Radboud Repository

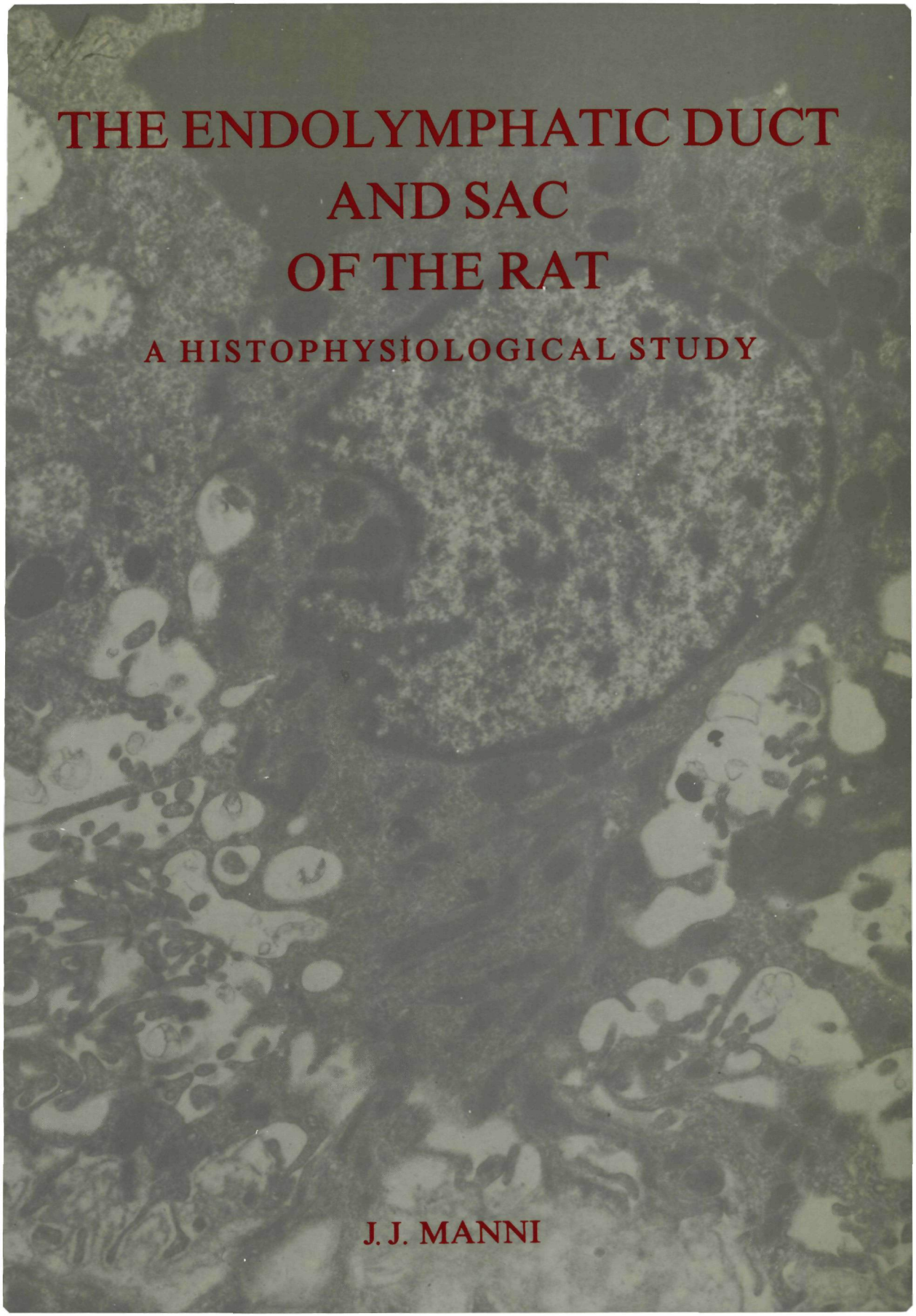
Radboud University Nijmegen

PDF hosted at the Radboud Repository of the Radboud University Nijmegen

The following full text is a publisher's version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/113392

Please be advised that this information was generated on 2017-12-06 and may be subject to change.



# THE ENDOLYMPHATIC DUCT AND SAC OF THE RAT

## A HISTOPHYSIOLOGICAL STUDY

J. J. MANNI

# THE ENDOLYMPHATIC DUCT AND SAC OF THE RAT

## A HISTOPHYSIOLOGICAL STUDY

Cover: The intermediate portion of the endolymphatic sac of the rat.

# THE ENDOLYMPHATIC DUCT AND SAC OF THE RAT

## A HISTOPHYSIOLOGICAL STUDY

PROEFSCHRIFT
ter verkrijging van de graad van
doctor in de geneeskunde
aan de Katholieke Universiteit van Nijmegen
op gezag van de Rector Magnificus Prof. Dr. B.M.F. van Iersel
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op maandag 15 juni 1987
des middags te 3.30 uur
door

JOHANNES JAN MANNI
geboren te Apeldoorn

1987
DRUK: SSN - NIJMEGEN

PROMOTOR: Prof. Dr. P. van den Broek
CO-REFERENTEN: Dr. W. Kuijpers
Dr. P.L.M. Huygen

# THE ENDOLYMPHATIC DUCT AND SAC OF THE RAT

## A HISTOPHYSIOLOGICAL STUDY

THESIS
to obtain the degree of
"doctor in de geneeskunde"
at the Catholic University Nijmegen
on the authority of the Rector Magnificus
Prof. Dr. B.M.F. van Iersel
and by the decision of the College of Deans.
The public defense will take place on
Monday June 15, 1987
at 15.30 hrs
by

JOHANNES JAN MANNI
born in Apeldoorn

Nijmegen 1987

The investigations presented in this thesis were performed under the direction of Dr. W. Kuijpers in the Research Laboratory of the Department of Otorhinolaryngology of the University of Nijmegen.

CIP-DATA KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Manni, Johannes Jan

The endolymphatic duct and sac of the rat: a histophysiological study/Johannes Jan Manni. – (S.l.: s.n.). – Ill.
Thesis Nijmegen – With ref. – With summary in Dutch.
ISBN 90-9001657-0
SISO 605 4 UDC 612 85 (043.3)
Subject headings endolymphatic duct and sac/endolymph circulation.

ISBN 90-9001657-0

The functional significance of the endolymphatic sac, an intradural appendix of the endolymphatic compartment, became a matter of speculation since its discovery in the 18th Century. A large diversity of functions has been ascribed to this structure, varying from being only a vestigial organ to an organ with a resorptive and/or secretory function.

The observation that in some animal species a hydrops of the endolymphatic space can be induced by obliteration of the endolymphatic sac, has focused attention to the role of this structure in endolymph circulation. However, this phenomenon seems to be species dependent and physiological evidence for such a role is still lacking.

This study was undertaken to determine the significance of the endolymphatic duct and sac in endolymph circulation as well as their functional relationship to the cochlea and the vestibular apparatus in the rat.

The first part of this thesis describes the morphological and physiological aspects of the various compartments of the mammalian inner ear and reviews the literature dealing with the various hypotheses on the function of the endolymphatic sac.

The second part deals with our experimental study on the inner ear of the rat, describes the morphological, surgical and physiological procedures and the results obtained.

CHAPTER 1

# THE MAMMALIAN INNER EAR

## 1.1 INTRODUCTION

The first comments on the anatomy of the mammalian inner ear were made by Galen (131-201). He applied the term "labyrinth" to the confusing number of holes and passages in the temporal bone (Guthrie, 1940).
The great anatomist Vesalius (1514-1564) merely mentioned the vestibule and bony canals. However Fallopius (1523-1562) divided the bony inner ear into two major cavities: the vestibule and canal system, named by him as labyrinth, and the cochlea. His pupil Casserio (1566-1616) further defined the three semicircular canals, which were found to end with five holes into the vestibule.
Duverney (1648-1730) has to be credited as the first anatomist to describe the currently known anatomy of the bony labyrinth in its full extent. Cotugno (1736-1822), who studied under Morgagni (1682-1771) at Padua, discovered in 1760 that the inner ear space contained fluid instead of air. This observation was first made in the horse and later in the human ear. He also discovered the endolymphatic sac, which was described by him as an intradural cavity. However his preparation technique was so poorly described that it was a long time before this discovery was accepted (Politzer, 1956). Moreover, he gave the first description of the vestibular and cochlear aqueduct.
The membranous labyrinth was not known at that time. It was left to Scarpa (1747-1832), another pupil of Morgagni, to demonstrate in 1789 that in higher animals the membranous labyrinth was virtually a replica of the bony labyrinth within the cochlea and semicircular canals. He distinguished the different fluid compartments of the membranous labyrinth and described the saccule and utricle ("stone sacs") as well as the ampullae and their cristae ("septum"). The names perilymph and endolymph were introduced by Breschet (1784-1845) in 1833.
With Scarpa's contribution the macroscopic anatomy of the mammalian inner ear was complete. The facility of light- and electronmicroscopic preparation techniques added further details to the anatomy.
Huschke (1824) first mentioned the "basilar papilla" and considered it the excitatory structure of the cochlea. In 1851 Corti provided the first description of the cytoarchitecture of this structure. This followed shortly after the Dutch professor Harting (1812-1885) of Utrecht showed him the technique of preservation of preparations in fluid.
Important contributions to the morphology of the utricle and saccule were made by Steifensand (1835) and Schultze (1858). The morphological structure of the cupula was first described in detail by Lang (1863). The introduction of decalcification by Reissner (1852) made it possible to refine the histological techniques. Later studies by Odenius (1867), Hasse (1867), Boettcher (1869), Hensen (1878), Retzius (1884), Kaiser (1891), Held (1926) and Kolmer (1927) completed the current knowledge of the lightmicroscopic features of the inner ear. Further contributions did not come until the advent of electron microscopy, first introduced by Engström and Wersäll in 1953[a,b] in inner ear studies. Their investigations were soon followed by others (Smith, 1955; Spoendlin, 1956; Friedmann, 1959; Iurato,1960[a,b], 1962; Lundquist, 1965; Kimura, 1966; Iurato, 1967; Duvall, 1969).

Scanning electron microscopy has further deepened the insight into the spacial aspects of the inner ear structures at submicroscopic level (Lim and Lane, 1969; Bredberg et al, 1970, 1972; Jahnke, 1975; Soudijn, 1976).
In the first part of this chapter the morphological features of the mammalian inner ear will be described.

## 1.2 GROSS ANATOMY OF THE MAMMALIAN INNER EAR

The mammalian inner ear (labyrinth), consisting of the cochlea and vestibular apparatus, is situated in the petrous part of the temporal bone (fig. 1). The bony labyrinth contains the membranous labyrinth.
The understanding of the relation of these parts is facilitated by reference to the embryology. A detailed account of the embryonic development of the inner ear is given by Bast and Anson (1949).
The membranous labyrinth begins as ectodermal thickenings, the otic placodes, on each side of the rhombencephalon at an early embryonic stage. The primitive otocyst is formed by invagination of the otic placode and becomes the inner epithelial layer of the whole membranous labyrinth. This otocyst is transformed through infoldings of its wall into a series of fluid (endolymph) filled compartments and canals which connect with each other. Finally the membranous labyrinth consists of the cochlear duct, saccule, utricle, semicircular canals, endolymphatic duct and sac. These compartments

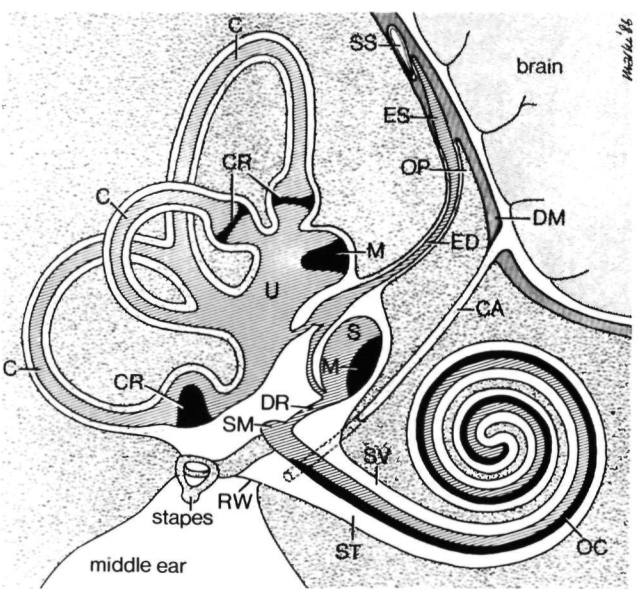
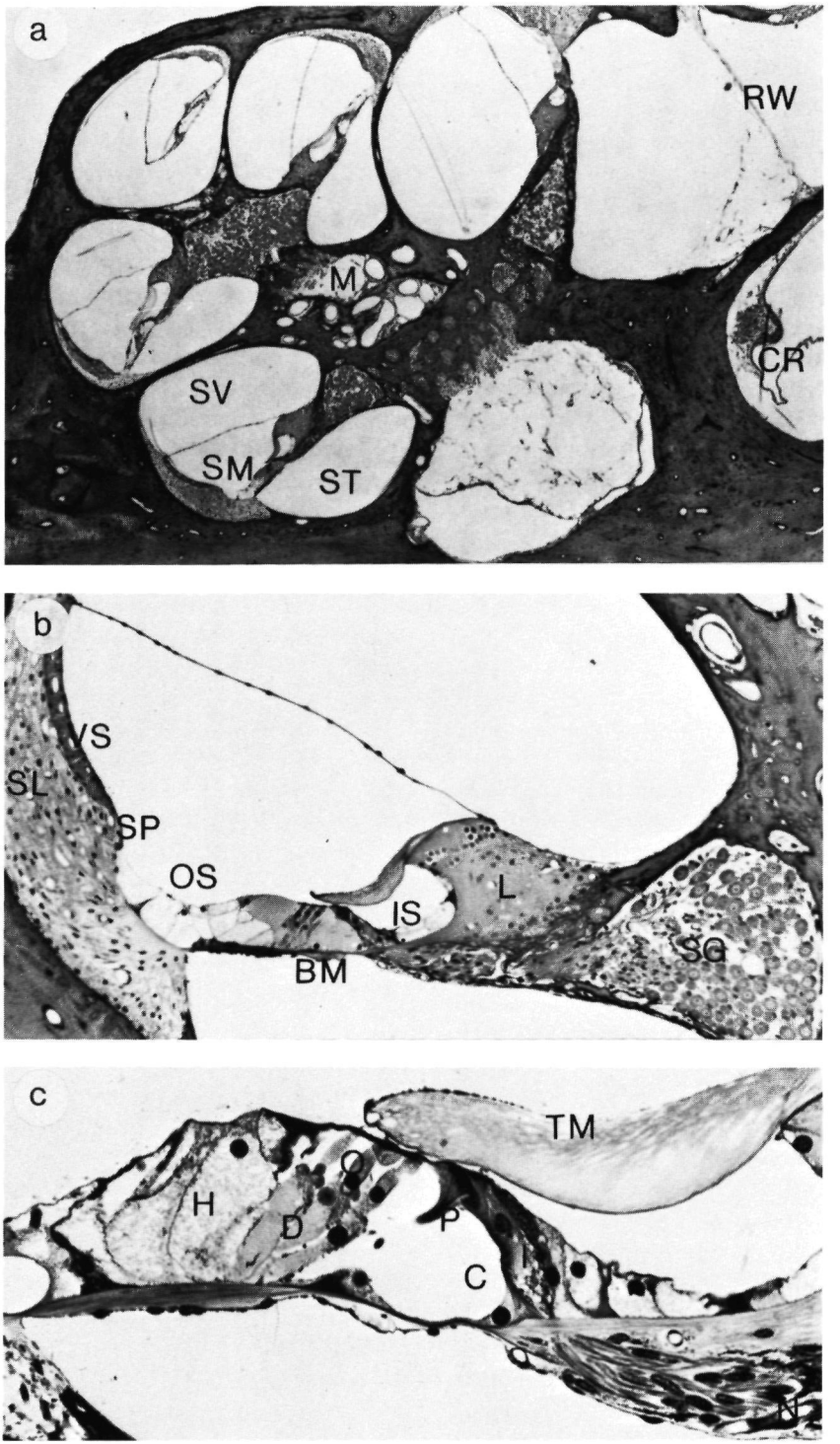



Fig. 1. Schematic drawing of the various compartments of the membranous labyrinth (modified from Kuijpers, 1969). Shaded areas are filled with endolymph, white areas with perilymph.
C: semicircular canals, CA: cochlear aqueduct, CR: crista, DM: dura mater, DR: ductus reuniens, M: macule, OC: organ of Corti, OP: operculum, RW: round window, S: saccule, ED: endolymphatic duct, ES: endolymphatic sac, SM: scala media, SS: sigmoid sinus, ST: scala tympani, SV: scala vestibuli, U: utricle.

are interconnected by the ductus reuniens, the utricular duct and the saccular duct (fig. 1).
The epithelial lining in these compartments is derived from the otocyst which differentiates into areas with specialized sensory epithelium, namely the organ of Corti, which contains the acoustic sensory cells concerned with perception of sound, the utricular and saccular maculae and ampullary cristae with vestibular sensory cells concerned primarily with the function of equilibrium.
The bony labyrinth is simply a condensation through cartilage to bone of the mesenchymal primitive otic capsule, which thus comes to form a hard protective case for the membranous labyrinth. During their development the bony and membranous labyrinths become separated by the perilymphatic spaces as a result of resorption of the mesenchymal tissue in between. The perilymphatic spaces surrounding the so called "pars superior" of the membranous labyrinth (utricle and semicircular canals) are filled with loosely textured connective tissue. In some species a limiting membrane separates the "pars superior" from the "pars inferior" (saccule and cochlea) which lacks this connective tissue (fig. 1). These perilymph filled spaces are connected with the cerebrospinal fluid space by means of the cochlear aqueduct.
The membranous endolymphatic duct and proximal part of the sac are situated in the bony vestibular aqueduct. The endolymph filled part is separated from the bony surrounding by the extension of the perilymphatic space. The internal aperture of the bony vestibular aqueduct is a small depression on the medial wall of the vestibule. The external aperture is usually a slit-like opening, which matches the adjacent margin of the sigmoid sulcus.
In the adult stage the membranous labyrinth is fully surrounded by the bony labyrinth apart from the major portion of the endolymphatic sac which extends beyond the limits of the bony vestibular aqueduct (fig. 1).
In this chapter the morphology of the epithelial lining of the membranous labyrinth will be described. Since this thesis is focused on the endolymphatic duct and sac, special attention will be paid to these structures.

### *1.2.1 Cochlear duct*

The cochlear duct or scala media is a spirally wound epithelial lined canal with a central bony axis (modiolus) which contains the cochlear nerve, spiral ganglion and main blood vessels (fig. 2[a]). The number of turns varies from species to species. The apical end of the cochlear duct ends blindly in the cupular cecum. The basal end terminates in the vestibular cecum, close to which the cochlear duct communicates with the saccule via the ductus reuniens. The cochlear duct is bounded on two sides by perilymphatic spaces, the scala vestibuli and the scala tympani. At the apex (helicotrema) the scala vestibuli passes into the scala tympani. In midmodiolar sections the cochlear duct has a triangular shape (figs. 2[a],[b]). Towards the scala tympani it is bordered by the tympanic wall, towards the scala vestibuli by the vestibular wall (Reissner's membrane) and laterally by the vascular stria and spiral prominence (fig. 2[b]).


a
RW
M
CR
SV
SM
ST
b
VS
SL
SP
OS
IS
L
SG
BM
c
TM
H
D
P
C
N

The epithelial lining of the tympanic wall rests on the basilar membrane which extends from the bony spiral lamina to the basilar crest of the spiral ligament (fig. 2[b]). The basilar membrane is constituted of bundles radiating collagen fibers embedded in ground substance. From the base towards the apex the basilar membrane increases in width, tapers in thickness and the number of collagen fibers increases (Iurato, 1962). On the scala tympani side this membrane is covered with mesothelial cells. The epithelial lining bordering the endolymphatic space is rather complex.

The main constituent is the organ of Corti which consists of sensory cells and supporting cells.

Following the radial organisation of the organ of Corti two different populations of sensory cells may be seen, a single row of inner hair cells (IHC) and usually three rows of outer hair cells (OHC) (fig. 2[c],5). The IHC are flask-shaped, the OHC are columnar in shape. The surface of each hair cell bears stereocilia which are embedded in a cuticular plate, the thickened upper surface of the hair cell. In man the stereocilia of one IHC number about 50-70 and of one OHC about 100-300. They are arranged in rows and form a "V" or "W" configuration.

Every hair cell has both afferent and efferent innervation distributed along the basal part of the cell (Engström, 1958). The afferent nerve endings are small containing few synaptic vesicles, the efferent nerve endings are much larger and contain many synaptic vesicles.

The afferent innervation is supplied by the cochlear nerve fibers connecting the bipolar ganglion cells, the cell bodies of which are located in the spiral ganglion (figs. 2[a],[b]) with the first synapse in the cochlear nuclei (Lorento de Nó, 1933). The afferent nerve endings synapse directly with the hair cells of which the majority innervate the IHC. By direct route or indirect relay circuit the axons travel to the superior olivary nucleus, the inferior colliculus and the medial geniculate body in order to terminate in the transverse temporal gyri of the cerebral hemispheres. One IHC is innervated by several afferent nerve fibers, while many OHC are supplied by one afferent nerve fiber (Spoendlin, 1969; Morrison et al, 1975)

The efferent innervation of the organ of Corti is supplied by the olivocochlear bundle of Rasmussen (Rasmussen, 1940), which originates in the superior olivary complex in the brainstem. The efferent nerve endings make no direct contact with the IHC, but form axo-dendritic synapses with the afferent fibers (Smith and Sjöstrand, 1961). The efferent fibers for the OHC cross the tunnel of Corti and make almost exclusively direct synaptic contact with the base of the OHC and may provide several collaterals (Spoendlin, 1969).

In addition to sensory cells the organ of Corti contains various types of supporting cells

←Fig. 2. Micrographs of rat cochlea.
a: midmodiolar section (X40)
b: scala media (X150)
c: organ of Corti (X425) (toluidin blue)
BM: basilar membrane, C: tunnel of Corti, CR: crista, D: cells of Deiter, H: cells of Hensen, I: inner hair cell, IS: inner sulcus, L: limbus, M: modiolus, N: nerve fibers, O: outer hair cells, OS: outer sulcus, P: pillar cells, RW: round window, SG: spiral ganglion, SL: spiral ligament, SM: scala media, SP: spiral prominence, ST: scala tympani, SV: scala vestibuli, TM: tectorial membrane, VS: vascular stria.

(pillar cells, Deiter's and Hensen's cells) (fig. 2c). The apical surfaces of the sensory and supporting cells, which are firmly interconnected by tight junctions, form the reticular membrane, providing a tight sealing of the endolymphatic space. Large intercellular spaces between the cells are present. They are filled with so-called cortilymph.
Medially the organ of Corti passes into the inner sulcus cells and limbus interdental cells. Laterally the organ of Corti passes into the epithelial lining of the spiral ligament. The spiral limbus, situated on top of the bony spiral lamina, contains loose connective tissue. The epithelial lining of the spiral limbus consists of T-shaped or interdental cells separated by bands of connective tissue, the so called teeth of Huschke. The apical parts of each interdental cell remain in close contact through thin horizontal plates or phalanges. The tectorial membrane extends from the interdental cells to the Hensen's and Claudius cells.
Electronmicroscopically the tectorial membrane consists of protofibrils type A and B bound together in a complex network.
Protofibrils A are unbranched, straight and predominate in the basal layer and in the entire middle zone of the tectorial membrane. Protofibrils type B are branched and coiled and occur throughout the entire tectorial membrane; these fibrils appearing in the middle zone are strongly hydrated. The contact surface between the tectorial membrane and inderdental cells is made up of tightly packed, weakly hydrated protofibrils type B, which essentially make up the border net (Kronester-Frei, 1978). The diameter

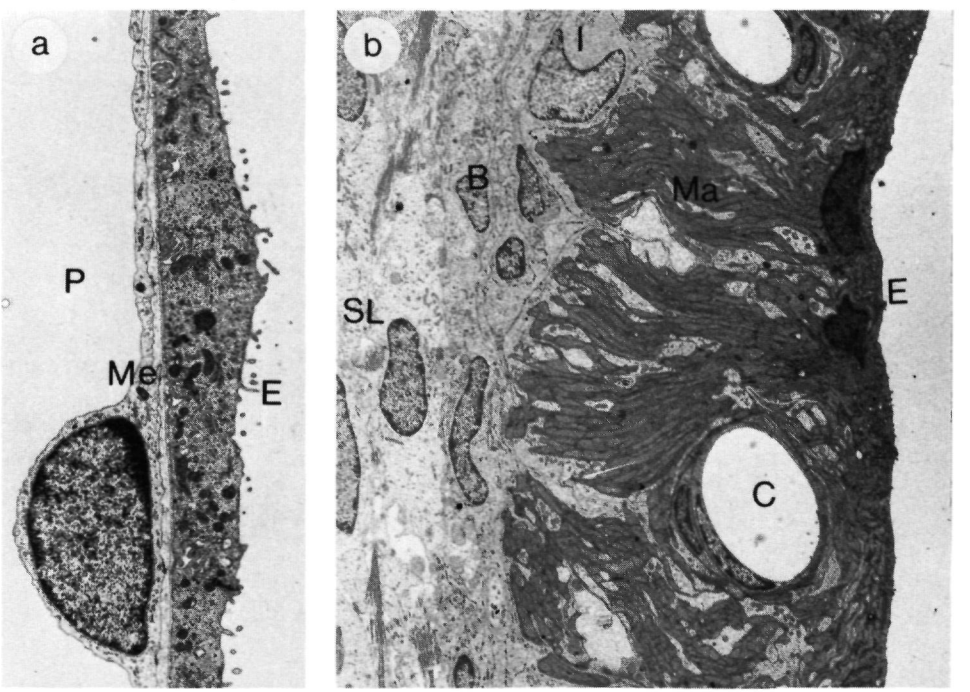



Fig. 3. Electronmicrographs of Reissner's membrane (a) (X5400) and vascular stria (b) (X1800).
B: basal cell, C: capillary, E: endolymph, I: intermediate cell, Ma: marginal cell, Me: mesothelial cell, P: perilymph, SL: spiral ligament.

of the protofibrils differ among species (Engström and Wersäll, 1958; Iurato, 1960[b]; Ross, 1974). Both histochemical and biochemical studies demonstrate that the tectorial membrane consists mainly of protein and to a minor extent of carbohydrate. The carbohydrates are presumably linked to proteins to form glycoproteins (Wislocki and Ladman, 1955; Dohlman et al, 1959; Schätzle, 1971; Tachibana et al, 1973; Ross, 1974; Steel, 1985).

#### 1.2.1.2 Vestibular wall

The separation of the cochlear duct from the scala vestibuli is performed by Reissner's membrane which courses from the spiral limbus to the vestibular crest of the spiral ligament (fig. 2[b]). On the endolymph side Reissner's membrane is composed of cuboidal cells, firmly attached to each other by tight junctions. At the endolymph surface these cells have microvilli and some coated invaginations. Their cytoplasm contains few mitochondria and some vesicles. The scala vestibuli side is lined by flat mesothelial cells with gaps in between. Both cell layers are separated by a distinct basement membrane. Reissner's membrane is avascular (fig. 3[a]).

#### 1.2.1.3 Lateral wall

The lateral wall of the cochlear duct is formed by the vascular stria and the spiral prominence (figs. 2[b], 3[b]). The separation between epithelium and bone is formed by the fibrous spiral ligament. The vascular stria is highly vascularized. Three cell types can be distinguished: the marginal cells, bordering the endolymphatic space, the intermediate cells and basal cells adjacent to the spiral ligament. The lateral and basal wall of the marginal cells show many infoldings contacting the intermediate cells. The marginal cells are interconnected with tight junctions. The apical cell membrane contains some coated invaginations. The cytoplasm of these cells contains numerous mitochondria in the basal part. In comparison with the marginal cells the intermediate and basal cells contain only a few cell organelles (Rodrigues-Echandia and Burgos, 1965). From both histochemical and biochemical studies the vascular stria shows an extremely high rate of oxidative metabolism, comparable with that of the kidney tubules (Chou and Rodgers, 1962). The functional aspects are discussed in paragraphs 1.3.3 and 1.4.2.

### *1.2.2 Vestibular labyrinth*

#### 1.2.2.1 Saccule and utricle

The saccule is a cylindrical space located in the anterior and medial inferior portion of the vestibule. The utricle is an oblong, slightly flattened sac which occupies the elliptical recess in the upper and posterior/superior portion of the vestibule (fig. 4). Part of the epithelial lining of the saccule and utricle consists of sensory cells with supporting cells in between, concentrated in the utricular and saccular macule. Each macule is divided in the pars interna and pars externa by a narrow curved zone through its middle, the

striola (Lindeman, 1969). The sensory cells consist of two types of hair cell. The type I hair cell is flask-shaped. The type II hair cell is columnar-shaped. Both cells have at their surface stereocilia firmly anchored in the cuticular plate. Each cell has a kinocilium emerging from a basal body which is located at the periphery of the bundle of stereocilia. This arrangement provides a morphological and functional polarization. In the utricular macule the stereocilia are polarized towards the striola and in the saccular macule away from it, inkeeping the morphological polarization towards the kinocilium. The apical surface of both hair cells and supporting cells are firmly held together by the reticular membrane,forming a tight closure of the endolymphatic space.

All hair cells have both afferent and efferent innervation along the basal part of the cell (Engström, 1958). The type I hair cells are enveloped by direct synapses of caliform afferent nerve endings, on the basal part of which bud-like synapses of efferent nerve endings are found. The type II hair cells are innervated by numerous direct synapses of bud-like afferent and efferent nerve endings. The afferent innervation is supplied by the saccular and utricular nerve fibers connecting the bipolar ganglion cells, the cell bodies of which are located in Scarpa's ganglion, with the first synapse in the vestibular nuclei in the brainstem where the signals are transmitted to secondary neurons. The efferent innervation of the vestibular system originates from the lateral vestibular nucleus. The vestibular nuclei are involved in vestibulo-cerebellar, vestibulo-spinal and vestibulo-ocular pathways (Gacek, 1980).

The cilia of the sensory cells are embedded in the otolithic membrane,a gelatinous structure which contains the otoconia (figs. 4, 7). The otoconia vary in size dependent on their location (Lindeman, 1969).

The otolithic membrane is composed of the otoconial layer, gelatinous layer and

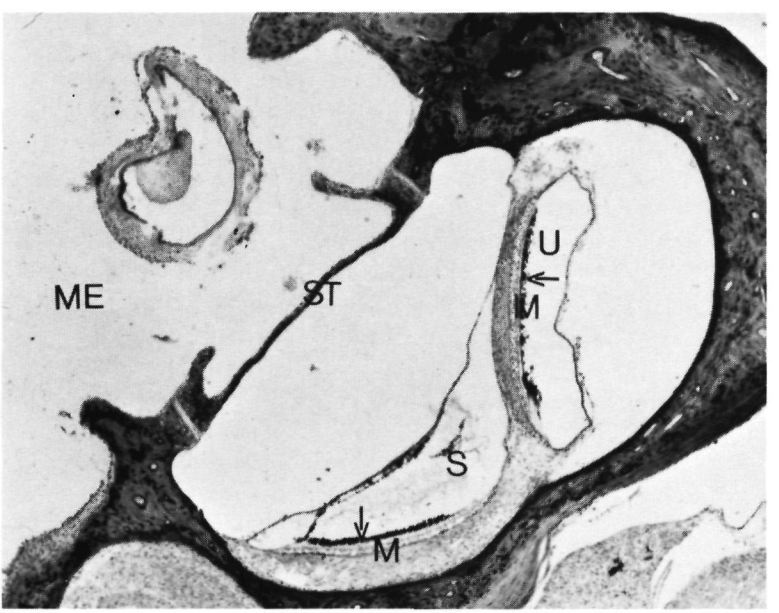



Fig. 4. Micrograph of otolith organs.
M: macule, ME: middle ear, S: saccule, ST: stapes footplate, U: utricle (→) otoliths (toluidin blue, X40).

subcupular meshwork. The gelatinous layer is formed of amorphous and fibrillar material and consists of two distinct parts; the upper layer, to which the otoconia are attached and the honeycomb layer, which houses the tall ciliary bundles of sensory cells. The underside of the honeycomb layer is attached to the supporting cells of the sensory epithelium by a gelatinous meshwork (Lim, 1980).
Mammalian otoconia contain an organic, filamentous-granular fraction (Igarashi and Kanda, 1969; Salamat et al, 1980) constituting the otoconial matrix and an inorganic fraction with $CaCO_3$ crystals in the form of calcite embedded in it (Carlström et al, 1953).
Histochemical studies have shown both in the gelatinous layer and the otoconia the presence of glycoproteins (Wislocki and Ladman, 1955; Ferreri and Crifo, 1956; Takahashi, 1961; Kurata, 1963; Veenhof, 1969).
The non-sensory epithelial lining of saccule and utricle consists of one layer of flattened to cuboidal cells.
In particular areas of the membranous wall of the utricle dark cells are found, the name based on their osmiophilic capacity. These cells have numerous infoldings of the basal cell membrane containing many mitochondria. In the saccule dark cells are absent (Kimura, 1969).
The macule of the saccule is hook-shaped and located on the medial saccular wall in an approximately vertical plane with a slight lateral tilt, perpendicular to the utricular macule. The utricular macule is a spade-shaped, thickened area situated anteriorly and laterally within the utricle.
A small duct leaves the saccule to connect with the endolymphatic duct. The utricle is connected with the endolymphatic duct by the utricular duct in which a valve-like fold over the orifice of the duct, the utriculo-endolymphatic valve, is situated (Bast and Anson, 1949; Schuknecht and Belal, 1975; Konishi, 1977).

### 1.2.2.2 Semicircular canals

The three semicircular canals are the horizontal, the anterior vertical and the posterior vertical canal (fig. 1). Each canal extends over about two thirds of a circle. The planes in which the three semicircular canals are located subtend approximately right angles. The membranous canals are almost circular in transverse section and separated from the bony wall by the perilymphatic space which is filled with a loosely textured connective tissue.
Near the utricular opening each canal is enlarged to form a membranous ampulla, the base of which is attached to bone. The non-ampullated ends of the anterior and posterior vertical canal form the common crus (fig. 1).
The ampulla of each duct contains a transverse ridge of sensory cells and supporting cells, the ampullary crista, projecting from the floor of the ampulla.
Like the otolith organs the sensory epithelium of the ampullary crista contains type I and II hair cells. Each hair cell is supplied by many stereocilia and one kinocilium. This arrangement provides the morphological and functional polarization that is for the lateral semicircular canal towards the utricle, for the superior and posterior semicircular canal away from the utricle.
As described for the macule the hair cells are in direct contact with surrounding

supporting cells and the apical surface of both are firmly held by the reticular membrane, forming a tight closure of the endolymphatic space. At the base of the crista the sensory epithelium passes via a zone of transitional cells into the more flattened epithelium of the semicircular canals. At distinct sites the transitional cells border irregular areas of dark cells which are continuous with those described for the utricle (Kimura, 1969).
On the ampullary walls at each end of the crista is a zone of cuboidal cells which form a half-moon-shaped area termed the semilunar planes.
The innervation is supplied by the ampullary nerve fibers. The cilia of the sensory cells are embedded in the cupula, which obliterates the space between the crista and the roof of the ampulla (figs. 2, 6). Electronmicroscopic studies of the cupula reveal an exceedingly loose acellular structure composed of filaments forming a three dimensional network and enclosing an amorphous interfibrillar substance. No separate outer membrane can be observed, but there is a dense outer layer forming a boundary between cupula and endolymph (Wersäll, 1956; Iurato, 1967).
Histochemical investigations revealed that glycoproteins are important chemical constituents of the cupula (Wislocki and Ladman, 1955; Ferreri and Crifo 1956; Jensen and Vilstrup, 1960; Takahashi, 1961; Igarashi and Kanda, 1969).

### *1.2.3 Endolymphatic duct and sac; morphological and functional aspects.*

#### 1.2.3.1 Introduction

About 100 years after its discovery (Cotugno, 1760) the first description of the lightmicroscopic structure of this "sac-like appendage of the membranous labyrinth" was described by Boettcher in 1869 for cat and human. The names endolymphatic duct and sac were introduced by Hasse (1873). Guild (1927[a]) defined the ductus endolymphaticus and saccus endolymphaticus proprius. The latter, on the basis of differentiation of the epithelial lining, was subdivided into three portions: 1. proximal part, lying entirely within the dilated portion of the vestibular aqueduct; 2. intermediate part, lying partly within the vestibular aqueduct and partly between the layers of the dura mater near its external aperture; 3. distal part, lying in the dura mater (fig. 1).
Bast and Anson (1949) introduced new nomenclature for the various portions of the human endolymphatic duct and sac. The endolymphatic duct was divided into two parts, the sinus portion and the isthmus portion, and the endolymphatic sac into two parts, the rugose or interosseous portion and the endolymphatic sac proper or smooth portion (fig. 1).
The first detailed description of the morphology of the endolymphatic duct and sac of animals was reported by Guild (1927[a]) for the guinea pig. The ultrastructural features have been described by Lundquist et al (1964), Lundquist (1965, 1976), Mitani et al (1973), Rask-Andersen and Stahle (1979) and Bagger-Sjöbäck et al (1981). In contrast to the comprehensive studies on the guinea pig, only sparse data are available on the morphology of the endolymphatic sac of other species including rabbit (Seymour 1954; Adlington, 1967, 1984; Friberg et al, 1985), chinchilla (Lim and Silver, 1974), mouse (Friberg et al, 1985), monkey (Friberg et al, 1985) and dog (Harada and Gaafar 1976).

