounced in the apex of the cell. The next stage showed extreme swelling of the cell body, nuclear pyknosis, abundant vacuolization throughout the cell and formation of myeloid bodies, ultimately leading to complete disappearance of the inner hair cells with replacement by supporting cells.

Nerve elements
Degenerative changes in the afferent and efferent nerve endings of the outer hairs cells were always found to be secondary to degeneration of the outer hair cells. Pathology of the afferent nerve endings seemed to precede degen-

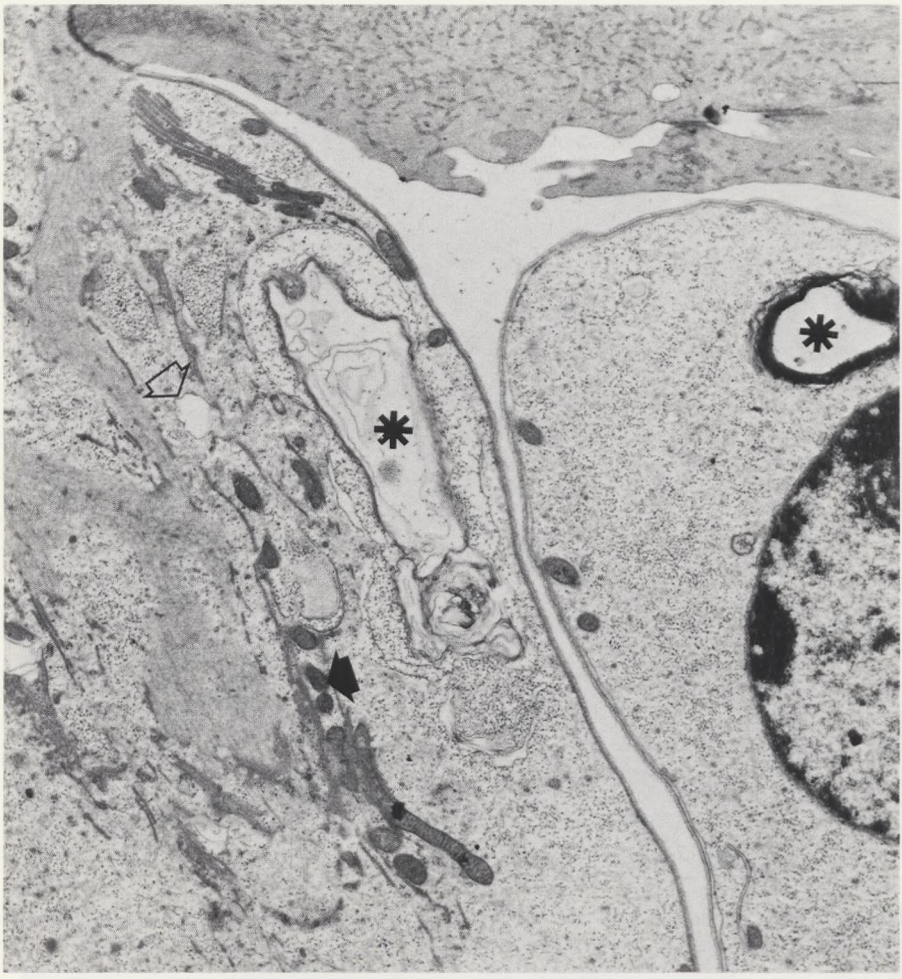

Fig. 5. Mitochondrial condensation (arrow), myeloid bodies (asterisks) and beginning vacuolization (unfilled arrow) in a 2-months hydrops outer hair cell. (12,000x).

eration of the efferent nerve endings. Degeneration was characterized by swelling of mitochondria with loss of cristae and vacuolization, followed by formation of myeloid bodies and extreme swelling of the axoplasm.

Degeneration of the inner hair cell nerve endings was observed together with minor morphological changes of the inner hair cell itself. (Fig. 7).

Despite the atrophy of inner hair cells in the most apical part of three months hydrops cochleae, no significant loss of myelinated neurons or spiral ganglia was found.

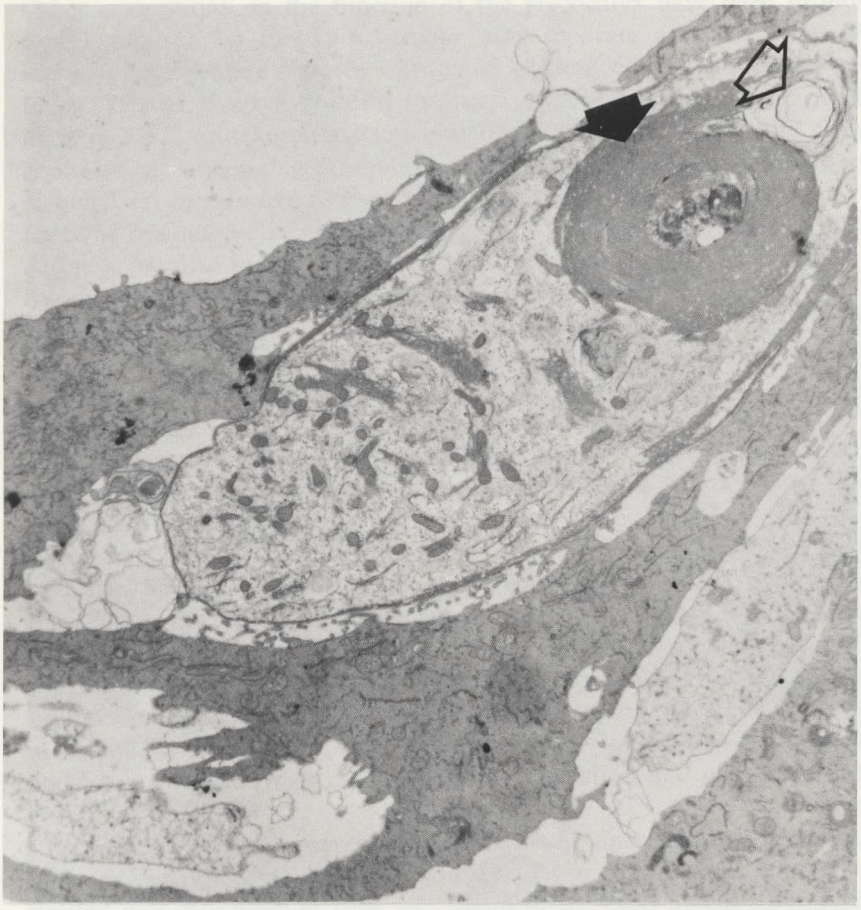

Fig. 6. Extensive proliferation of lamellar bodies (arrow) and formation of myeloid bodies (unfilled arrow) in a 2-month hydrops outer hair cell. (12,000x).