### 1.2.3.2 Endolymphatic duct

The endolymphatic duct in mammals begins at the site where the utricular and saccular ducts unite, as a sinusoid enlargement in the proximal part of the vestibular aqueduct (fig. 1). As it enters this duct it narrows to form a constricted tube, the isthmus. From here the duct courses through the vestibular aqueduct towards the posterior surface of the petrous pyramid. Close to the external aperture of the vestibular aqueduct the endolymphatic duct dilates to form the endolymphatic sac. Both lightmicroscopic and ultrastructural features have been reported for the endolymphatic duct in man and guinea pig, while lightmicroscopic data are available for the cat. The morphology of the endolymphatic duct of these species do not differ fundamentally, but there are differences in degree.
In the cat and the guinea pig the epithelial lining of the endolymphatic duct consist of simple squamous or low cuboidal epithelium, resting on a smooth basal lamina. According to Boettcher (1869) and Lundquist (1965) the mucosa has a rather smooth appearance. However Rask-Andersen et al (1981[b]) reported a more irregular epithelium with crypts and protruding papillae. In man the epithelial lining shows several folds, luminal projections and epithelial invaginations (Friberg et al, 1984).
The epithelial cells are polymorphic, varying from flat to cylindrical. The cytoplasm is dense, with a few scattered ribosomes, occasional lysosome like structures and endoplasmic reticulum. Mitochondria are sparse and evenly distributed throughout the cytoplasm. The luminal surfaces of the cells possess a few short microvilli. The cells are connected by junctions which have been classified as "leaky" (Bagger-Sjöbäck et al, 1981; Friberg et al, 1984; Bagger-Sjöbäck and Rask-Andersen, 1986). Lateral and basal infoldings of the membranes of the epithelial cells are quite extensive in the human duct (Friberg et al, 1984; Rask-Andersen et al, 1984) but they are only rarely found in the guinea pig (Lundquist, 1965; Rask-Andersen et al, 1981[b]).
Usually the lumen of the duct is optically empty but in some human specimens the lumen was found to be filled with a stainable colloid like substance, while occasionally geometrical bodies resembling otoconia were observed (Friberg et al, 1984). In the guinea pig the duct is surrounded by a rather loose connective tissue which is traversed by small thin walled capillaries and lymph vessels (Rask-Andersen and Stahle, 1979; Rask-Andersen et al, 1981[b]; Friberg et al, 1984).

### 1.2.3.3 Endolymphatic sac

The proximal part of the endolymphatic sac is situated in the vestibular aqueduct while the distal part ends blindly intradurally (fig. 1).
Fundamental morphological differences in the epithelial lining of the endolymphatic sacs of the species studied so far have not been observed.
For the description of the morphological features of the endolymphatic sac the subdivision of this structure proposed by Guild (1927[a]) will be followed.
For all animals studied the epithelial lining of the proximal portion of the sac consists of one celllayer with a rather smooth appearance which gradually changes from flattened to cuboidal towards the intermediate portion. Small microvilli are found on the luminal surface. The cytoplasm is rather dense and granular and contains more inclu-

sion bodies than the cells of the duct. The junctional complexes are of the "leaky" type (Bagger-Sjöbäck et al, 1981; Bagger-Sjöbäck and Rask-Andersen, 1986).
The subepithelial tissue contains many fibroblasts, lymph vessels and capillaries, some of them fenestrated (Lundquist et al, 1964; Rask-Andersen et al, 1983).
The epithelial lining of the intermediate portion is very irregular with large epithelial infoldings in all mammals studied. The number of these epithelial foldings have been found to increase from birth until 30 years of age in man (Gussen, 1971). In adults the epithelial cells of the foldings may become pseudostratified (Zechner and Altmann, 1969), sometimes containing a central core of connective tissue with a small blood vessel (Arenberg et al, 1970). The degree of folding may vary greatly between different specimens (Lundquist, 1965; Bagger-Sjöbäck et al, 1986). According to Antunez et al (1980) the human endolymphatic sac consists of a complicated system of interconnecting canaliculi rather than a single lumen with epithelial folds.
In the intermediate portion the cells range from cuboidal to tall columnar. Light and dark cells can be distinguished on account of their electron density in the guinea pig (Lundquist, 1965). These cells correspond to the type I and type II cells described for the endolymphatic sac of the rabbit (Adlington 1967; 1984) and man (Schindler, 1980; Bagger-Sjöbäck et al, 1986).
The light cells have a homogeneous pale granular matrix, with many ribosomes and mitochondria. The luminal cell surface is provided with numerous microvilli and pinocytic vesicles. The basal and lateral parts of these cells exhibit extensive infoldings which can vary from species to species (Friberg et al, 1985). The epithelium is separated from the underlying connective tissue by a basement membrane.
The dark cells have a more condensed cytoplasm. The number of ribosomes and mitochondria is less than in the light cells. The large nucleus is elongated and often irregular due to invaginations. The apical surfaces have few microvilli in guinea pig, chinchilla and human specimens (Lundquist 1965; Lim and Silver, 1974; Schindler, 1980; Bagger-Sjöbäck et al, 1986) but none in rabbit (Adlington, 1984), which instead occasionally show club like protrusions.
The junctional complexes between the cells are also of the "leaky" type (Bagger-Sjöbäck et al, 1981; Bagger-Sjöbäck and Rask-Andersen, 1986).
The subepithelial alveolar connective tissue, which can vary in density between the interosseous and extraosseous part, (Lim and Glasscock, 1981) reveals a rich capillary network, partly fenestrated (Rask-Andersen et al, 1983), in close proximity to the epithelial lining. Thin walled lymph vessels are also found close to the sac (Arnvig, 1951).
The distal lining of the endolymphatic sac is smooth, with cuboidal cells and, at its extreme end, squamous cells. The cells resemble those of the proximal portion, although light and dark cells can be differentiated in the guinea pig (Lundquist, 1965). The apical surface reveals few microvilli (Mitani et al, 1973; Harada and Gaafar, 1976), but not in man (Schindler, 1980). The basal cell lining is smooth.
The junctional complexes between the cells appear as a very extensive network of continuous sealing strands forming extensive tight junctions (Bagger-Sjöbäck and Rask-Andersen, 1986).
The subepithelial tissue resembles that of the intermediate portion, but appears more dense in man (Schindler, 1980). In the distal portion a true lumen has never been observed in the guinea pig; the epithelial surfaces of the walls are firmly adherent to one

another (Lundquist, 1965).
In contrast to other areas of the membranous labyrinth, the lumen of the endolymphatic sac of the mammals studied is endowed with a multitude of free floating cells (FFC) (Guild, 1927[a],[b]; Doi, 1939). FFC comprise mainly macrophages and leucocytes. These cells are present in the proximal portion, but are more frequent in the intermediate part of the sac.
According to Lundquist (1965) FFC could be derived from the connective tissue and the dark epithelial cells or from migration of perisaccular cells as suggested by van Egmond and Brinkman (1956). Sörnas (1971), Rask-Andersen and Stahle (1980) proposed their origin from haematogenous sources and Guseo (1977) and Morse and Low (1972) from cerebrospinal fluid. The macrophages seem to exhibit both pinocytic and phagocytic activity (Guild, 1927[a],[b]; Lundquist, 1965).
The luminal fluid in the guinea pig is assumed to be a proteinaceous precipitate which stains heavily with osmium and toluidin blue (Rask-Andersen and Stahle, 1979).
In man the luminal fluid contains a granular or floccular precipitate, while at the bottom of crypts in the intermediate portion a PAS and alcian blue staining granular substance has been described (Zechner and Altmann, 1969; Bagger-Sjöbäck et al, 1986). Adlington (1967) observed in rabbits a dense homogenous coagulum consisting of aggregates of cell debris, mitochondria, lysosomes and myelin figures.

### 1.2.3.4 Functional aspects

Speculation as to the possible function and significance of the endolymphatic sac soon followed its discovery and a variety of sometimes contradictory functions has been proposed. In addition to the original description of this structure, Cotugno (1760) suggested a pressure regulating function. This was also proposed by Hasse (1873), Kolmer (1923), Siirala (1942), Secrétan (1944), Mygind (1952), Allen (1964), Bosher and Hallpike (1965), Partsch (1966), Adlington (1967, 1984) and Bagger-Sjöbäck et al (1986) although without clear experimental evidence.
Sterzi (1909) and Siebenmann (1919) considered this structure as a vestigial organ.
The presence of free floating cells and the data obtained from electronmicroscopic studies have given rise to the assumption that the endolymphatic sac could be involved in resorption of endolymph (Iwata, 1924; Guild, 1927[b]; Yamakawa, 1929; Anson and Nesselrod, 1936; Rollin, 1940; Siirala, 1942; Secrétan, 1944; Andersen, 1948; Arnvig, 1951; Saxén, 1951; van Egmond and Brinkman, 1956; Lundquist, 1965; 1976; Kimura and Schuknecht, 1965; Adlington, 1967, 1968, 1984; Zechner and Altmann, 1969; Rudert, 1969[a]; Lim and Silver, 1974; Kimura, 1976; Rask-Andersen and Stahle, 1979; Rask-Andersen et al, 1981[a],[b]; Bagger-Sjöbäck and Rask-Andersen, 1986).
This assumption was supported by enzyme histochemical studies demonstrating that the epithelial lining of the sac was richly provided with enzymes involved with protein degradation and the presence of phagocytes. (Ishii et al, 1966; Schätzle and Haubrich, 1966; Silverstein, 1966[b]; Haubrich, 1975).
Further evidence for a resorptive function was derived from the observation that a large variety of foreign substances introduced into the endolymphatic space could be recovered from the endolymphatic sac (table 1).
Another finding that seems to speak in favour of a resorptive function is the emergence of an endolymphatic hydrops after obliteration of the endolymphatic sac and duct, as

Table 1

Substances applied into or outside the endolymphatic space and recovered from the endolymphatic sac

| Site | Material | Author |
|---|---|---|
| Endolymphatic space | Ferrosalts 1–4%<br>Indian inc<br>Tryphan blue<br>Colloidal silver 0,25%<br>Haemolytic streptococci<br>Peroxidase 1, 2, 4%<br>Tryphan blue 0,5%<br>$^{3}$H lysine<br>Horse Radish Peroxidase 3%<br>Ferritin 1,10%<br>Lanthanium citrate 4%<br>Rhodamine | Guild (1927[a])<br>Doi (1939)<br>Engstrom and Hjorth (1950)<br>Lundquist (1965)<br>Lundquist (1965)<br>Ishii et al (1966)<br>Rudert (1969[a])<br>Rudert (1969[b])<br>Jahnke and Rudert (1973)<br>Jahnke and Rudert (1973)<br>Rask-Andersen et al (1981[a])<br>Giebel (1982) |
| Perilymphatic | Trypan blue<br>Ferrosalts | Engstrom and Hjorth (1950)<br>Altmann and Waltner (1950) |
| Intraperitoneal | Trypan blue<br>$^{3}$H tyrosine | Andersen (1948)<br>Koburg et al (1967) |
| Intravenous | Trypan blue | Andersen (1948) |
| Subcutaneous | Trypan blue | van Egmond and Brinkman (1956) |

will be discussed in paragraph 1.3.4. However it must be noted that the occurrence of this phenomenon appeared to be largely dependent on the animal species used.

An exclusive secretory function or a combination of secretory and resorptive functions for the endolymphatic sac has been proposed by Boettcher, 1871; Hasse, 1873; Seymour, 1954; van Egmond and Brinkman, 1956; Cimino, 1964; Koburg et al, 1967; Adlington, 1967, 1984.

The assumption of a secretory function was based on the presence of intraluminal secretory granules and on the observation that foreign substances administered elsewhere outside the inner ear could be seen afterwards in the lumen of the endolymphatic sac (table 1).

Adlington (1984) and Friberg et al (1986) proposed some form of secretion based on the observation that the saccus did not show evidence of change of epithelium or collapse of its lumen when isolated from any source of endolymph following disruption of the endolymphatic duct or labyrinthectomy.

Apart from this general involvement of the endolymphatic sac in resorption and secretion of endolymph, two very specialised functions have been ascribed to the endolymphatic sac. A possible role in the formation and/or degradation of otoconia was derived from the presence of such structures in the endolymphatic sac and duct (Imoto et al, 1983; De Vincentiis and Marmo, 1968; Yamane et al, 1984; Ohashi and Igarashi, 1985; Bagger-Sjöbäck et al, 1986).

Finally, very recent studies may support an involvement of the endolymphatic sac in

the immune defence of the inner ear. Arnold et al (1984) demonstrated the presence of immunoglobulins both in the epithelial lining and in the lumen of the endolymphatic sac, while Tomiyama and Harris (1986) showed that endolymphatic sac obstruction resulted in a significantly suppressed antibody level in the inner ear after antigen challenge.
In contrast to the vast number of studies on the endolymphatic sac, studies on the endolymphatic duct are scarce. Rask-Andersen et al (1981[a]), from the course taken by ionic lanthanum introduced into the cochlear duct, concluded the existence of an intercellular fluid pathway between the epithelial lining of the endolymphatic duct in the guinea pig.
Summarizing, this review of the literature refers to a multifunctional role of the endolymphatic sac in inner ear physiology, of which a resorptive function seems to be most prominent.

## 1.3 INNER EAR FLUIDS

### *1.3.1 Introduction*

Cell physiological processes are ultimately related to the properties of the fluids that surround the various tissues. The inner ear fluids serve the purpose of providing the transmission mechanism for auditory and vestibular stimuli to the sensorineural receptor cells and of providing the proper environment for the transformation of these stimuli into action potentials in the afferent nerve fibers (Konishi et al, 1966; Duvall and Rhodes, 1968; Kuijpers, 1969).
The three fluid compartments defined for the mammalian inner ear are the endolymphatic, perilymphatic and cortilymphatic spaces, containing endolymph, perilymph and cortilymph respectively. In this chapter the composition of inner ear fluids, their possible origin and function will be discussed. Finally the current endolymph flow theories will be reviewed.

### *1.3.2 Perilymph*

The perilymphatic space originates, as already discussed, as a result of the resorption of the mesenchymal tissue between the bony and membranous labyrinth. It is not surrounded by true epithelium but by so-called mesothelial cells, separating the space from bone and the endolymphatic space. The perilymphatic space has free communication with the cerebrospinal fluid (CSF) through the cochlear aqueduct (fig. 1). Initially bulk inflow of CSF through the cochlear aqueduct was thought to be the source of perilymph.
At present perilymph is considered essentially a local ultrafiltrate from inner ear blood vessels, whereas influx of CSF makes a minor contribution (Kley, 1951; Rauch, 1964; Kellerhals, 1976; Sterkers et al, 1982).
Perilymph closely resembles serum in ionic composition (Arnold, 1974; Makimoto and Silverstein, 1974; Kellerhals, 1976) but the protein content is less than 5% of that of serum (Fernández, 1967). Uptake of perilymph takes place through capillaries of the

spiral ligament and the spiral prominence.
Initially, cortilymph (the fluid surrounding the cells of the organ of Corti) and perilymph were thought to originate from different sources (Kley, 1951) but later studies demonstrated that both fluid spaces are in full communication with each other (Schuknecht and El Seifi, 1963; von Ilberg, 1968).

### *1.3.3 Endolymph*

The earliest investigation of the ionic composition of inner ear fluids was carried out by Kaieda (1930) who pooled the inner ear fluids of freshly killed sharks and found sodium, potassium and chloride to be about twice as concentrated in endolymph as in perilymph.
In 1954 Smith et al were the first to examine the ionic composition of mammalian endolymph. Most remarkably the endolymph was found to have a much higher concentration of potassium than of sodium. The similarity of endolymph – itself an extracellular fluid – to intracellular fluid in terms of cationic composition, has since been convincingly demonstrated by other authors.
The protein concentration of the endolymph is rather low. Miyamoto and Morgenstern (1981) reported 120 mg% for the guinea pig. For the guinea pig the $Na^+$ concentration varies between 1 and 5 mmol/l, the $K^+$ concentration between 150 and 152 mmol/l (Johnstone et al, 1963; Sellick and Johnstone, 1975; Konishi and Kelsey, 1976).
The maintenance of this peculiar cationic concentration against the reverse concentrations in perilymph and serum was found to be provided by the $Na^+$-$K^+$ activated adenosine triphosphatase system, which was shown to be present in extremely high concentration in the vascular stria (Kuijpers, 1969).
In the vestibular portion of the membranous labyrinth a rather high concentration of this enzyme was found to be present in the dark cells (Nakai and Hilding, 1968; Kuijpers, 1969). These cells are comparable with the marginal cells of the vascular stria and can therefore be assumed to contribute to the maintenance of the intracellular like cation composition of the vestibular endolymph.
The composition of the endolymph in the endolymphatic sac appears to be essentially different from that in other compartments of the membranous inner ear as far as can be concluded from the sparse data shown in table 2. These show a reversed cation concentration and an extremely high protein content.

Table 2

Composition of endolymph of the endolymphatic sac.

| Species | $Na^+$ (mmol/l) | $K^+$ (mmol/l) | Cl (mmol/l) | Protein (mg%) | Author |
|---|---|---|---|---|---|
| cat | 153 | 8 | – | 5200 | Silverstein, 1966[a,b] |
| guinea pig | 127,4 | 16,7 | 85 0 | 1750 | Miyamoto and Morgenstern, 1981 |

Endolymph circulation has received considerable attention since Corti described the vascular stria in 1851 and suggested that this structure might be the source of the inner ear fluids. Over the years the circulation of endolymph has provoked a vast amount of speculation and controversy.

The different concepts of endolymph flow proposed are the longitudinal flow theory, the radial flow theory and the dynamic flow theory.

The **longitudinal flow theory**, attributed to Guild (1927[b]), describes endolymph, secreted by the vascular stria, flowing from the cochlea through the reunien duct into the saccule and thence into the endolymphatic duct and sac where resorption takes place. This theory is based on the observation that a large variety of foreign substances, when introduced directly into the endolymphatic space, could be recovered from the endolymphatic sac after various periods of time (table 1). However these experiments are not without fault. Application of these nonphysiological substances, which are often toxic, into the cochlear duct may give rise to a pressure increase in the endolymphatic space, resulting in membrane ruptures and deleterious effects on the inner ear structures and metabolism. Moreover, rigid criteria to establish whether these substances were really introduced into the endolymphatic space are usually lacking.

Further arguments for the existence of a longitudinal endolymph flow and a resorptive function of the endolymphatic sac have been derived from the observation that obliteration of the endolymphatic sac can result in endolymphatic hydrops.

Kimura and Schuknecht reported in 1965 the consistent development of endolymphatic hydrops after surgical obliteration of the endolymphatic duct and sac in the guinea pig. These authors were unaware of the first report of Naito in 1950, which described the production of endolymphatic hydrops in one out of five guinea pigs by surgical obliteration of the endolymphatic sac. With refinement of the technique Kimura (1967) produced hydrops in 100% of the animals. These findings have subsequently been confirmed by other investigators (Nakamura, 1967; Konishi and Shea, 1975; Suh and Cody, 1977). The cochlea and saccule appeared expanded within 24 hrs after obliteration of the endolymphatic duct and sac in guinea pigs. Within two weeks the saccus can touch the stapes footplate. Distension of Reissner's membrane continued slowly with progression of time.

The most obvious ultrastructural change in the distended Reissner's membrane was the frequent lack of mesothelial cells over a wide area in the apical turns, while the epithelial cells were greatly enlarged. Outpouchings and holes were observed (Kimura, 1967; Shinosaki and Kimura, 1980).

Freeze-fracture studies revealed no change in the intercellular junctions of the cells of Reissner's membrane (Jahnke et al, 1985).

Ruptures of Reissner's membrane were occasionally observed in the apical turn, but they have not been demonstrated in the vestibular membranous wall.

Atrophic changes in sensorineural receptor cells, spiral ganglion cells and vascular stria were usually observed in the apical turns after one postoperative month. OHC were more often atrophic than IHC. However, the vestibular sensory cells remained normal in all cases (Kimura, 1967).

Reports on electrolyte concentrations in endolymphatic hydrops revealed no significant change in sodium, potassium and chloride concentration (Cohen and Morizono,

Table 3

Results of endolymphatic sac obliteration in various animals.

| Animal | Source, yr. | Endolymphatic hydrops | Comments |
|---|---|---|---|
| Rabbit | Beal (1968)<br>Martin et al (1983) | 7/7<br>7/7 | |
| Monkey | Lindsay (1947)<br>Kimura (1968<br>Suh and Cody (1977) | 0/4<br>0/11<br>0/3 | |
| Cat | Suh and Cody (1977)<br>Lindsay et al (1952)<br>Schuknecht and Kimura (1953)<br>Schuknecht and El Seifi (1963) | 0/6<br>0/9<br>0/5<br>0/3 | Short survival time |
| | Kimura (1968)<br>Schuknecht et al (1968)<br>Beal (1968)<br>Kerr and Smith (1976) | 5/6<br>12/15<br>5/6<br>1/1 | Long survival time |
| Chinchilla | Kimura (1968)<br>Suh and Cody (1977) | 2/2<br>2/9 | |
| Guinea pig | Naito (1968)<br>Kimura and Schuknecht (1965)<br>Kimura (1967)<br>Nakamura (1967)<br>Konishi and Shea (1975)<br>Suh and Cody (1977) | 1/6<br>14/14<br>47/47<br>31/31<br>10/10<br>19/19 | |

1984; Konishi and Kelsey, 1976; Konishi et al, 1981; Miyamoto and Morgenstern, 1981). Although most experimental studies on endolymphatic hydrops are confined to the guinea pig, production of endolymphatic hydrops by endolymphatic sac obliteration has also been attempted in other mammals. It has been reported to be successful in the rabbit (Beal, 1968; Martin et al, 1983) and in the cat (Kimura, 1968; Schuknecht et al, 1968; Kerr and Smith, 1976) but only after longer observation periods, and to a minor extent in chinchillas (Kimura, 1968; Suh and Cody, 1977) but not in monkeys (Lindsay, 1947; Kimura, 1968; Suh and Cody, 1977). The currently published data on the effect of saccus obliteration in various mammals are summarized in table 3. These data seem to suggest a species dependency as well as a difference in the time required to evoke endolymphatic hydrops by saccus obliteration.

Detailed morphological descriptions of the cochlear duct and the membranous vestibular labyrinth following endolymphatic hydrops produced by sac obliteration are not available for species other than the guinea pig.

In addition to the longitudinal flow theory a radial flow of endolymph has also been suggested. This **radial flow theory**, proposed by Naftalin and Harrison (1958), suggests a flow proceeding from the perilymphatic space through Reissner's membrane to the endolymphatic space. The vascular stria in this hypothesis would extract the sodium and exchange potassium for it, while Reissner's membrane is thought to prevent the

flow of potassium from the scala media to the scala vestibuli.
Support for this hypothesis was derived from the observation that degeneration of the organ of Corti and vascular stria after local rupture of Reissner's membrane remained mainly confined to the traumatized area (Lawrence et al, 1961; Lawrence, 1966; Duvall and Rhodes, 1967). The failure of this destruction to progress along the cochlear duct was assumed to indicate that perilymph, entered in toxic concentrations into the lymphatic space, was not carried along the cochlear duct.
At present there is convincing evidence, both from biochemical and physiological studies, that maintenance of the intracellular-like cationic composition of the endolymph against the passive leakage through the lining of the endolymphatic space seems to be chiefly a local event, governed by the $Na^+$-$K^+$ activated ATPase system in the vascular stria and in the dark cell area in the vestibular portion (Kuijpers and Bonting, 1969, 1970[a],[b]).
In an attempt to combine the abovementioned conflicting concepts of endolymph circulation Lundquist (1976) introduced **the dynamic flow theory**. According to this theory the local radial flow (Naftalin and Harrison, 1958) could explain the maintenance of the characteristic cationic composition of the endolymph, while the longitudinal flow (Guild, 1927[b]) was supposed to be the underlying mechanism for the transport of cellular debris and high molecular weight waste products towards the endolymphatic sac.

Although the existence and character of the longitudinal flow under physiological conditions still needs to be proven, the abovementioned experimental data seem to be in favour of the dynamic flow theory.

## 1.4 ELECTROPHYSIOLOGY OF THE MAMMALIAN INNER EAR

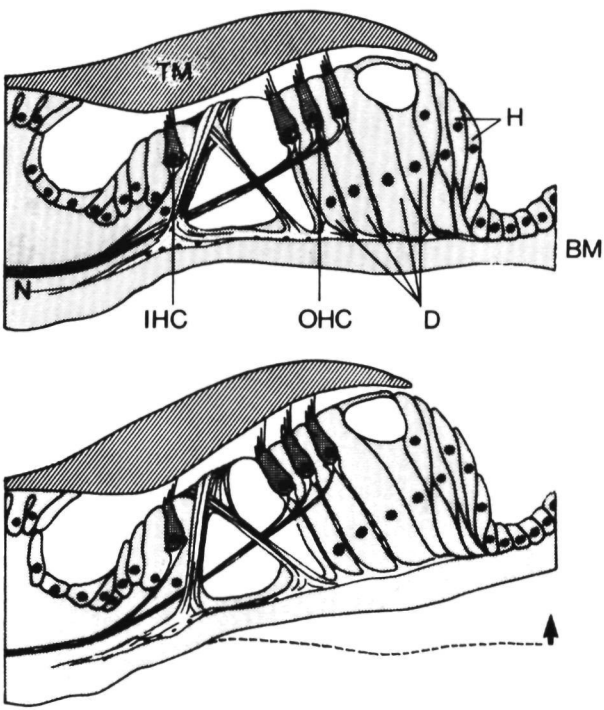
### *1.4.1 Introduction*

The electrical phenomena in the membranous labyrinth can be divided into resting or DC potentials and evoked or AC potentials. The resting potentials include the endolymphatic DC potential to be measured in the cochlear duct and various parts of the vestibular labyrinth as well as the intracellular DC potentials of the sensory cells.
The acoustic evoked AC potentials comprise cochlear microphonics, summating potentials and compound action potentials, all of which can be measured outside the cochlea by means of electrocochleography.
The vestibular evoked reflexes on eye position are recorded by means of electronystagmography.

### *1.4.2 Resting potentials*

The endolymphatic resting potential (EP) discovered by von Békésy (1952) is a positive DC potential in the cochlear duct which averages about 80 mV with respect to perilymph. This is an unusual situation since, on account of the existing gradients for sodium and potassium between endolymph and perilymph, a negative diffusion poten-

tial of the same magnitude should be expected. Such a negative diffusion potential mainly determined by the potassium gradient across the cell membrane can be found in the sensory cells of the organ of Corti (von Békésy, 1952; Russell and Sellick, 1978).
It was found that the endocochlear DC potential rapidly decreased to zero during anoxemia and after prolonged anoxemia it falls below zero to negative values of about -20 to -50 mV (Konishi et al, 1961; Matchinsky and Thalmann, 1967; Konishi and Kelsey, 1968).
Kuijpers (1969) and Kuijpers and Bonting (1970[a],[b]) demonstrated that by perilymphatic perfusion with ouabain, a specific inhibitor of $Na^+$-$K^+$-ATPase, a comparable effect on the EP could be obtained. On account of these observations and the presence of a high amount of $Na^+$-$K^+$-ATPase activity in the vascular stria it was concluded that the EP originated from an ouabain sensitive electrogenic potassium pump represented by the $Na^+$-$K^+$-ATPase in the vascular stria. The negative potential was considered a potassium diffusion potential.
In contrast, the vestibular parts of the endolymphatic system, having the same ionic composition as the cochlear endolymph, remarkably have a resting DC potential of only a few millivolts (Smith et al, 1958; Sellick et al, 1972). Both in the utricle and ampullae the DC potential probably originates from the dark cells, which can be considered analogous to the vascular stria. The saccule contains no dark cells and its DC potential has been found to depend on the DC potential of the cochlea (Sellick and Johnstone, 1972).
According to Miyamoto and Morgenstern (1981) and Amano et al (1983) the DC potential of the endolymphatic sac varies between 0 and + 16 mV.



Three different types of electrical responses may be recorded after acoustic stimulation; the cochlear microphonics (CM), the summating potential (SP) and the compound nerve action potential (AP).

The CM is an alternating current (AC) potential, first described by Wever and Bray (1930), generated by sound synchronous modulated changes in the ohmic resistance of the mechanosensitive portion of the hair cell membrane. This results in change of current flow between the positive endolymphatic potential and the negative hair cell potential, as originally postulated by Davis (1965) in the "resistance modulation" theory.

The differences in the ionic environment between the top and the body of the hair cells provide the asymmetry required for a standing current flow carried by potassium, as was demonstrated by Russel (1983). Sound induced deflection of the apical surface of the hair cells (fig. 5) likely is to be followed by opening of transduction channels on the apical surface of the hair cells, driving ions down their potential gradient into the cell, depolarizing the cell (Russel, 1983).

The CM can be measured close to or at a short distance from the hair cells. In the case of a round window electrode, CM mainly originating from the first turn of the cochlea is recorded and includes contributions of the lower frequency range in a weighted average (Eggermont, 1976). The CM response has the same frequency as the stimulating tone frequency and its amplitude is correlated linearly with sound pressure up to intensities of 80 dB.

The SP, first described by Tasaki et al (1954), being a stimulus related DC potential, like the CM, is generated in the hair cells. It appears as a DC shift and is supposed to be the result of non-linearities in the movement of the basilar membrane, or in mechano-electrical transduction (Davis, 1976).

The compound nerve action potential AP is the summation of the contribution of many individual stimulus related AC responses of the cochlear nerve fibers. The AP recorded from the round window has a diphasic form, the first deflection being negative (Eggermont et al, 1974). The shape, amplitude and latency of the AP are related to the synchronisation of the responses of stimulated nerve fibers. Synchronisation requires rapid onset stimuli, while the number of stimulated nerve fibers increaseswith increasing stimulus intensities and frequency. The AP latency decreases with increasing stimulus intensity and is largest for lower frequencies. At low frequency, with a sound intensity above 40 dB, nerve fibers in the high frequency range are also stimulated (Eggermont et al, 1974).

The pattern of the AP response, as recorded with a round window electrode, is usually composed of two negative deflections, $N_1$ and $N_2$ in order of their appearance (fig. 38). $N_1$ and $N_2$ deflections are assumed to originate from the eighth nerve and the cochlear nucleus respectively (Eggermont, 1976). With use of electrocochleography the stimulus

←Fig. 5. Diagram of hair cell stimulation in the organ of Corti. The stereocilia of the hair cells anchored in the tectorial membrane are deflected by basilar membrane displacement which results in depolarisation of the hair cells.
BM: basilar membrane, D: cells of Deiter, H: cells of Hensen, IHC: inner hair cells, OHC: outer hair cells, N: nerve fibers, TM: tectorial membrane.

related potentials can be recorded outside the cochlea (Eggermont et al, 1974). This method is widely practiced as a diagnostic tool for hearing disorders and for experimental studies (Eggermont, 1976; Brackmann, 1977).
In response to acoustic stimuli CM, SP and AP appear simultaneously in the recorded response. By presenting the stimuli alternately in phase and counterphase and averaging the recorded response, a separation can be obtained between CM on the one hand and AP and SP on the other. High pass filtering or increasing the repetition rate of stimuli, which results in adaptation of AP only, separates SP and AP (Eggermont et al, 1974).

### *1.4.4 Electronystagmography and assessment of otolith function*

Owing to problems of stimulus triggering, vestibularly evoked electrical responses cannot be measured in a convenient way outside the vestibulum. Fortunately the neural reflex pathways between the vestibular sensory cells and the extra-ocular muscles provide a useful alternative. The responses evoked by stimulation of the vestibulo-ocular reflex can be evaluated by direct examination of the position or movement of the eye, or by using electrodes positioned near the eye to record eye displacement potentials which can be registered by means of electronystagmography (ENG).
The semicircular canals were recognised as the receptor organs for angular acceleration by Flourens (1842) and Ewald (1892). Breuer (1874) established the stimulus response; when the head undergoes an angular acceleration or abrupt deceleration the inertia of

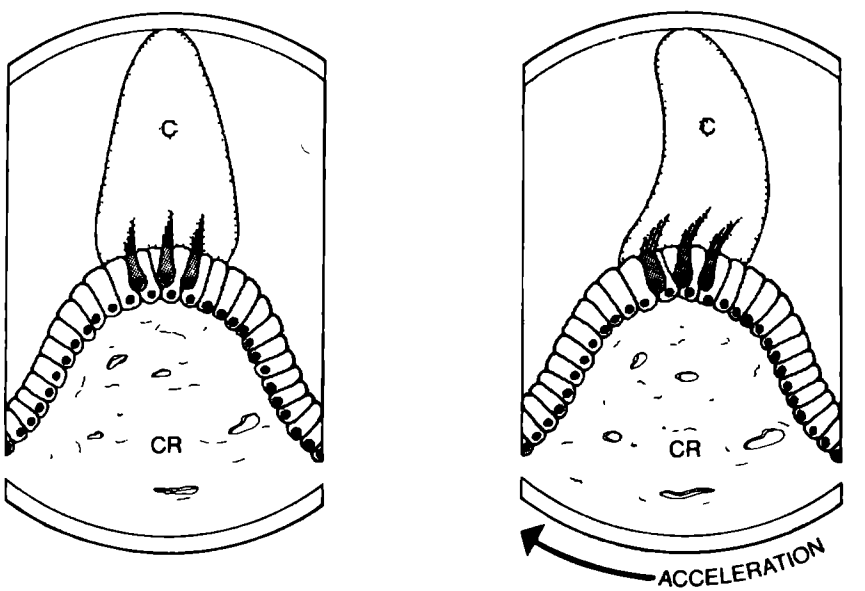



Fig 6 Schematic drawing of stimulation of crista (CR) ampullaris At acceleration to the left the endolymph lags behind resulting in opposite deviation of the cupula (C) and stereocilia embedded in it This stimulates the sensory cells

the endolymph in the canal causes the fluid to lag behind the walls of the canal. The fluid presses on the cupula, which thereby becomes deflected (fig. 6). This deflection of the cupula causes the stereocilia embedded in it to bend, which leads to depolarisation of hair cells and gives rise to action potentials in the neurons of the ampullary nerve, vestibular nuclei and central pathways. A nystagmus is established by means of vestibulo-ocular pathways. Nystagmus is a particular type of eye movement consisting of alternating slow and fast movements in opposite directions. The slow phase of the nystagmus is of vestibular origin, the fast phase is of "central" origin. The slow phase usually is directed opposite to the direction of rotation while the fast phase is in the same direction. By convention the direction assigned to the nystagmus is the direction of the fast phase. During steady rotation there is, eventually, no difference in velocity between endolymph and canal wall and nystagmus does not arise. After stopping the rotation the relative motion of the endolymph elicitates a nystagmus response.

The utricular and saccular maculae were designated by Breuer (1891) as the receptor organs for horizontal and vertical linear acceleration respectively. At present it is well established that the utricles and saccules continuously monitor gravity or linear acceleration; they are crucially important in the control of posture, gait and equilibrium. As the density of the otoconia is somewhat greater than that of the surrounding endolymph, linear acceleration causes displacement by inertial lag of the otoconia. This is transmitted to the stereocilia by the otolithic membrane, resulting in deflection of the stereocilia, depolarisation of the hair cells and action potentials in the utricular and saccular nerve fibers (fig. 7). Impulses are directed to similar central pathways as mentioned above for the semicircular canals. As a result several labyrinthine reflexes may follow, some of which will be described below.

The utricle and saccule are involved in the static labyrinthine reflexes (de Kleyn and Magnus, 1921; de Kleyn and Versteegh, 1933) such as the compensatory eye deviation (van der Hoeve and de Kleijn, 1917) and tonic head and body righting reflexes on lateral tilt (Magnus, 1924). Lateral or vertical deviations of the head are accompanied by a torsional eye deviation. In lateral tilt position animals with laterally placed eyes show compensatory eye shifts downward of the upper eye and upward of the lower eye.

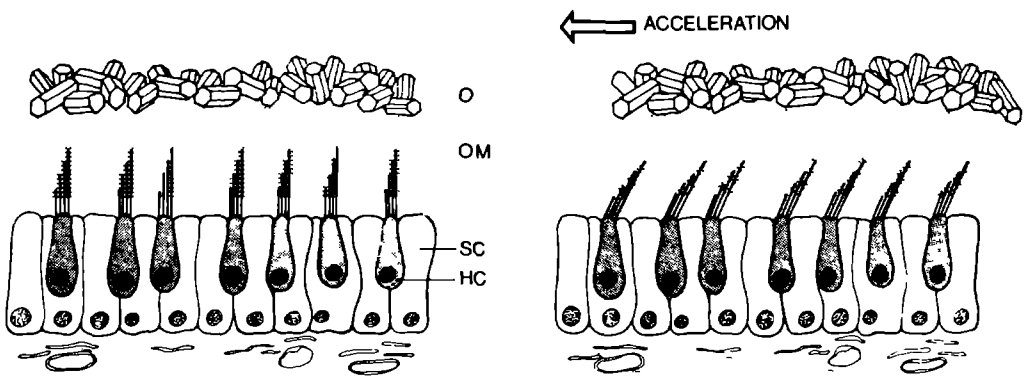



Fig 7. Schematic representation of stimulation of the otolith organs. During horizontal acceleration the otolithic membrane (OM) is displaced by the inertia of the otoconial mass. This stimulates the sensory cells (HC) by deflection of the stereocilia
O: otoliths, SC. supporting cells.

In nose up (pitch) position the upper pole of the cornea of both eyes is shifted forward and downward. In nose down pitch these poles are shifted backward and upward and the conjunctiva appears at the inner canthus. These deviations are accompanied by a torsional eye deviation (countertorsion).
The "tonic" eye deviations maintained in stationary body and head positions are to be distinghuished from the dynamic movements occurring with positioning and head righting, as these latter reflexes originate from the semicircular canals.
Besides vestibulo-ocular pathways, the vestibular nuclei are connected to vestibulo-cerebellar, vestibulo-vestibular and vestibulo-spinal neural tracts giving rise, apart from nystagmus, to rotatory sensations, vestibulo-collic and vestibulo-spinal reflexes.