Discussion

Selective contrast-enhancement of the endolymphatic glycocalyx on the external surfaces of the cochlear sensory cells after osmium tetroxide-potassium ruthenium cyanide post-fixation has been reported earlier by De Groot[6]. The uniform contrast-staining of the glycocalyx of both the sensory cells and supporting cells is in accordance with previous studies using different techniques[4,5]. By contrast, Lim[7] and also Santi and Anderson[8] found glycocalyx contrast-staining after ruthenium red post-fixation to be limited to the sensory cells. However, other factors involved in tissue processing, such as differences in primary fixation, may be responsible for this discrepancy.

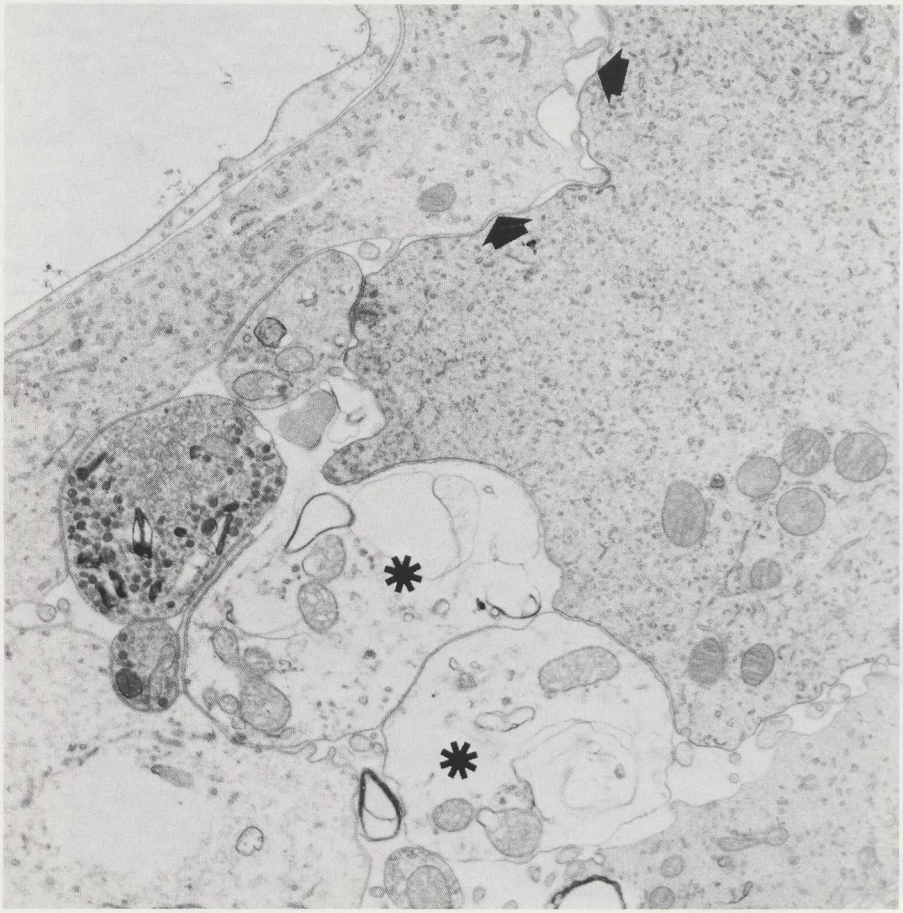

Fig. 7. Degeneration of afferent nerve endings of inner hair cell (asterisks) and irregular shape of inner hair cell body (arrows) in a 3-month hydrops cochlea. (12,000x).

An interesting observation in this study is the less prominent contrast-staining of the endolymphatic glycocalyx of the sensory cells in the 2- and 3-month hydrops cochleae. (see also Chapter 6). Recent evidence suggests that the glycocalyx lining the apical surfaces of the cochlear hair cells may play an active role in the transduction process by creating a cation-rich micro-environment along the apical cell surfaces[4,5]. Ultrastructural studies of the stria vascularis and Reissner's membrane in experimental hydrops indicate an early involvement of structures significant in the osmoregulation and volume control of the inner ear fluids[12]. Subtle shifts in the ion and fluid balance of the inner ear in experimental hydrops might influence the transduction process indirectly by changing the micro-environment of the apical surfaces of the sensory cells.

Other suggestions on the function of the glycocalyx have been proposed in earlier reports. Its electro-negativity may separate adjacent stereocilia by repulsion, preventing contact and fusion of stereociliary membranes[4]. Simultaneously, the glycocalyx seems to play a significant role in the cross-linking of stereocilia, thus keeping them in a bundle[2,3]. Reduction of the glycocalyx could be the underlying mechanism for ciliary fusion and irregularity of the stereociliary membranes as reported in this study.

Extreme bending of inner hair cell stereocilia, suggesting reduction of ciliary stiffness, has also been observed following acoustic trauma and aminoglycoside ototoxicity[11,13,14]. Disorganization of the cuticular plate with disruption of the anchoring system of the stereociliary rootlets has been described as a possible mechanism to explain the reduction of ciliary stiffness in acoustic trauma and aminoglycoside intoxication[11]. Similar morphological changes in the cuticular plate have been found in this study, suggesting a common basis for these morphological features. An increased number of lamellae in the subsurface cisternae and proliferation of lamellar bodies (Hensen's bodies) in the outer hair cells is not only found in experimental hydrops[15], but also after acoustic hyperstimulation[11]. The subsurface cisternae are connected to the apical cisternae, the subsynaptic cisternae and the lamellar bodies. The function of these interconnecting structures is not yet clear, although a possible involvement in neural conduction or in mediating excitation-contraction coupling has been suggested[7,9,10]. The number of lamellae in the subsurface cisternae and lamellar bodies seems to be dependent on the functional state of the cell. The previously mentioned morphological changes may indicate an extremely high metabolic activity in the outer hair cell, leading to exhaustion and ultimately to degeneration of the cell.

Loss of outer hair cells precedes degeneration of inner hair cells in experimental hydrops, as reported in several studies[15–18]. In this experiment only a very limited loss of inner hair cells was found in the most apical part of 3-month hydrops cochleae. No distinct retrograde degeneration of cochlear neurons was observed in any of the three hydrops groups. This finding is in complete agreement with the fact that 95% of the cochlear neurons are associated with the inner hair cells[19,20].