### *1.4.5 Cochlear and vestibular function after obliteration of endolymphatic duct and sac*

As described in paragraph 1.3.4. obliteration of the endolymphatic duct in experimental animals can result in a hydrops of the endolymphatic system. This hydrops is assumed to result from blockade of the resorption of endolymph by the endolymphatic sac.
This experimentally evoked hydrops has received special attention, since endolymphatic hydrops has been found in temporal bones of patients with Ménière's disease (Hallpike and Cairns, 1938; Rollin, 1940; Lindsay, 1942; Altmann and Fowler, 1943; Schuknecht et al, 1962; Antoli-Candela, 1976; Saad, 1984; Paparella, 1985). This experimental model has therefore been used for studying the effects of hydrops on cochlear and vestibular function.
Ménière's disease is an idiopathic disorder involving the inner ear and is generally characterized by episodes of true vertigo and a sensorineural hearing loss accompanied by roaring tinnitus.
The vestibular and auditory symptoms may begin simultaneously, or one may precede the other by days or years. The frequency of attacks is highly variable and long remissions may occur. Vestibular tests may show a decreased response in the involved ear. Frequently, in the early stages of the disease, the hearing loss is greater for low frequencies, whereas at later stage the audiogram is flat. Characteristically the hearing loss fluctuates at least in early stages of the disease. The most characteristic findings during electrocochleography, apart from an increase in threshold, are a broad action potential complex, enhanced negative summating potential and an excessive increase of action potential amplitude with stimulus level (Eggermont et al, 1974, 1976; Eggermont, 1979; Gibson et al, 1977; Coats et al, 1984; Eggermont and Schmidt, 1985).
Generally, the morphological features in experimental hydrops after obliteration of the endolymphatic sac and in temporal bones of patients with Ménière's disease are comparable, but the question whether the cause of this disease lies in an abnormal function of the endolymphatic sac is still unanswered. In some instances radiological studies have revealed partial atresia or obliteration of the vestibular aqueduct (Clemis and Valvasori, 1968; Oigaard et al, 1976; Wilbrand and Stahle, 1981; de Groot, 1987) while measurements on sections of temporal bones demonstrated that a small vestibular aqueduct was more often observed in patients with Ménière's disease than in patients without this disease (Egami et al, 1978; Sando and Ikeda, 1984).
Morphological studies of autopsy material and biopsy specimens of the sac obtained

during surgery are conflicting. A case of atrophy of the sac was reported by Shambough et al (1969), while Schuknecht (1976) reported a case in which the endolymphatic duct was absent. Perisaccular fibrosis associated with thickening of the basilar lamina and loss of cellular elements and blood vessels in the perisaccular connective tissue was observed by Hallpike and Wright (1940), Cawthorne (1947), Schindler (1980), Lim and Glasscock (1981) and Arenberg et al (1985). Although these observations seem to support an impaired function of the endolymphatic sac, they disagree with the observations made by Plantenga and Browning (1979). These authors failed to find any pathology of the endolymphatic duct and sac in specimens from patients with Ménière's disease in a blind comparison with normal control specimens. A possible explanation for the discrepancy in the reported data might be that the interpretation of autopsy and biopsy specimens is hampered by processing artefacts and postmortem autolysis, as proposed by Schindler (1980).
The clinical symptom complex of Ménière's disease and morphological findings described as characteristic for Ménière's disease, including hydrops, have also been reported in syphilis (Schuknecht, 1974; Paparella et al, 1980) bacterial and viral infections and inflammation (Nadol et al, 1975; Paparella et al, 1983), trauma (Paparella and Mancini, 1983) and otosclerosis (Liston et al, 1984). The specific mechanisms causing these changes are still not understood, but there are no indications that in these instances the function of the endolymphatic sac is disturbed.
In the next section the effects of experimental saccus obliteration on auditory and vestibular function will be discussed.

**Auditory function**

A wide range of changes in auditory responses after surgical obliteration of the endolymphatic sac for CM, AP and SP were reported by several investigators for the guinea pig (Kimura, 1967; Yanagi, 1973; Konishi and Kelsey, 1976; Kumagami et al, 1981; Kitahari et al, 1982; Kumagami and Miyazaki, 1983; Inamori, 1984, Aran et al, 1984; Morizono et al, 1985; van Deelen, 1986) cat and rabbit (Beal, 1968). The number of experimental animals tested other than guinea pigs is very small, three cats and seven rabbits respectively. The results of all these studies reveal a large variation in auditory responses from normal to severe changes in the electrical output. This is at least partly related to the use of different modes of stimulation, positioning of recording electrodes and postoperative survival times, as well as to the lack of reliable control values. Further, a substantial number of guinea pigs have been tested only by Kitahari et al (1982), Inamori (1984), Morizono et al (1985) and van Deelen (1986). Therefore no general conclusion can be drawn.
Moreover, apart from the assessment of hydrops in some studies, advanced histological studies on the integrity of the cochlear structures are lacking. Only recently a comprehensive combined physiological and morphological study on hydropic ears of the guinea pig has been reported by van Deelen (1986). He observed that AP threshold increase was nearly always associated with loss of outer hair cells and spiral ganglion cells. In addition, an increase of the negative summating potential was consistently found during the first months after obliteration of the endolymphatic sac.
The results of studies of endocochlear potentials in endolymphatic hydrops also failed to show unanimity. Konishi and Kelsey (1976), Konishi et al (1981), Cohen and Morizono (1984) and Kusakari et al (1986) reported decrease in endolymphatic resting

potential in the cochlea (EP), attributed to dysfunction of the vascular stria, but no change in EP was found by Morgenstern and Miyamoto (1979).

**Vestibular function**

Vestibular function tests and behavioural observations after endolymphatic sac obliteration have only occasionally been performed. However the description of the methods used is scanty or absent and the number of animals tested limited, while histologic confirmation of endolymphatic hydrops of the vestibular system is usually lacking or not clearly stated. Moreover the majority of these experiments have been performed during the early postoperative period and one has to be aware that the observed vestibular disturbances are likely to reflect the effect of the intracranial surgical procedures.

Among the large number of guinea pigs operated upon by Kimura (1967) imbalance and nystagmus were observed in one animal only, three weeks after operation. One out of ten guinea pigs in a report by Suh and Cody (1974) showed mild unsteadiness for seven days postoperatively.

Matsunaga et al (1972), in a preliminary report on sinusoidal acceleration of guinea pigs, found directional preponderance in the absence of spontaneous or positional nystagmus in the first postoperative week only, while slight directional preponderance was demonstrated in the presence as well as in the absence of endolymphatic hydrops in two out of twelve guinea pigs by Aran et al (1984). They explained this as "recruitment", which in the usual, i.e. audiological sense is an indication of a peripheral lesion

A significant decrease in caloric excitability was reported for guinea pigs by Morgenstern and Lamprecht (1984) but method and correlation with histological findings were not reported.

Summarizing, it can be concluded that the value of these observations is very limited.

### *1.4.6 Purpose of this study*

From an evaluation of the literature it became obvious that a multitude of functions has been ascribed to the endolymphatic sac. The possible role in endolymph secretion and resorption was the subject of many experimental studies, but direct proof of its involvement under normal physiological conditions is still lacking.

Remarkably, little or no attention has been paid to the possible role of the endolymphatic duct.

Surgical obstruction of the endolymphatic sac, an important technique in the investigation of the functional significance of this structure in inner ear physiology, has been shown to result in a hydrops of the endolymphatic system; a result which seemed to be species dependent.

No attention has been paid to the effect of saccus obstruction on secretion and resorption of endolymph, nor the behaviour of the endolymphatic sac and duct under these artificially induced conditions.

Studies of the function of the inner ear after saccus ablation are mainly confined to the cochlea, but they largely lacked a simultaneous histological assessment of the character of the induced lesions. The function of the vestibular apparatus has only been incidentally investigated under these conditions but without using appropriate experimental

methods, and correlations with the histological observations are absent.
The aims of the present study were:*

1. To study the morphology of the endolymphatic duct and sac in the rat.
2. To study the involvement of the endolymphatic duct and sac in endolymph circulation under pysiological conditions and after surgical obstruction of the endolymphatic sac.
3. To study the morphological changes in the cochlea and the vestibular system induced by obstruction of the endolymphatic sac.
4. To obtain more information on the physiological changes in the cochlea and vestibular apparatus induced by surgical obstruction of the endolymphatic sac and to correlate these findings with the observed histological changes.

* Parts of this study have been published in: Arch Otolaryngol (1986) 112: 423; Acta Otolaryngol (1986) Suppl 429: 35; Hearing Res (1987) 26: 229.

CHAPTER 2

# MATERIAL AND METHODS

## 2.1 EXPERIMENTAL ANIMAL

The Wistar rat (laboratory bred) was chosen as experimental animal. This animal has been the subject of various experiments for both electrophysiological and morphological studies on the auditory and vestibular system in our laboratory. Throughout this study newborn (age 1-10 days) and adult rats (body weight 120-200 g) were used. The adult animals were screened for the presence of otitis media by otoscopy.

## 2.2 MORPHOLOGICAL METHODS

### *2.2.1 Light microscopy*

For light microscopic studies of the inner ear the animals were deeply anaesthetised with an intraperitoneal injection of Nembutal (50 mg/kg body weight). A thoracotomy was performed and the animals were perfused intracardially with saline and subsequently fixed by perfusion of phosphate buffered (0.1M; pH 7.4) glutaraldehyde (2%). The temporal bones were dissected and stored for one day in the same glutaraldehyde solution and subsequently decalcified in EDTA (10%, pH 7.4).
Newborn rats were fixed by immersion fixation in the same solution. The specimens were dehydrated in graded alcohols, embedded in glycol methacrylate (JB 4, Polysciences) and sectioned (2$\mu$m). Each tenth section was studied. The following staining procedures were used: toluidin blue, hematoxylin-eosin, alcian blue (pH 2.5), Periodic Acid Schiff (PAS) and a combination of PAS and alcian blue (Pearse, 1972).

### *2.2.2 Electron microscopy*

For electron microscopy the inner ear structures were fixed in the same way as for light microscopy. For transmission electron microscopy decalcification was performed in EDTA (10%, containing 1,25% glutaraldehyde). Relevant parts of the inner ear were dissected and postfixed in 1% osmium tetroxide in phosphate buffer (pH7.4) for one hour, rinsed in buffer and after dehydration in graded alcohols embedded in Epon (Luft, 1961). Ultrathin sections were contrasted with a saturated aqueous solution of uranyl acetate (Watson, 1958) and subsequently with lead citrate (Reynolds, 1963). The sections were examined with a Philips EM 300 electron microscope.
For scanning electron microscopy the fixed specimens were dehydrated in acetone and dried by the critical point procedure. Subsequently the bony wall of the cochlea was carefully removed with a diamond burr to expose the organ of Corti. The specimens were coated with gold and examined with a scanning electron microscope at 12 kV (PSEM 500, Philips).

## 2.3 AUTORADIOGRAPHY

Radioactive sulphur was injected intraperitoneally as sodium sulphate ($Na^{35}SO_4$) (1 $\mu$Ci per gram body weight; S.A. 50 mCi/mmol, Radiochemical Centre, Amersham, England). After varying periods the animals were sacrificed and specimens were processed as described under light microscopy. Glycol methacrylate(JB 4) sections (2m$\mu$) were coated with Ilford K-5 emulsion by the dipping technique. After an exposure time at 4°C varying from 6 to 12 weeks, slides were developed with Amidol and fixed. Staining was performed either before dipping with Periodic Acid Schiff (PAS) or with toluidine blue, hematoxylin-eosin or methylgreen pyronin after exposure.

## 2.4 SURGICAL OBSTRUCTION OF THE ENDOLYMPHATIC SAC

Surgical obstruction of the endolymphatic sac was performed on the right ear under Nembutal anaesthesia (50 mg/kg body weight), administered intraperitoneally. The left ear served as control. Operations were carried out under clean conditions with the aid of a binocular operating microscope.

A midline incision was made extending from the top of the head to the neck. The occipital muscles were detached at the lamboid ridge and retracted, exposing the dorsal surface of the occipital bone and foramen magnum and part of the bone was removed with use of drill and forceps (Kimura, 1968). The dura mater was incised. A piece of cotton soaked with saline and covered with a sheet of silastic was gently pushed between the dura mater and the brain. By suction of cerebrospinal fluid through the

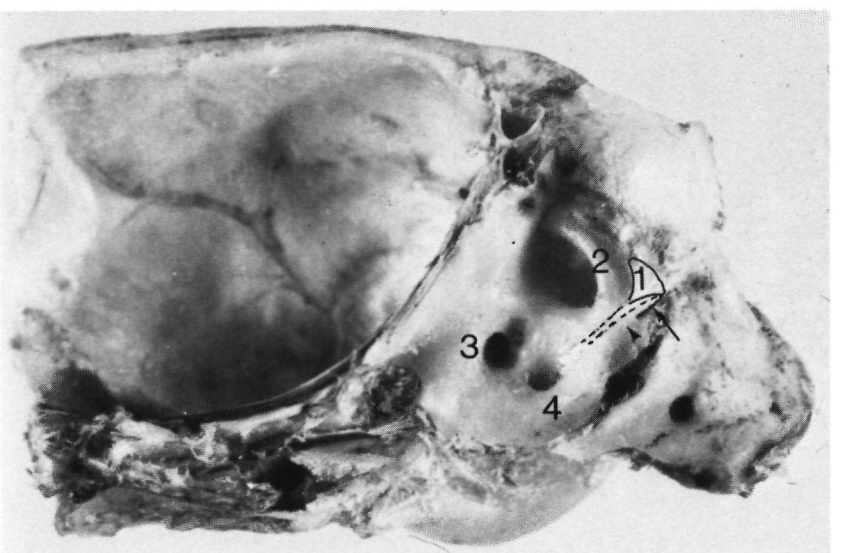



Fig. 8. Medial wall of right temporal bone of rat (X7). The dotted line indicates the course of the endolymphatic duct.
1: endolymphatic sac, 2: posterior semicircular canal, 3: internal acoustic porus, 4: arcuate fossa.
Arrow indicates bony operculum covering ventral border of endolymphatic sac. Arrow head: site of obstruction.

cotton and careful medial displacement of the cerebellum, the medial surface of the temporal bone was exposed. After penetrating the dura covering the bony operculum the endolymphatic duct was disrupted just rostrally from the operculum with the use of a small burr and fine hooks (fig. 8). Occasionally, a small amount of bone wax or gelfoam was used to seal this site. Finally, a piece of gelfoam was placed in the defect in the occipital bone. The neck muscles were sutured with catgut and the skin closed with clamps.

## 2.5 ELECTROCOCHLEOGRAPHY

### *2.5.1 Positioning of electrodes*

For electrocochleography (ECoG) teflon insulated bipolar electrode units (Pt, diameter 0.1 mm) with a head plug were used. The animals were anaesthetized with Nembutal (50 mg/kg body weight), injected intraperitoneally. After shaving and cleansing the skin over the skull a midline incision was made. By retraction of the skin and the external meatus the bony middle ear was exposed. Using a dental burr, a one mm hole was made in the postero-superior part of the bony wall, giving full view of the round window. A second one mm hole was drilled a few mm distant from the first. Through this second hole the recording electrode for ECoG was inserted into the middle ear and positioned in the round window niche with the use of the operating microscope. The electrode was fixed on the bony wall with dental cement. The second electrode was placed close to the bony annulus. Thereafter, the headplug was fixed on the skull with dental cement and the skin closed with clamps.

### *2.5.2 Stimulation and recording*

For ECoG recording the anaesthetized animal was situated in an electrically shielded cage, placed in a sound proof room. Anaesthesia was performed using a gas mixture of oxygen (0.6 l/min, nitrous oxide (0.4 l/min) and Fluothane (0.75-1.5%), given by mask. The animal was positioned on the left side with the head fixed. A modified headphone with ear speculum was placed just at the entrance of the right external ear canal. The body temperature, maintained by an infra red light, was monitored by a digital display, using a rectal probe.
Compound action potential recordings from the cochlea (ECoG) were performed between the round window (active electrode) and the annulus electrode.
During the recordings the animals were observed by closed circuit television.

#### 2.5.2.1 Stimulation equipment

The stimulation apparatus consisted of a generator system with attenuator and a power amplifier to drive the transducer (fig. 9). The generator system was composed of a remotely controlled oscillator (HP 3300 A), a pulse-generator (HP 8005 B), a trigger unit and a tone-gate. A sinewave was generated with a trapezoid envelope with variable

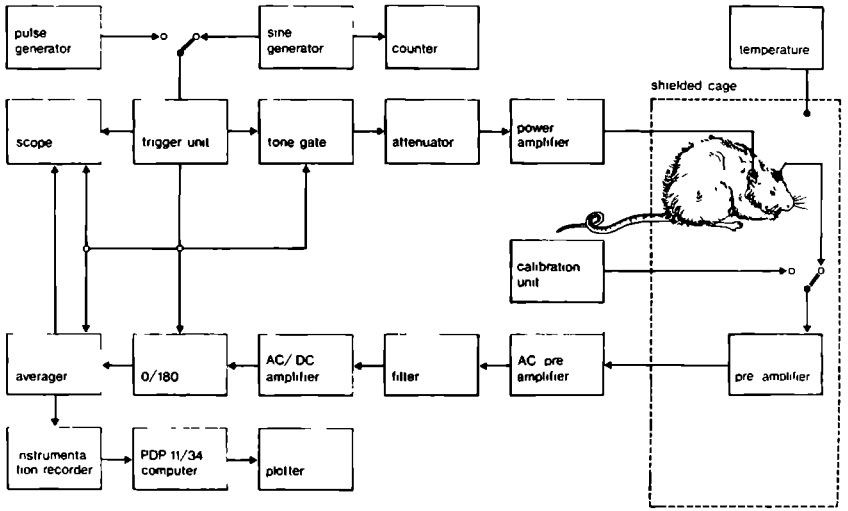



Fig. 9. Block diagram of stimulation and recording equipment for electrocochleography.

rise and fall times and plateau duration. The sine wave was phase-locked to the start of the burst and sign-reversed in continuous alternation. The ECoG-responses were evoked by tone-bursts with a rise- and fall-time of 1 ms., a plateau-duration of 4 ms. and with frequencies of 1, 2 and 4 kHz.
The signal reached the power amplifier through an attenuator (HP 350 D) with 1 dB steps from 0 to 120 dB.
The transducer was a modified headphone (shielded TD-48; Beyer Dynamic) with ear speculum.
The stimulus intensity (dB SPL) was defined as the intensity level of a continuous tone with the same sine wave amplitude. The attenuator of the stimulation equipment was calibrated by measuring the sound pressure in the external ear canal of the rat (after inserting the speculum of the transducer) by means of a calibrated probe tube-microphone ensemble (B&K UA-0040).

### 2.5.2.2 Recording equipment

The recording system consisted of an amplifier, averager and recording apparatus (fig. 9). The high input impedance pre-amplifier (NL 100) was used in combination with an AC pre-amplifier (NL 103). The pre-amplifier was of the differential type. This means that in the ideal case only the difference betweenthe input signals is amplified and the common components cancelled. It was possible to adjust the balance for asymmetrical inputs with a gain of 1000 and a low cut-off frequency of 0.1 or 10 Hz. The output of this amplifier circuit was connected to a band pass filter (NL 115) with a low cut-off frequency of 0.1 or 10 Hz and a high cut-off frequency of 10 kHz. The amplification of the AC/DC amplifier (NL 106) was adjustable from 1 to 100. The input DC signal could be zeroed by using the offset adjustment. A calibration unit could be plugged directly

into the head electrode connection box so that the entire system integrity could be checked and proper calibration guaranteed. The unit could deliver a stable waveform of precisely known shape and amplitude, triggered by the stimulus. The output signal of the AC/DC amplifier was processed by the averager (NL 750), a digital computer with a memory of 256 addresses.
The start of the sweep of sampling was time-locked with the start of the stimulus by the trigger unit. The sweep time was set at 10 ms. The accumulated average response was retrieved and displayed on a scope during each input sweep.
The output was recorded in digital code on a FM taperecorder (Bell & Howell Data tape 4010) for transport to a PDP-11/34 computer for waveform manipulation (such as digital filtering) and subsequent analysis. The computer-analysis comprised determination of latency and amplitude of evoked potential waveform components and hard-copy output of the graphics produced.
The digital waveforms were stored in retrievable format on hard disk together with the relevant stimulus, recording and animal parameters.

## 2.6 ELECTRONYSTAGMOGRAPHY AND ASSESSMENT OF OTOLITH FUNCTION

**Electronystagmography**
For electronystagmography, the awake animal was placed in a special restraining frame (Fischer et al, 1980). Stainless steel hypodermic needles were inserted near the outer and inner canthi of the eyes and vertex for the registration of eye movements (Fischer et al, 1979).
For vestibular stimulation a Tönnies rotation chair was used. The frame was placed on the chair with an anterior declination of 30 deg for appropriate positioning of the horizontal canal in the plane of rotation.
For cupulometry tests velocity steps (40, 60, 90, 120, 150, 180 and 250 deg/s, in that sequence; in both directions) were applied with a subthreshold acceleration of 4 deg/s$^2$ and 200 deg/s$^2$ deceleration (Huygen et al, 1986). Sinusoidal oscillation was performed at various maximum velocities and frequencies for the qualitative evaluation of response asymmetry. A Tönnies nystagmograph amplifier (AC-coupled with 2.5 s time constant) was used with an Elema Mingograph 81 ink-jet recorder. From the nystagmus record, the duration of the postrotatory nystagmus after the velocity step was measured.
The cupulogram was constructed by plotting nystagmus duration against the logarithm of the velocity step amplitude. The threshold velocity step and the time constant were derived with a graphical method.
The gain of the vestibulo-ocular reflex was obtained from the inverse of the threshold step. From these parameters the directional preponderance (DP) was calculated as a measure of asymmetry: DP = 100% (R-L)/(R+L), R and L being the values of any parameter for nystagmus to the right and left, respectively. The cupulograms were fitted by eye: an overlay was used with a set of regression lines with integer values of the time constant (T) which was adjusted at the nearest threshold (Thr) value which represented an integer multiple of 5 (30, 35, 40 .....) deg/s. A common regression line for both nystagmus directions was arbitrarily established if the difference in time constant was equal to or smaller than one second and/or the difference in threshold was equal to

or smaller then 10 deg/ s. The responses were then assumed to be symmetrical for both nystagmus directions. The parameters used for the assessment of cupulograms are mentioned in Chapter 6.

**Otolith function**

Otolith function was assessed by direct examination of vertical tonic eye deviations with an otoscope during 90 deg lateral tilt positions and counter-rolling of the eyes during nose up and down pitch positions (Fischer, 1980).
Posture and gait were observed by direct examination of the spontaneous behaviour of the animal.

**Righting reflexes**

The position of the head, body and limbs was observed when the animal was lifted by the tail; it was assessed whether it showed "Sprungbereitschaft" and/or lift reaction (spreading of toes on downward acceleration) (Magnus, 1924). Normal rats, when lifted by the tail, elevate the head in the long body axis while at the same time the extremities are stretched, the forelegs "reaching" forwards and downwards, and the hindlegs outwards.

**Swimming ability**

The behaviour of the animal was evaluated when it was gently placed into lukewarm water and after pushing the animal under water, it was observed whether it showed normal underwater orientation and the ability to resurface. Normal rats are perfect swimmers. The upright position of the head just above the water surface is easily maintained. When pushed under the surface, normal rats resurface quickly. Posture and gait were also observed just after the removal of the animal from the water (Fischer, 1980; Huygen et al, 1986).

# MORPHOLOGY OF THE ENDOLYMPHATIC DUCT AND SAC OF THE RAT

## 3.1 INTRODUCTION

Comprehensive morphological studies have previously only been made on the endolymphatic sac of the guinea pig and man.
For this study the rat was chosen as an experimental animal. This animal has been the subject of several experimental studies on middle and inner ear in our laboratory, but no data are available on the morphology and function of the endolymphatic duct and sac in this animal.
This chapter describes the morphology of the endolymphatic duct and sac of the rat as seen by light and electron microscopy; 15 rats were studied using light microscopy and 10 rats by electron microscopy.
For comparison lightmicroscopical studies of the endolymphatic duct and sac were made in five guinea pigs and these findings are included.

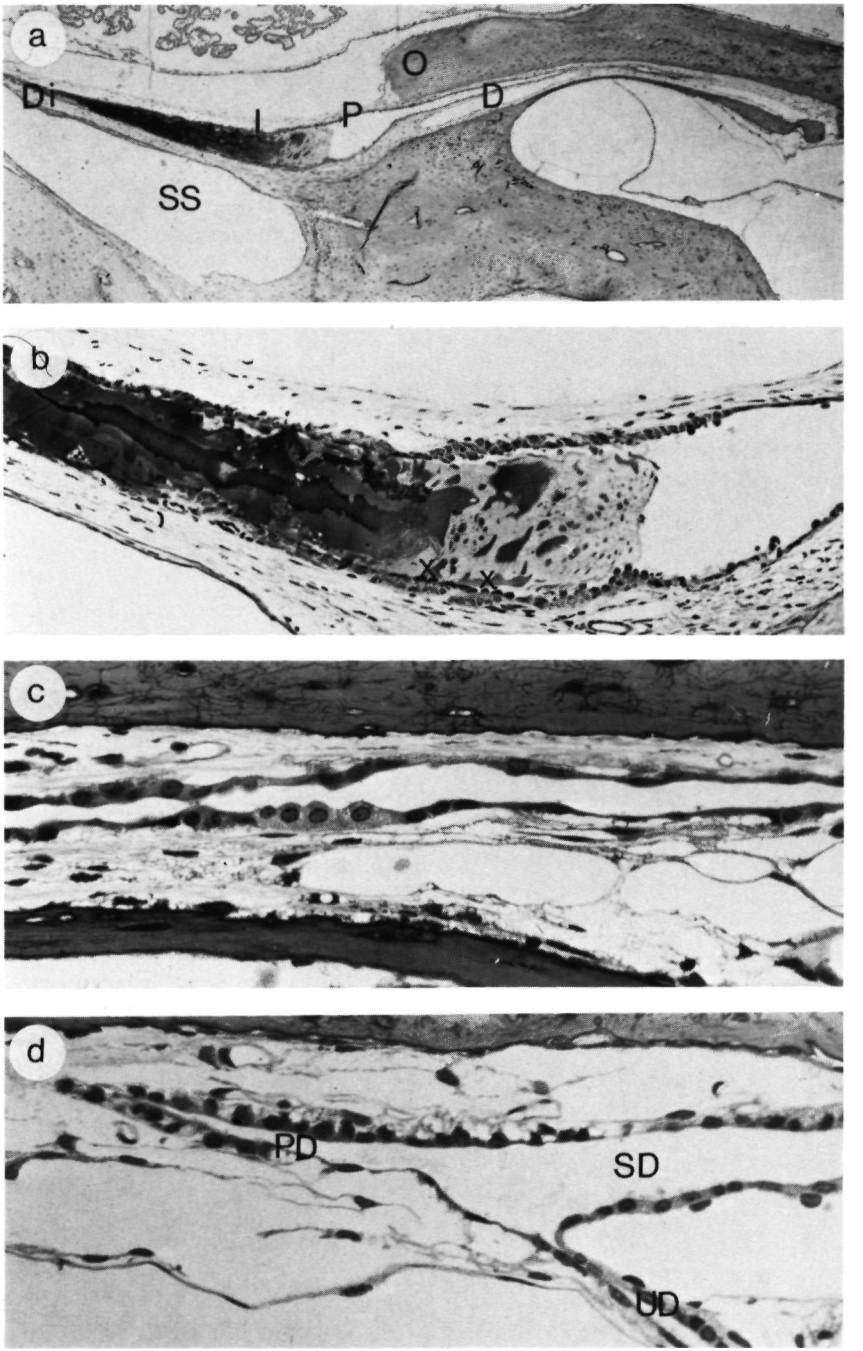
## 3.2 GROSS ANATOMY

The endolymphatic duct of the rat begins at the confluence of the saccular and utricular duct as a dilated sinus on the medial wall of the vestibule. After its entrance into the vestibular aqueduct, the duct narrows and widens again at the end of the vestibular aqueduct, where it merges into the funnel-shaped endolymphatic sac (fig. 8).
The endolymphatic sac can be divided into three parts (Guild, 1927[a]): proximal, intermediate and distal portion. The proximal portion is mainly located within the aqueduct. The intermediate and distal portion lie intradurally and are bordered on their lateral sides by the sigmoid sinus. The flattened distal portion has a very narrow lumen and ends blindly. The ventral margin of the intermediate portion is covered by a small bony process, the operculum, which can greatly vary in size (fig. 8). Comparison of the dimensions of the endolymphatic duct and sac in rat and guinea pig have revealed a similar length of the duct in both species, but the dimensions of the sac in both length and width appears to be twice as great in the rat.

## 3.3 LIGHT MICROSCOPY

### *3.3.1 Endolymphatic duct*

**Rat**
The proximal portion of the endolymphatic duct of the **rat** in the vestibular region is lined by a one layered cuboidal epithelium, similar to the lining of the utricular and saccular ducts. This part of the duct is surrounded by a very loose connective tissue with many capillaries (fig. 10[d]).
The epithelial lining of the intraosseous part of the duct is mainly flat, but local areas of cuboidal epithelium are also present. At the distal end of the duct, the epithelium


a
Di
I
P
O
D
SS
b
c
d
PD
SD
UD

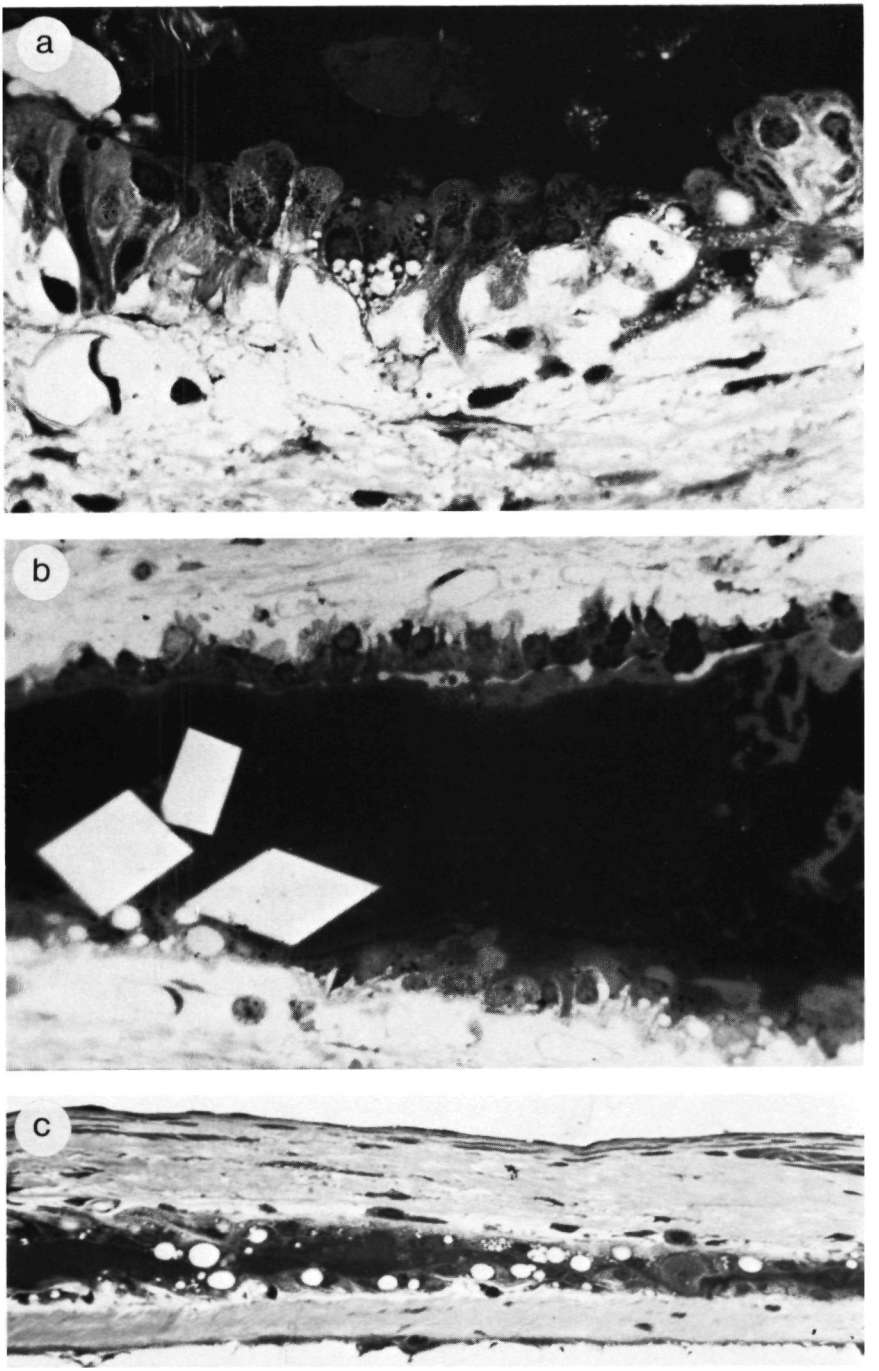
merges into the cuboidal epithelium of the proximal portion of the endolymphatic sac. The basal surface of the epithelial lining of the duct shows at distinct sites numerous infoldings some of which are in close contact with capillaries.
The lumen of the duct is usually optically empty, although occasionally cellular debris can be found. The endolymphatic duct is separated from the bony aqueduct by a rather loose connective tissue which contains many capillaries (fig. 10[c]).

**Guinea pig**
The endolymphatic duct of the **guinea pig** is lined by a one layered irregular epithelial lining with deep crypts, giving it a tortuous appearance (fig. 12[a,b]). The epithelial cells are mainly flat. The basal cell membrane is rather smooth although occasionally protrusions penetrating in the connective tissue are found. A relatively dense connective tissue with a few capillaries separates the duct from the bony wall (fig. 12[b], 13[c]).
Within the distal lumen free floating cells and cell debris can be observed.

### *3.3.2 Endolymphatic sac*

**Rat**
The distal part of the endolymphatic duct of the **rat** gradually widens to form the proximal portion of the sac which passes into the wide intermediate portion. The distal part shows a gradually narrowing lumen and ends blindly (fig. 10[a]).
No distinct boundary exists between the endolymphatic duct and proximal portion of the sac or between the various parts of the endolymphatic sac in the rat.
The proximal portion of the endolymphatic sac is lined with a rather smooth flat or cuboidal epithelium.
The epithelium of the intermediate portion varies from cuboidal to columnar and sometimes has a pseudostratified appearance. The cells of this portion show numerous inclusions, some of which stain with PAS. The basal surface of these cells show many infoldings (figs. 11[a,b]).
The epithelial cells lining the distal portion are cuboidal except at its extreme end where the epithelium is flat. The cells often contain optically empty vacuoles (fig. 11[c]). The luminal surface of the intermediate portion is rather irregular but without real crypts or intraluminal projections.
The subepithelial connective tissue is loosely textured, containing many capillaries. Macrophages and lymphocytes are regularly found in this area and also occasionally in between the epithelial cells of the sac. This loose connective tissue gradually passes into the dense tissue of the dura.
The lumen of the endolymphatic sac is filled with a dense strongly PAS-positive sub-

←Fig. 10. Micrographs of endolymphatic sac (a,b) and duct (c,d) of the rat.
The epithelial lining of both structures is rather smooth. The lumen of the sac is filled with a PAS-staining substance. The epithelial cells of the duct vary from flat to cuboidal (c) and show basal infoldings which are especially pronounced in the proximal portion (d). The surrounding connective tissue is loose and contains many capillaries.
D: duct, Di: distal portion of the sac, I: intermediate portion of the sac, O: operculum, P: proximal portion of the sac, PD: proximal portion of the duct, SD: saccular duct, SS: sigmoid sinus, UD: utricular duct. PAS-positive cells in the intermediate portion (X). a (PAS, X40); b (PAS, X150); c,d (toluidin blue, X425).


a
b
c

stance which also stains with alcian blue (pH2.5) (figs. 10[a],[b]). Especially in the transitional area between the proximal and intermediate portion it has a very inhomogeneous appearance with locally PAS-positive areas. Towards the distal portion it gradually changes into a more homogeneous PAS staining area. This substance always contains one or more crystals of varying size (fig. 11[b]). Besides cell debris occasionally a few free floating cells can be found.

**Guinea pig**

The epithelial lining of the proximal portion of the endolymphatic sac of the **guinea pig** gradually changes from flat to cuboidal cells.

The epithelial lining of the intermediate portion consists of cuboidal cells passing distally into the columnar epithelium in the distal part, where locally extensive villous like formations and deep infoldings are found (pars rugosa). The basal cell membrane has many infoldings.

The epithelial lining of the distal portion of the sac is smooth without villous like formations and crypts. The cells are cuboidal in shape except at the very end where they are flat.

The connective tissue close to the sac is rather loose in the intermediate and distal portion, containing a varying number of capillaries which are especially numerous in the pars rugosa (figs. 12, 13).

The lumen of the sac gradually widens from the proximal towards the intermediate portion. In the distal portion it is slit like and often no clear lumen can be distinguished (fig. 12[d]). The content of the sac filling the whole proximal and intermediate portion is far less dense than in the rat and stains faintly both with PAS and alcian blue. In contrast to the rat free floating cells are abundant in the proximal and intermediate portion, but absent in the distal portion.

These cells are filled with vacuoles of varying size and dense inclusion bodies (figs. 12[a], 13[a]).

←Fig. 11. Endolymphatic sac of the rat.
a: epithelial cells of the intermediate portion containing many vacuoles and granules. Note the irregular basal cell surface and capillary rich connective tissue (toluidin blue, X600)
b: intermediate/distal portion with large crystals (toluidin blue, X425)
c: distal portion with a very narrow lumen and vacuolated epithelium (toluidin blue, X425).

Fig. 12. Endolymphatic duct and sac of the guinea pig. ——————————→
a: survey showing numerous free floating cells (FFC) in the proximal]ntermediate portion of the sac (toluidin blue, X40)
b: duct showing irregular epithelial lining with many crypts. Note the rather dense connective tissue (toluidin blue, X425)
c: rugose part of the intermediate portion with many epithelial crypts and capillary rich connective tissue (toluidin blue, X425)
d: distal portion with very narrow lumen (toluidin blue, X600)
D: duct, Di: distal portion of the sac, I: intermediate portion of the sac, P: proximal portion of the sac, SS: sigmoid sinus.

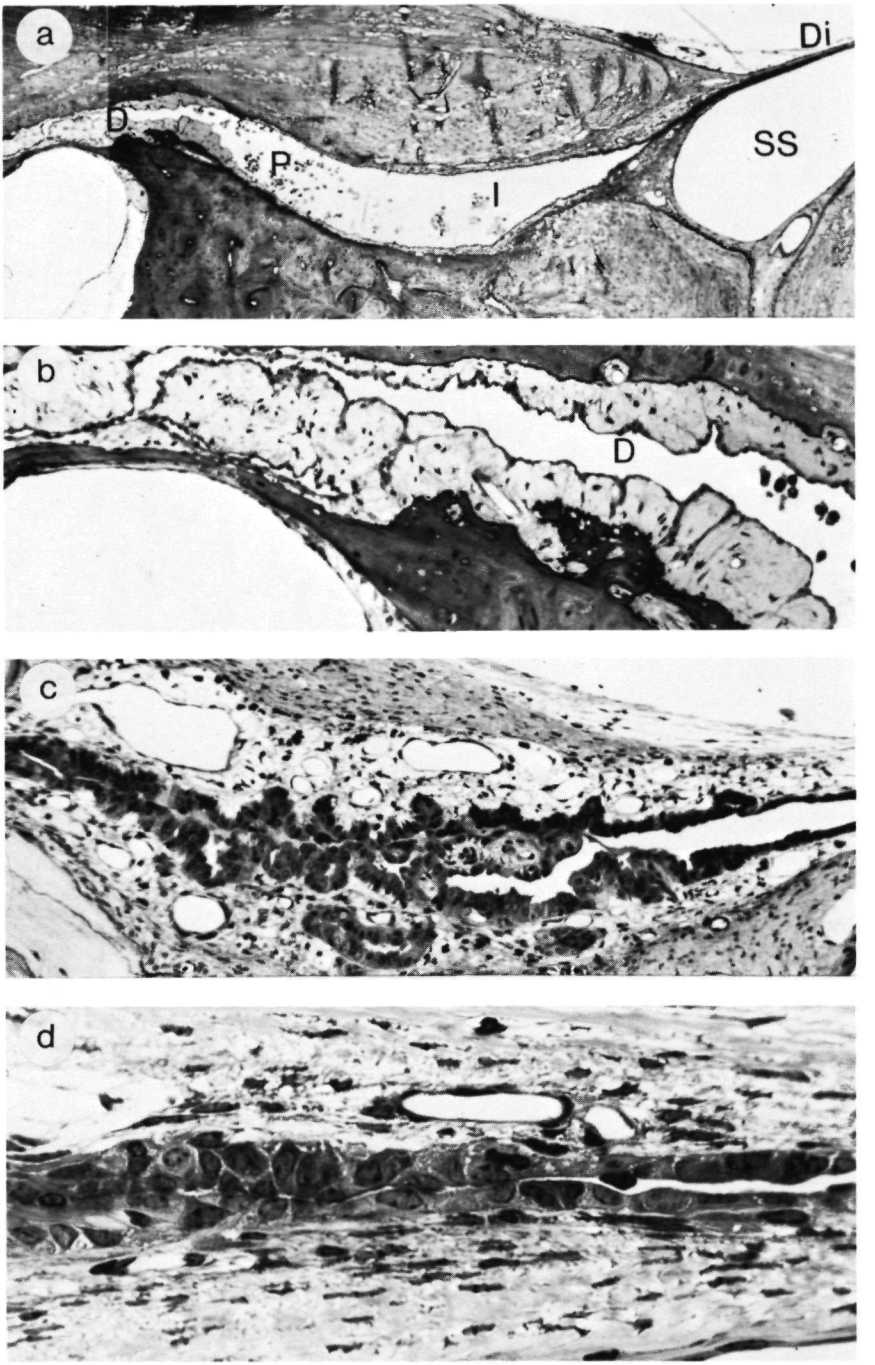



Fig. 12. See p.49 for legend.

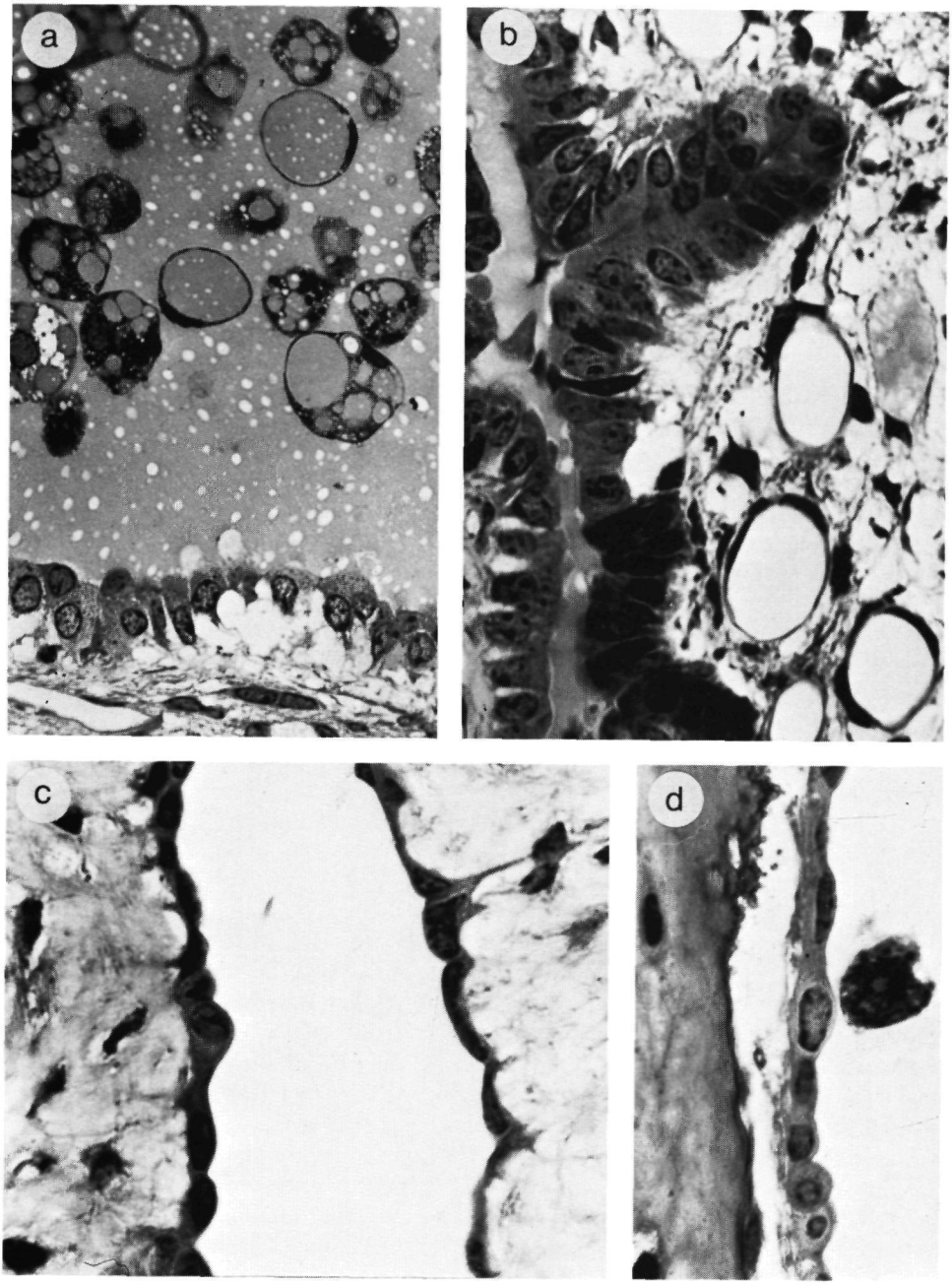



Fig. 13. High magnification of epithelial lining of endolymphatic sac and duct of the guinea pig.
a: intermediate portion with vacuolated epithelium. Free floating cells contain many vacuoles and dark granules
b: rugose part of intermediate portion with dark and light cells and many capillaries
c: flat epithelial lining of the duct, surrounded by avascular connective tissue
d: flat epithelial lining of the proximal part of the sac. (toluidin blue, X600).

### *3.4.1 Endolymphatic duct*

**Rat**

Electron microscopy of the epithelial lining of the endolymphatic duct of the **rat** reveals the presence of electron lucent and electron dense (light and dark) cells (figs. 14[a],[b]). The luminal surfaces of both light and dark cells have microvilli, although their number largely varies from cell to cell. Occasionally a rudimentary kinocilium can be found. The cells are connected by junctional complexes. The basal part of the cell mainly consists of long slender projections penetrating deep into the subepithelial tissue in focal areas (figs. 14[a],[b]). The basal lamina which follows the basal cell membrane infoldings separates the cells from the richly vascularized subepithelial loose connective tissue. The capillaries are non-fenestrated.

The intercellular spaces are generally narrow but at certain sites and notably at the distal part of the duct they may be greatly widened, forming large lacunae (fig. 14[b]).

The cytoplasm contains scattered mitochrondria and many ribosomes. The amount of RER differs from cell to cell and is sometimes found to be highly dilated in both light and dark cells. Golgi complexes and lysosomes are few in number. Small coated vesicles are present throughout the cell and they are frequently found in close association with the apical and basal cell membrane, as well as with the lateral cell membrane at the widened intercellular spaces. They can be often seen pinching off from the plasma membrane (fig. 14[c]).

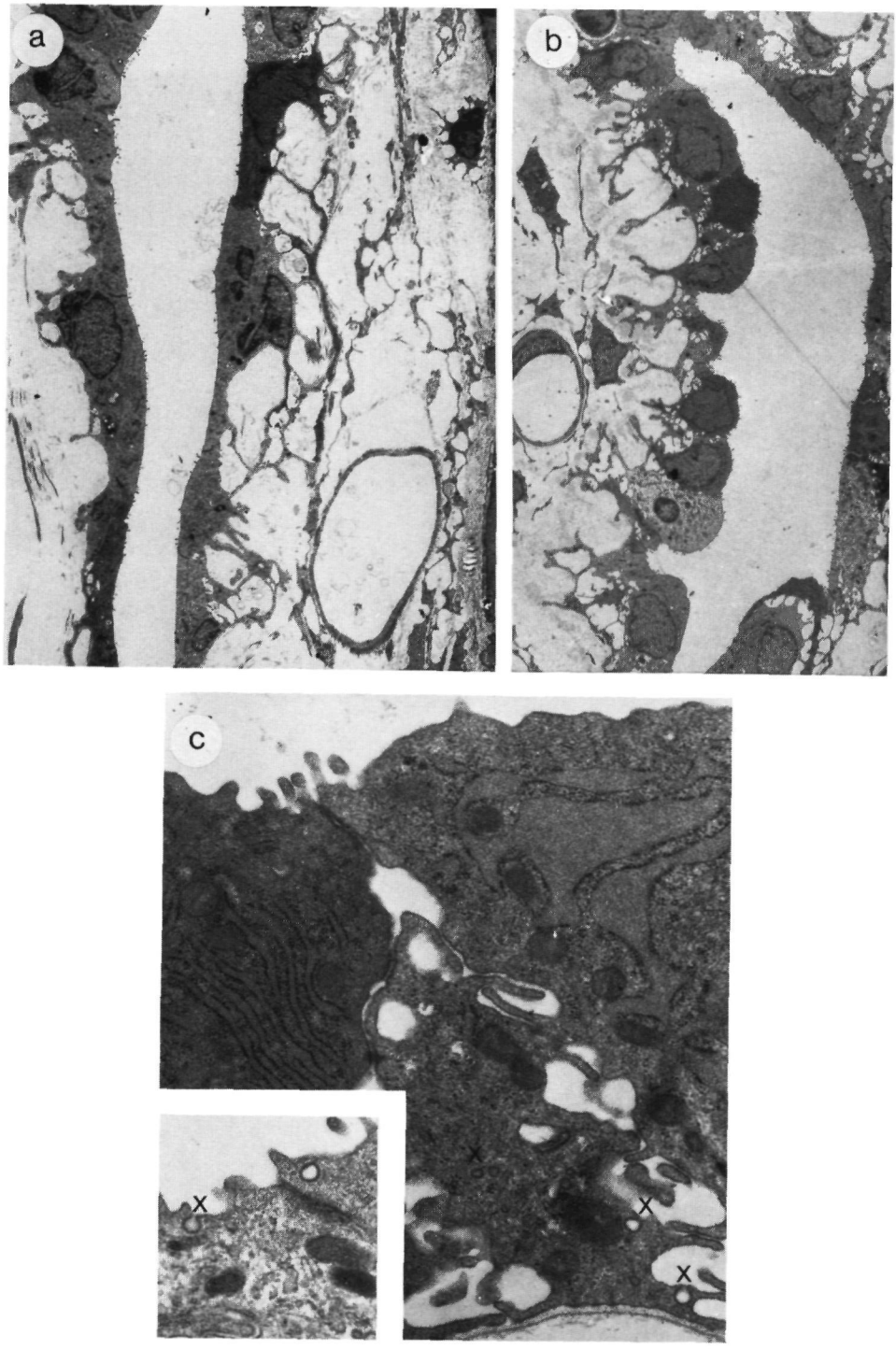
### *3.4.2 Endolymphatic sac*

The cells lining the proximal portion of the endolymphatic sac of the rat are cuboidal in shape, interconnected with juntional complexes. Light and dark cells can be distinghuished. The apical surfaces are rather convex.

The epithelium of this portion of the sac forms the transition between the epithelium of the duct and that of the intermediate portion of the sac and this is reflected in its cytoarchitecture. Close to the duct its cytological features are very similar to those of the duct epithelium. More distally it gradually gains the characteristics of the intermediate cells with an increased number of cell organelles and inclusions, while at the basal membrane the number of cytoplasmatic projections was increased.

The epithelium of the intermediate portion is composed of light and dark cells, interconnected by junctional complexes. The apical surface of the cell is convex with scattered microvilli and small (coated) and macropinocytotic invaginations. The basolateral cell membrane shows interdigitations creating intercellular spaces which can vary in size. Some cells reveal large outpouchings into the lumen. The basal cell membrane

Fig 14 Electronmicrographs of the epithelium of the endolymphatic duct of the rat showing dark and light cells (a: longitudinal, b. transverse section) Note the numerous projections of the basal cell membrane and the intercellular spaces. The apical cell surface reveals many microvilli. c: dark and light cells with RER which is highly dilated in the right cell Coated vesicles (X) are seen in the cytoplasm, fused with the lateral cell membranes and in the apical cell membrane (inset) (a, X1800; b, X1200; c, X24500).


a
b
c
X
X
X
X

displays extensive infoldings partly lined by a basal lamina.
The cytoplasm contains a well developed Golgi apparates and a varying amount of RER. Also the number of mitochondria can vary between different cells and their size is different in light and dark cells. Both light and dark cells contain many coated vesicles, many lysosomal structures including multivesiculated bodies and resorption vacuoles occasionally are found to coalesce. These vacuoles of varying size contain granular material of different density; lamellar inclusions were also found (figs. 15, 16). Coated vesicles are also numerous in the basal cytoplasmatic projections and they are often

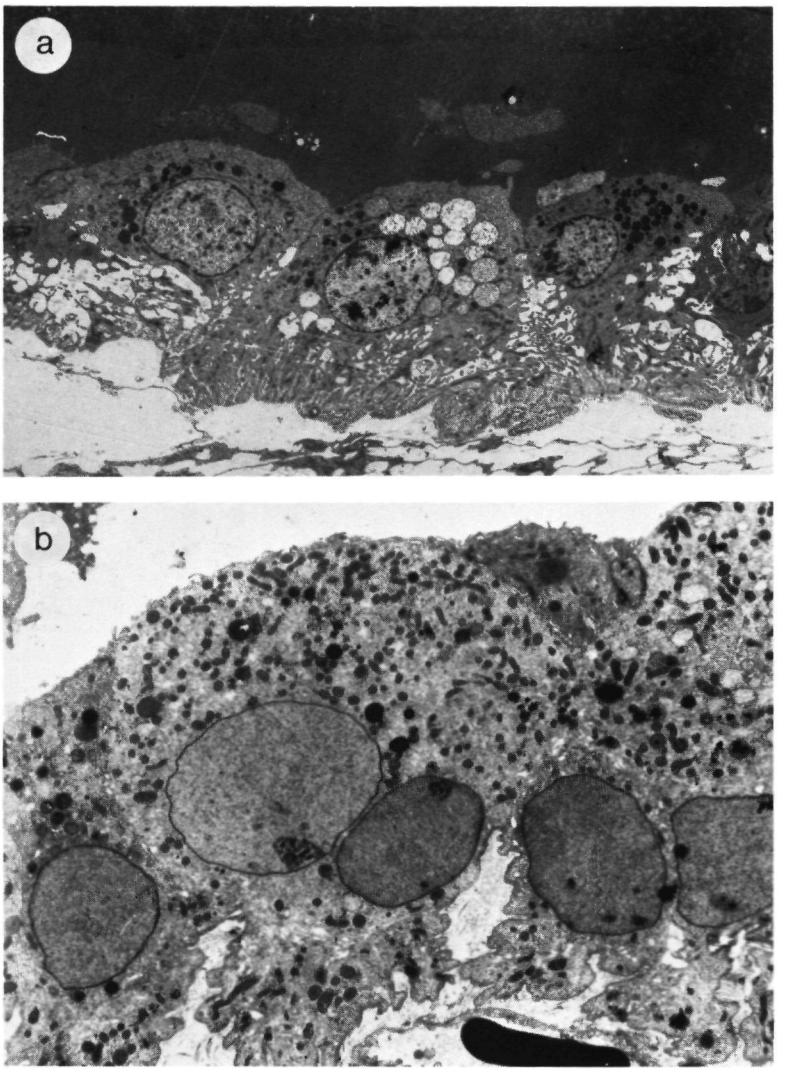
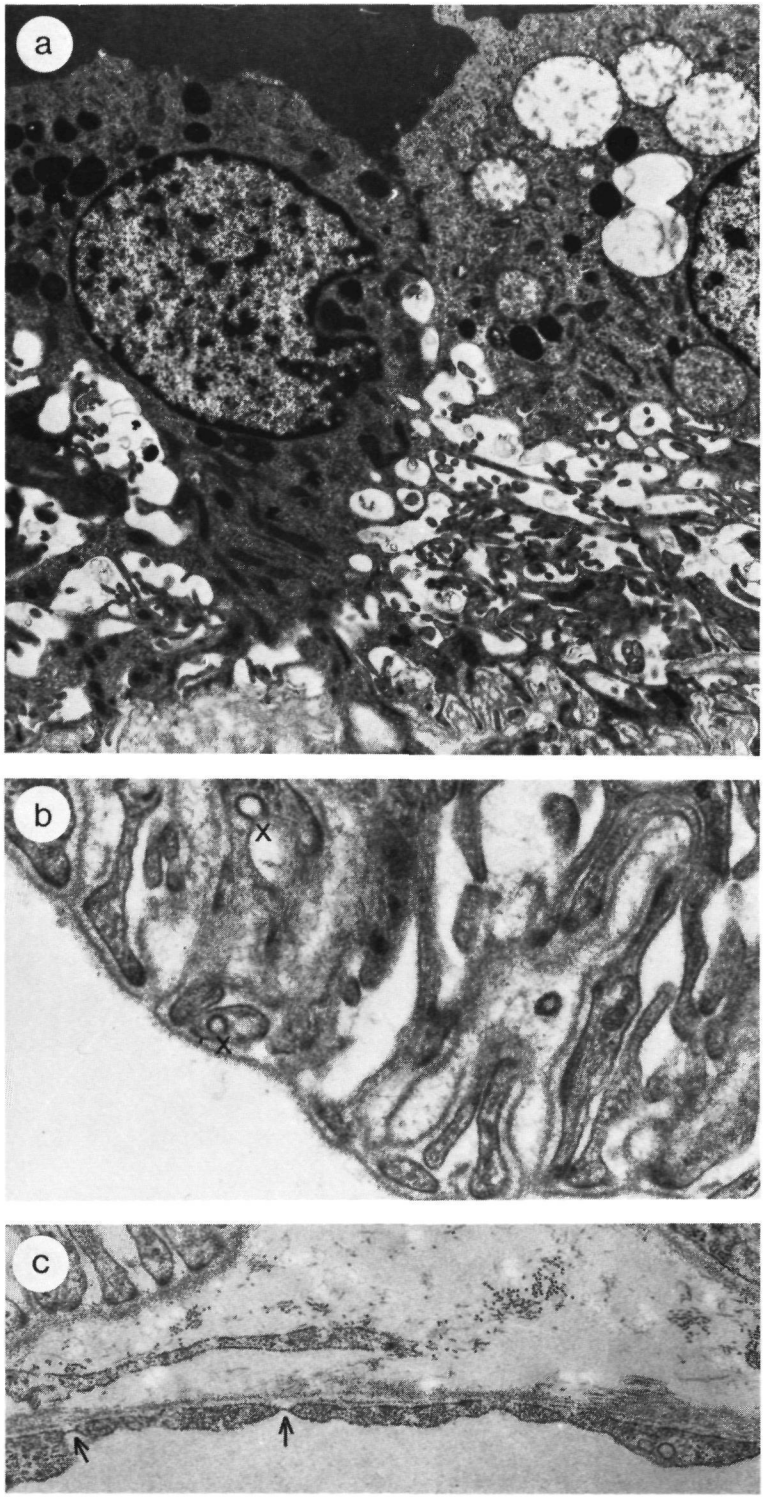



Fig. 15. Micrographs of epithelial cells in the intermediate (a) and distal portion (b) of the rat endolymphatic sac. The epithelium in the intermediate portion shows numerous basal infoldings and intercellular spaces. The cytoplasm contains many lysomes and resorption vacuoles. The cells in the distal portion contain numerous mitochondria and a few lysosomes (a, X1800; b, X3700).

found in close association with the basolateral cell membrane (fig. 16[b]).
The loose areolal subepithelial connective tissue contains many fenestrated capillaries (fig. 16[c]), lymph vessels and scattered macrophages. Macrophages and lymphocytes are also occasionally observed between the epithelial cells.
The epithelial lining of the distal portion of the sac has a rather uniform appearance and is composed of cuboidal cells, changing to squamous cells at the extreme end. Light and dark cells cannot be distinguished, while lateral cell membrane interdigitations are much less pronounced than in the other parts of the sac or may be absent. The basal membrane reveals many infoldings. The luminal surface shows a varying number of microvilli and small coated invaginations.
Mitochondria-rich and mitochondria-poor cells can be distinguished. Resorption vacuoles and lysosomal bodies are scarce in the distal portion, but small coated vesicles are present (fig. 15[b]). Occasionally cells with one or two large vacuoles occupying nearly the whole cell were observed. The subepithelial connective tissue resembles that of the intermediate portion, although it is more dense at the end.

## 3.5 DISCUSSION

The observations made in this morphological study demonstrate that the gross anatomy of the endolymphatic duct and sac of the rat does not fundamentally differ from the present observation in the guinea pig and those reported by other authors in guinea pig (Lundquist, 1965; Rask-Andersen et al, 1981[b]) rabbit (Adlington, 1968) and man (Zechner and Altmann, 1969; Rask-Andersen et al, 1984; Bagger-Sjöbäck et al, 1986), but there are differences in degree.
The endolymphatic duct in the rat is straight, while in guinea pig and other mammals it is very irregular with many crypts. The fine morphology of the duct epithelium reveals the presence of many coated vesicles associated with the apical and basolateral membranes. The basolateral cell membranes obviously show many projections, while the lateral intercellular spaces are often found widened. These morphological features together with the presence of subepithelial blood and lymph capillaries are indicative of the existence of a transepithelial water and solute transport as established in many other epithelia with comparable morphological characteristics (Diamond and Bossert, 1967). The presence of focal areas of cells with comparable features has recently been reported in the endolymphatic duct of guinea pig (Rask-Andersen et al, 1981[b]) and man (Wackym et al, 1986).
Apart from a much smaller surface area, the structure of the endolymphatic sac of the rat shows several striking differences when compared with the findings made in the guinea pig and in other mammals, both with respect to the epithelial lining and the saccular content. The difference in the epithelial lining is most pronounced in the intermediate portion. In the rat this lining is rather smooth, lacking the many infoldings and villous like formations supplied with many capillaries, present in the guinea pig (Guild, 1927[a]; Lundquist, 1965; Rask-Andersen and Stahle, 1979) rabbit (Adlington, 1967, 1984) and man (Zechner and Altmann, 1969; Bagger-Sjöbäck et al, 1986).
Generally, the epithelial lining of the sac in the rat shows the same basic morphology as the epithelium of the duct, but the basolateral infoldings as well as the number of cell


a
b
X
X
c

organelles and inclusion bodies are much more extensive. These morphological data refer to a transport of water and ions across the epithelium of both endolymphatic duct and sac. By this process resorption of water and solutes from the endolymph can be assumed to occur. Furthermore, the abundant presence of large resorption vacuoles and numerous lysosomal bodies especially in the intermediate portion are highly suggestive of an involvement in bulk resorption and breakdown of endolymph by these cells. This fits in with the presence of high concentrations of proteolytic enzymes in this area in the guinea pig (Ishii et al, 1966).

Free floating cells (phagocytic cells) found to be present in high numbers in the guinea pig (figs. 12[a], 13[a]) (Lundquist, 1965) rabbit (Adlington, 1968) and man (Zechner and Altmann, 1969) are only incidentally found in the rat. This makes it unlikely that in the rat, in contrast to the guinea pig and some other mammals, phagocytic cells play an important role in the removal of cell debris and high molecular waste products. A most unusual feature is the presence of one or two large crystals of unknown composition in the saccular lumen. To our knowledge no such structures have ever been demonstrated in other mammalian species. The lumen of the intermediate and distal portion of the rat sac is filled with a dense rather homogeneous substance, containing cell debris. The affinity of this substance for PAS and alcian blue indicates the presence of (acid) glycoproteins.

The origin of the glycoproteins is still a matter of debate. According to the longitudinal flow concept (Lundquist, 1965) they should originate entirely from the other parts of the membranous labyrinth, although the sites of production are as yet unknown. Other authors believe that they are at least partly secreted by the epithelial lining of the endolymphatic sac (Arenberg et al, 1976; Rask-Andersen et al, 1981[b]; Adlington, 1984; Friberg et al, 1986). These locally produced glycoproteins are assumed to play a facilitating role in the transepithelial fluid transport (Arenberg et al, 1976). Friberg et al (1986) suggested an important role of the glycoproteins in the regulation of the labyrinthine fluid volume or pressure, a sieving effect for high molecular waste products and a possible phagocytosis stimulating function.

However, in the present study no distinct morphological evidence was found for secretion of glycoproteins by the epithelium of the endolymphatic sac. This matter will further be discussed in the next chapter.

←Fig. 16.
a: epithelial cell of intermediate portion containing mitochondria, lysosomes and many (fusing) resorption vacuoles. The basal and lateral cell membranes show numerous infoldings. Note coated invaginations in the apical membrane (X5400)
b: basal part of cell in intermediate portion. Note coated vesicles (X) fused with cell membranes (X24500)
c: fenestrated capillary (→ fenestrae) (X24500).

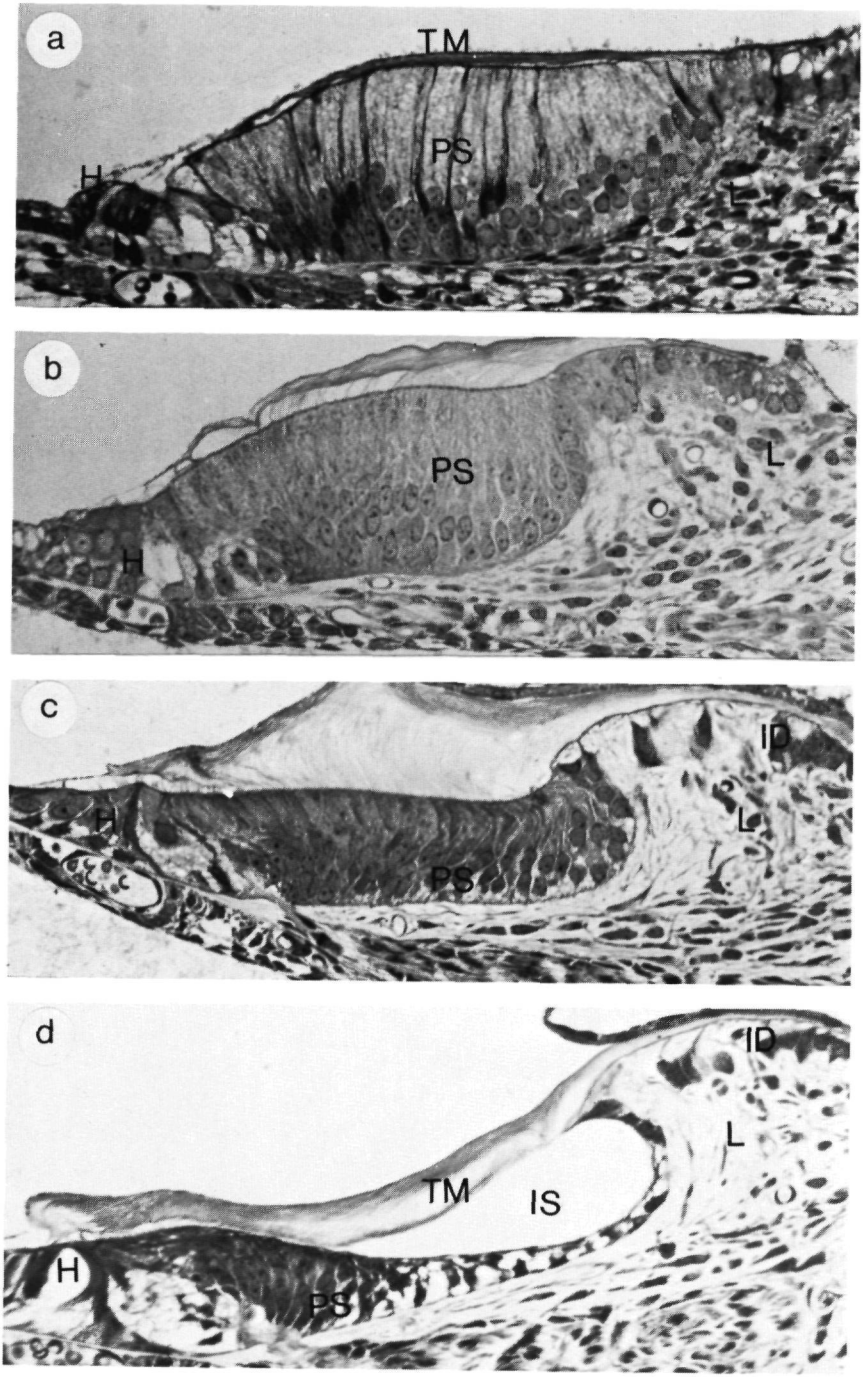



Fig. 17. Postnatal development of the organ of Corti of the rat showing formation of the inner sulcus (IS) by disappearance of the embryonal sulcus cells (PS) and maturation of tectorial membrane (TM) and hair cell area (H). All micrographs are of the second cochlear turn.
a: 1 day, b: 3 days, c: 6 days, d: 9 days. (toluidin blue, X425). L: limbus, ID: interdental cells.

CHAPTER 4

# AUTORADIOGRAPHIC STUDIES ON THE ROLE OF THE ENDOLYMPHATIC SAC IN ENDOLYMPH CIRCULATION

## 4.1 INTRODUCTION

Attempts to shed some light on the function of the endolymphatic duct and sac and their role in endolymph circulation have been limited to the use of foreign substances as tracers, introduced directly into the endolymphatic space or outside the inner ear (Chapter 1).
In this chapter a physiological method is introduced to study these problems.
In the previous chapter high concentrations of (acid) glycoproteins were shown to be present in the endolymphatic sac of the rat. According to some authors (Allen, 1964; Rask-Andersen et al, 1981[b]; Friberg et al, 1986) this substance is secreted by the epithelial lining of the endolymphatic sac, although convincing experimental evidence is lacking.
Histochemical studies in the guinea pig and mouse revealed the presence of glycoproteins in other parts of the inner ear, notably in the cupulae, the tectorial and otolithic membranes (Wislocki and Ladman, 1955; Dohlman et al, 1959; Veenhof, 1969; Lim, 1980) but their origin is unknown.
With these data in mind it was attempted to study the origin and fate of the inner ear glycoproteins with the aim of obtaining some insight into endolymph circulation and function of the endolymphatic sac.
The observations of Bélanger (1953) who demonstrated that intraperitoneal injection of $^{35}SO_4$ in newborn rats resulted in isotope incorporation into the tectorial membrane, cupulae and otolithic membranes served as a guideline for these experiments. Since inorganic sulphate is selectively incorporated into sulphated glycoproteins, $^{35}SO_4$ was assumed to be a suitable precursor for studying the secretion and fate of this group of glycoproteins in the rat inner ear autoradiographically.
In this study both newborn (1-9 days old, n = 20) and adult rats (n = 10) were used. The newborn rats were killed after periods varying from 30 minutes to four days and the adult rats after periods varying from two hours upto eight weeks. Because the inner ear of the rat is still immature at birth a brief account of its postnatal development will be given.

## 4.2 SECRETION AND MIGRATION OF $^{35}S$-LABELLED GLYCOPROTEINS IN THE ENDOLYMPHATIC SPACE

### *4.2.1 Newborn rat*

At birth the organ of Corti of the rat is still immature while the sensory epithelium of the vestibulum shows a mature configuration. The postnatal development of the organ of Corti is illustrated in fig. 17. During the first days after birth the hair cell region (Köllikers organ) lacks the fluid filled spaces, while the inner sulcus is densely packed by tall slender cells. The apical surface of these cells shows many long microvilli

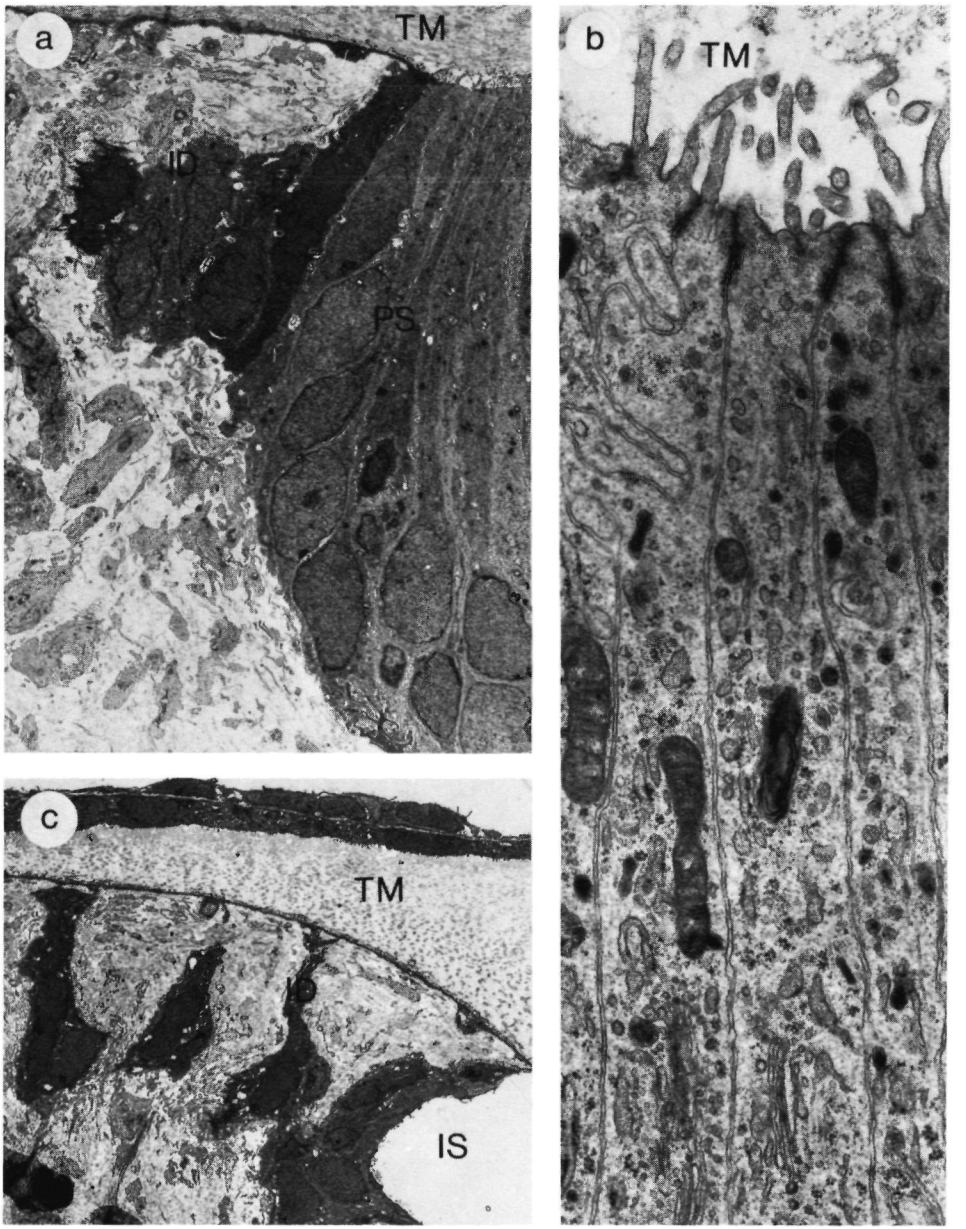



Fig. 18. Electronmicrographs of developing inner sulcus (IS) of the second cochlear turn. a: 4 days after birth; the sulcus is filled with tall embryonal cells (PS) while interdental cells (ID) are formed (X2100), b: close-up view of embryonal inner sulcus cells, with long microvilli embedded in the developing tectorial membrane (TM). The cytoplasm contains active Golgi complexes, vesicles and RER of the smooth type (X12000), c: 8 days after birth; the inner sulcus (IS) is formed and lined by cuboidal epithelium (X1200).

embedded in the developing tectorial membrane. The cytoplasm contains Golgi complexes, scarcely RER and more ER of the smooth type, polysomes, few mitochondria and many small vesicles (figs. 18[a,c]). These features are highly suggestive of a secretory function. During the late prenatal and early postnatal period the tectorial membrane consists of a small pale staining strip and is closely apposed to the underlying epithelium.
In the subsequent days cell differentiation progresses from base to apex, leading to the typical adult architecture of the hair cell region. Simultaneously, the tall embryonal inner sulcus cells disappear, resulting in the fluid filled inner sulcus, lined with cuboidal epithelium (figs. 17[d], 18[c]). In the adult animal the cytoplasm is optically empty (fig. 2) and only contains a few scattered mitochondria. It couldnot be established if these cells represent transformed tall sulcus cells or are newly formed. This maturation process proceeds from base to apex.
Parallel to this transformation the tectorial membrane grows thicker and becomes gradually released from the inner sulcus cells. In the mean time the spiral limbus is covered by the cell protrusions of the T-shaped interdental cells (figs. 17[c,d], 18[c]). These cells appeared to be formed in close connection with the inner sulcus cells as illustrated in fig. 18[a].
In contrast to the organ of Corti, the sensory epithelium of the vestibular part of the inner ear, including the cupulae and otolithic membranes already show at birth a nearly morphologically mature configuration.