Degeneration of nerve endings associated with the outer hair cells was always secondary to outer hair cell degeneration. The afferent nerve endings of the outer hair cells seemed to degenerate at an earlier stage than the efferent nerve endings. These observations strongly suggest that the pathological processes in experimental hydrops initially affect the vital functions of the outer hair cells and only at a later stage the associated nervous system.

Pathology of the inner hair cells and degeneration of the inner hair cell nerve endings seemed to occur at the same time. This is in contrast with the findings of Kimura, who observed degeneration of inner hair cell nerve endings while the inner hair cells were not yet damaged[16]. However, in the present study only a limited investigation in the degeneration pattern of the inner hair cells

was possible, since the longest survival time did not exceed a three months period.

In conclusion, subtle changes in the micromechanics of the auditory transduction mechanism seem to play a significant role in the early pathophysiology of endolymphatic hydrops.

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CHAPTER 6

EFFECTS OF ENDOLYMPHATIC SAC OBLITERATION ON THE COCHLEAR DUCT GLYCOCALYX

Published in: Albers FWJ, De Groot JCMJ, Veldman JE, Huizing EH. Effects of endolymphatic sac obliteration on the cochlear duct glycocalyx. ORL 1987; 49; 277-281.

Introduction

Bennett introduced the term glycocalyx in 1963 to describe cell surface coating material, which he suspected to contain glycoconjugates[1]. Ultrastructural investigations using various cytochemical techniques have demonstrated qualitative and quantitative differences in the biochemical composition of the glycocalyx. The significance of cell surface glycoconjugates with regard to the specialized biological activities of the various cell types has been emphasized by Spicer[2].

The cochlear duct glycocalyx has been described in previous studies using different TEM techniques[3–7]. Possible implications of glycoconjugates in stereociliary cross-link systems have been suggested recently[3–5].

Despite detailed light microscopical and ultrastructural studies of the cochlear duct in experimental hydrops[8–11] only limited information is available with regard to cell surface glycoconjugates under these pathological conditions. Therefore, in this study an ultrastructural investigation of the endolymphatic glycocalyx has been performed after surgical obliteration of the endolymphatic duct and sac.

Material and methods

Nine healthy, female albino guinea pigs (CPB-TNO, Zeist, The Netherlands; strain GpHi65 Himalayan, weight: 350-450 g) with a positive Preyer reflex were used for this experiment. Surgical obliteration of the endolymphatic sac and duct through the extradural posterior fossa approach was performed on the left ear, whereas the right ear served as control. The animals were sacrified one month (N = 3), two months (N = 3) and three months (N = 3) after sac obliteration. Tissue fixation and specimen processing for light and electron microscopy was performed as described in Chapter 4[10,11]. Ultra-thin sections of re-embedded quarter turns were stained with methanolic uranyl acetate and Reynold's lead citrate and examined by transmission electron microscopy (Zeiss EM109; 50 kV).

Results

All cochleae of the operated side showed a distension of Reissner's membrane, indicating an endolymphatic hydrops.

In all control cochleae an uniform electron-dense layer was observed, covering the endolymphatic surfaces of the sensory cells and the supporting cells, the marginal cells of the stria vascularis and the epithelial lining of the spiral prominence and Reissner's membrane. (Figs. 1A, 2A, 3A). The contrast of the stereociliary glycocalyx and the cross-links between the stereocilia of the

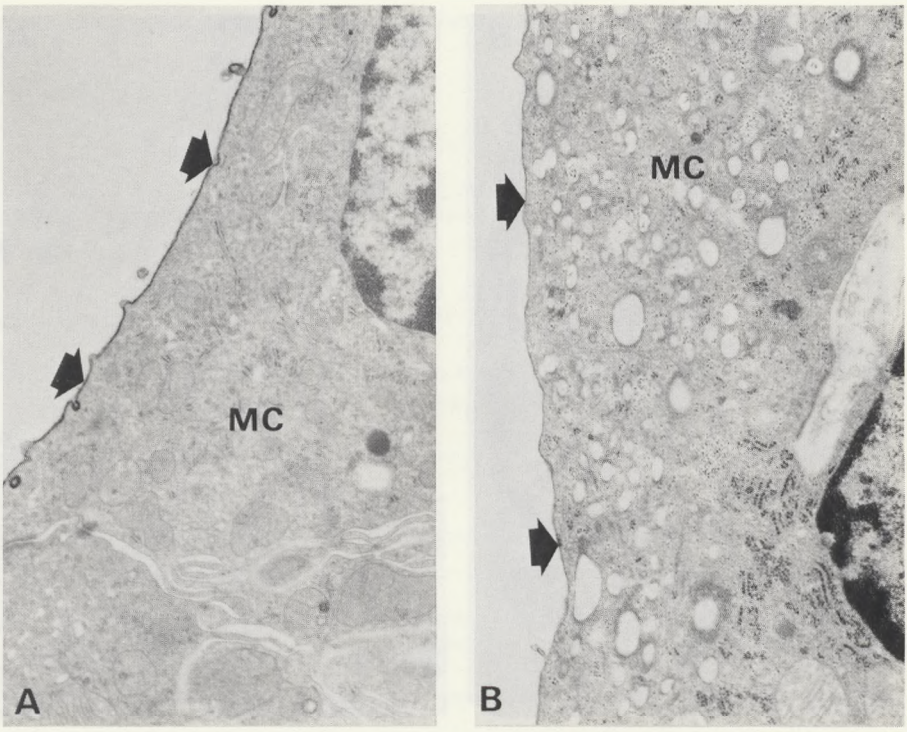

Fig. 1.
(A). Glycocalyx (arrows) on the endolymphatic surface of the marginal cells (MC) of the stria vascularis in a control cochlea. (14,000x).
(B). Less prominent glycocalyx staining (arrows) of the stria vascularis in a 2-month hydrops cochlea. MC = marginal cell. (13,000x).

inner and outer hair cells also appeared to be enhanced. (Fig. 3A). No glycocalyx contrast-staining was observed on the basolateral margins of the sensory cells and the supporting cells nor on the epithelial cells delineating the perilymphatic compartments. A slight individual variation in glycocalyx contrast-staining was found in both hydrops and control cochleae.