Autoradiography of the cochlear portion revealed a marked labelling of the primitive sulcus cells 30 minutes after intraperitoneal injection of radioactive sulphate. No significant uptake of isotope could be detected in other parts of the epithelial lining of the cochlear duct. One hour after injection, labelling was also found in the adjacent part of the tectorial membrane. In the subsequent hours the tectorial membrane became heavily labelled while labelled material was frequently found in the area of the endolymphatic space bordering the tectorial membrane (fig. 19).
At six hours the apical part of the sulcus cells was still labelled. The presence of radioactive material was confined to the tectorial membrane, one day after isotope administration. This course of events was observed in all newborn animals studied, irrespective of their age.
Concomitantly with the replacement of the tall sulcus cells by cuboidal cells, the uptake of $^{35}S$ decreased. At nine days after birth, only the apical turn and part of the second turn showed distinct labelling (figs. 20[a-c]). The observations made two and four days after $^{35}SO_4$ administration appeared to be highly dependent on the degree of maturity of the tectorial membrane at the time of injection. If this structure was still immature at that time a major part of the mature membrane was still labelled (fig. 20[d]).However if the tectorial membrane had reached mature or nearly mature conditions no labelling was found.
With respect to the epithelial lining of the vestibular part of the inner ear, the sensory epithelium of both cristae and maculae and a small area of non-sensory epithelium bordering these structures revealed incorporation of $^{35}S$ within 30 minutes after injection. After four hours also the cupulae and otolithic membranes showed distinct labelling (fig. 21). From this time this material could also be found in the endolymphatic space. With progression of time labelling became gradually confined to the cupulae and

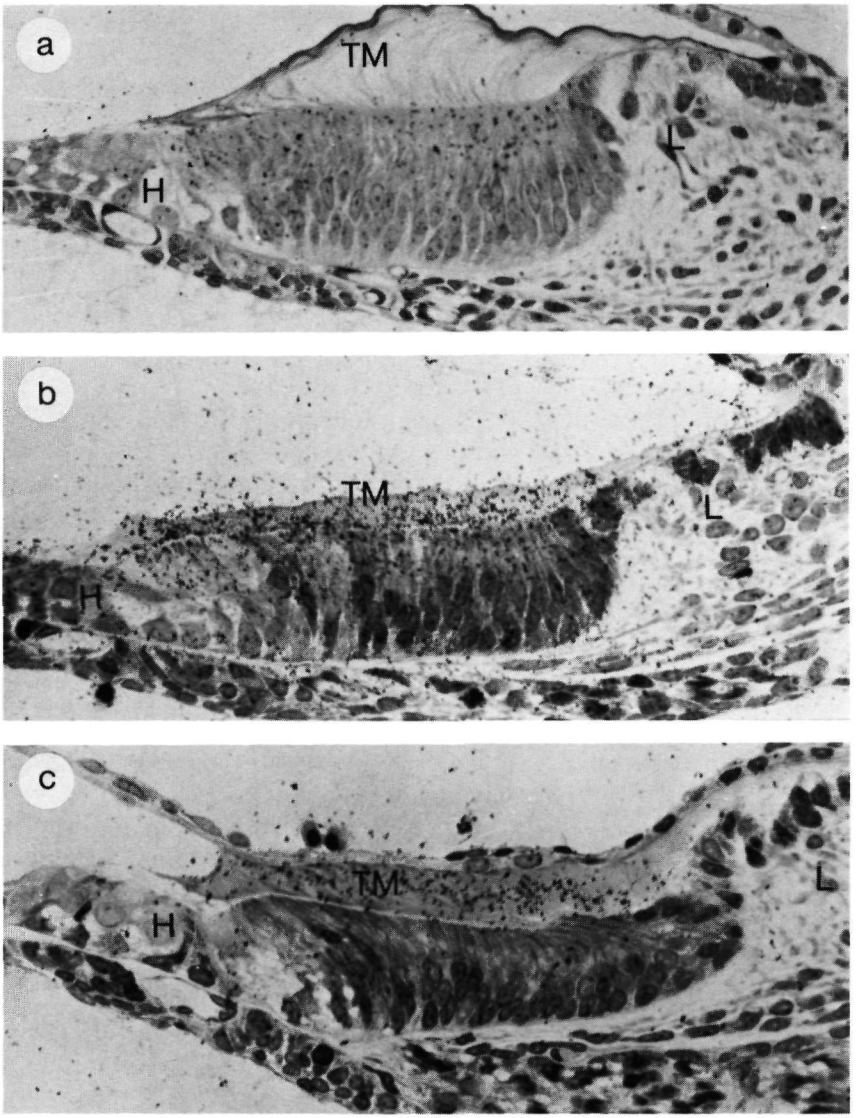
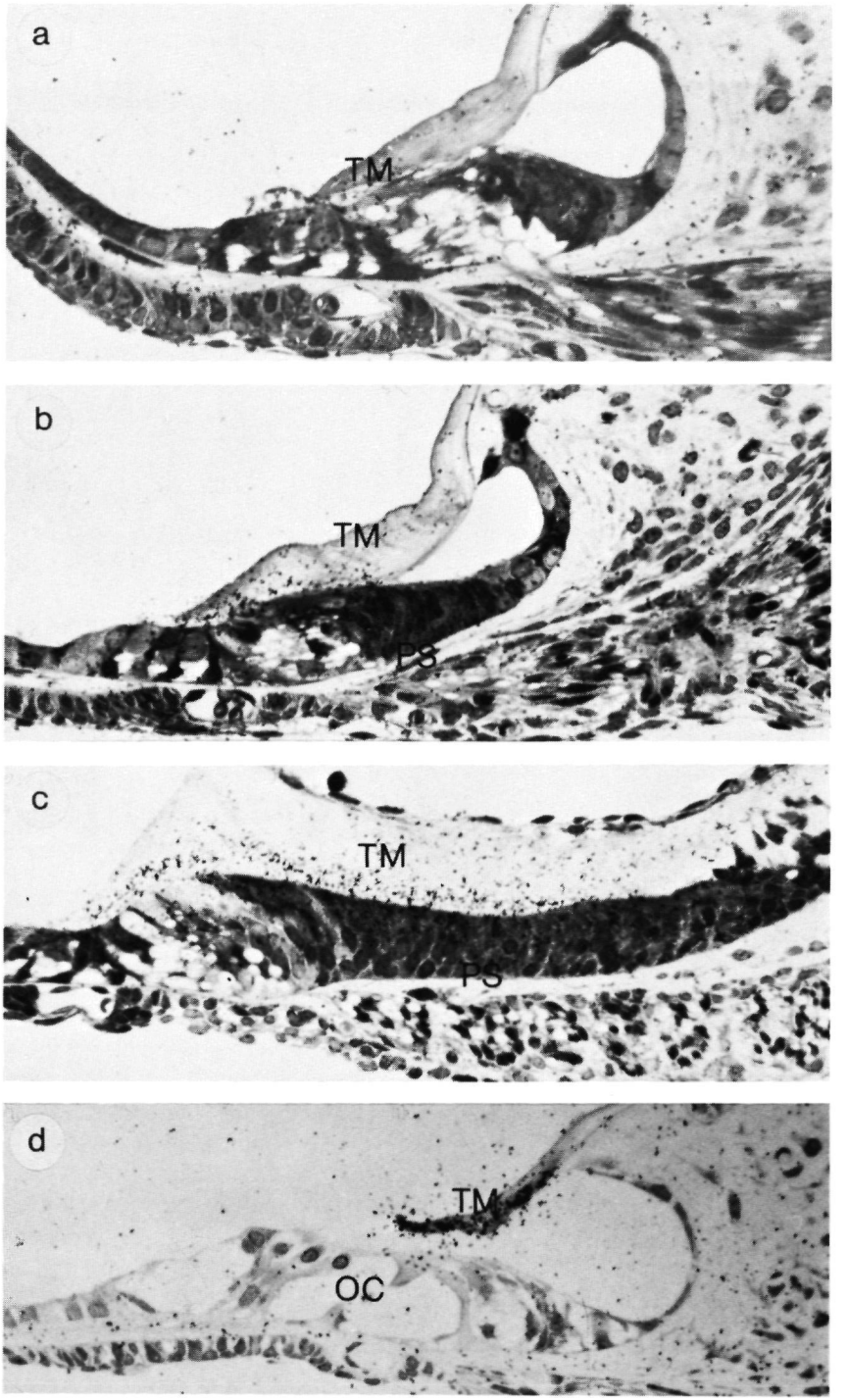



Fig. 19. Autoradiographs of organ of Corti.
a: 30 min, b: 6 hours and c: 4 days after i.p. injection of $^{35}SO_4$ of 2 day old rats. The embryonal sulcus cells show selective incorporation of isotope, which is subsequently secreted into the developing tectorial membrane. Note labelling of endolymph in (b). L: limbus with interdental cells, H: hair cell area, TM: tectorial membrane (toluidin blue, X450).

Fig. 20. Autoradiographs of organ of Corti in various cochlear turns of 9 day old rats, 6 hours after i.p.→ injection of $^{35}SO_4$.
a: basal turn (no labelling)
b: second turn (slight labelling)
c: apical turn (heavy labelling). The labelling is associated with the presence of embryonal sulcus cells (PS)
d: labelling of lateral edge of tectorial membrane (TM) of mature organ of Corti (OC) 4 days after isotope injection of 9 day old rat (toluidin blue, X450).


a
TM
b
TM
PS
c
TM
PS
d
TM
OC

otolithic membranes and persisted there up to four days after injection (figs. 21[b,d]). Occasionally a labelled coagulum was found in the proximal part of the endolymphatic duct (fig. 22[c]).

During this period of postnatal development the endolymphatic sac was lined by a rather smooth cuboidal epithelium, while the lumen was filled with a homogeneous coagulum. Distinct labelling of the saccus content but not of the epithelial lining was first observed after six hours. Heavy labelling was found after one day (figs. 22[a,b]) and this picture persisted up to the observation period of four days. These observations were made in all age groups studied.

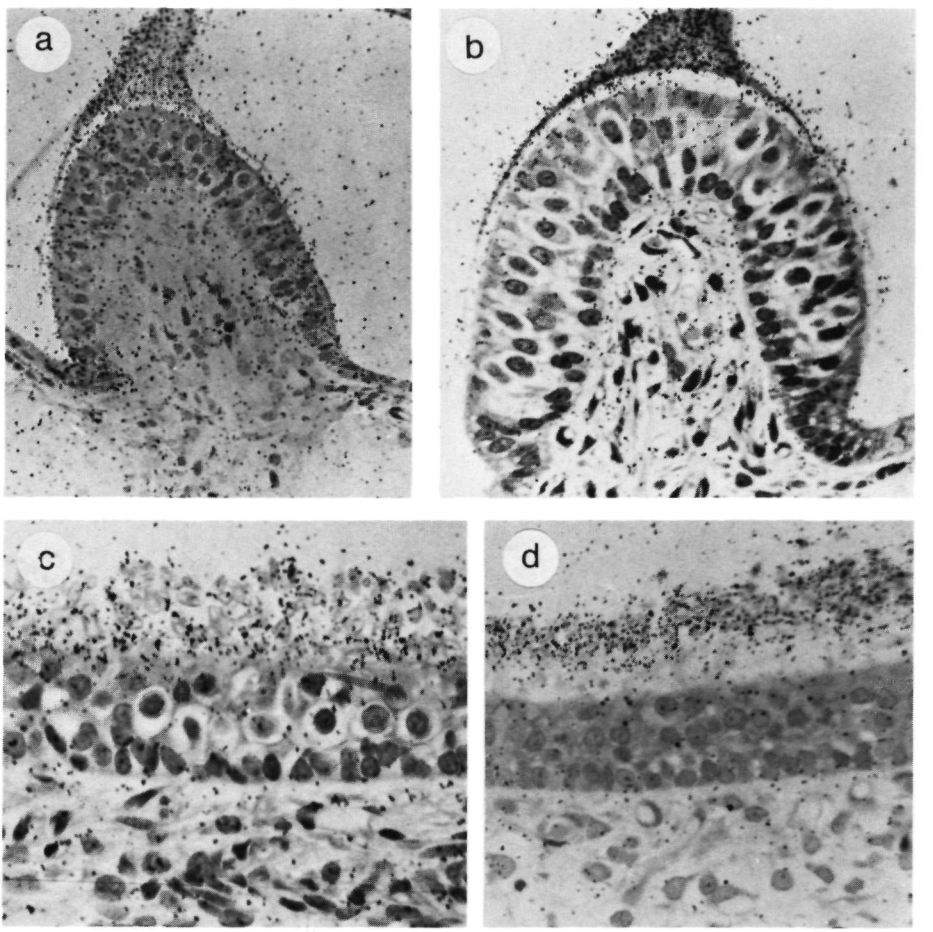



Fig. 21. Autoradiographs of cristae and maculae. Four hours (a,c) and 4 days (b,d) after i.p. injection of $^{35}SO_4$. After 4 hours both epithelium, cupula and otolithic membrane are labelled. After 4 days labelling is confined to cupula and otolithic membrane (toluidin blue, X400).

Autoradiographs of sections of the inner ear of the rat revealed an increasing uptake of $^{35}S$ in distinct parts of the epithelial lining of the vestibular portion within eight hours after intraperitoneal injection. Generally isotope incorporation was much less pronounced than in the newborn animals. The epithelial lining of the cristae ampullares showed slight labelling four hours after isotope administration. This was more pronounced after eight hours. At that time labelled material could also be detected in the cupular area adjacent to the epithelium. The cupula is usually severely shrunken and distorted due to the fixation and dehydration procedure.
Labelling was not confined to the neurosensory area, but also the area between the sensory epithelium and the dark cell area at the base of the crista revealed substantial uptake of $^{35}S$ (fig. 23[a]).
Moreover in the side walls of the ampullae a small rim of columnar to cuboidal cells

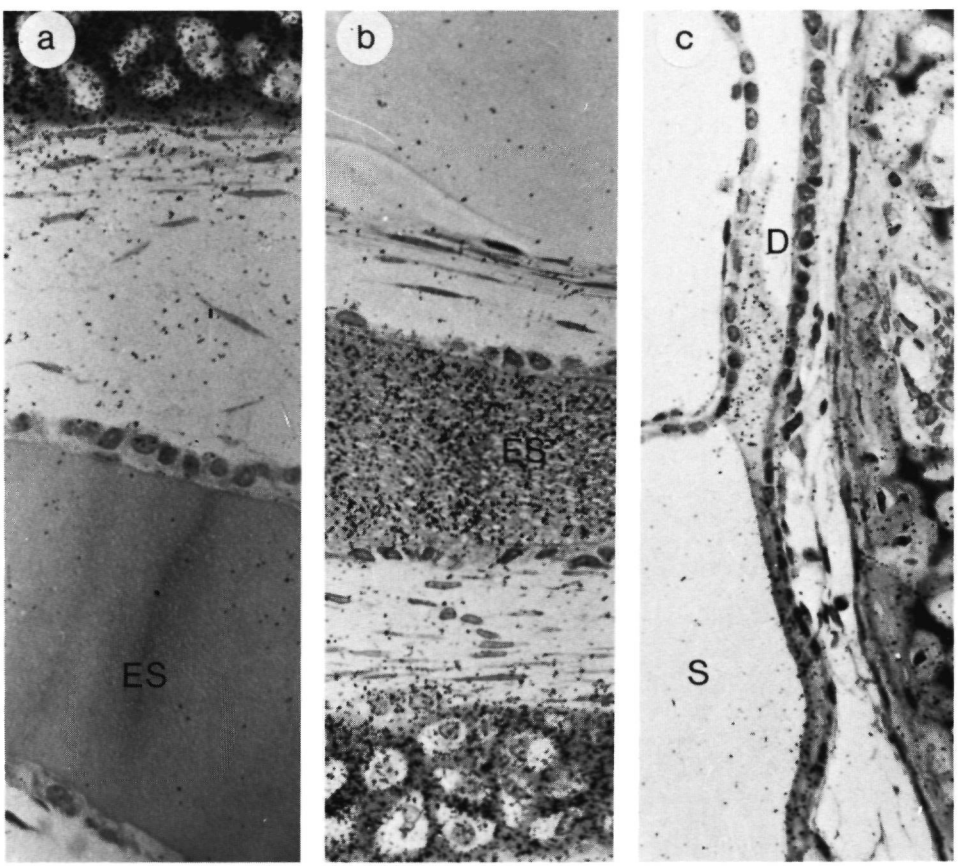



Fig. 22. Autoradiographs of endolymphatic sac (ES).
a: 4 hours and b: 1 day after $^{35}SO_4$ injection of 2 day old rats. After 4 hours, there is no isotope uptake in the sac. After 1 day the content of the sac shows heavy labelling (note labelled cartilage). c: labelled coagulum at the entrance of the endolymphatic duct (D). S: saccule (toluidin blue, X400).

bordering the sensory epithelium showed isotope incorporation (fig. 23[b]). Electron microscopy showed the presence of abundant dilated RER fragments with scattered ribosomes and filled with a flocculent substance, small vesicles and active Golgi complexes in the apical cytoplasm of these cells and the sensory supporting cells (fig. 25[a]). This strongly suggests a secretory function. After 24 hours the bulk of the radioactive material was found in the cupulae and after two days no $^{35}S$ could be detected in the epithelium. During subsequent days the grain density gradually diminished and after one week only a few grains were present over the cupula (fig. 23[c]).
A comparable course of events could be established in the macula utriculi and sacculi, although the incorporation of $^{35}S$ was considerably less than in the cristae, since a nearly

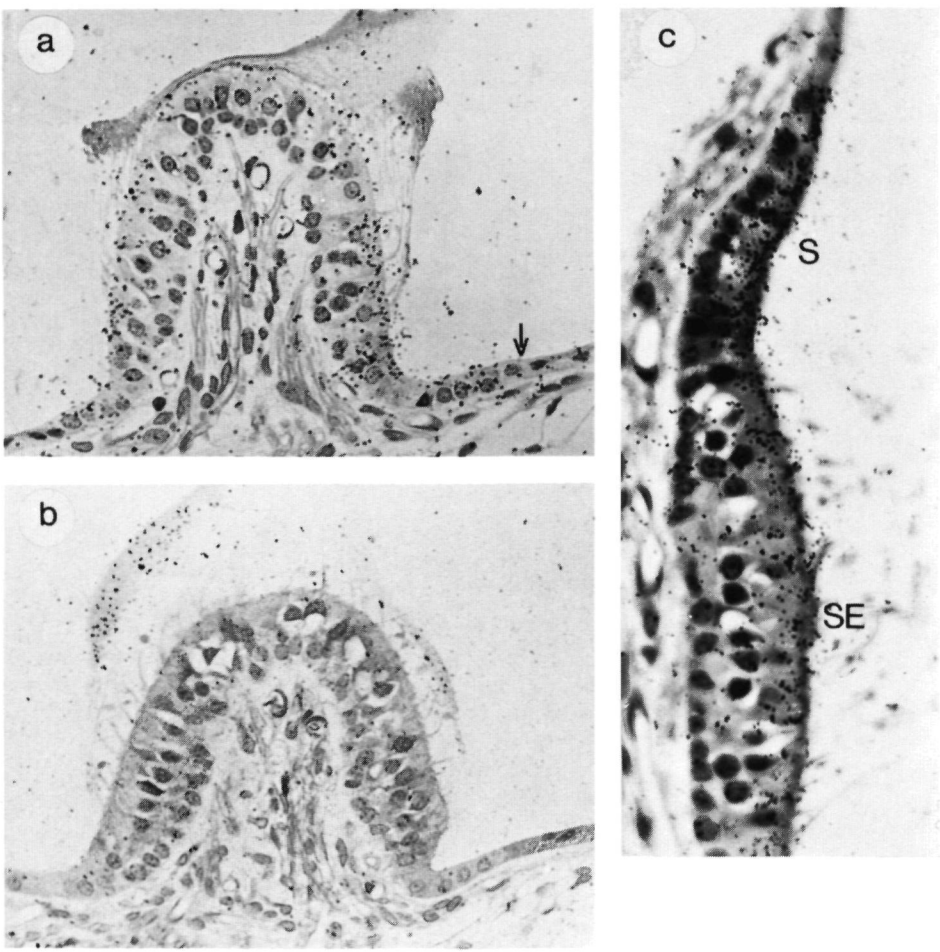



Fig. 23. Autoradiographs of crista ampullaris various times after $^{35}SO_4$ injection of adult rats.
a: synthesis of $^{35}S$-labelled glycoproteins by the sensory and non-sensory epithelium up to the dark cell area (→) after 8 hours (toluidin blue, X400)
b: slight labelling of cupula after one week (toluidin blue, X400)
c: upper part of crista in the lateral ampullar wall showing labelled sensory cell area (SE) and adjacent area of supporting cells (S), after 8 hours (toluidin blue, X400).

double exposure time of the photographic emulsion was needed to obtain a comparable grain density.

In the maculae incorporation of $^{35}S$ was initially found in the sensory area and in part of the cuboidal perimacular epithelium bordering the extra-macular extension of the otolithic membrane (fig. 24).

The sensory supporting cells and the bordering cells revealed a similar cytoarchitecture as the comparable cells in the ampullae (fig. 25[b]).

Thereafter the labelled material accumulated in the otolithic membrane, followed by a gradual diminution during subsequent days. After one week labelled substance could no longer be detected.

The epithelial lining of the cochlear part of the membranous labyrinth failed to show any incorporation of $^{35}S$ at any point in the observation period. Only the connective tissue of the spiral ligament and the spiral limbus revealed slight labelling. A similar observation was made in the connective tissue of the vestibular part.

During the first eight hours following $^{35}SO_4$ administration, no isotope incorporation was registered in the endolymphatic sac and duct, either in the epithelium or in the lumen. Only the surrounding connective tissue of the sac and the periosteal membrane of the adjacent bone revealed slight labelling (fig. 26).

Twentyfour hours after isotope injection the proximal part of the saccus content showed slight labelling. This was more marked after two days. The content of the sac, especially in the proximal part, had a very inhomogeneous structure and labelling appeared to be particularly confined to those areas which stained with PAS (fig. 27[a]).

After one week extensive labelling was established in the whole proximal area, while the more homogeneous distally located PAS staining area showed diffuse labelling (fig. 27[b]).

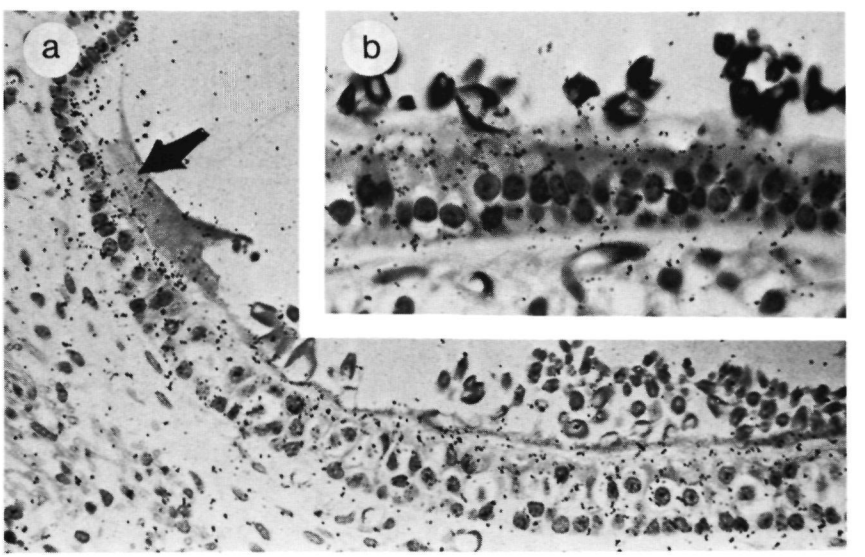



Fig. 24. Autoradiographs of utricular (a) and saccular macule (b) 8 hours after $^{35}SO_4$ injection of the adult rat showing synthesis and secretion of labelled glycoproteins by the macular epithelium and perimacular non-sensory cells (→) into otolithic membranes. a (toluidin blue, X225); b (toluidin blue, X400).

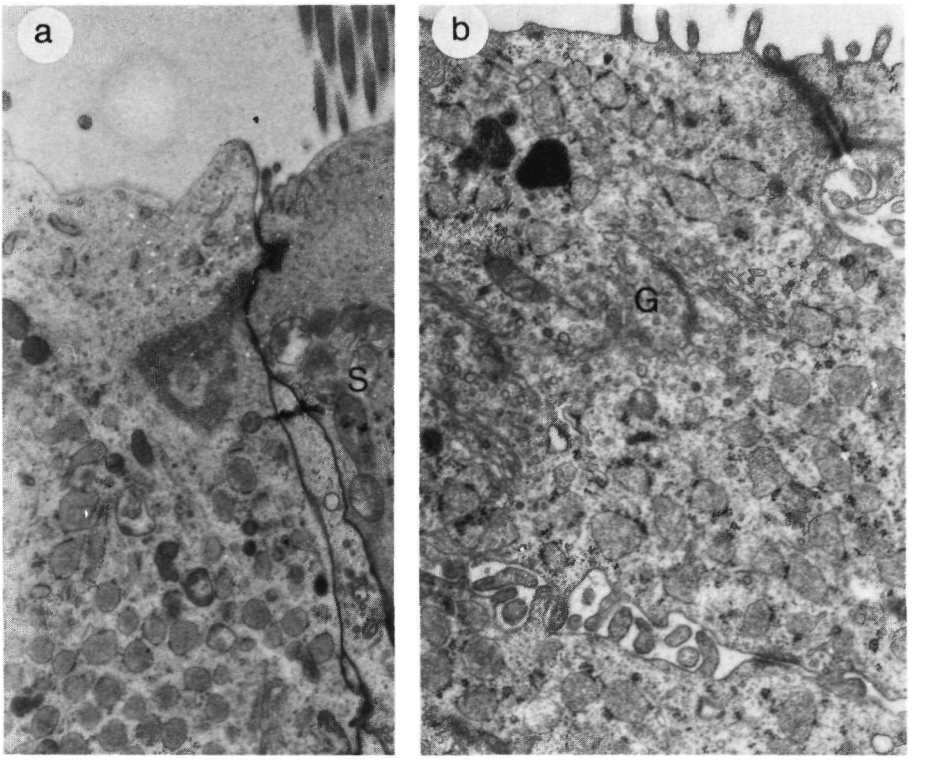



Fig. 25. Electronmicrograph of sensory supporting cell in crista epithelium (a) and of perimacular cell (b). Both cells reveal abundant dilated RER. Note the active Golgi complex (G). s: sensory cell. (a, X9900; b, X15500).

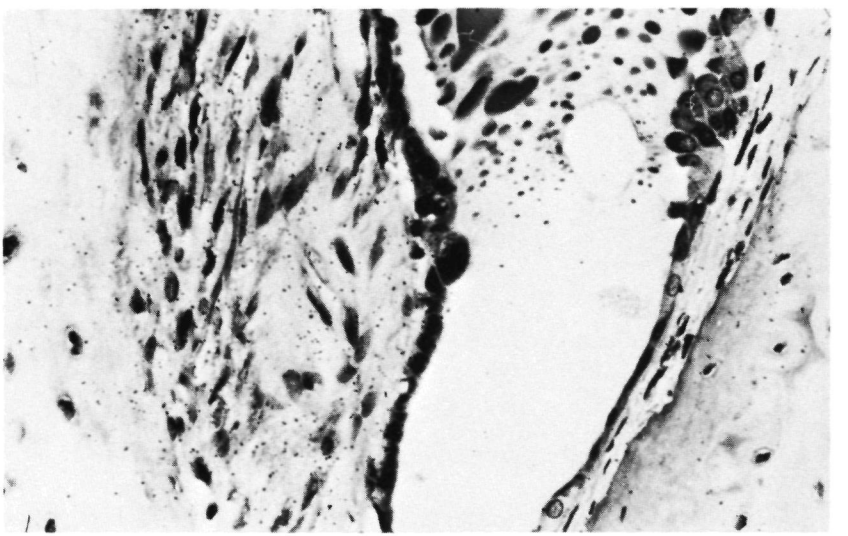
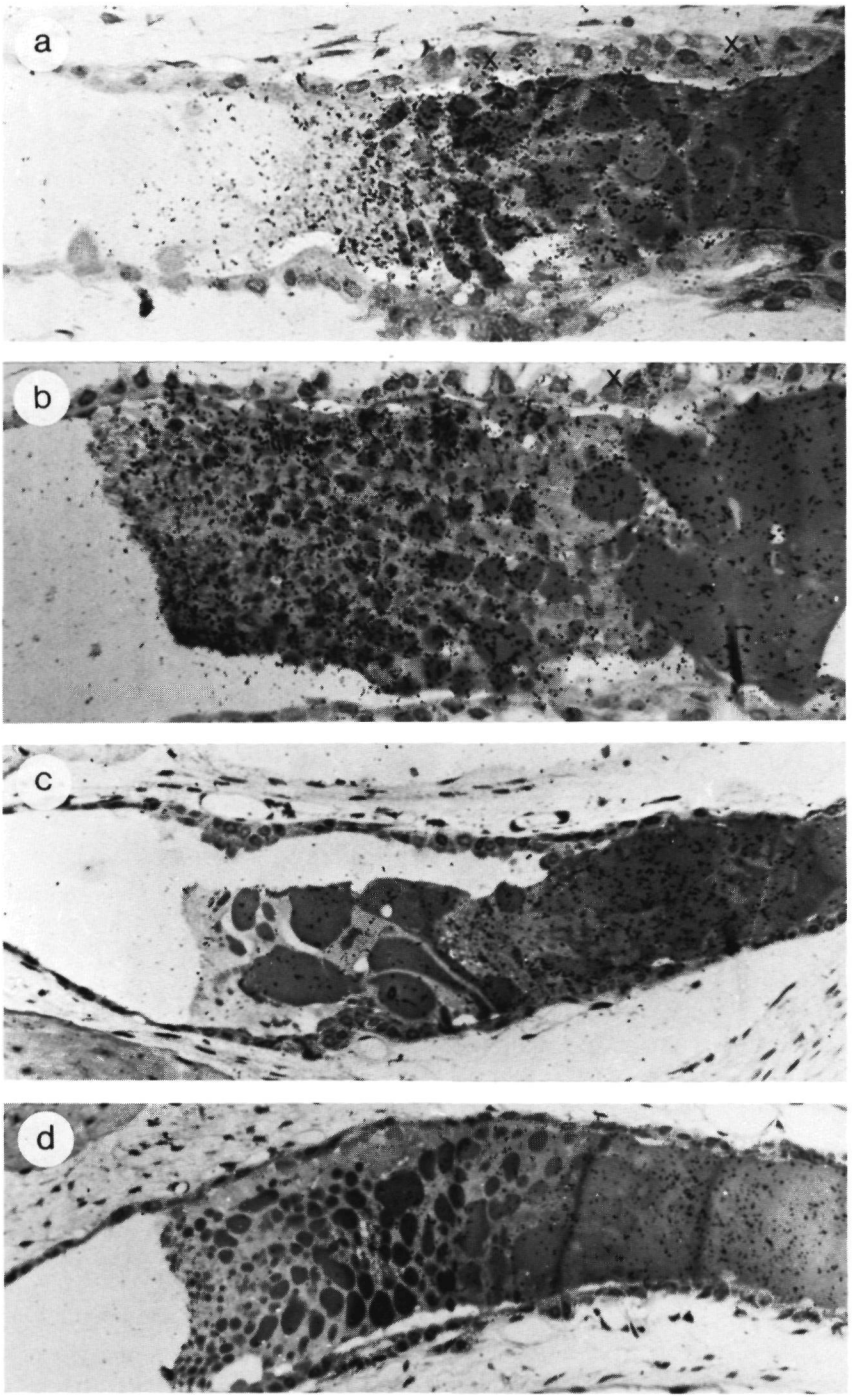


Fig. 26. Proximal portion of endolymphatic sac of the adult rat 8 hours after $^{35}SO_4$ injection showing isotope uptake in the perisaccular connective tissue but not in the endolymphatic sac (toluidin blue, X400).

Generally it was very difficult to draw conclusions on the presence of radioactive material in the epithelium bordering the heavily labelled contents, because of the rather high radiation energy of the used isotope, but occasionally distinct labelling of some epithelial cells could be established. During subsequent weeks the bulk of the radioactive material moved distally, accompanied by a decrease in grain density (fig. 27). Still a significant number of grains were found scattered throughout the distal part of the saccus lumen in those animals which survived for eight weeks after isotope administration.

## 4.3 DISCUSSION

The results obtained in this study demonstrate that radioactive sulphur administered as sodium sulphate seems to be an appropriate precursor for tracing both secretion and migration of sulphated glycoproteins in the endolymphatic space of the rat. Secretion of this substance appeared to be confined to the areas of neurosensory epithelium.
The observations demonstrate a striking difference between the cochlear and vestibular parts of the inner ear during maturation and when mature conditions are reached. Secretion of sulphated glycoproteins virtually ceases in the cochlear part as soon as functional maturation is achieved.
In the cochlea a remarkable role in the final stage of functional maturation of the tectorial membrane is played by the embryonal sulcus cells. During the postnatal period, in which the tectorial membrane strongly increases in size, sulphated glycoproteins are synthesized by the embryonal sulcus cells and subsequently secreted into the tectorial membrane. This process decreases with progressing maturation of the organ of Corti. It practically stops when the embryonal sulcus cells are replaced by the cuboidal lining of the developing inner sulcus, leading to detachment of the tectorial membrane when the organ of Corti reaches mature conditions. At that time the tectorial membrane has only contact with the newly formed interdental cells. This occurs in the organ of Corti in the rat for the basal turn at about the 9th day after birth and coincides with the mature composition of the endolymph (Bosher and Warren, 1971) and with the appearance of the first electrophysiological responses (Uziel et al, 1981).
According to Iurato (1962), Arnold and Vosteen (1973), Kronester-Frei (1976), Anniko (1980), the tectorial membrane should originate from secretion of the interdental cells. Anniko (1980) observed during development of the mouse inner ear, which parallels that of the rat (Schmidt and Fernández, 1963), that the main activity of the embryonal interdental cells occurred on the 18th gestational day. Thereafter it decreased sharply and was very low after birth. On the 18th gestational day a fibrillar network was formed, which except for a tighter packing did not change after birth. These observations and the findings from the present study suggest a two stage development: first the formation of filaments by the embryonal interdental cells followed by incorporation of glycoproteins, produced by the embryonal inner sulcus cells. The lack of any observable uptake of $^{35}S$ in the tectorial membrane when the tall sulcus cells are replaced by the adult cuboidal cells and the organ of Corti has reached functional maturity, differs from the situation in the vestibular part of the labyrinth. In this part of the inner ear synthesis and secretion of sulphated glycoproteins continue when these structures are morphologically mature.


a
b
c
d

Both in the newborn and the adult rat secretion of sulphated glycoproteins is restricted to the sensory epithelium and specialised parts of the adjacent non-sensory epithelial lining of the ampullae, utricle and saccule.

However it was practically impossible to decide which type of cell was involved in this process. Taking into account the electronmicroscopical data this secretion is most likely performed by the sensory supporting cells, a small band of peripheral supporting cells and transitional cells at the base of the crista, and in the side wall of the ampulla by a narrow band of cylindrical to cuboidal peripheral supporting cells, extending between the sensory cells and the semilunar planes. Cells with a similar cytoarchitecture have been described at the same sites in the guinea pig (Wersäll, 1956; Bairati, 1960; Kimura et al, 1964). An involvement of the cells at the base of the crista in the formation of the cupula in the early stages of development can be derived from the electronmicroscopical studies of Anniko and Nordemar (1982). Although the precise in vivo position of the cupula is still unknown, it seems likely that it also covers this area of non-sensory cells, although a direct secretion into the endolymphatic space cannot be excluded.

Part of the non-sensory epithelium in the utricle and saccule also shows secretion of sulphated glycoproteins. The position of these cells having the same architecture as those bordering the cristae coincides with the extramacular extension of the otolithic membrane, which is very pronounced in rat and mouse (Lim and Erway, 1974).

The secretion of sulphated glycoproteins by specialised cells into cupulae and otolithic membranes in the adult rat indicates that these structures are continuously renewed after functional maturation. This deviates from the observation made in the cochlea where a continuing turnover could not be established in the tectorial membrane, which has been shown to have the same basic structure as the cupula and otolithic membranes (Spoendlin, 1957; Iurato, 1960[b]; Johnson and Hawkins, 1967; Ross, 1974). However these data seem to be in conflict with the observations made in the adult cochlea where interdental cells have been shown to secrete an amorphous substance into the tectorial membrane (Voldrich, 1967; Lim, 1970). Arnold and Vosteen (1973) established the presence of glycoproteins in this secretion. This discrepancy might be explained by assuming that the observed secretion of sulphated glycoproteins by the primitive sulcus cells during maturation is taken over by the interdental cells, but at such a low level that it cannot be detected by the method employed in the present study. Otherwise, the existence of a different secretion process in the adult, when compared to the developing animal, cannot be fully excluded.

One can only guess at the functional role of glycoproteins in these structures. Based on their chemical properties immobilization of water and increase of viscosity can be assumed to contribute significantly to the maintenance of the mature configuration required for optimal coupling of the applied stimuli to the stereocilia of the hair cells.

← Fig. 27. Accumulation of $^{35}S$-labelled glycoproteins in the adult rat endolymphatic sac various times after i.p. injection of $^{35}SO_4$.
There is a gradual shift of labelled substance from the proximal part (left) to the distal part (right) of the saccus lumen with progression of time. Note exclusive labelling of PAS-staining areas.
a: after 2 days (PAS, X400)
b: after 1 week (PAS, X400)
c: after 2 weeks (PAS, X150)
d: after 6 weeks (PAS, X150). X: labelled epithelial cells.

Why this substance is continuously renewed in cupulae and otolithic membranes and virtually not in the tectorial membrane remains obscure.

The results obtained in the autoradiographic study on the adult rat inner ear deviate from the data reported for a comparable study on the avian inner ear by Dohlman and Ormerod (1960). They established in the pigeon that after parenteral injection of $^{35}SO_4$ isotope, incorporation in the normal inner ear was exclusively limited to the semilunate plane, followed by secretion into the endolymph and cupula. Comparable observations were made in the cochlea (lagena), saccule and utricle, where sulphated glycoproteins were secreted by the tegmentum vasculosum in the lagena and similar structures in saccule and utricle into the endolymph and were assumed to pass subsequently into the tectorial and otolithic membranes. This might be explained by the presence of more specialised epithelium in the avian inner ear. However in a second study Dohlman and Boord (1964) showed after experimental removal of the cupula that secretion of $^{35}S$-labelled glycoproteins was also found in the transitional epithelium and to a lesser extent in the sensory epithelium of the crista agreeing with the present observations in the adult rat.

The course of events, recorded both in the newborn and adult rat, shows that the secretion of $^{35}S$-labelled glycoproteins into cupula, tectorial and otolithic membranes is followed after a short delay by the first appearance of labelled substance in the lumen of the endolymphatic sac after six and 24 hours respectively. As before that time no isotope uptake was found in the epithelial lining of the endolymphatic duct and sac, it can be concluded that a mechanism (longitudinal flow) exists which transports these substances from the peripheral parts of the endolymphatic space towards the endolymphatic sac under physiological conditions. Removal of the macromolecules from the saccus lumen seems to be performed by the saccus epithelium, but this process is rather slow. These observations make a local secretion of glycoproteins by the epithelial lining of the endolymphatic sac as proposed by Allen (1964), Rask-Andersen et al (1981[b]) and Friberg et al (1986) very unlikely. In the newborn animal it indicates the existence of endolymph flow towards the endolymphatic sac before endolymph has reached its mature composition.

In a comparable experiment in the adult pigeon, Dohlman and Ormerod (1960) observed accumulation of $^{35}S$-labelled glycoproteins in the endolymphatic sac after four days.

The mechanism underlying the longitudinal flow is still unclear.

CHAPTER 5

# HISTOLOGICAL EFFECTS OF OBSTRUCTION OF THE ENDOLYMPHATIC SAC

## 5.1 INTRODUCTION

In this chapter the effects of surgical obstruction of the endolymphatic sac in rats on endolymph circulation and morphology of the cochlea and vestibular system will be discussed.
For that purpose a total number fo 107 Wistar rats both male and female (body weight 140-200 g) underwent obstruction of the endolymphatic sac of the right ear. The left ear served as control.
Ninety-one of these animals were available for morphological studies: 66 for light microscopy, 15 for transmission electron microscopy and 10 for autoradiography. Sixteen animals were excluded from this study for several reasons. Eight animals died intercurrently either from complications of the surgical procedures (6), or unknown cause (2). In addition, those animals which developed otitis media during the observation period (5) were excluded from this study and three animals were used for preliminary scanning electronmicroscopical studies. These data are summarized in table 4.

## 5.2 MORPHOLOGY

For light microscopy sections of the whole os petrosum including the endolymphatic sac were studied. Electron microscopy was focused on the endolymphatic duct and sac. Since dissection of these structures could not be performed without destruction of the remaining parts of the ear, these latter parts could not be used for morphological evaluation.
Because scanning electron microscopy has been shown to be superior to sections for studying the integrity of the hair cells of the organ of Corti, it was initially attempted to use this technique to establish the effect of endolymphatic sac obstruction on the hair cells. However it appeared to be rather difficult to determine the presence of hydrops by using this procedure. Moreover the anatomical position of the rat cochlea makes it less accessible for routine exposure of the organ of Corti by peeling off the thick bony

Table 4

Distribution of morphological techniques of rats with obstructed endolymphatic sac.

| Total | Light microscopy | Transmission electron microscopy | Autoradiography | Excluded |
|---|---|---|---|---|
| 107 | 66* | 15 | 10 | 16 |

* 47 of these animals were also used for physiological studies (Chapter 6).

medial capsule without damaging the delicate inner ear structures. Therefore this technique was soon abandoned.

### *5.2.1 Cochlear and vestibular portion*

Light microscopy of 66 operated ears revealed successful obstruction of the endolymphatic sac in 57 specimens. A micrograph of the site of obstruction is shown in fig. 28. Incomplete obstruction was found in four animals. Labyrinthitis was observed in two ears, while the posterior vertical canal appeared to be fractured during surgery in three specimens, although without damage to the membranous wall.
All those ears were excluded, so that finally 57 specimens remained for lightmicroscopical evaluation of the effects of obstruction of the endolymphatic sac.
In 28 (47%) animals a hydrops of the vestibular and/or cochlear portion was established. The microscopical observations are summarized in table 5. From these data it appears that no clear correlation was found between the occurrence and severity of hydrops and the time after obstruction. Hydrops in the vestibular portion was slightly more frequent than in the cochlea.
In the cochlea endolymphatic hydrops was characterized by distension of Reissner's membrane. This distension was usually seen in all turns, but was often most pronounced in the apical turn (figs. 29, 30) sometimes with outpouching of the helicotrema. In cases of extreme distension Reissner's membrane was apposed to the bony cochlear wall or showed extensive infoldings (fig. 30). In two cases a fistula of the membrane could be established (fig. 30[a]).
In addition to the distension of Reissner's membrane cell loss was found in the spiral ganglion. It was confined to the apical turn in two animals (fig. 29[a]) but in eight animals also the middle and basal turn were involved (fig. 30). Ganglion cell loss was mostly associated with atrophy of the organ of Corti. Sometimes only the outer hair cells were absent but occasionally the whole organ of Corti was involved (figs. 29[b], 30[a]). In one animal a distinct atrophy of the vascular stria was established. In most animals with endolymphatic hydrops dilatation of the saccular wall, which was often found to be folded, was very pronounced (fig. 31). In some cases the distended membrane touched the stapes footplate. Distinct fistulae in the saccular wall with clear signs of repair were

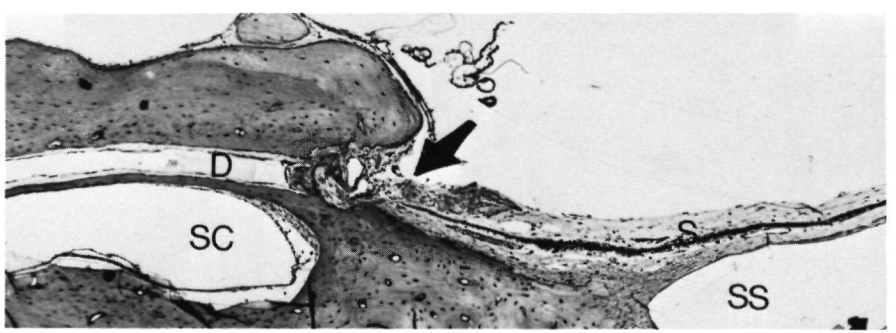



Fig. 28. Site of endolymphatic sac obstruction (→) after 6 weeks. Note dilated duct (D) and collapsed sac (S). SS : sigmoid sinus, SC : semicircular canal (toluidin blue, X40).

Table 5

Overview of the site and extent of endolymphatic hydrops following obstruction of the endolymphatic sac in rat (n = 57)

| Survival time in months | nr of rats | Endolymphatic hydrops | | | | | | | | | | | Spiral ganglion cell loss and/or atrophy organ of Corti | | | Fistulae | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cochlea | | | Saccule | | | Utricle | | | Canal | | | | | Reissner's membrane | Saccule |
| | | + | ++ | +++ | + | ++ | +++ | + | ++ | +++ | + | ++ | + | ++ | +++ | | |
| 1–5 | 20 | 2 | 5 | 4 | 6 | 5 | 4 | 1 | 2 | 2 | 1 | 3 | 1 | 1 | 1 | 1 | 5 |
| 10–15 | 37 | 3 | 6 | 6 | 6 | 4 | 3 | 5 | 3 | – | 1 | 2 | 1 | 3 | 3 | 1 | 3 |
| | | 5 | 11 | 10 | 12 | 9 | 7 | 6 | 5 | 2 | 2 | 5 | 2 | 4 | 4 | 2 | 8 |
| TOTAL | 57 | 26 | | | 28 | | | 13 | | | 7 | | 10 | | | 10 | |

Extent of – endolymphatic hydrops
– spiral ganglion cell loss and/or atrophy organ of Corti

\+ slight
++ moderate
+++ extensive

seen in eight specimens (fig. 32[a]). Dilatation of the saccule without hydrops in the cochlea was observed in two specimens. In only 13 ears distinct hydrops of the utricle and/or semicircular canals could be established (figs. 32[b,c]). However it must be noted that the membranous wall of the canals was often found collapsed both in the operated and control ears so that no conclusion could be made as to the presence of hydrops. After longer survival in most operated ears the utriculosaccular valve appeared to be closed. With respect to the integrity of the sensory epithelium, no degeneration of hair cells was found in the vestibular organs. In two animals the saccular otolithic membrane was found to be displaced (fig. 31[b]).

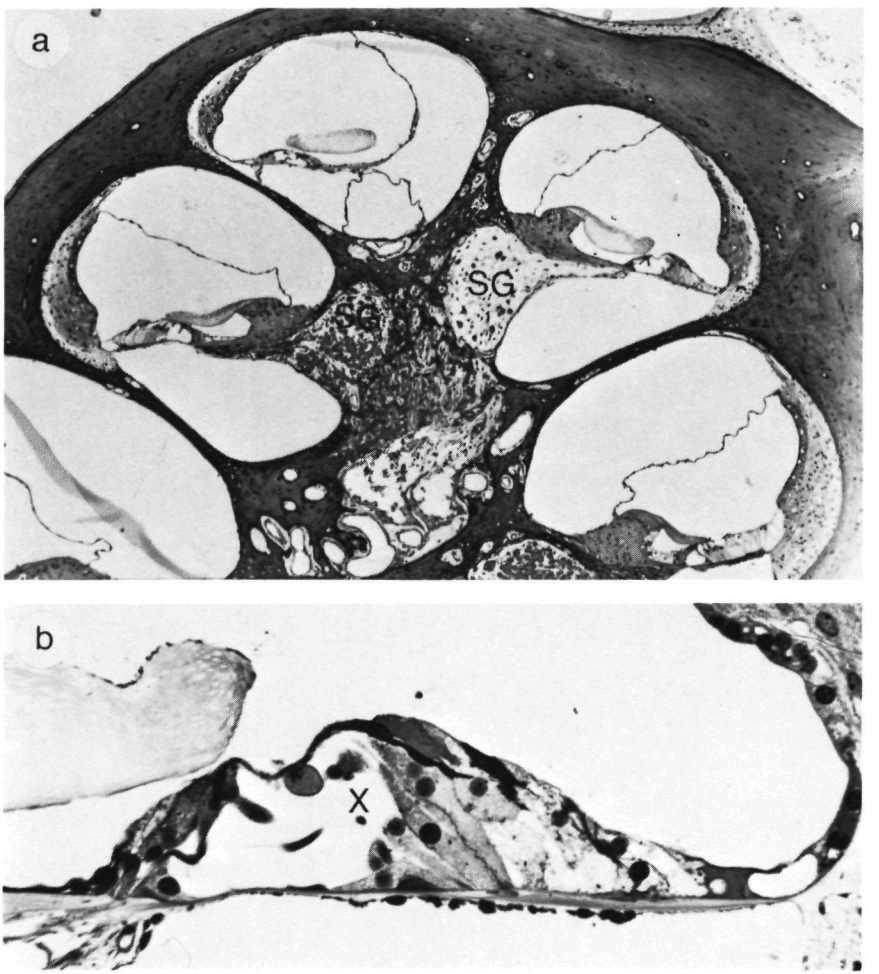



Fig. 29. a: Moderate hydrops of the cochlea 4 months after obstruction of the endolymphatic sac. Note the severe loss of nerve fibers and spiral ganglion (SG) cells in the upper turn and loss of outer hair cells (X) in the same area (b). For comparison see fig. 2c. (toluidin blue, X425).

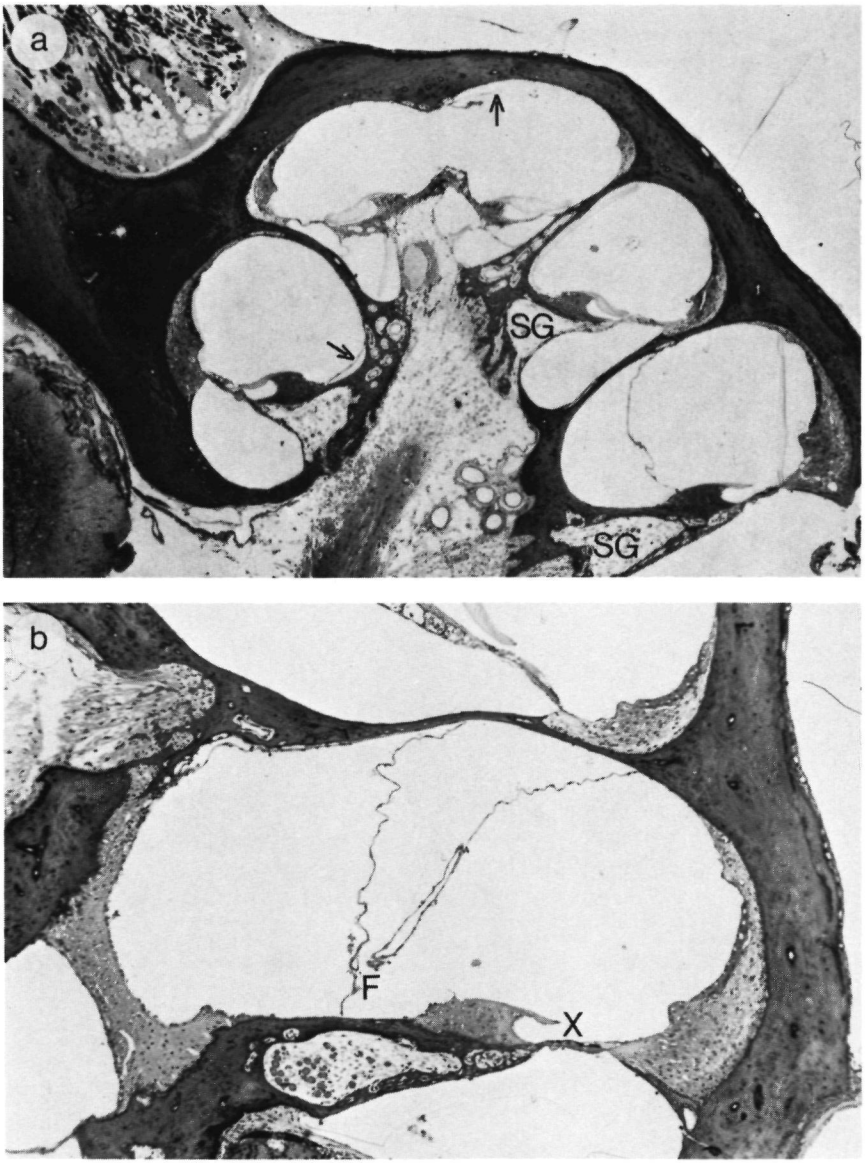



Fig. 30.
a: severe hydrops of cochlea 6 months after endolymphatic sac obstruction.
Reissner's membrane is apposed to the bony wall (→). Severe atrophy of the organ of Corti and loss of nerve fibers and spiral ganglion cells (SG) in all turns (toluidin blue, X40)
b: extensive dilation and fistula (F) of Reissner's membrane of the same animal. Note the complete absence of the hair cells (X) (toluidin blue, X150).

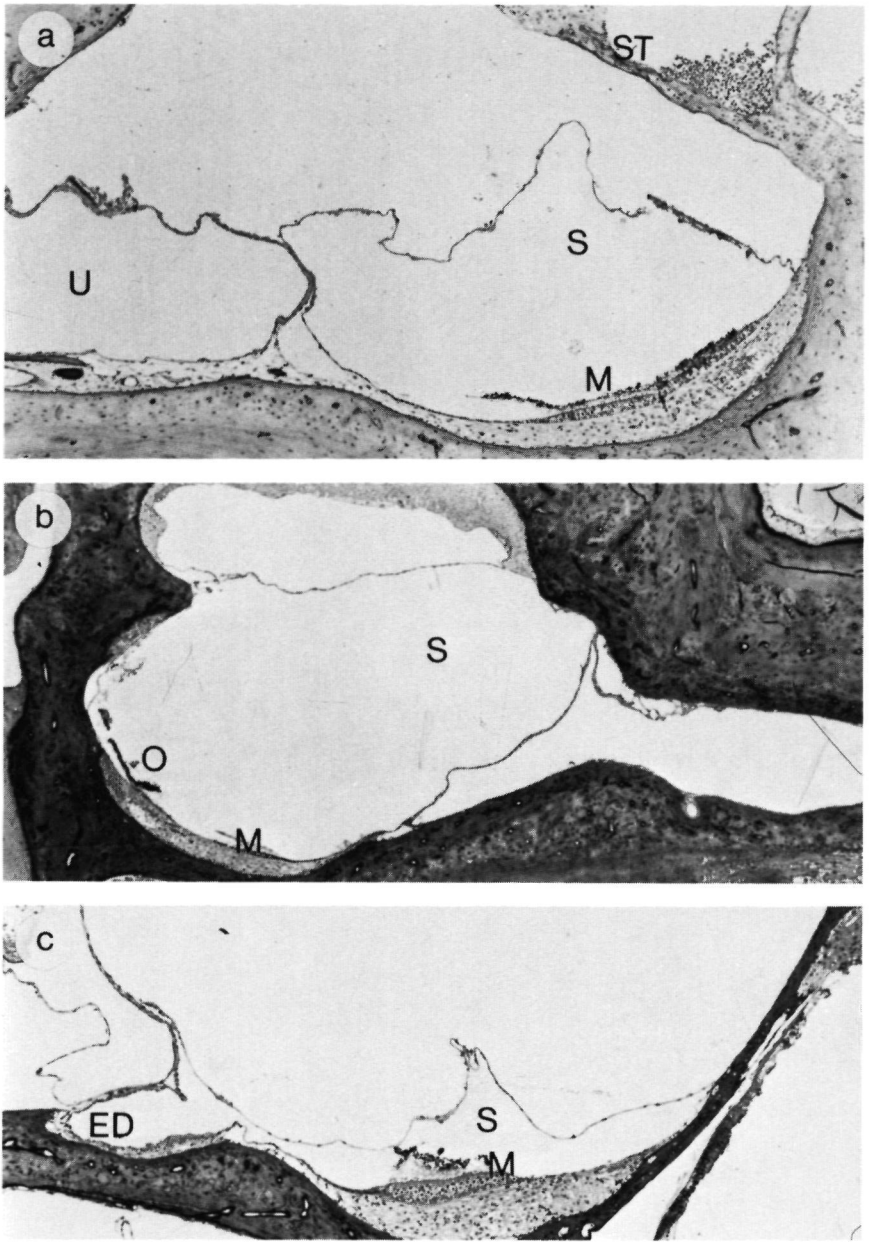



Fig. 31. Severe hydrops of saccule (s) various times after obstruction of the endolymphatic sac. a: 4 months, b: 8 months, c: 10 months.
The saccular membrane is strongly dilated. (b) shows dislocated otoliths (o) and (c) a dilated and collapsed saccular membrane. Note widened sinus of the endolymphatic duct (ED). M: macula, ST: stapes, U: utricle. For comparison see fig. 4 (toluidin blue, X40).

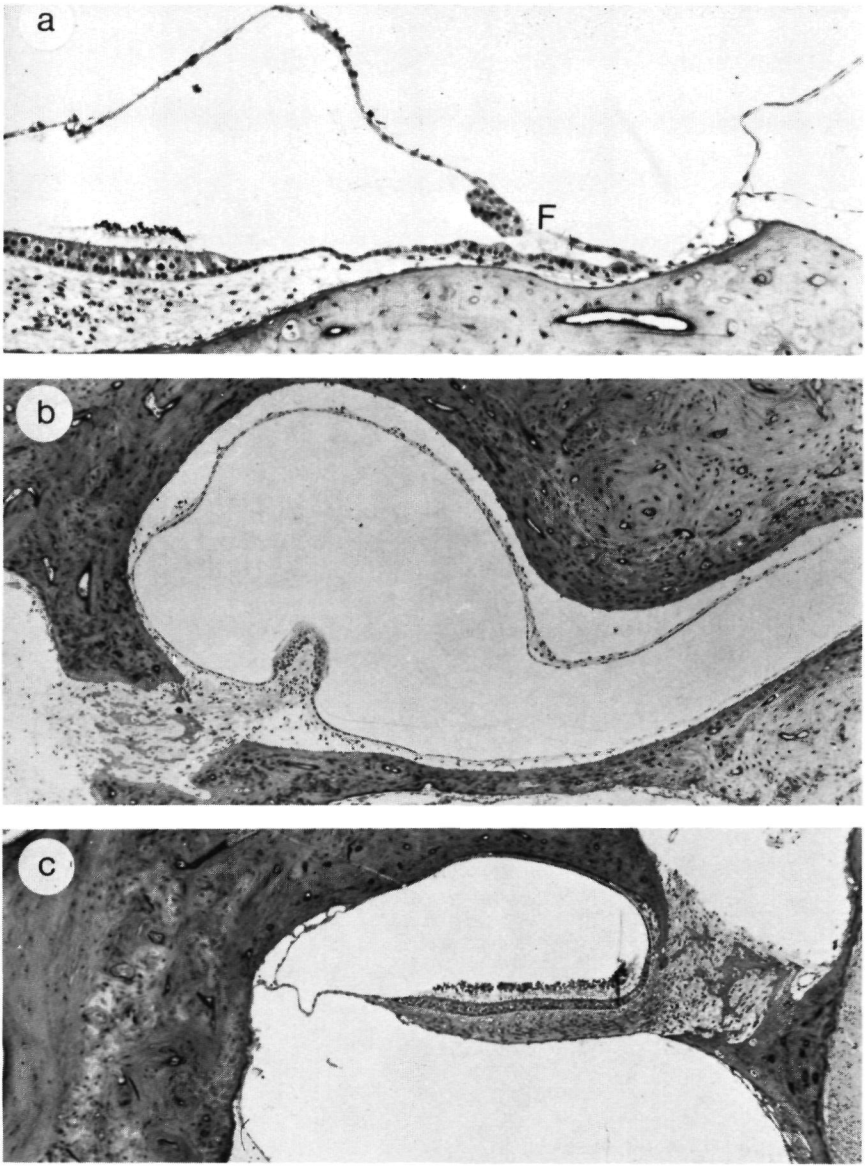



Fig. 32. Micrograph of rupture of saccular wall with signs of repair (F) 6 months after saccus obstruction (a); hydrops of the ampulla 3 months after obstruction (b) and hydrops of the utricle 6 months after obstruction (c). a (toluidin blue, X150); b (toluidin blue, X60); c (toluidin blue, X40).

The lumen of the endolymphatic duct revealed no marked changes during the first weeks after obstruction. After a postoperative period of more than six weeks, a clear distension of the duct, especially of its distal part, was observed. Distension of the duct, including the proximal part (sinus) was usually more marked in the animals with a severe hydrops (figs. 31[c], 33[a]). In the occluded ducts of animals that survived for more than two months the presence of a varying amount of a PAS-positive coagulum could be established. This coagulum sometimes filled the major part of the duct, but was often limited to the distal part. Occasionally some free floating cells (FFC) could be identified (fig. 33[a]). These findings were irrespective of the presence or absence of a hydrops in the cochlear or vestibular portion.

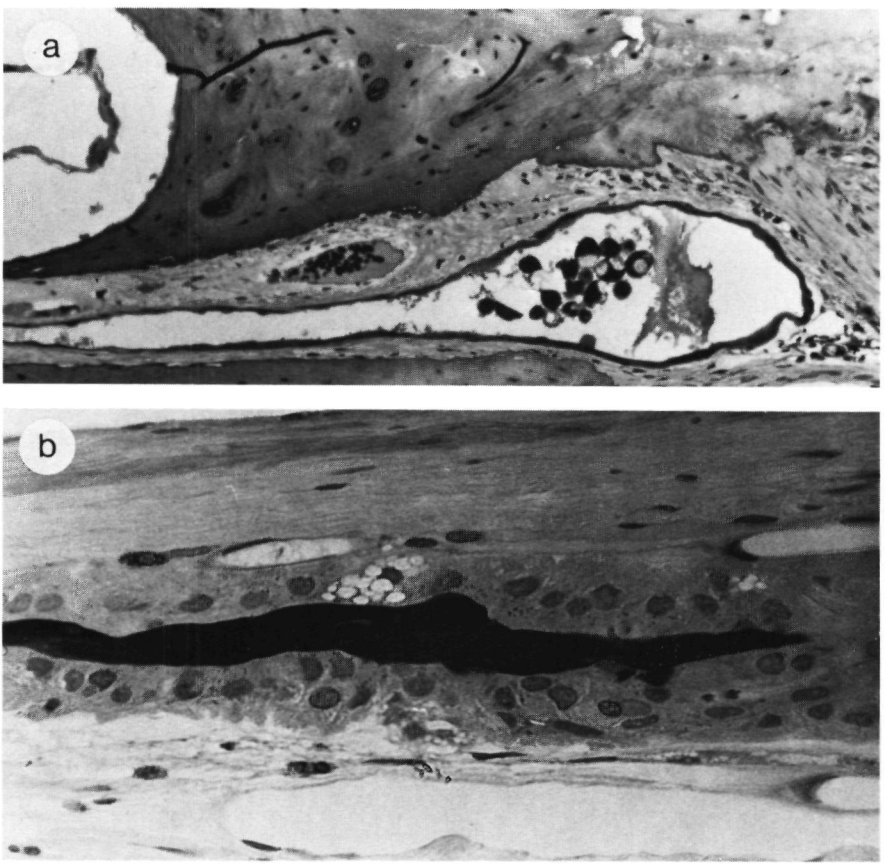
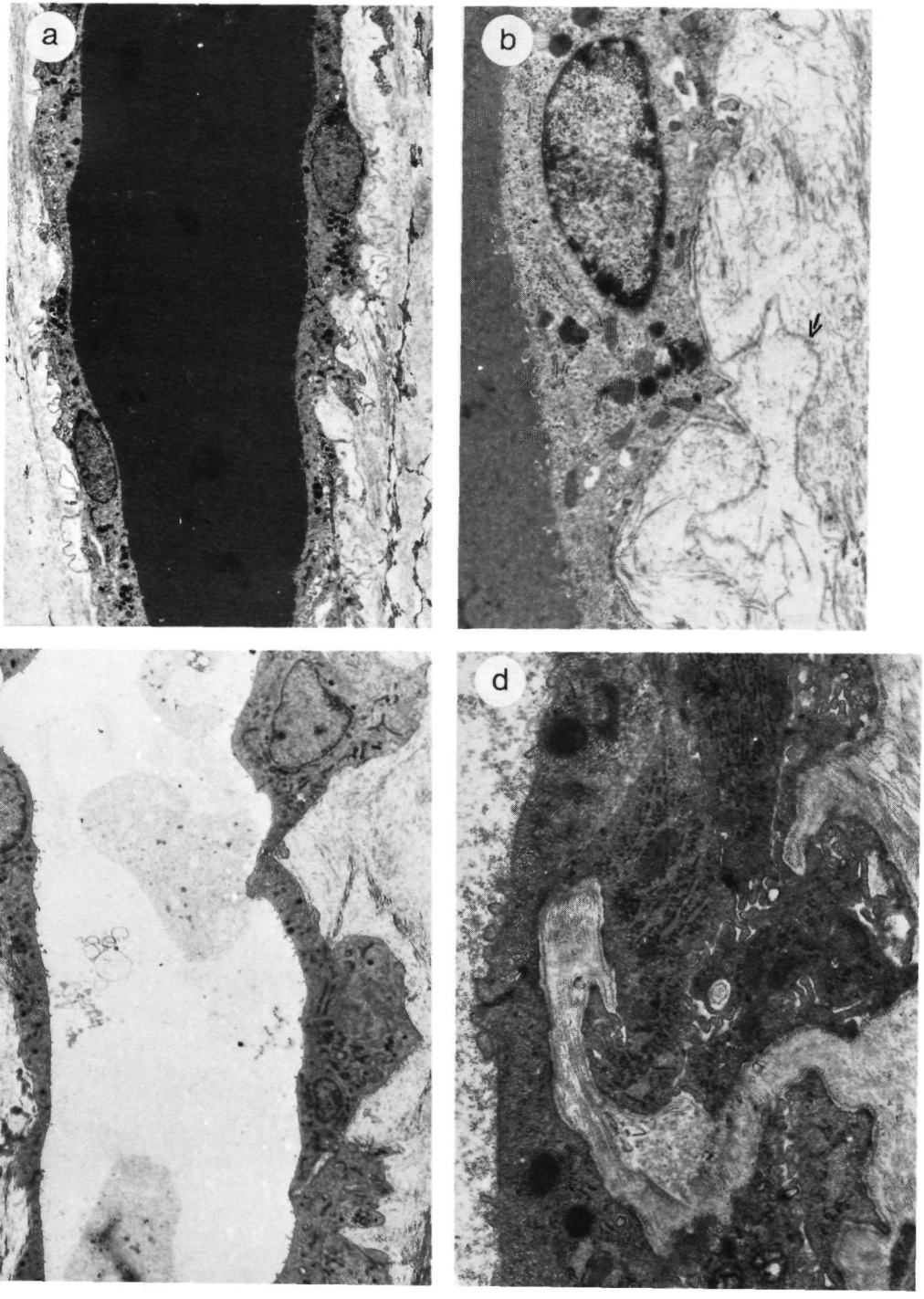



Fig. 33.
a: endolymphatic duct with dilated distal end filled with coagulum and phagocytic cells 6 months after endolymphatic sac obstruction. The epithelium is flat and surrounded by dense connective tissue (PAS, X150)
b: collapsed endolymphatic sac with a strongly PAS staining substance in the lumen, 6 months after sac obstruction. The epithelium has a rather uniform appearance except for two cells with numerous light vacuoles (PAS, X600).

Light microscopy of the epithelium of the occluded duct showed no distinct changes in the character of the epithelial cells during the first month. Occasionally cells were found to contain a varying amount of PAS-staining granules. Generally the impression was obtained that after prolonged survival the epithelial lining of the duct appeared to be more flattened (fig. 33[a]).
Electron microscopy of the epithelium of the duct did not show marked differences as compared to the control specimens during the first weeks after occlusion. After six weeks the epithelium appeared flattened and more granular due to increase of lysosomal bodies. Except for a few cells, the slender basal projection disappeared. The apical cell cytoplasm showed an increased number of light and dark vesicles of the same density as the luminal content together with irregular cytoplasmic protrusions. Between adjacent cells junctional complexes were present. The cytoplasm displayed an increased number of cell organelles, especially the number of coated vesicles and lysosomes was increased considerably.
The basal lamina revealed peculiar outpouchings and duplications, while the subepithelial space showed marked fibrosis, consisting of thin collagenous fibers (figs. 34[a],[b]). This fibrosis could be established along the entire osseous part of the duct, although the severity could largely differ between the various specimens and from site to site in the same specimen. Also the capillaries became enclosed by this process. After more than six months the epithelium was rather flat with a few large basolateral invaginations bordered by fibrous tissue. In the cytoplasm lysosomal structures were still detectable. Occasionally signs of autophagy were observed. The luminal content which was initially rather homogeneous electron dense became more granular and condensed after prolonged survival (figs. 34[c],[d]).

### *5.2.3 Endolymphatic sac*

During the first weeks after obstruction the endolymphatic sac was mostly found to be severely collapsed, although in two cases the sac had almost retained its normal dimensions.
The luminal surface varied from smooth to very irregular. The epithelium was rather uniform consisting of cuboidal cells with some PAS-positive inclusions. With prolonged survival in all specimens the sac appeared almost completely collapsed, except for some small areas where the remaining lumen was filled with a PAS-staining substance (figs. 28, 33[b], 37[a],[c]).
Electron microscopy showed that the cytoplasmatic content could largely differ from cell to cell, both with respect to the cell organelles and cellular inclusions. The number of lysosomes was generally less than in the normal sac and they decreased further after longer survival times. The same applied to the number of resorption vacuoles, which could largely vary in size and content (fig. 35).
The luminal surface displayed only a few microvilli and some coated invaginations, which were only occasionally found in the laterobasal cell surface. Lateral intercellular spaces were often narrow, sometimes filled with a granular substance, or absent (fig. 35).
As observed in the endolymphatic duct, the subepithelial area revealed increasing fibrosis with progression of time. This tissue was composed of abnormal collagen and


a
b
c
d

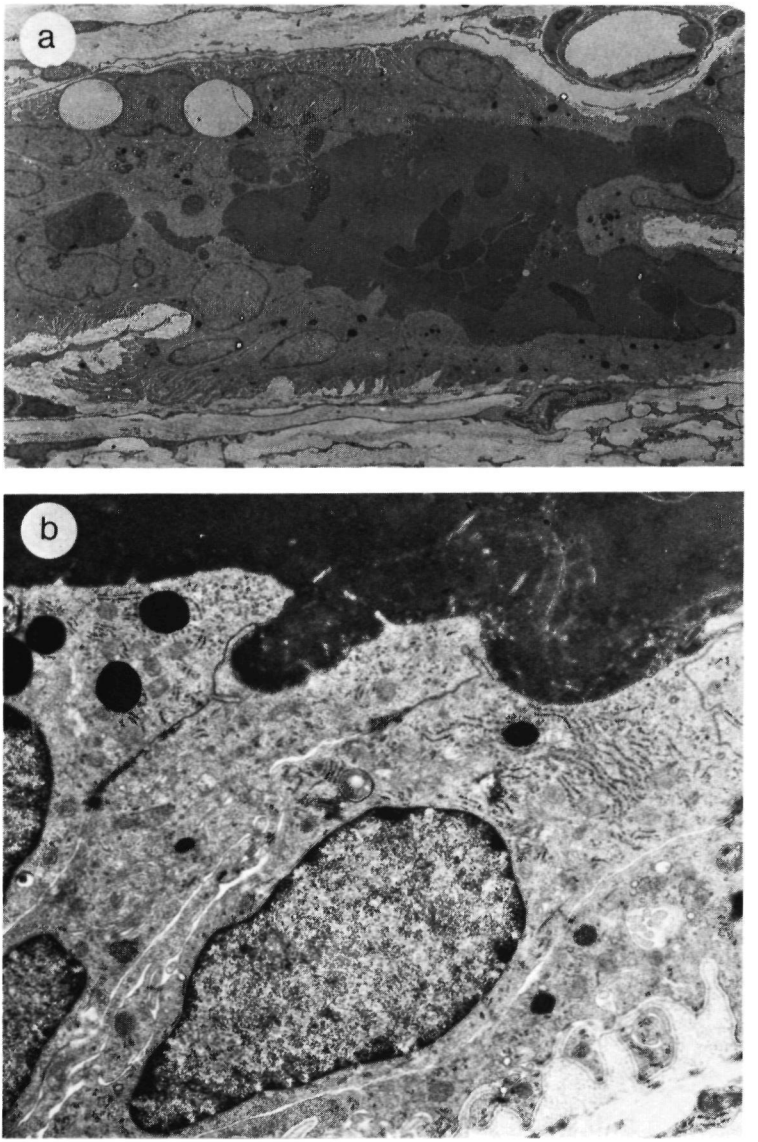



Fig. 35. Electronmicrographs of obstructed endolymphatic sac after 3 months (a: survey; b: detail). The apical surface of the rather uniform epithelium is irregular. The cytoplasm contains few lysosomes and RER lamellae. Note absence of intercellular spaces. (a, X1800; b, X7750).

←Fig. 34.
a,b: endolymphatic duct 2 months after endolymphatic sac obstruction.
The lumen is filled with a homogeneous substance. The epithelial cells contain many lysosomes and lack the slender basal projections. Note subepithelial fibrosis and peculiar outpouchings and duplications of the basal lamina (→) (a, X1800; b, X6660)
c,d: six months after sac obstruction. The luminal content is inhomogeneous. Note extensive subepithelial fibrosis. For comparison (see fig. 14) (c, X2700; d, X9900).

elastic fibers, penetrating between the basal cell infoldings and enclosing the capillaries. The basal lamina of the blood capillaries was largely thickened (fig. 36).
In those animals where occlusion failed, no collaps of the endolymphatic sac was observed.

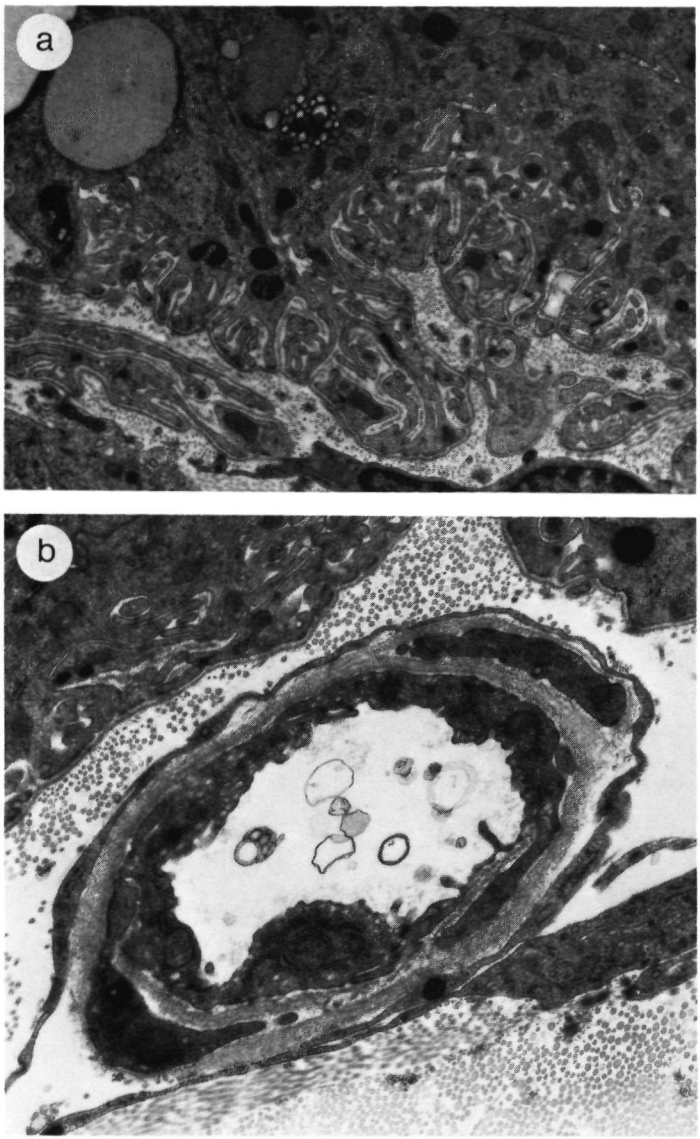



Fig. 36. Close up view of basal portion of epithelial cell of the sac (a) and subepithelial capillary (b) 6 months after sac obstruction. Note fibrosis consisting of collagen and elastic fibers in between the basal cell infoldings and around capillary. The basal lamina of the capillary is largely thickened (a, X9900; b, X15500).