In the 2- and 3-month hydrops cochleae a less prominent glycocalyx contrast-staining was found as compared to the control cochleae. The endolymphatic surfaces of the marginal cells of the stria vascularis as well as the epithelial cells of the spiral prominence and Reissner's membrane showed the most significant decrease in contrast-staining. (Figs. 1B, 2B). In addition, a less prominent contrast-staining of the stereociliary membranes was frequently found together with disappearance of positive staining material connecting adjacent stereocilia. (Fig. 3B). No regional differences in contrast-staining were observed between the apices and the bases of the cochleae.

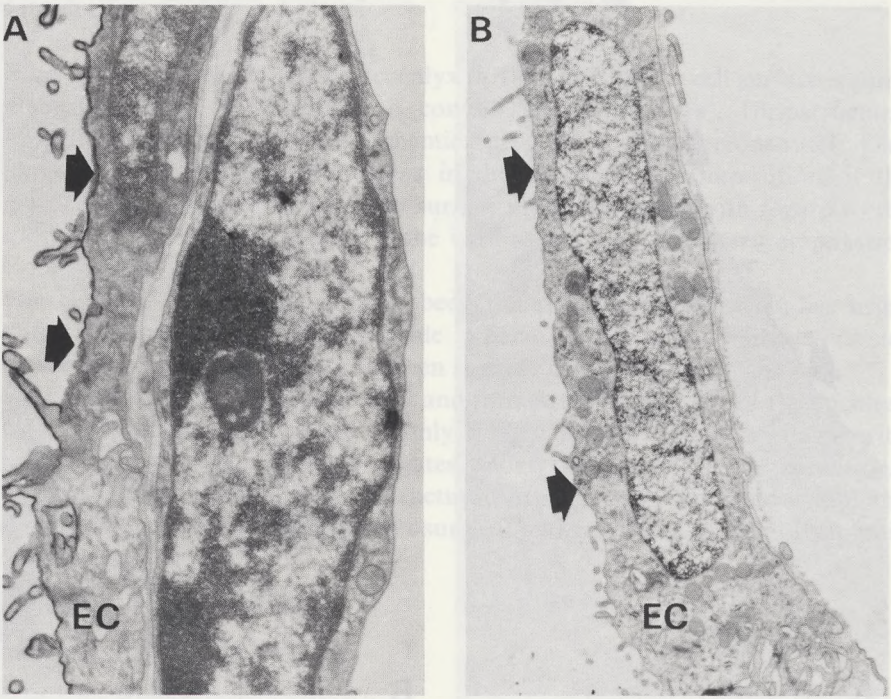

Fig. 2.
(A). Glycocalyx (arrows) on the endolymphatic surface of the epithelial cells (EC) of Reissner's membrane in a control cochlea. (14,000x).
(B). Less marked glycocalyx contrast (arrows) of Reissner's membrane in a 2-month hydrops cochlea. EC = epithelial cell. (15,000x).

Discussion

Selective contrast-enhancement of the cochlear duct glycocalyx after osmium tetroxide-potassium rutheniumcyanide post-fixation has been reported previously[6,11]. The uniform distribution of positive staining material on the endolymphatic surfaces of both sensory cells and supporting cells as found in this investigation is in accordance with other studies using different TEM techniques[3,6]. Changes in the endolymphatic fluid balance after obliteration of the endolymphatic sac and duct might influence the biochemical composition of the cochlear duct glycocalyx explaining the less prominent contrast-staining throughout the entire cochlea in experimental hydrops as described in this study. The cell surface plays an important role in maintaining the intracellular biochemical composition by continuous molecular and ion exchange between the intra- and extracellular compartments[2]. Alterations in the cochlear duct

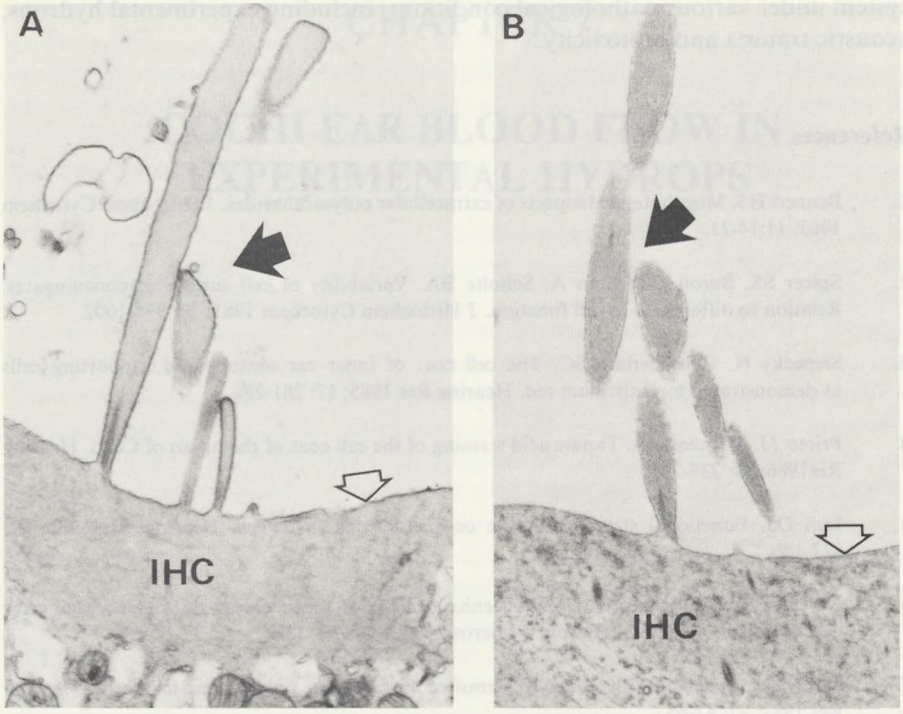

Fig. 3.
(A). Glycocalyx (unfilled arrow) on the endolymphatic surface of an inner hair cell (IHC) with positive staining material (arrow) between adjacent stereocilia in a control cochlea. (20,000x). (B). Less prominent glycocalyx staining (unfilled arrow) of an inner hair cell (IHC) with disappearance of interconnecting material (arrow) between the stereocilia in a 3-month hydrops cochlea. (18,000x).

glycocalyx might interfere with the secretory/reabsorptive capability of the structures which are involved in ion and fluid exchange such as the stria vascularis and Reissner's membrane. Further disturbances in the fluid balance could lead to reversible and ultimately to irreversible changes in these structures as previously suggested[8–10].