In Chapter 4 it was described that with the use of $^{35}SO_4$, specifically incorporated into glycoproteins, the existence of an endolymph flow from the cochleo-vestibular compartments towards the endolymphatic sac (longitudinal flow) could be established.
In order to establish the separate role of the endolymphatic sac and duct in this longitudinal flow, the same approach was used in rats in which the endolymphatic sac

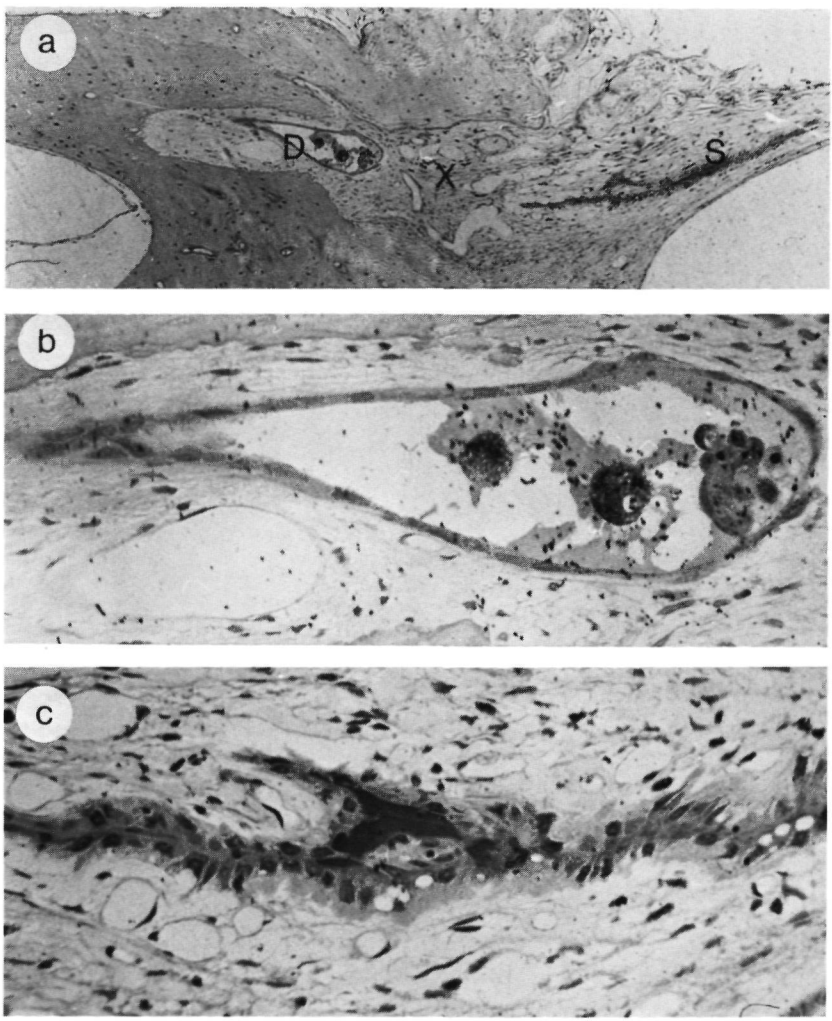



Fig. 37. Accumulation of $^{35}S$-labelled glycoproteins in the occluded endolymphatic duct (D), 2 weeks after i.p. injection of $^{35}SO_4$. At the time of injection the sac(S) was obstructed for 6 weeks.
a: survey (PAS, X40); X site of obstruction
b: close up view of the distended distal portion of the duct with a PAS-positive $^{35}S$-labelled coagulum and labelled phagocytic cells (PAS, X300)
c: no labelling is found in the collapsed endolymphatic sac (PAS, X300).

of the right ear was occluded six weeks earlier. The animals were sacrificed after survival times varying from two days to eight weeks after intraperitoneal injection of $^{35}SO_4$. The left ear served as a control.
Sections showed a collapsed endolymphatic sac, containing a varying amount of PAS-staining substance on the operated side (fig. 37). In one rat sacrificed after eight weeks signs of a cochlear hydrops were found.
Autoradiographs failed to disclose any difference in isotope incorporation of crista, cupula, macula and otolithic membrane, described in Chapter 4, between operated and control ears. No labelling of the epithelium or residual content of the occluded endolymphatic sac was found throughout an observation period of eight weeks. Distinct accumulation of labelled material was established in the occluded distal part of the endolymphatic duct, but only in those animals which survived for more than two weeks after isotope injection. The amount of labelled substance in the duct was considerably less than in the sac of the unoperated ear. Labelling was mainly confined to a PAS-staining coagulum, but labelled phagocytic cells, occasionally present, in the occluded duct, were observed (fig. 37[b]). Throughout the observation period of eight weeks no isotope incorporation into the epithelium could be established.

## 5.4 DISCUSSION

The results obtained demonstrate that a hydrops of the endolymphatic system of the inner ear of the rat can be induced by obstruction of the endolymphatic sac. These observations generally agree with those described for some other animal species, although the incidence of hydrops is rather low (47%) in comparison to 100% obtained in the guinea pig by Kimura (1967). This discrepancy is difficult to explain and will be discussed later on.
The underlying mechanism for the development of endolymphatic hydrops is still questionable.
There is strong evidence that secretion of endolymph with its peculiar high $K^+$ concentration is performed by the sodium-potassium activated ATPase in the vascular stria and presumably also in the dark cell area of the utricle (Kuijpers, 1969). At those sites there will be a continuous inflow of ions and water. For maintaining the homeostasis in the endolymph compartments any surplus of endolymph has to be eliminated. According to the longitudinal flow concept resorption of endolymph is thought to take place in the endolymphatic sac. If this is true hydrops can be simply explained by blockade of the outflow system. This assumption is supported by the findings made in the present study.
The existence of an endolymph flow from the membranous labyrinth towards the endolymphatic sac has convincingly been demonstrated in Chapter 4 with the use of $^{35}SO_4$.
The diminished accumulation of $^{35}S$-labelled glycoproteins in the duct after saccus obstruction demonstrates that the flow is severely impaired by this intervention, but not completely blocked. These observations indicate that the force underlying the directional flow of endolymph must be an active mechanism, mainly located in the endolymphatic sac. However the duct alsocontributes to the maintenance of this flow although to a minor degree.

Since the endolymph in the endolymphatic sac has the same ionic composition as the perisaccular fluid, this mechanism is most likely represented by an active sodium transport system in the epithelial lining. By this system absorption of water and solutes from the endolymph can be assumed to be performed in a similar way as established for other absorptive epithelia (Spring, 1982).
This assumption is supported by the morphological features of the epithelial lining of the endolymphatic duct and sac (Chapter 3) which have been shown characteristic for epithelia involved in active fluid transport (Diamond and Bossert, 1967).
Moreover, the existence of a fluid transport across the epithelium of the duct can be derived from the observations made by Rask-Andersen et al (1981[a]). Using ionic lanthanum, introduced into the endolymphatic space, they provided morphological evidence for the existence of an intercellular pathway in the duct of the guinea pig.
An additional explanation for the development of hydrops can be derived from the severely impaired longitudinal transport of glycoproteins induced by saccus obstruction while it was found that the secretion of glycoproteins in the membranous labyrinth remained virtually unaffected. This implies that this secretion will accumulate in the membranous labyrinth and can cause an increase of the colloid osmotic pressure, resulting in an increased inflow of water and hydrops. A comparable hypothesis, assuming an augmentation of the colloid osmotic pressure by the accumulation of cell debris, has been proposed by Dohlman (1965). However, this assumption seems to be in conflict with the only available data on endolymph protein concentration after saccus obstruction (Vosteen and Morgenstern, 1986). These authors, remarkably, found a decrease of the protein concentration in hydropic ears of the guinea pig, in comparison with controls.
The same authors suggested that hydrops might be due to a changed osmolarity of the endolymph caused by an increased concentration of ions. Although already small changes in the ionic content suffice to develop hydrops, as calculated by Johnstone and Robertson (1981), this assumption is not supported by measurements of the ionic content, because several authors failed to find changes in the concentrations of $Na^+$, $K^+$ and $Cl^-$ in hydropic ears of the guinea pig (Konishi et al, 1981; Giebel, 1982). Moreover no change in the $K^+$ permeability of the endolymph-perilymph barrier was established (Konishi et al, 1981).
As already mentioned, the small but distinct accumulation of $^{35}S$-labelled glycoproteins in the endolymphatic duct after saccus occlusion refers to a contribution of the duct in the maintenance of the longitudinal flow. However, the flattening of the epithelium, the disappearance of the slender basal cell membrane protrusions and the subepithelial fibrosis indicate that this role of the duct seems to diminish with time. These observations deserve further attention with respect to the rather low percentage of hydrops obtained in this and other studies as compared with the 100% score obtained by Kimura (1967). Comparison of Kimura's and our approach teaches that in his case a large part of the duct is destroyed while in the present study the major part of the duct is left intact. These observations suggest that the inconsistency and the time dependency in the arousal of hydrops as discussed in Chapter 1 might be attributed to the degree of destruction of the endolymphatic duct.
However, in this context also a possible species dependent contribution to the removal of excess endolymph by other parts of the membranous labyrinth has to be taken into account. In addition to the role of the duct epithelium in the resorption of water and

solutes, the increased number of lysosomes and vesicles found in the epithelium of the duct after obstruction of the sac, refers to a distinct involvement of the epithelial cells in the bulk phagocytosis of the accumulated endolymph. Moreover, the presence of $^{35}S$-labelled phagocytic cells, normally absent in the duct, demonstrates their contribution to the removal of high molecular waste products after saccus obliteration.

The course of events established in the endolymphatic sac after its obstruction confirms the resorptive role of this structure. The lack of uptake of $^{35}S$ after obstruction corroborates the findings made in Chapter 4 that the epithelial cells lining the sac do not secrete glycoproteins.

During the first period after obstruction the epithelial lining remains active in resorbing the luminal content. Thereafter a loss in resorptive activity is found although the last remnants of the luminal content can persist for more than one year. The perisaccular fibrosis including capillary changes can be considered a sign of deteriorated resorption. Both perisaccular and periductular fibrosis most likely result from the surgical intervention. Notably this observation makes benefit of the saccus shunt operation to relieve the presumed endolymphatic hydrops in patients with Ménière's disease (House, 1962) highly questionable , because it can adversely affect the resorption of endolymph by inducing perisaccular fibrosis.

The morphology of hydrops is chiefly characterized by distention of the membranous walls of the various parts of the inner ear. However atrophy of the sensory cells was limited to the organ of Corti, while the sensory epithelium of the vestibular partition remained apparently normal. These observations are generally in line with those described in the guinea pig by Kimura (1968) although there are some differences. No fistulae of the saccule have been reported for the guinea pig. To our knowledge their presence has only been mentioned, at the same site, in the saccule in the squirrel monkey after saccus obstruction but in the absence of distinct hydrops (Kimura, 1968). A time-related progression in the pathological changes in the cochlea as established by Kimura (1968) in the guinea pig could not be confirmed in this study on the rat, mainly because of the inconsistency of hydrops induction.

How damage to the inner ear structures is effected is difficult to decide. The most obvious explanation seems to be a longterm pressure effect as suggested by Kimura (1981). However when ruptures in Reissner's membrane occur, changes in the ionic composition of the endolymph, resulting from intermixing of endolymph with perilymph, can be assumed to be an important cause of injury. This has been demonstrated by Lawrence et al (1961), Lawrence (1966) and Duvall and Rhodes (1967) by artificially induced ruptures of Reissner's membrane. Loss of hair cells is usually followed by degeneration of nerve fibers and ganglion cells.

Although dictinct fistulae of Reissner's membrane were only established in two cases in our study, one must keep in mind that small fistulae could easily have been overlooked, since no serial sections were studied.

In contrast to the cochlea neither hydrops nor the presence of fistulae in the vestibular organs results in observable changes in the sensory cells as concluded from light microscopy. So it must be assumed that these structures are less vulnerable to continuing pressure and changes in ionic composition than the organ of Corti.

CHAPTER 6

# PHYSIOLOGICAL EFFECTS OF OBSTRUCTION OF THE ENDOLYMPHATIC SAC

## 6.1 INTRODUCTION

The effects of obstruction of the endolymphatic sac on the function of the auditory and vestibular systems are discussed in this chapter.
For evaluation of the auditory system the compound action potential recorded from the round window was used as a parameter (Chapter 2.2.4).
The function of the vestibular system was assessed by means of electronystagmography and behavioural studies (Chapter 2.2.5).

## 6.2 PHYSIOLOGY

### *6.2.1 Auditory system*

For combined morphological and physiological studies on the auditory and vestibular system 47 rats operated upon the right endolymphatic sac were available. On five occasions the physiological data had to be excluded as histology revealed either incomplete occlusion, labyrinthitis or fracturation of the posterior vertical canal (Chapter 5.2).
An example of the compound action potential evoked for a 4000 Hz stimulus at different intensities prior to obliteration of the endolymphatic sac is given in fig. 38. The compound action potential appears as a double peaked negative deflection ($N_1$ and $N_2$). The amplitude of $N_1$ as a function of the stimulus intensity (input-output curve) is shown in fig. 38. The threshold is determined by extrapolation.
A summating potential (SP) is usually not observed in normal rats, except at higher intensities. When present it appears as an inconsistent small negative shift of the baseline.
The mean value and standard deviation for the $N_1$ amplitudes as a function of stimulus intensity (4000 Hz) of 10 normal ears before endolymphatic sac obstruction are presented in fig. 40.
No deterioration of the auditory threshold for the used frequencies (1000, 2000 and 4000 Hz) could be established within the observation period.
Fifteen rats were supplied with longterm electrodes for ECoG recording (Chapter 2.4) before sac obstruction. However, this method was abandoned during the course of this study. It turned out that in many cases the measured deterioration of the auditory responses (compound action potential) after long survival times had to be ascribed to a spontaneous change in electrode position, presumably caused by continuing growth of the skull, because repositioning resulted in a substantial improvement of the responses, in some cases even to the preoperative level. An example of such a recording is given in fig. 39. For that reason the electrocochleograms derived from those animals where no check up for electrode shift was performed, are scored as "not available" in table 6. On account of this experience, the remaining rats were supplied with an electrode four

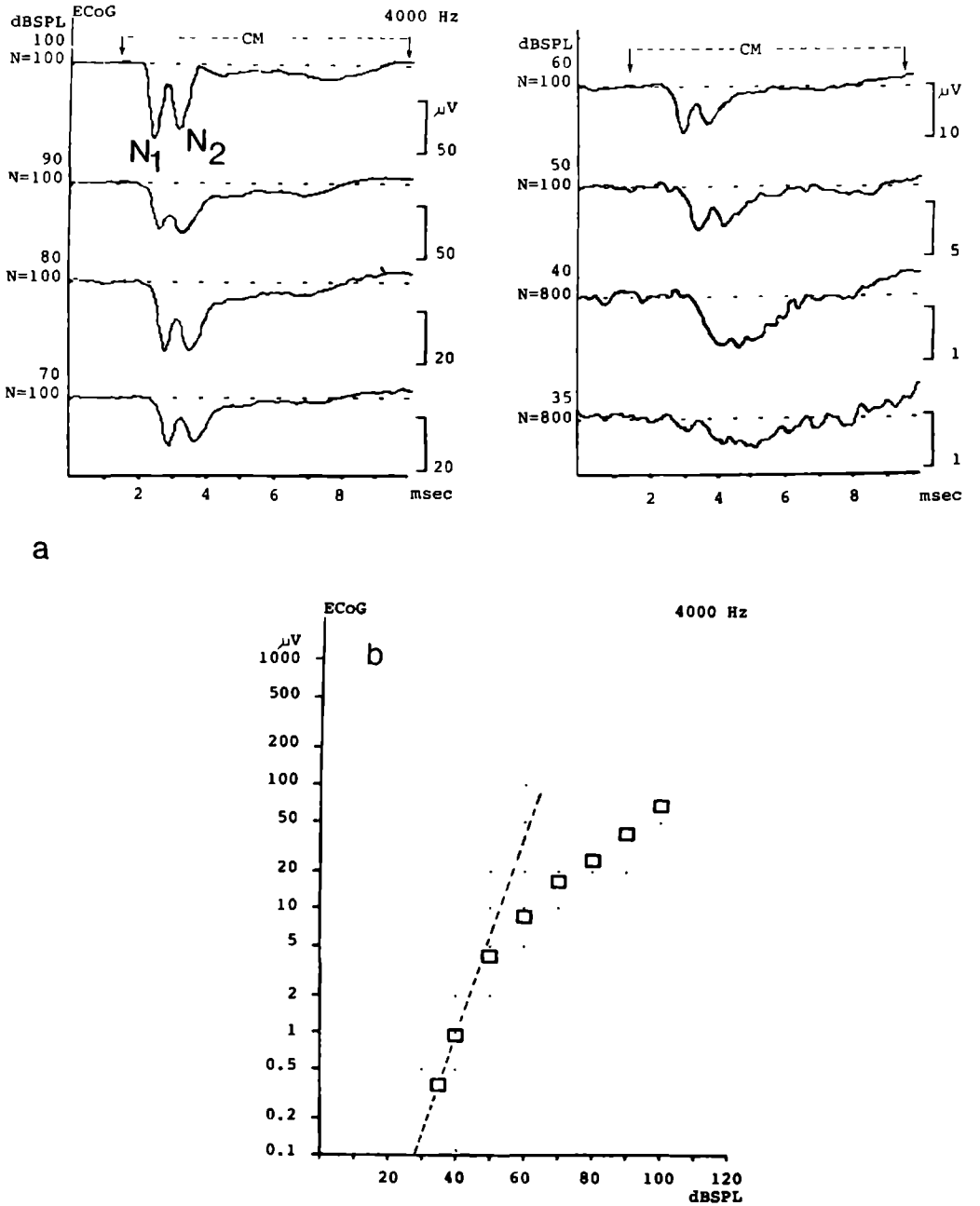



Fig. 38. Cochlear action potentials for tone bursts at 4000 Hz at different intensities (a) and amplitude-intensity function (b) of a normal rat. CM: indicates length of tone burst.

Table 6

Summary of morphological and electrophysiological findings in 42 rats after obstruction of the endolymphatic sac

| Rat nr | Morphology | | | | | Physiology | |
|---|---|---|---|---|---|---|---|
| | Cochlea | | Saccule | Utricle | Semi-circular canal | ENG | ECOG |
| | Hydrops | Atrophy go | | | | | |
| survival time 3–5 months | | | | | | | |
| 1 | ++ | | +++ f | n | n | n | n |
| 2 | +++ | 2,3+++ | +++ o | +++ | ++ | type I | thr incr 45 dB SP recruitm |
| 3 | +++ | 3++ | +++ | c | + | type II | thr incr 40 dB |
| 4 | + | | f | n | n | type I | n |
| 5 | n | | + | n | n | n | n |
| 6 | n | | c | n | n | n | n |
| 7 | n | | + | n | n | n | thr incr 30 dB |
| 8 | ++ | | + | n | n | n | n |
| survival time 10–15 months | | | | | | | |
| 9 | + | | n | n | n | n | n |
| 10 | + | 1,2,3+ | cf | n | n | n | thr incr 20 dB |
| 11 | ++[1]) | 1,2,3++ | cf | n | n | n | na |
| 12 | +++ | | +++ | + | n | n | n |
| 13 | +++ | | ++ | n | c | n | na |
| 14 | +++ | | +++ | + | n | n | na |
| 15 | +++ f | 1,2,3+++ | +++ o | ++ | c | n | thr incr 35 dB |
| 16 | ++ | | ++ | n | n | n | n |
| 17 | + | | + | n | n | n | na |
| 18 | ++ | 1,2,3,++ | + | + | c | n | na |
| 19 | ++ | 1,2,3+++ | + | n | n | n | thr incr 65 dB |
| 20 | ++ | 1,2,3+++ | + | + | ++ | type I | na |
| 21 | ++ | | ++ | + | + | type II | na |
| 22 | +++ | 1,2,3++ | + f* | ++ | ++ | type I | thr incr 45 dB SP |
| 23 | n | | n | c | c | n | na |
| 24 | n | | ++ | + | n | n | n |
| 25–38 | n | | n | n | n | n | n |
| 39–42 | n | | n | n | n | n | na |

c – collapse of endolymphatic space
extent of hydrops – spiral ganglion cell loss and/or atrophy organ of Corti
+ slight, ++ moderate, +++ extensive
f – fistulae Reissner's membrane or saccular wall *healed fistula
go – ganglion cell loss and/or atrophy organ of Corti turn 1, 2 and/or 3
n – normal
recruitm – recruitment
na – not available
thr incr – threshold increase
SP – Summating Potential
o – otolith displacement from macule
type I, II – see table 7
[1]) – atrophy vascular stria

weeks before sacrifice. During that period ECoG recording was performed twice. In this short period no artificial deterioration of the hearing threshold was observed.
The effects of endolymphatic sac obstruction on the compound action potentials are summarized in table 6 and fig. 40. In this table the morphological and vestibular findings are included. Seven out of 30 animals tested demonstrated a threshold increase varying from 20 to 65 dB. All other animals failed to show significant changes of AP thresholds although sac obstruction was successful.
No relationship could be established between the presence and degree of hearing loss and the time after saccus obstruction. In the group that survived for 3-5 months, three out of eight animals revealed a threshold elevation, and only four out of 22 that survived for 10-15 months. Comparison of the audiological data with the morphological findings demonstrates that in all animals, except one, the threshold increase was associated with a cochlear hydrops of differing severity and a varying loss of spiral ganglion cells. The loss of spiral ganglion cells was associated with degeneration of the organ of Corti. With respect to the stimulus frequencies used (1000, 2000 and 4000 Hz) no significant differences between the respective thresholds could be observed, not even in the animals where spiral ganglion cell loss was limited to the apical turn. In fig. 40 the auditory threshold changes of the six rats with cochlear morphological changes in

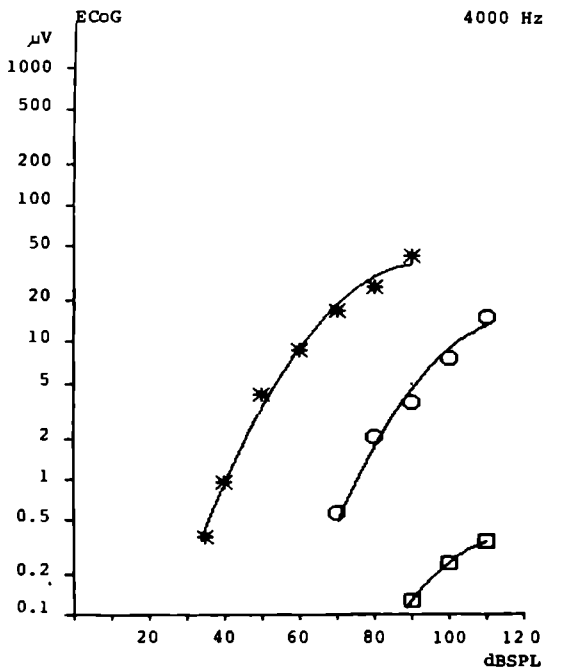



Fig 39 Typical example of artificial deterioration of auditory responses measured after endolymphatic sac obstruction This phenomenon was due to a spontaneous change in the position of a long term electrode. Repositioning of the electrode resulted in substantial improvement of the response
* before obstruction
□ 4 months after obstruction
○ after electrode repositioning

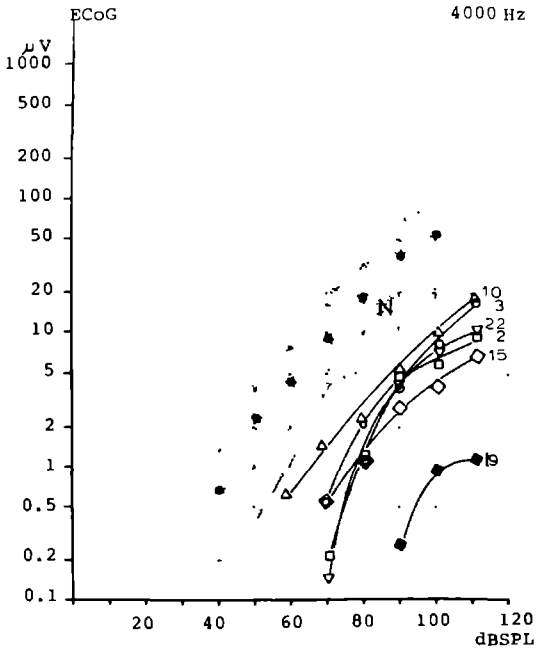



Fig. 40. Compilation of amplitude-intensity function of 6 rats with threshold increase for 4000 Hz and cochlear morphological changes at various time intervals after obstruction of the endolymphatic sac. The shaded area (N) represents the mean values with standard deviations of 10 normal rats. The arabic figures refer to the rat numbers in table (6).

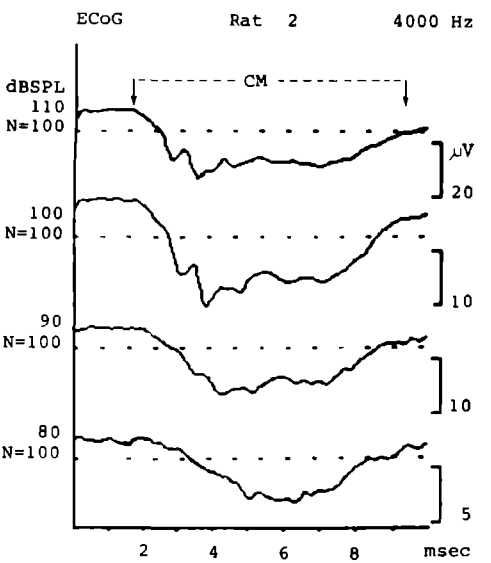


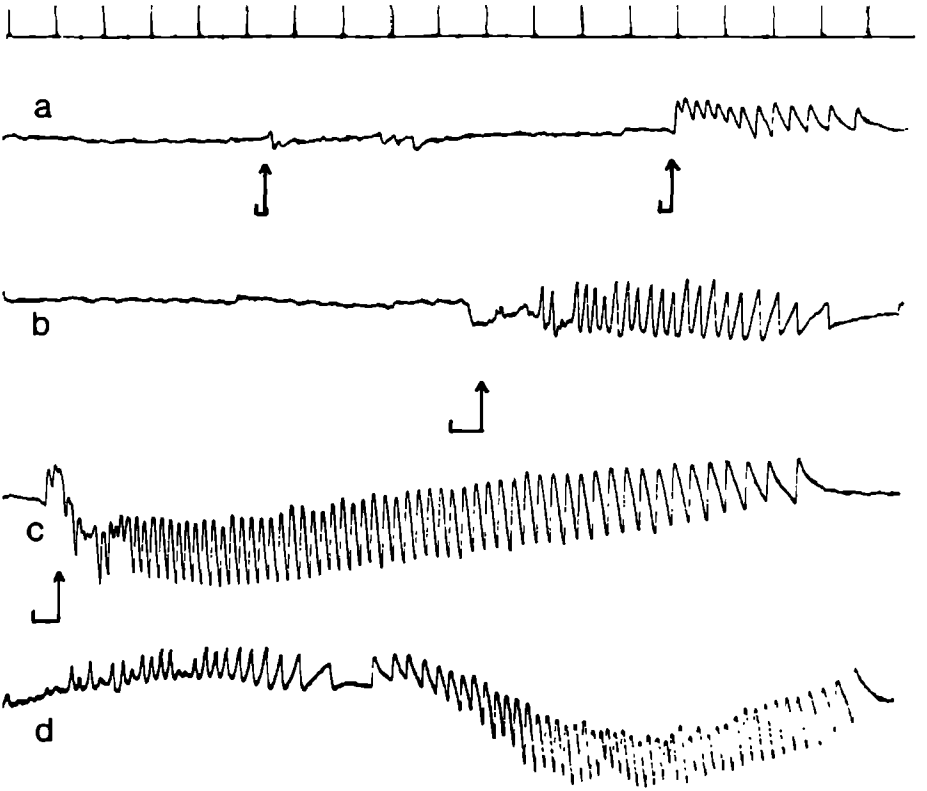

Fig. 41. Cochlear action potentials of rat nr 2 (table 6) showing distinct increase of the negative summating potential (SP) (negative shift of baseline).

comparison to normal values are summarized for a stimulus frequency of 4000 Hz.
In two animals threshold elevation of the compound action potential appeared to be accompanied by an increase of the negative summating potential (fig. 41). In one animal a more rapid increase of the AP amplitude with increasing sound pressure intensities in comparison with the control curve was found. This phenomenon is suggestive of the existence of recruitment.
Apart from the animals showing a distinct correlation between morphological changes and threshold elevation, two animals with a slight and four with a moderate to severe hydrops but without loss of spiral ganglion cells, failed to show any change in the AP threshold. Furthermore, one animal (nr 7) without observable morphological defects in the cochlea but with a slight saccular hydrops had a threshold shift of 30 dB.

### *6.2.2 Vestibular system*

Testing of the vestibular system was performed once before saccus obstruction and twice during the last two months. The last measurement was done within one week prior to sacrifice. Among the total number of 42 rats evaluated for vestibular function six were found to have abnormal canal reflexes as assessed from the electronystagmograms pertaining to the horizontal canal reflexes. Examples of recordings of typical abnormal nystagmic responses provoked by rotatory stimulation are shown in figs. $42^{a-d}$ and figs. $43^{a-c}$. Velocity step responses are presented in figs. $42^{a-c}$ and figs. $43^{a-c}$, whereas fig. $42^{d}$ is an example of a perrotatory response during sinusoidal stimulation. The cupulograms derived from the nystagmus records of these rats are illustrated in fig. $42^{e}$ and fig. $43^{d}$. Asymmetry in the duration of postrotatory nystagmic responses (figs. $42^{a-c}$, $43^{a-c}$) was always accompanied by asymmetry of sinusoidal responses (fig. $42^{d}$). In all animals examined, the results obtained were confirmed at repeat examinations.
For the determination of abnormalities in cupulograms, five different parameters were used (table 7): nystagmus duration (nr 1), relative difference in duration (nr 2) or directional preponderance (DP), DP of threshold and/or time constant (nr 3), threshold value (nr 4) and time constant (nr 5), as defined in Chapter 2. The criteria for

Fig 42 Mount of postrotatory nystagmus responses of rat nr 22 (table 6,7) ——————→
a,b,c responses to velocity steps of 90 deg/s (a) and 250 deg/s (b,c) Note the increased duration of nystagmus to the right
d response to sinusoidal stimulation (250 deg/s, period 20s) showing directional preponderance for nystagmus to the right The horizontal movement of both eyes (electronic summation) is shown for nystagmus to the left (b) and nystagmus to the right (c) Time scale (upper trace) in seconds The arrows and attached lines indicate the time interval covered by the step in velocity (deceleration 200 deg/s²). The duration of nystagmus is measured between the arrow and the peak of the last observed nystagmus beat
e cupulogram of rat nr 22 (Type 1) The duration of the postrotatory nystagmus is plotted against the logarithm of the velocity step amplitude Note the directional preponderance of nystagmus to the right (operated) side at higher stimulus amplitudes The straight (regression) lines are fitted for amplitudes100 deg/s only, the extrapolation to the abscissa is to establish the threshold
○ - nystagmus to the right
● - nystagmus to the left
Thr - threshold velocity step (deg/s)
T - time constant in seconds (s)


a
b
c
d

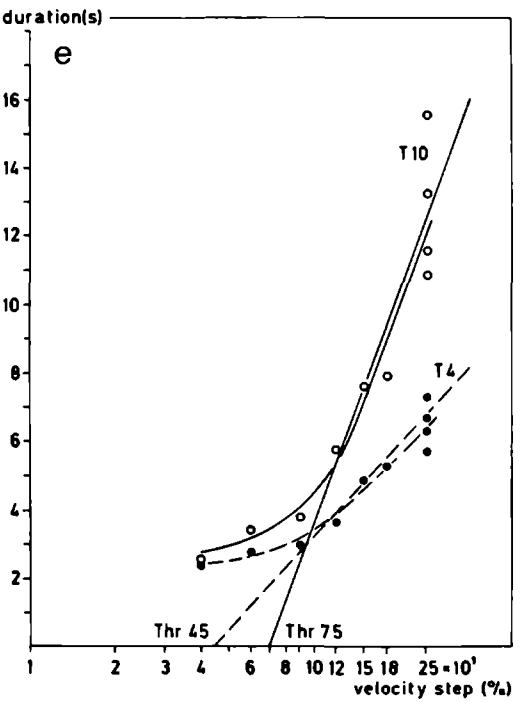

duration(s)
e
16
14
12
10
8
6
4
2
T10
T4
Thr 45
Thr 75
1
2
3
4
6
8
10
12
15
18
25
velocity step (%)

Table 7

Abnormal ENG findings in 6 rats out of 42 with obstruction of the endolymphatic sac.

| Criterion | | Nystagmus type I | | | | Nystagmus type II | |
|---|---|---|---|---|---|---|---|
| | Rat nr | 2 | 4 | 20 | 22 | 3 | 21 |
| 1 duration 8s or longer (180 deg/s)* for nystagmus to | | R | R | – | R | – | L |
| 2 relative difference in duration[a] DP* towards | | R | R | R | R | L | L |
| 3 DP of threshold[b] and/or time constant | | + | + | + | + | + | + |
| 4 threshold equal to/or greater than 65 deg/s[c] for nystagmus to | | R | R | R | R | – | R |
| 5. time constant equal to or greater than 7[c] for nystagmus to. | | R | – | – | R | – | – |

* confidence limit established at 5% upper tail probability in all animals measured
a equal to or greater than 25% at step amplitude 150 deg/s or higher for at least two observations
b equal to or greater than 20% (arbitrary limit)
c normal values obtained from Fischer et al (1979)
DP directional preponderance

parameter nrs 1 and 2 were derived from the values obtained in all animals measured. Each criterion corresponded with the exclusion of 5% of the (upper) most extreme values. Twenty percent DP for parameter nr 3 was taken as an arbitrary normal limit. The criteria for parameters nrs 4 and 5 were based on the normal values obtained in our laboratory (Fischer et al, 1979). The duration criterion (nr 1) was met in four of the six rats with abnormal responses, whereas four of the 36 animals with normal ENG also met this criterion. Criteria nrs 2 and 3 were met in all six presented rats but not in any other rat. Of the criteria nr 4 and nr 5, which are related to normal values (Fischer et al, 1979), only nr 4 (threshold) appeared to be useful. Table 7 demonstrates that response asymmetry and threshold increase were prominent features of the abnormal responses. In the cupulograms (figs. 42[e], 43[d]) these features can be clearly observed. An increased threshold was found for nystagmus to the operated side.

Two different types of response asymmetry were found. The one with increasing difference in nystagmus duration, i.e. directional preponderance to the operated side, at increasing amplitude (higher than 100 deg/s) (fig. 42[e]), the other with a constant difference in duration i.e. directional preponderance to the intact side, at all amplitudes (fig. 43[d]).

On account of these considerations two types of abnormal responses were defined: Type I response, designated as "Recruitment of nystagmus to the obliterated side", was characterized by an extremely long duration of postrotatory nystagmus in the indicated direction at higher stimulus amplitudes (fig. 42[e]). The type II response was characterized by directional preponderance (DP) to the non-obliterated side (fig. 43[d]). Comparison of the ENG findings with the morphological data indicates that all six

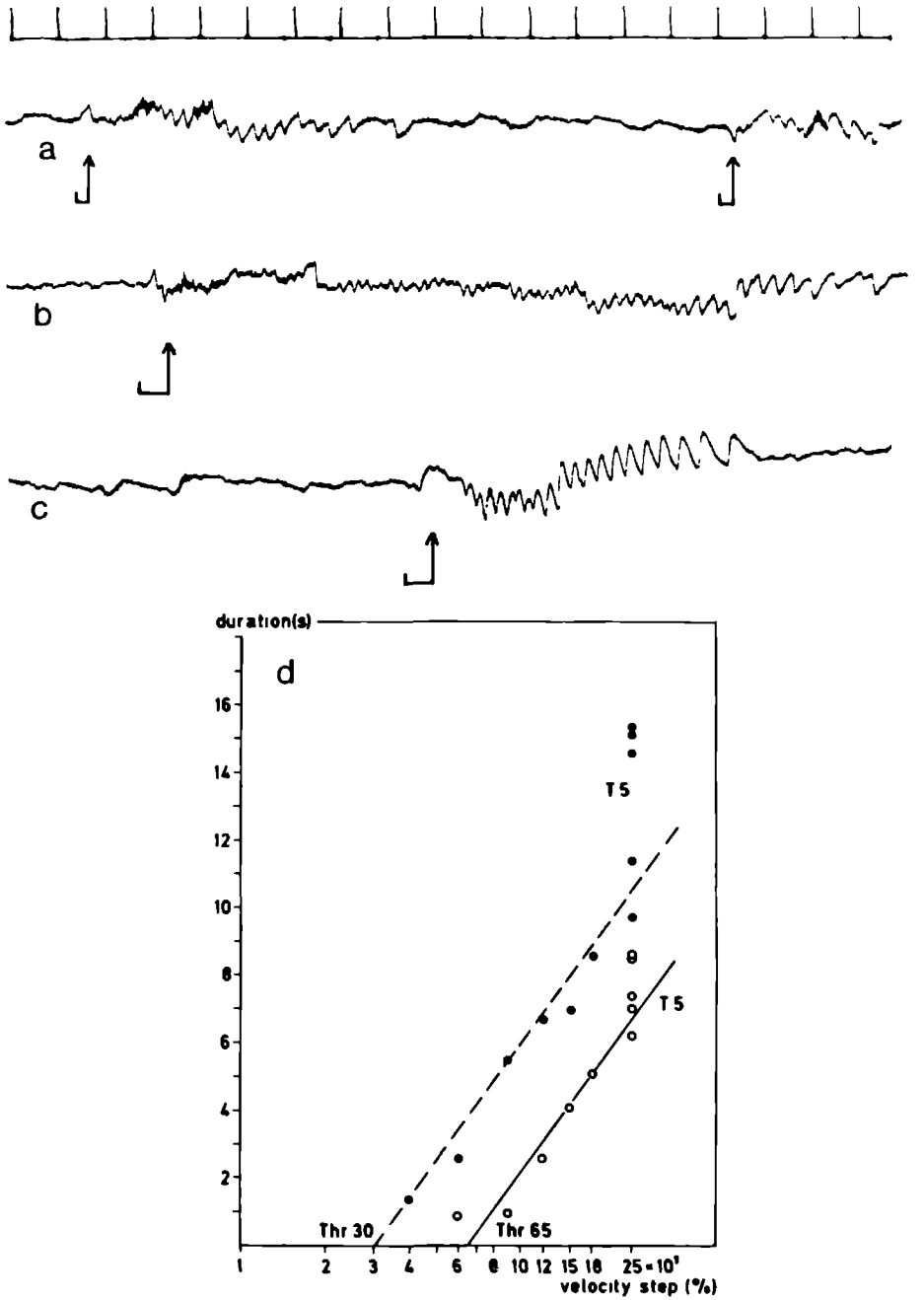



Fig. 43. Mount of postrotatory nystagmus responses of rat nr 21 (table 6,7).
a,b,c: responses to velocity steps of 120 deg/s (a) and 250 deg/s (b,c). The response with nystagmus to the left lasts longer than to the right; the difference is constant.
d cupulogram of the same rat (type II). Note the directional preponderance of nystagmus to the left (intact) side. See fig. 42 for further explanation.

animals with abnormal responses (type I or II) had a varying degree of hydrops of the endolymphatic system in both the cochlear and vestibular portions, combined with a fistula of the saccular wall in two animals. In all but one case a hydrops of the semicircular canals was present. This feature was not found in any of the other animals (table 6). In the three animals with abnormal ENG, where electrocochleograms were available, abnormal vestibular responses were associated with a threshold shift of the auditory responses.
A normal ENG was found in 36 animals, despite the presence of slight to extensive hydrops in the cochlear and/or vestibular parts, notably in the saccule, in 16 animals. Moreover three of these animals exhibited a fistula of the saccular wall. In none of these 36 animals a distinct distension of the membranous wall of the semicircular canals was established. Four specimens had a collapse of the canals.