Recent studies of the stereociliary linkage systems have revealed new hypotheses to explain the auditory transduction mechanism[5,12,13] (see also Chapter 5). The frequent absence of positive staining material between adjacent stereocilia in 2- and 3-month hydrops cochleae, as observed in this study, may indicate a disruption of stereociliary cross-links under these experimental conditions. However, the less prominent staining of the glycocalyx in hydrops cochleae makes interpretation of these observations difficult.

Combined SEM and TEM analyses using different cytochemical techniques are needed to obtain more detailed information on the stereociliary linkage

system under various pathological conditions, including experimental hydrops, acoustic trauma and ototoxicity.

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CHAPTER 7

COCHLEAR BLOOD FLOW IN EXPERIMENTAL HYDROPS

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Introduction

Anatomical and histopathological aspects of the cochlear vasculature have been the subject of various studies in an attempt to find an explanation for certain clinical disorders. The vascular micro-anatomy of the mammalian cochlea has been described in a number of in vivo and in vitro studies. An extensive research of the vascular architecture of the human and guinea pig cochlea was reported by Axelsson in his monograph in 1968[1].

The main vascular supply of the guinea pig inner ear is provided by the labyrinthine artery (also called the internal auditory artery), which most commonly is a ramification of the anterior inferior cerebellar artery. In the internal acoustic meatus the labyrinthine artery divides into two branches: the common cochlear artery and the anterior vestibular artery. At the cochlear base the common cochlear artery diverges in the posterior vestibular artery and the cochlear artery (or spiral modiolar artery). The cochlear artery has a spiral course in the modiolus from the basal turn to the apex. It provides the corresponding capillary regions except the most basal part of the cochlea, which is supplied by the posterior vestibular artery. Three main vascular areas can be identified in the guinea pig cochlea: the modiolus, the cochlear partition and the lateral cochlear wall. In the modiolus the capillary regions are located in the spiral ganglion, the cochlear nerve and the modiolus wall, forming irregular networks. The vascular region of the cochlear partition consists of an arcadic system with peripherally radiating arterioles, a capillary network in the osseous spiral lamina and the spiral limbus, and spirally running vessels of the tympanic lip and the basilar membrane. The external wall of the scala media is supplied by radiating arterioles running along the external wall of the scala vestibuli. The vascular system of the lateral wall consists of a spirally running vessel of the spiral prominence and capillary networks in the apical part of the spiral ligament and stria vascularis. Externally to the stria vascularis arterio-venous anastomoses connect the radiating arterioles in the scala vestibuli with the collecting venules in the scala tympani.

Despite the detailed knowledge of the vascular anatomy of the mammalian cochlea, limited information is available with regard to the dynamics of the cochlear blood supply under normal and pathological conditions. Qualitative information of the cochlear blood flow and micro-circulation have been obtained in experiments using different research techniques including direct in vivo microscopy[2,3] and impedance plethysmography[4,5]. Quantitative investigations of the total cochlear blood flow have been reported using radioactively labelled microspheres[6,7]. Recently, modifications of the radioactive microsphere technique have been applied to measurements of the regional cochlear blood flow in different compartments using non-radioactive microspheres[8–11].

Surgical obliteration of the endolymphatic duct and sac in the guinea pig results in a disturbance of the inner ear fluid balance, ultimately leading to a sequence of histopathological changes in the cochlear duct[12–16]. As mentioned earlier,

the lateral cochlear wall including the stria vascularis is one of the main capillary regions in the cochlear blood supply of the guinea pig. The early ultrastructural changes in the stria vascularis after endolymphatic sac and duct obliteration as recently described[15] could indicate that disturbances in the cochlear microcirculation are also responsible for certain pathophysiologal changes in experimentally induced hydrops. In the present series of experiments the regional blood flow in specific vascular areas of the guinea pig cochlea was studied after surgical obliteration of the endolymphatic duct and sac using the microsphere method in combination with serial sectioning of plastic embedded cochleae.

Material and methods

Nine healthy, female albino guinea pigs (CPB-TNO, Zeist, The Netherlands; strain GpHi65 Himalayan, weight: 350-450 g) with a positive Preyer reflex were used for this experiment. Surgical obliteration of the endolymphatic sac and duct was performed on the left ear, whereas the right ear was used as control. The animals were sacrified two months (N = 3), four months (N = 3) and eight months (N = 3) after endolymphatic sac and duct obliteration.
The non-radioactive microsphere technique applied for measuring the cochlear blood flow has recently been published in detail by Larsen et al.[10] and by Angelborg et al.[11] and can be summarized as follows.
The weight of the guinea pigs at the time of the blood flow measurements was 650-950 g. The experimental animals were anesthesized by intravenously injected Saffan® after intraperitoneally premedication with Ketalar®. The animals were tracheotomized and artificially ventilated. Before sacrificing the animals the left cardiac ventricle was transcutaneously catheterized with a metal cannula and the correct position was ensured by analyzing the blood acid-base balance. The femoral arteries were catheterized to obtain reference blood samples and blood pressure measurements. Non-radioactive microspheres ($5x10^6$) with a diameter of 11.7 ± 1.7 μm were dispersed in saline and injected into the heart over a period of 10 seconds. Within two minutes after injection of the microspheres the animals were put to death by intracardiac injection of saturated potassium chloride. After decapitation the temporal bones were dissected and fixed in phosphate-buffered 1.5% glutaraldehyde (pH 7.4). After primary fixation the temporal bones were decalcified in 10% EDTA, postfixed in phosphate-buffered 1% OsO_4 (pH 7.4), dehydrated in a graded ethanol and embedded in Araldit resin.
Serial sections of 35 μm thickness were obtained of each cochlea in a plane parallel to the modiolus. Each section was examined using a light microscope and the distribution of the microspheres in the capillary beds was noted. (Fig.1 A-B). The total and regional cochlear blood flow could be calculated after quantification of the microspheres in the reference blood samples using a light

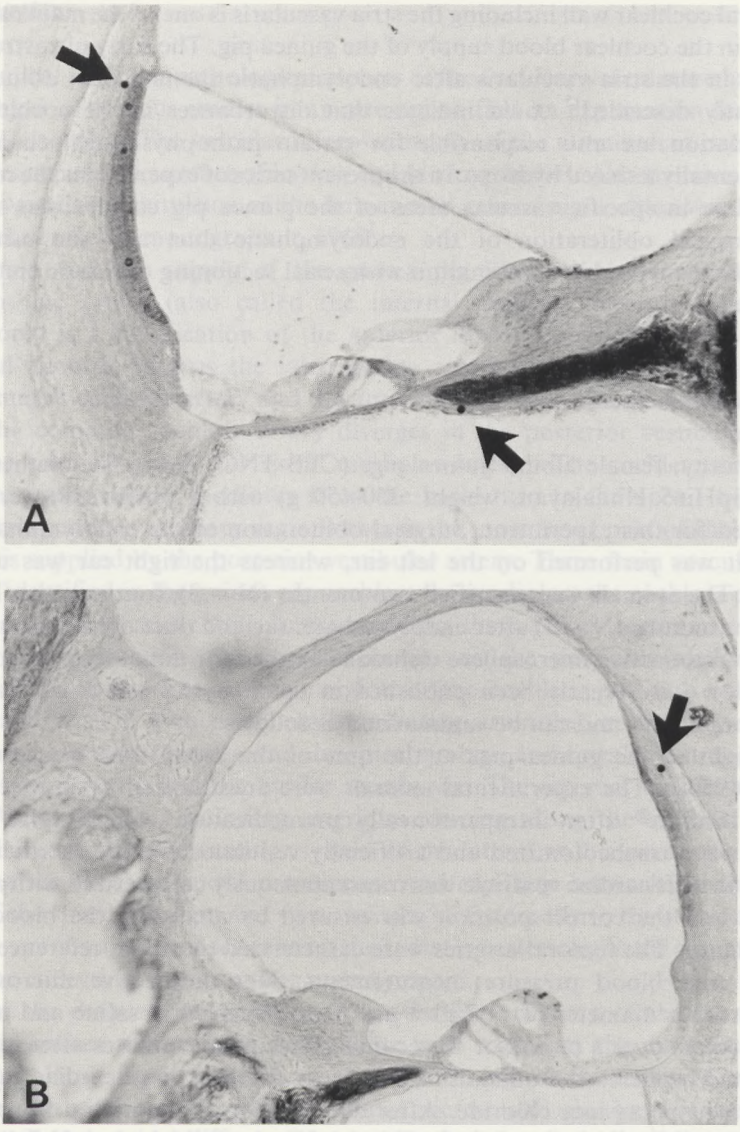

Fig. 1. (A-B). Microspheres (arrows) in 35 μm cross-sections of a normal (A) and hydrops (B) guinea pig cochlea. (400x).

microscope and a Buerger counting chamber according to the method previously described[17,18].

In order to calculate the regional cochlear blood flow the capillary beds were divided into the following vascular areas: 1. the modiolus (M) including the capillary regions of the spiral ganglion, the cochlear nerve and the modiolus

wall; 2. the cochlear partition (CP) including the vascular area of the osseous spiral lamina and the spiral limbus, and the vessels of the tympanic lip and the basilar membrane; 3. the lateral cochlear wall (LW) including the radiating arterioles in the roof of the scala vestibuli, the vessel of the spiral prominence and the capillary regions of the spiral ligament and the stria vascularis.

Results

Hydrops

Histological examination of all nine operated cochleae showed a distension of Reissner's membrane indicating an endolymphatic hydrops. (Fig. 1B).

Total cochlear blood flow

The arterial acid-base balance and the mean arterial blood pressure (MAP) during the experimental procedures were found to be within normal range in all nine animals.

After quantification of the total number of microspheres in each cochlea the total cochlear blood flow was calculated. The mean values ± the standard deviation for the hydrops and control cochleae of the two-month, four-month and eight-month group are presented in Table I.

TOTAL COCHLEAR BLOOD FLOW (mg/cochlea/min)

	control	hydrops
2 months (N = 3)	1.49 (± 0.84)	1.33 (± 0.34)
4 months (N = 3)	1.20 (± 0.06)	0.96 (± 0.59)
8 months (N = 3)	1.44 (± 0.80)	1.29 (± 0.60)

Table I. Total cochlear blood flow of the hydrops and control cochleae two months, four months and eight months after surgical obliteration of the endolymphatic duct and sac.

Regional cochlear blood flow

In order to study the regional blood flow the capillary areas were divided in three vascular regions: the modiolus, the cochlear partition and the lateral cochlear wall. In the hydrops cochleae as well as in the control cochleae the greatest quantity of microspheres was found in the lateral cochlear wall, less in the modiolus and least in the cochlear partition. The quantitative distribution of the microspheres in the three different vascular regions is presented in Table II.

REGIONAL COCHLEAR BLOOD FLOW

	Modiolus	Cochlear partition	Lateral wall
	Control / Hydrops	Control / Hydrops	Control / Hydrops
2 months	38% / 37%	6% / 6%	56% / 57%
4 months	36% / 46%	3% / 8%	61% / 46%
8 months	36% / 37%	8% / 8%	56% / 55%

Table II. Regional cochlear blood flow of the hydrops and control cochleae in the modiolus, the cochlear partition and the lateral cochlear wall.

Discussion

Quantification of the total cochlear blood flow by direct observation of non-radioactive microspheres in serially sectioned cochleae gives results which correlate well with those obtained with the radioactive microsphere technique[8,10]. Regional blood flow calculations of large vascular compartments show a relative constant distribution in the normal guinea pig cochlea[8-10]. In the present study the total and regional cochlear blood flow during progression of endolymphatic hydrops was calculated using the non-radioactive microsphere technique. It should be noted that the small number of experimental animals did not allow statistical evaluation of the results.

The arterial blood supply of the apical part of the cochlea demonstrates an increasing simplification of the vasculature in comparison to the more basal part of the cochlea[1]. This simplification is particularly marked in the stria vascularis and the arterio-venous anastomoses external to the stria vascularis. The histopathological changes in the cochlear duct after surgical obliteration of the endolymphatic duct and sac are found to start in the most apical part of the cochlea[12-16].

It is tempting to assume that a disturbance of the cochlear blood supply plays an important role in experimental hydrops, thus contributing to the apex-basis differences in cochlear histopathology as observed and described in Chapters 3-5. However, the arterial blood supply to the endolymphatic sac is provided by the posterior meningeal artery[19], while the main blood supply of the cochlea comes from the cochlear artery[1]. It is therefore unlikely that destruction of the vasculature of the endolymphatic sac and duct by surgical obliteration will have any direct effects on the cochlear blood supply. This is in agreement with the findings of the present study, in which no significant changes in the total cochlear blood flow were found between hydrops and control cochleae at two, four and eight months after sac and duct obliteration. Disturbances in the cochlear fluid balance after obliteration of the endolym-

phatic sac and duct cause reversible and ultimately irreversible changes in the stria vascularis indicating an increased functional activity followed by a hypofunction of the stria vascularis[15] (see also Chapter 4). Changes in stria vascularis activity may influence the local blood flow of the strial vessels. However, in the present study no indications were found of either an increase or a decrease in the regional blood flow of the lateral cochlear wall at two, four and even eight months after sac and duct obliteration, although the number of animals investigated is unsufficient for a reliable statistical evaluation. In order to assess the more subtle changes in the blood flow of the stria vascularis itself a further subdivision of the lateral cochlear wall into more specific vascular regions and a statistically sufficient number of experimental animals is needed.

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CHAPTER 8

SUMMARY AND CONCLUSIONS

Recent advances in inner ear research have led to a considerable growth in our understanding of the structure-related function of the auditory organ. In the experiments of this study surgical obliteration of the endolymphatic sac and duct in the guinea pig is used as a method of investigating the early histopathologal changes in the hydrops cochlea with special emphasis on the functional structures of the inner ear.

In Chapter 2 the current knowledge of the light microscopical and ultrastructural cytoarchitecture of the cochlea under non-pathological conditions is described. The specimens used as illustrations in this chapter had the same tissue fixation and specimen processing as described in Chapters 4-6.

In Chapter 3 a qualitative and quantitative documentation is presented of the early degenerative changes in the organ of Corti in experimental hydrops by means of interference differential (Nomarski) microscopy in specimens processed according to the block surface technique. The sequence of morphological changes in the organ of Corti was studied one, two and four months after surgical obliteration of the endolymphatic sac and duct. The hair cell losses were calculated and mapped in cytocochleograms. One month postoperatively a minimal loss of outer hair cells only was observed in the apical cochlear turn. At two months a progression of outer hair cell loss was seen, which had proceeded further in the 4-month group. At four months the inner hair cells showed a slight tendency to degenerate, again starting in the most apical part of the cochlea. A characteristic pattern in hair cell degeneration could be distinguished. Initial degeneration was marked by disappearance of the stereocilia, followed by swelling of nuclei and cell bodies. The next phase showed shrinkage and collapse of the cells with the formation of a phalangeal scar. Ultimately the phalangeal scars were concealed by overgrowth of the supporting cells, resulting in an irregular surface on the organ of Corti. In conclusion, in experimentally induced endolymphatic hydrops initial degeneration of outer hair cells is followed by inner hair cell loss, both starting in the apical part of the cochlea.

In Chapter 4 a time-sequence study is reported of the initial ultrastructural changes in the stria vascularis and Reissner's membrane of the guinea pig after endolymphatic sac and duct obliteration. Pathological alterations in both the stria vascularis and Reissner's membrane were found to start in the apex of the cochlea. In the stria vascularis they were characterized by an increase of vesicles in the marginal cells and by intercellular edema, followed by vacuolization and atrophy of the marginal and intermediate cells. In Reissner's membrane extensive gaps in the mesothelial cell layer were observed together with intracellular pathology of the epithelial cells and a fuzzy appearance of the basement membrane. Up to three months after endolymphatic sac and duct obliteration no ruptures of Reissner's membrane were found. The stria

vascularis and Reissner's membrane, which are essential for the maintenance of inner ear fluid balance, seem to be directly involved in the pathophysiology of experimental hydrops.

In Chapter 5 the early ultrastructural changes in the organ of Corti of the guinea pig after obliteration of the endolymphatic sac and duct are examined in a time-sequence study. Initial loss of outer hair cells was followed at three months by inner hair cell degeneration, both starting in the apical part of the cochlea. The morphological changes in the sensory cells started on the endolymphatic surface and were characterized by a variety of stereociliary lesions (such as irregularity of the stereociliary membrane followed by fusion, disruption and loss of stereocilia) and by distortion of the cuticular plate. Further degeneration of the hair cells was marked by pathological changes in intracellular organelles such as mitochondrial degeneration, formation of myeloid bodies and increase of Hensen bodies. Degeneration of the nerve endings of the outer hair cells was always secondary to outer hair cell degeneration and was characterized by swelling of mitochondria, formation of myeloid bodies and swelling of the axoplasm. Degeneration of inner hair cell nerve endings seemed to occur together with pathology of the inner hair cells itself. The ultrastructural pathology of the sensory cells indicates that changes in the micromechanics of the auditory transduction mechanism may occur even at an early phase of experimental hydrops.

In Chapter 6 the ultrastructural effects of surgical obliteration of the endolymphatic duct and sac on the cochlear duct glycocalyx using osmium tetroxide-potassium rutheniumcyanide post-fixation are described. It has been proposed that the glycoconjugates on the apical surfaces of the sensory cells may play an active role in the auditory transduction process. After two and three months a less prominent glycocalyx contrast-staining was found on the endolymphatic surfaces of the sensory cells together with disappearance of interconnecting material between the stereocilia of the inner as well as the outer hair cells. A less prominent contrast-staining was also observed on the endolymphatic surface of the marginal cells of the stria vascularis, the epithelial cells of Reissner's membrane and the spiral prominence. Experimental hydrops seems to have an early impact on the endolymphatic glycocalyx, which may have a direct effect on the biochemical and micromechanical processes of auditory transduction.

In Chapter 7 the dynamics of the cochlear blood supply after surgical obliteration of the endolymphatic sac and duct are described in a time-sequence study. The total cochlear blood flow and the regional blood flow in specific vascular regions of the cochlea were studied using the non-radioactive microsphere method in combination with serial sectioning of plastic embedded cochleae. No differences in the total cochlear blood flow were found between hydrops

and control cochleae two, four and eight months after endolymphatic sac and duct obliteration. Further investigation of the local blood flow in the lateral cochlear wall, the modiolus and the cochlear partition failed to show any indication of an increase or a decrease in the regional cochlear blood flow at any time postoperatively. Despite the atrophy observed in the stria vascularis, disturbances in the cochlear blood supply cannot be regarded as a major factor in the pathophysiology of experimental hydrops.

SAMENVATTING

Recente ontwikkelingen in het binnenoor-onderzoek hebben geleid tot de huidige inzichten ten aanzien van de structuur en functie van het gehoorsorgaan. In de experimenten van dit proefschrift werden de vroege histopathologische veranderingen in de hydrops cochlea van de cavia na chirurgische obliteratie van de ductus en saccus endolymphaticus bestudeerd met speciale aandacht voor de correlatie tussen structuur en functie van het binnenoor.

In Hoofdstuk 2 wordt de lichtmicroscopische en ultrastructurele micro-anatomie van het niet-pathologische binnenoor bij de cavia beschreven. De histologische opwerkingsmethode van de preparaten, die gebruikt zijn voor de illustratie van dit hoofdstuk, is beschreven in Hoofdstuk 4-6.

In Hoofdstuk 3 wordt een kwalitatieve en kwantitatieve inventarisatie gegeven van de vroege degeneratieve veranderingen in het orgaan van Corti ten gevolge van een endolymphatische hydrops, waarbij oppervlakte-preparaten bestudeerd werden met behulp van interferentie-microscopie volgens Nomarski. De morphologische veranderingen in het orgaan van Corti werden een, twee en vier maanden na chirurgische obliteratie van de ductus en saccus endolymphaticus bestudeerd. Het verlies van binnenste en buitenste haarcellen werd geregistreerd in cytocochleogrammen. Na een maand werd een gering verlies van buitenste haarcellen in de apex van de cochlea waargenomen. Twee maanden na de operatie bestond er een toename van het verlies aan buitenste haarcellen, welke zich voortzette in de hydropsgroep van de viermaandsdieren. Na vier maanden werd eveneens een geringe neiging tot verlies van binnenste haarcellen geconstateerd. Ten gevolge van de endolymphatische hydrops werd een karakteristieke opeenvolging van degeneratieve veranderingen in het orgaan van Corti waargenomen. Verlies van stereocilia tesamen met zwelling van nucleus en cellichaam werd gevolgd door een collaps van de haarcel, hetgeen resulteerde in een phalangeaal litteken. Ten slotte ontstond overgroeiing van het defect door steuncellen, waardoor het orgaan van Corti een onregelmatig oppervlak met phalangeale uitlopers vertoonde. In een experimenteel geïnduceerde endolymphatische hydrops wordt verlies van buitenste haarcellen gevolgd door degeneratie van binnenste haarcellen, waarbij de eerste pathologische veranderingen gelocaliseerd zijn in de apex van de cochlea.

In Hoofdstuk 4 worden de vroege submicroscopische veranderingen van de stria vascularis en de membraan van Reissner na obliteratie van de ductus en saccus endolymphaticus beschreven. De eerste pathologische veranderingen van zowel de stria vascularis als de membraan van Reissner werden waargenomen in de apex van de cochlea. Morphologische veranderingen in de stria vascularis werden gekenmerkt door een toename van vesiculae in de marginale cellen alsmede door intercellulair oedeem, gevolgd door vacuolisatie en atrofie van de marginale en intermediaire cellen. In de membraan van Reissner werden omvangrijke openingen in de mesotheliale cellaag waargenomen tesamen met

intracellulaire pathologie en een onregelmatig verloop van de basaal membraan. Tot drie maanden na obliteratie van de ductus en saccus endolymphaticus werden geen rupturen van de membraan van Reissner gevonden. De stria vascularis en de membraan van Reissner lijken in een vroeg stadium betrokken te zijn bij de pathofysiologie van de experimentele hydrops.

In Hoofdstuk 5 worden de vroege ultrastructurele veranderingen van het orgaan van Corti na obliteratie van de ductus en saccus endolymphaticus weergegeven. Verlies van buitenste haarcellen werd na drie maanden gevolgd door degeneratie van binnenste haarcellen. De morphologische veranderingen van de haarcellen werden gekarakteriseerd door een aantal degeneratieve kenmerken van de stereocilia (zoals een onregelmatig verloop van de ciliaire membraan, gevolgd door samensmelting, ontworteling en verlies van stereocilia) en door verplaatsing van de cuticula. Toenemende degeneratie van de haarcellen werd gekenmerkt door pathologische veranderingen van intracellulaire organellen zoals degeneratieve afwijkingen van mitochondria, de vorming van myeloïde structuren en een toename van Hensen lichamen. Pathologie van zenuwuiteinden aangrenzend aan de buitenste haarcellen werd altijd voorafgegaan door degeneratieve veranderingen van de buitenste haarcellen en werd gekarakteriseerd door zwelling van mitochondria, de vorming van myeloïde structuren en zwelling van het axoplasma. Degeneratie van de zenuwuiteinden van de binnenste haarcellen bleek gelijktijdig op te treden met pathologische veranderingen van de binnenste haarcellen zelf. De bovengenoemde ultrastructurele pathologie in het orgaan van Corti suggereert, dat micromechanische veranderingen in het auditieve transductie mechanisme reeds kunnen voorkomen in een vroege fase van de experimentele hydrops.

In Hoofdstuk 6 worden de ultrastructurele gevolgen van obliteratie van de ductus en saccus endolymphaticus op de glycocalyx in het endolymphatische compartiment na osmium tetroxide-kalium rutheniumcyanide post-fixatie beschreven. Na twee en drie maanden werd een verminderde glycocalyx contrastering van de endolymphatische oppervlakten van de haarcellen waargenomen tesamen met de verdwijning van de verbindingen tussen aangrenzende stereocilia van zowel de binnenste als buitenste haarcellen. De endolymphatische oppervlakte van de marginale cellen van de stria vascularis, de epitheliale cellen van de membraan van Reissner en van de prominentia spiralis vertoonden eveneens een verminderd glycocalyx contrast. De vroege veranderingen van de endolymphatische glycocalyx in een experimentele hydrops hebben mogelijk directe consequenties voor biochemische en micromechanische processen in het auditieve transductie mechanisme.

In Hoofdstuk 7 worden de effecten van obliteratie van de ductus en saccus endolymphaticus op de cochleaire bloedvoorziening in een tijdreeks-studie beschreven. De totale bloedstroom in de cochlea alsmede de regionale bloed-

stroom in specifieke vasculaire gebieden werden bestudeerd met behulp van een methode, waarbij niet-radioactieve microspheren in de bloedbaan gebracht werden. Twee, vier en acht maanden na obliteratie van de ductus en saccus endolymphaticus werden geen veranderingen in de totale en regionale cochleaire bloedstroom waargenomen in vergelijking met de niet-geopereerde cochleae. Dientengevolge lijkt het onwaarschijnlijk, dat lokale stoornissen in de cochleaire bloedvoorziening van oorzakelijke betekenis zijn voor de pathofysiologie van de experimenteel geïnduceerde endolymphatische hydrops.

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