**Assessment of otolith function**
Static otolith reflexes of the eyes and posture and gait were normal in all 42 animals studied, irrespective of the presence or absence of hydrops of the otolith organs.
The same applied to the righting reflexes and lift reaction. None of the animals showed either abnormal swimming behaviour or abnormal posture and gait just after removal from the water, not even in two animals where the otoliths were displaced from the saccular macule.

## 6.3 DISCUSSION

Evaluation of the data on cochlear function in hydropic ears as assessed by electrocochleography, demonstrates that hydrops does not necessarily affect sound processing. Increase of action potential threshold was only observed when hydrops was associated with atrophy of the organ of Corti and loss of spiral ganglion cells. In one rat the observed hearing loss cannot be explained, because no morphological changes except for a slight distension of the saccular membrane could be established. Impairment of the cochlear function after saccus obliteration has been reported by several authors, but only related to the postoperative period or to the presence of hydrops without histological establishment of cochlear lesions (Kimura, 1968; Konishi et al, 1981; Morgenstern and Lamprecht, 1984; Aran et al, 1984; Morizono et al, 1985). Recently a comparable relation between hair cell and ganglion cell loss and AP threshold elevation has been reported in hydropic ears of the guinea pig (van Deelen, 1986).
In contrast to the guinea pig, the summating potential (SP) recorded from the round window in the rat is extremely small. This might be explained by a different electroanatomy between the rat and guinea pig cochlea. Such a relationship has been suggested by Eggermont (1976). The large negative SP observed in one rat with cochlear hydrops is in line with the observations of Davis et al (1958) and Durrant and Dallos (1974) that displacement of the basilar membrane towards the scala tympani, which is the case in cochlear hydrops, resulted in an increase of the negative SP. This phenomenon was also found by van Deelen (1986) in cochlear hydrops in the guinea pig.

The behavioural studies on otolith function and otolith reflexes on head positioning indicate that the static labyrinthine function is apparently not affected by unilateral

obstruction of the endolymphatic sac, despite of the presence of hydrops in most of these animals. This is rather suprising since no normal signal processing can be expected when the otolithic membrane is dislocated from the sensory cells or in the presence of saccular fistulae (table 6), which will result in destruction of the resting (generator) potential. A possible explanation might be that the methods used are not sensitive enough to detect abnormalities. Otherwise, because both utricle and saccule are involved in the control of static equilibrium, it might be assumed that the saccular deficit can be overcome by the information collected by the utricle, which was found to be morphologically intact in animals with otolith dislocation in the sacculus.

Abnormal electronystagmograms were found only in rats with a hydrops of the semicircular canals, except for one case where only a fistula in the saccule was found. Type I response can be interpreted as a kind of vestibular recruitment, type II as a sequel of a vestibular deficit. Both types can be considered as a sign of vestibular dysfunction of the operated ear. It is conceivable that the semicircular canals because of their specific shape and function are very sensitive to an increase in dimensions. Due to the hydrops (distension) of the ampulla the cupula will become either detached from the ampullar wall or crista (sensory epithelium) or damaged otherwise by which the stimulus response relationship may be influenced.

Comparison of these vestibular findings with the few data reported on guinea pigs is rather difficult, because there is no clear presentation of the methods used and response evaluation in terms of biophysically interpretable parameters. Moreover no correlation with morphological findings in the vestibular organs are given.

Aran et al (1984), using sinusoidal stimulation only found inconsistent directional preponderance in animals with or without unilateral or bilateral hydrops, which does not justify, in our opinion, any conclusion concerning "vestibular recruitment". Morgenstern and Lamprecht (1984) described reduced cold water caloric responses in guinea pigs with saccus obliteration on the operated side. In that test, nystagmus beats away from the stimulated side. This can be also explained as a directional preponderance to the operated side, which then would agree with our interpretation of the type I response.

**Summary**
The sensory part of the inner ear consists of a fluid-filled compartment, the endolymphatic space. It is filled with endolymph and lined with various types of specialised epithelium including the sensorineural cells of cochlea and vestibular organs. It is connected by means of the endolymphatic duct with an intradural appendix of the endolymphatic space, the endolymphatic sac.
The functioning of both auditory and vestibular apparatus are ultimately related to the properties of the endolymph, which is in close contact with the sensory cells. The endolymph has a unique ionic composition, high potassium and low sodium content, which is usually found only inside cells. The secretion of this fluid is assumed to be performed by specialised epithelial cells in the cochlea and vestibular compartments, but resorption of the endolymph is still a matter for debate.
According to the dynamic flow theory the removal of high molecular products from the endolymph is thought to be governed by the longitudinal flow, which should transport these substances towards the endolymphatic sac. However, the existence and the mechanics of such a flow under physiological conditions are unknown.
In this thesis we have looked at the significance of the endolymphatic duct and sac in endolymph homeostasis. In addition, the role was studied of these structures in the functioning of the cochlea and vestibular organs.
In Chapter 1 the current knowledge of the morphology and physiology of the epithelial lining of the various compartments of the endolymphatic space, including the endolymphatic duct and sac is described. In addition various hypotheses concerning the function of the endolymphatic sac are discussed.
The morphological, surgical and physiological procedures used in this study are reported in Chapter 2.
Chapter 3 describes the light- and electronmicroscopical findings in the endolymphatic duct and sac of the rat and guinea pig. It was concluded that the morphological features of the epithelial lining are highly suggestive of the existence of a transepithelial fluid transport in both the endolymphatic duct and sac. Meanwhile the intermediate portion of the sac reveals notable features which suggest involvement in the bulk resorption of endolymph. In contrast to the guinea pig, phagocytic cells seemed to play no part in the uptake of endolymph in the case of the rat.
In Chapter 4 an autoradiographic investigation is described for the study of endolymph circulation in both the adult and developing inner ear. Themethod utilised the presence of high concentrations of glycoproteins in the endolymphatic sac. Radioactive sulphate ($^{35}SO_4$), known to be selectively incorporated in sulphated glycoproteins, was used as a tracer. It was established that sulphated glycoproteins are essential components of the tectorial and otolithic membranes and cupulaeand that they are secreted into these structures by specialised cells in the sensory areas.
It is worth noting that the secretion of glycoproteins into the tectorial membrane virtually ceased at the same time as the embryonal sulcus cells disappeared, that is at the time that the organ of Corti matured. However glycoprotein secretion into the otolithic membranes and cupulae continued after maturation. As the gradual disappearance of the labelled substance from these structures was followed by its appearance in the endolymphatic sac, it was concluded that a flow of endolymph from the cochlear and vestibular compartments towards the endolymphatic sac existed. Resorption, was found to take place in the endolymphatic sac at a very slow rate. Because this directio-

nal flow was not completely prevented by obstruction of the endolymphatic sac, it was assumed that the duct itself also contributed to a minor degree to this longitudinal flow. The mechanism underlying this flow was considered to be an active sodium transport system.

The morphological effects of endolymphatic sac obstruction on the inner ear are described in Chapter 5. Obstruction produced a hydrops in both the cochlear and vestibular parts of the endolymphatic space and this hydrops was frequently associated with fistulae in the membranous wall and loss of hair cells and ganglion cells in the cochlea. No such loss was established in the sensory epithelium of the vestibular organs. It was concluded that hydrops resulted from blockage of the outflow system.

After saccus obstruction a gradual development of periductal and perisaccular fibrosis was seen and ascribed to the surgical intervention. It was thought that this fibrosis may seriously interfere with endolymph resorption and thereby limit the benefit of the saccus shunt operation in the relief of the presumed endolymphatic hydrops. Consequently the use of this operation in Ménière's disease is highly questionable.

The functional implications of induced hydrops for cochlear and vestibular function are discussed in Chapter 6. It was found that hydrops alone does not necessarily affect cochlear function for it was only when the hydrops was associated with loss of hair cells and ganglion cells that a threshold increase of the compound action potential became established.

In the vestibular portion using behavioural studies no obvious change of the otolith organs was observed in the presence of hydrops. However a functional change of the semicircular canals as measured by electronystagmography was always associated with hydrops in the vestibular portion. This is thought to be due in part to the increased dimensions of the canals and ampullae which would alter the stimulus-response relationship.

**Samenvatting**
In het binnenoor vindt de signaaltransductie plaats in een volledig gesloten met vloeistof gevuld compartiment, de endolymphatische ruimte. Dit compartiment dat met endolymphe is gevuld, is bekleed met verschillende soorten gespecialiseerd epitheel, waaronder de zintuigcellen van gehoor- en evenwichtsorgaan. Via de ductus endolymphaticus is de endolymphatische ruimte verbonden met de saccus endolymphaticus, een in de dura mater gelegen appendix.
De endolymphe heeft een unieke kationensamenstelling – hoog kalium- en laag natriumgehalte – een situatie die normaliter alleen intracellulair wordt aangetroffen. Het functioneren van gehoor- en evenwichtsorgaan is sterk gerelateerd aan de samenstelling van de endolymphe, die in nauw contact staat met de zintuigcellen.
De secretie van endolymphe wordt toegeschreven aan gespecialiseerde epitheliale cellen in de cochlea en in het evenwichtsorgaan, maar de plaats waar de resorptie plaats vindt en de wijze waarop dit gebeurt is nog steeds onderwerp van discussie. Volgens de "dynamic flow" theorie zou de verwijdering van hoog-moleculaire stoffen uit de endolymphe geregeld worden door een zogenaamde "longitudinale" stroming die deze stoffen naar de saccus endolymphaticus transporteert. Tot op heden ontbreekt echter het bewijs voor het bestaan van een dergelijke stroming onder fysiologische condities.
In dit proefschrift is de betekenis van de ductus en saccus endolymphaticus voor de endolymphe homeostasis bestudeerd. Daarnaast is nagegaan of en hoe deze structuren betrokken zijn bij de functie van gehoor- en evenwichtsorgaan.
In hoofdstuk 1 wordt een overzicht gegeven van de morfologie en fysiologie van de epitheliale bekleding van de verschillende compartimenten van de endolymphatische ruimte, inclusief die van de ductus en saccus endolymphaticus. Tevens worden de verschillende hypothesen over de functie van de saccus endolymphaticus besproken.
De morfologische, chirurgische en fysiologische methodieken die in dit onderzoek gebruikt zijn worden besproken in hoofdstuk 2.
In hoofdstuk 3 wordt een beschrijving gegeven van de licht- en elektronenmicroscopische bevindingen aan de ductus en saccus endolymphaticus van de rat en de cavia. De morfologische kenmerken van de epitheliale bekleding van zowel ductus als saccus wijzen op een betrokkenheid bij transepitheliaal vloeistof transport, terwijl met name het intermediaire deel van de saccus een belangrijke functie vervult bij de bulkresorptie van endolymphe. Bij de rat werd, in tegenstellling tot de cavia, geen overtuigende deelname gevonden van phagocyterende cellen aan de opname van endolymphe uit het lumen van de saccus endolymphaticus.
In hoofdstuk 4 worden de resultaten van een autoradiographisch onderzoek naar de circulatie van endolymphe in het volwassen en het zich ontwikkelende binnenoor van de rat beschreven. Dit onderzoek was gebaseerd op de aanwezigheid van hoge concentraties glycoproteinen in de saccus endolymphaticus. Radioactief sulfaat ($^{35}SO_4$) dat selectief wordt opgenomen in gesulfateerde glycoproteinen, werd gebruikt als tracer. Uit dit onderzoek kwam naar voren dat gesulfateerde glycoproteinen essentiële componenten zijn van de membrana tectoria, de otolithen membranen en de cupulae. Deze stoffen worden in de genoemde structuren gesecreteerd door gespecialiseerde cellen in en rondom het zintuigepitheel. Tijdens de ontwikkeling van het orgaan van Corti houdt de secretie van gesulfateerde glycoproteinen in de membrana tectoria op tegelijk met het verdwijnen van de embryonale sulcus cellen en de functionele rijping van de zintuigcellen. In de otolithen membraan en de cupula gaat de secretie echter door in het

volwassen binnenoor. Het geleidelijk verdwijnen van het radioactieve materiaal uit deze structuren gevolgd door het verschijnen ervan in de saccus endolymphaticus, wijst op het bestaan van een endolymphe stroming vanuit het cochleaire en vestibulaire compartiment naar de saccus endolymphaticus. In de saccus kon een langzame resorptie van deze macromoleculen worden vastgesteld.
Na obliteratie van de saccus bleef deze endolymphe stroming nog gedeeltelijk bestaan. Hieruit werd de conclusie getrokken dat naast de saccus ook de ductus ingeringe mate bijdraagt aan het instand houden van deze longitudinalestroming. Verondersteld wordt dat een actief Na-transport systeem de drijvende kracht is achter deze stroming.
De morfologische veranderingen, die het gevolg zijn van obstructie van de saccus endolymphaticus, zijn beschreven in hoofdstuk 5. Obstructie resulteerde in een hydrops van zowel het cochleaire als het vestibulaire compartiment van de endolymphatische ruimte. Hydrops was vaak geassocieerd met fistels van de endolymphatische ruimte en met degeneratie van haar- en ganglioncellen in de cochlea. In het evenwichtsorgaan werd geen degeneratie van zintuigcellen vastgesteld. Het ontstaan van hydrops is vermoedelijk het gevolg van de blokkade van het systeem dat verantwoordelijk is voor de afvoer van endolymphe.
Na obstructie van de saccus endolymphaticus werd een geleidelijke periductulaire en perisacculaire fibrose vastgesteld. Dit verschijnsel, dat vermoedelijk het gevolg is van de chirurgische ingreep, kan de resorptie van endolymphe nadelig beinvloeden. Deze waarneming wekt twijfel aan het nut van shuntoperaties van de saccus endolymphaticus ter verlichting van de veronderstelde hydrops bij patiënten met de ziekte van Ménière.
De gevolgen van de opgewekte hydrops voor de functie van gehoor- en evenwichtsorgaan worden besproken in hoofdstuk 6. Hydrops alleen bleek de functie van de cochlea niet aan te tasten. Alleen in die gevallen waar hydrops gepaard ging met verlies van haarcellen en ganglioncellen kon een drempelverhoging van de samengestelde aktiepotentiaal worden vastgesteld.
Bij hydrops van het evenwichtsorgaan kon geen verandering van de functie van de otolithenorganen worden vastgesteld met behulp van gedragstudies. Echter met electronystagmografie werd een functieverandering van het kanalensysteem waargenomen die altijd geassocieerd was met hydrops van het vestibulaire compartiment. Deze functie verandering kan ten dele verklaard worden als het gevolg van de toename van de afmetingen van kanalen en ampullae, waardoor de stimulusrespons relatie verandert.

ADLINGTON P (1967), The ultrastructure and the functions of the saccus endolymphaticus and its decompression in Meniere's disease J Laryngol Otol 81 759

ADLINGTON P (1968), The ultrastructure and functions of the saccus endolymphaticus II J Laryngol Otol 82 101

ADLINGTON P (1984), The saccus endolymphaticus in the rabbit Further studies J Laryngol Otol 98 857

ALLEN GW (1964), Endolymphatic sac and cochlear aqueduct Arch Otolaryngol 79 322

ALTMANN F, FOWLER EP (1943), Histological findings in Meniere's symptom complex Ann Otol 52 52

ALTMANN F, WALTNER JG (1950), Further investigations on the physiology of the labyrinthine fluids Ann Otol 59 657

AMANO H, ORSULAKOWA A, MORGENSTERN C (1983), Intracellular and extracellular ion content of the endolymphatic sac Arch Otorhinolaryngol 237 273

ANDERSEN HC (1948), Passage of trypan blue into the endolymphatic system of the labyrinth Acta Otolaryngol 36 273

ANNIKO M (1980), Embryogenesis of the mammalian inner ear III, Formation of the tectorial membrane of the CBA/CBA mouse in vivo and in vitro Anat Embryol 160 301

ANNIKO M, NORDEMAR H (1982), Formation of the cupula Comparative studies on development in vivo and in vitro Am J Otolaryngol 3 31

ANSON BJ, NESSELROD JP (1936), Endolymphatic and associated ducts in man Arch Otolaryngol 24 127

ANTOLI-CANDELA F Jr (1976), The histopathology of Meniere's disease Acta Otolaryngol Suppl 340 1

ANTUNEZ JCM, GALEY FR, LINTHICUM FH, MCCANN GD (1980), Computer-aided and graphic reconstruction of the human endolymphatic duct and sac Ann Otol 89 Suppl 76 23

ARAN JM, RAREY KE, HAWKINS JE (1984), Functional and morphological changes in experimental endolymphatic hydrops Acta Otolaryngol 97 547

ARENBERG IK, MAROVITZ WF, SHAMBAUGH GE Jr (1970), The role of the endolymphatic sac in the pathogenesis of endolymphatic hydrops in man Acta Otolaryngol Suppl 275 1

ARENBERG IK, MURRAY JP, RAUCHBACH E, SCHENK N (1976), An experimental model for the study of endolymphatic hydrops in sharks implications for the clinician Laryngoscope 86 1426

ARENBERG IK, NORBACK DH, SHAMBAUGH GE Jr (1985), Distribution anddensity of subepithelial collagen in the endolymphatic sac in patients withMeniere's disease Am J Otol 6 449

ARNOLD W, VOSTEEN KH (1973), Zur sekretorischen Aktivität der Interdentalzellen des Limbus spiralis Acta Otolaryngol 75 192

ARNOLD W (1974), Zur Frage der Produktion und Resorption der Perilymphe (Lymphabfluss des Innenohres) Laryng Rhinol Otol 53 774

ARNOLD W, ALTERMATT HJ, GEBBERS JO (1984), Qualitativer Nachweis von Immunoglobulinen im menschlichen Saccus endolymphaticus Laryng Rhinol Otol 63 464

ARNVIG J (1951), Lymph vessels in the wall of the endolymphatic sac Arch Otolaryngol 53 290

BAGGER-SJÖBACK D, RASK-ANDERSEN H, LUNDQUIST PG (1981), Intercellular junctions in the epithelium of the endolymphatic sac a freeze-fracture and TEM study on the guinea pig's labyrinth In Vosteen KH et al (eds) Meniere's disease Pathogenesis, Diagnosis and Treatment Thieme, New York, p 127

BAGGER-SJÖBÄCK D, FRIBERG H, RASK-ANDERSEN H (1986), The human endolymphatic sac Arch Otolaryngol 112 398

BAGGER-SJOBACK D, RASK-ANDERSEN H (1986), The permeability barrier of the endolymphatic sac A hypothesis of fluid and electrolyte exchange based on freeze fracturing Am J Otol 7 134

BAIRATI A (1960), Recentes connaissances sur la structure submicroscopique des organes du vestibule Acta Otolaryngol Suppl 163 91

BAST T, ANSON BJ (1949), The temporal bone and the ear CC Thomas, Springfield, III

BEAL DD (1968), Effect of endolymphatic sac ablation in the rabbit and cat Acta Otolaryngol 66 333

BÉKÈSY G von (1952), DC resting potentials inside the cochlear partition J Acoust Soc Amer 24 72

BELANGER LF (1953), Autoradiographic detection of $^{35}S$ in the membranes of the inner ear of the rat Science 118 520

BOETTCHER A (1869), Über den Aquaductus vestibuli bei Katzen und Menschen Arch Anat Physiol 36 372

BOETTCHER A (1871), Der Aquaductus vestibuli Über Entwicklung und Bau des Gehorlabyrinths nach Untersuchungen an Säugethieren Engelmann W, Leipzig, pp 34

BOSHER SK, HALLPIKE CS (1965), Observations on the histological features, development and pathogenesis of the inner ear degeneration of the deaf white cat Proc Roy Soc Lond (biol) 162 147

BOSHER SK, WARREN RL (1971), A study of the electrochemistry and osmotic relationships of the cochlear fluids in the neonatal rat at the time of the development of the endocochlear potential J Physiol 212 739
BRACKMANN DE (1977), Electric response audiometry in a clinical practice Laryngoscope 87 Suppl 5 1
BREDBERG G, LINDEMAN H, ADES H, WEST R, ENGSTROM H (1970), Scanning electron microscopy of the organ of Corti Science 170 861
BREDBERG G, ADES HW, ENGSTROM H (1972), Scanning electron microscopy of the normal and pathological altered organ of Corti Acta Otolaryngol Suppl 301 1
BRESCHET G (1833), Études anatomiques et physiologiques sur l'organe de l'ouie et sur l'audition, dans l'homme et les animaux vertebres Thuan, Paris
BREUFR J (1874), Über die Funktion der Bogengänge des Ohrlabyrinthes Med Jahrb (Wien) 4 72
BREUER J (1891), Über die Funktion der Otolithen-Apparate Pflug Arch ges Physiol 48 195

CARLSTROM B, ENGSTRÒM H, HJORTH S (1953), Electron microsopic and X-ray diffraction studies of statoconia Laryngoscope 63 1052
CAWTHORNE T (1947), Meniere's disease Ann Otol 56 18
CHOU JTY, RODGERS K (1962), Respiration of tissues lining the mammalian membranous labyrinth J Laryngol Otol 76 341
CIMINO A (1964) La Pars Rugosa del Sacco Endolinfatico nellembrione di Coniglio Il Valsalva 40 91
CLEMIS J, VALVASSORI GE (1968), Recent radiographic and clinical observations on the vestibular aqueduct Otolaryngol Clin N Am 1 339
COATS AC, JENKINS HA, MONROE B (1984), Auditory evoked potentials – the cochlear summating potential in detection of endolymphatic hydrops Am J Otol 5 443
COHEN J, MORIZONO T (1984), Changes in EP and inner ear ionic concentrations in experimental endolymphatic hydrops Acta Otolaryngol 98 398
CORTI A (1851), Recherches sur l'organe de louie des mammiferes Z Wiss Zool 3 7
COTUGNO D (1760), De aqueductibus auris humanae internae Neapoli
DAVIS H (1965), A model for transducer action in the cochlea Cold Spring Harbor symp Quant Biol 30 181
DAVIS H, DEATHERAGE BH, ELDRIDGE DH, SMITH CA (1958), Summating potentials of the cochlea Am J Phyiol 195 251
DAVIS H (1976) Principles of electric response audiometry Ann Otol 85 Suppl 28 1
DEELFN GW van (1986), Studies on cochlear endolymphatic hydrops and Meniere's disease Thesis Utrecht
DE VINCENTIIS M, MARMO F (1968), Inhibition of the morphogenesis of the otoliths in the chick embryo in the presence of carbonic anhydrase inhibitors Experientia 24 818
DIAMOND JM, BOSSERT WH (1967), Standing-gradient osmotic flow A mechanism for coupling water and solute transport in epithelia J Gen Physiol 50 2061
DOHLMAN GF, ORMEROD FC, MCLAY K (1959), The secretory epithelium of the internal ear Acta Otolaryngol 50 243
DOHLMAN GF, ORMEROD FC (1960), The secretion and absorption of endolymph Acta Otolaryngol 51 435
DOHLMAN GF, BOORD R (1964), The effects of cupular removal on the activity of ampullary structures in the pigeon Acta Otolaryngol 57 507
DOHLMAN GF (1965), The mechanism of secretion and absorption of the endolymph in the vestibular apparatus Acta Otolaryngol 59 275
DOI S (1939), Über die freien Zellen im Saccus endolymphaticus der Meerschweinchen Zentr Blatt f Hals-Nas-Ohrheilk 31 329
DURRANT JD, DALLOS P (1974), Modification of DIF summating potential components by stimulus biasing J Acoust Soc Am 56 562
DUVALL AJ, RHODES VT (1967), Ultrastructure of the organ of Corti following intermixing of cochlear fluids Ann Otol 76 688
DUVALL AJ, III (1969), The ultrastructure of the external sulcus in the guinea pig cochlear duct Laryngoscope 79 1

EGAMI T, SANDO I, BLACK FO (1978), Hypoplasia of the vestibular aqueduct and endolymphatic sac in endolymphatic hydrops Trans Am Acad Ophthalmol Otolaryngol 86 327
EGGERMONT JJ, ODENTHAL DW, SCHMIDT PH (1974), Basic principles for electrocochleography Acta Otolaryngol Suppl 316 7

EGGERMONT JJ (1976); Electrocochleography (Chapter 15). In: Handbook of sensory physiology. ed. Keidel WD, Neff WD, Springer, Berlin, V3: p 625.
EGGERMONT JJ (1979); Summating potential in Ménière's disease. Arch Otorhinolaryngol 222: 63.
EGGERMONT JJ, SCHMIDT PH (1985), Ménière's disease: A longterm follow-up study of hearing loss. Ann Otol 94: 1.
EGMOND VAN AAJ, BRINKMAN WFB (1956); On the function of the saccus endolymphaticus. Acta Otolaryngol 46: 285.
ENGSTRÖM H, HJORTH S (1950); On the distribution and localization of injected dyes in the labyrinth of the guinea pig. Acta Otolaryngol Suppl 95: 149.
ENGSTRÖM H, WERSÄLL J (1953[a]); The structure of the organ of Corti. I. Outer hair cells. Acta otolaryngol 43: 1.
ENGSTRÖM H, WERSÄLL J (1953[b]); Supporting structures and their relations to sensory cells and nerve endings. Acta Otolaryngol 43: 323.
ENGSTRÖM H (1958); On the double innervation of the sensory epithelia of the inner ear. Acta Otolaryngol 49: 109.
ENGSTRÖM H, WERSÄLL J (1958); The ultrastructural organization of the organ of Corti and of the vestibular sensory epithelia. Exp Cell Res 5: 460.
EWALD JR (1892); Physiologische Untersuchungen über das Endorgan des Nervus octavus. Bergmann, Wiesbaden. Cited by Wodak E (1956).

FERNANDEZ C (1967); Biochemistry of labyrinthine fluids. Inorganic substances. Arch Otolaryngol 86: 222.
FERRERI G, CRIFO S (1956); Mucopolisaccaridi acidi dei tessuti labirintici. Valsalva 32: 79.
FISCHER AJEM, HUYGEN PLM, KUIJPERS W (1979); Electronystagmography in the laboratory rat. Acta Otolaryngol 88: 412.
FISCHER AJEM, KUIJPERS W, HUYGEN PLM (1980); Fixation frame for electronystagmography in the rat. ORL 42: 142.
FISCHER AJEM (1980); The vestibular system of the manganese deficient rat. Thesis, Nijmegen.
FLOURENS P (1842); Recherches expérimentales sur les propriétés et lesfonctions du système nerveux dans les animaux vertébrés. 2nd ed Crevot, Paris.
FRIBERG U, RASK-ANDERSEN H, BAGGER-SJÖBÄCK D (1984); Human endolymphatic duct. An ultrastructural study. Arch Otolaryngol 110: 421.
FRIBERG U, BAGGER-SJÖBÄCK D, RASK-ANDERSEN H (1985); The lateral intercellular spaces in the endolymphatic sac. A Pathway for fluid transport? Acta Otolaryngol Suppl 426: 1.
FRIBERG U, WACKYM PA, BAGGER-SJÖBÄCK D (1986); Effect of labyrintectomy on the endolymphatic sac. Acta Otolaryngol 101: 172.
FRIEDMANN I (1959); Electron microscope observations on in vitro cultures of the isolated fowl embryo otocyst. J Biophys Biochem Cytol 5: 263.

GACEK RR (1980); Neuroanatomical correlates of vestibular function. Ann Otol 89: 2.
GIBSON WPR, MOFFAT DA, RAMSDEN RT (1977); Clinical electrocochleography in the diagnosis and management of Ménière's disease. Audiology 16: 389.
GIEBEL W (1982); Das dynamische Verhalten der Innenohrflüssigkeiten. Laryng Rhinol Otol 61: 481.
GROOT DE JAM (1987); Computed tomography of the petrous bone in otosclerosis and Ménière's disease. Thesis, Utrecht.
GUILD SR (1927a); Observations upon the structure and normal contents of the ductus and saccus endolymphaticus in the Guinea pig (Cavia cobaya). Am J Anat 39: 1.
GUILD SR (1927b); The circulation of endolymph. Am J Anat 39: 57.
GUSEO A (1977); Classification of cells in the cerebrospinal fluid. Eur Neurol 15: 169.
GUSSEN R (1971); Ménière's disease: New temporal bone findings in two cases. Laryngoscope 81: 1695.
GUTHRIE D (1940); Early textbooks of otology. J Laryngol Otol 55: 158.

HALLPIKE CS, CAIRNS H (1938); Observations on the pathology of Ménière's syndrome. J Laryngol Otol 53: 625.
HALLPIKE CS, WRIGHT AJ (1940); On the histological changes in the temporal bones of a case of Ménière's disease. J Laryngol Otol 55: 59.
HARADA Y, GAAFAR H (1976); Scanning electron microscopy of the endolymphatic sac epithelium. ORL 38: 257.

HASSE C (1867), Der Bogengangapparat der Vogel Ztschr f wissensch Zool 17 598
HASSE C (1873), Die Lymphbahnen des inneren Ohres der Wirbelthiere Anat Stud 1 765
HAUBRICH J (1975), Tierexperimentelle histologisch histochemische Untersuchungen zur Formalgenese bei hypothyreotisch bedingten Schwerhorigkeit Acta Otolaryngol Suppl 332 10
HELD H (1926), Die Cochlea der Sauger unter der Vogel, ihre Entwicklung und ihr Bau In Bethe et al Handbuch der Normalen und Pathologischen Physiologie XI 1 467, Berlin
HENSEN V (1878), Bemerkungen gegen die Cupula terminalis (Lang) Arch Anat Physiol 2 486
HOEVE J VAN DER, KLEIJN DE A (1917), Tonische Labyrinthreflexe auf die Augen Pflug Arch ges Physiol 169 241
HOUSE WF (1962), Subarachnoid shunt for drainage of endolymphatic hydrops Laryngoscope 72 713
HUSCHKE E (1824), Über die Horwerkzeuge Beitr Physiol 35
HUYGEN PLM, FISCHER AJEM, KUIJPERS W (1986), The vestibular functions of the Manganese-deficient rat Acta Otolaryngol 101 19

IGARASHI M, KANDA T (1969), Fine structure of the otolithic membrane in the squirrel monkey Acta Otolaryngol 68 43
ILBERG VON C (1968), Elektronenmikroskopische Untersuchungen uber Diffusion und Resorption von Thoriumdioxyd an der Meerschweinchenschnecke Arch klin exp Ohr- Nas- Kehlk Heilk 190 415
IMOTO T, RASK-ANDERSEN H, BAGGER-SJÖBÄCK D (1983), The role of the endolymphatic sac in statoconial formation and degradation Acta Otolaryngol 96 227
INAMORI T (1984), Experimental and clinical study of endolymphatic hydrops by means of electrocochleogram Jap J Otol 87 325
ISHII T, SILVERSTEIN H, BALOGH K Jr (1966), Metabolic activities of the endolymphatic sac An enzyme histochemical and autoradiographic study Acta Otolaryngol 62 61
IURATO S (1960a), Structure submicroscopique des cellules ciliees de l'organe de Corti J Franç Oto-Rhino-Laryngol 9 67
IURATO S (1960b), Submicroscopic structure of the membranous labyrinth I The tectorial membrane Z Zellforsch 52 105
IURATO S (1962), Submicroscopic structure of the membranous labyrinth III The supporting structure of Corti's Organ (basilar membrane, limbus spiralis and spiral ligament) Z Zellforsch 56 40
IURATO S (1967), Submicroscopic structure of the inner ear Pergamon Press, Long Island City, N Y p 61
IWATA N (1924), Über das Labyrinth der Fledermaus mit besonderer Berücksichtigung des statischen Apparates Aichi J Exp Med 1 41

JAHNKE K, RUDERT H (1973), Elektronen mikroskopische Untersuchungen uber die Aufnahme von Proteinen aus der Endolymphe Arch klin exp Ohr- Nas- KehlkHeilk 205 179
JAHNKE K (1975), The fine structure of freeze-fractured intercellular junctions in the guinea pig inner ear Acta Otolaryngol Suppl 336 1
JAHNKE K, MORGENSTERN C, MORI N (1985), Freeze-fracture studies on the perilymph-endolymph barrier in experimentally induced hydrops Arch Otorhinolaryngol 241 175
JENSEN C, VILSTRUP T (1960), On the chemistry of human cupulae Acta Otolaryngol 52 383
JOHNSON LG, HAWKINS JE (1967), Otolithic membranes of the saccule and utricle in man Science 157 1454
JOHNSTONE CG, SCHMIDT RS, JOHNSTONE BM (1963), Sodium and potassium in vertebrate cochlear endolymph as determined by flame microscpectrophotometry Comp Biochem Physiol 9 335
JOHNSTONE BM, ROBERTSON D (1981), The physiology and biophysics of hydrops In KH Vosteen et al (eds) Meniere's disease Pathogenesis Diagnosisand Treatment Thieme, New York p 44

KAIEDA J (1930), Biochemische Untersuchungen des Labyrinthwassers und der Cerebrospinalflussigkeit der Haifische Hoppe Seylers Z physiol Chem 188 193
KAISER O (1891), Das Epithel der Cristae und Maculae acusticae Arch f Ohrenheilk 32 181
KELLERHALS B (1976), Quantitative assessment of perilymph sources ORL 38 193
KERR AG, SMITH GDL (1976), Destruction of the endolymphatic sac in the cat Laryngoscope 86 841
KIMURA RS, LUNDQUIST P, WERSALL J (1964), Secretory epithelial linings in the ampullae of the guinea pig labyrinth Acta Otolaryngol 57 517
KIMURA RS, SCHUKNECHT HF (1965), Membranous hydrops of the inner ear of the guinea pig after obliteration of the endolymphatic sac Pract Otorhinolaryngol 27 343

KIMURA RS (1966), Hairs of the cochlear sensory cells and their attachment to the tectorial membrane Acta Otolaryngol 61 55
KIMURA RS (1967), Experimental blockage of the endolymphatic duct and sac and its effect of the inner ear of the guinea pig Ann Otol 76 664
KIMURA RS (1968), Experimental production of endolymphatic hydrops Otolaryngol Clin North Am 1 457
KIMURA RS (1969), Distribution, structure, and function of dark cells in the vestibular labyrinth Ann Otol 78 542
KIMURA RS (1976), Experimental pathogenesis of hydrops Arch Otorhinolaryngol 212 263
KIMURA RS (1981), Recent observations on experimental endolymphatic hydrops In Vosteen KH et al (eds) Meniere's disease Pathogenesis, diagnosis and treatment Thieme, New York, p 15
KITAHARA M, TAKEDA T, YAZAWA Y, MATSUBARA H, KITANO H (1982), Experimental study on Meniere's disease Otolaryngol Head and Neck Surg 90 470
KLEY E (1951), Zur Herkunft der Perilymphe Laryng Rhinol Otol 30 486
KLEYN A DE, MAGNUS R (1921), Über die Funktion der Otolithen II Mitteilung Isolierte Otolithenausschaltung bei Meerschweinchen Pflüg Arch ges Physiol 186 61
KLEYN A DE, VERSTEEGH C (1933), Labyrinthreflexe nach Abschleuderung der Otolithenmembranen bei Meerschweinchen Pflüg Arch ges Physiol 232 454
KOBURG E, HAUBRICH J, KERNBACH B (1967), Autoradiographische Untersuchungen zum Stoffwechsel des Ductus und Saccus endolymphaticus Acta Otolaryngol 64 146
KOLMER W (1923), Mikroskopische Anatomie des nervosen Apparates des Ohres In Alexander G (ed) Handbuch d Neurologie d Ohres Bd I Urban u- Schwarzenberg, Berlin, p 101
KOLMER W (1927), Gehororgan In v Mollendorff Handbuch des mikroskopischen Anatomie des Menschen III 1 250
KONISHI T, BUTLER RA, FERNANDEZ C (1961), Effect of anoxia on cochlear potentials J Acoust Soc Amer 33 349
KONISHI T, KELSEY E, SINGLETON GT (1966), Effects of chemical alteration in the endolymph on the cochlear potentials Acta Otolaryngol 62 393
KONISHI T, KELSEY E (1968), Effects of cyanide on cochlear potentials Acta Otolaryngol 65 381
KONISHI S, SHEA JJ (1975), Experimental endolymphatic hydrops in guinea pigs and its relief by interruption of the lateral semicircular duct J Laryngol Otol 89 577
KONISHI T, KELSEY E (1976), Cochlear potentials and electrolytes in endolymph in experimentally induced endolymphatic hydrops in guinea pigs In Ruben RJ et al (eds) Electrocochleography University Park Press, Baltimore, p 295
KONISHI S (1977), The ductus reuniens and utriculo-endolymphatic value in the presence of endolymphatic hydrops in guinea pigs J Laryngol Otol 91 1033
KONISHI T, SALT AN, KIMURA RS (1981), Electrophysiological studies of experimentally induced endolymphatic hydrops in guinea pigs In Vosteen KH et al (eds) Meniere's Disease Pathogenesis, Diagnosis and Treatment Thieme, New York, p 47
KRONESTER-FREI A (1976), Postnatale Entwicklung des Randfasernetzes der Membrana tectoria beim Kaninchen Laryng Rhinol Otol 53 687
KRONESTER-FREI A (1978), Ultrastructure of the different zones of the tectorial membrane Cell Tissue Res 193 11
KUIJPERS W (1969), Cation transport and cochlear function Thesis Nijmegen
KUIJPERS W, BONTING SL (1969), Studies on ($Na^+$-$K^+$) activated ATPase no XXIV Localization and properties of ATPase in the inner ear of the guinea pig Biochim Biophys Acta 173 477
KUIJPERS W, BONTING SL (1970a), The cochlear potentials I The effect of ouabain on the cochlear potentials of the guinea pig Pflüg Arch 320 348
KUIJPERS W, BONTING SL (1970b), The cochlear potentials II The nature of the endocochlear resting potential Pflug Arch 320 359
KUIJPERS W, MANNI JJ (1986), Developmental aspects of glycoprotein secretion and migration in the endolymphatic space Acta Otolaryngol Suppl 429 35
KUMAGAMI H, NISHIDA H, MORIUCHI H (1981), Changes of the action potential, the summating potential and cochlear microphonics in experimental endolymphatic hydrops ORL 43 314
KUMAGAMI H, MIYAZAKI M (1983), Chronological changes of electrocochleogram in experimental endolymphatic hydrops ORL 45 143

KURATA A (1963), The morphological and histochemical studies on the otolith membrane of the Guinea pig macula utriculi J Otolaryngol Jap 66 453

KUSAKARI J, KOBAYASHI T, ARAKAWA E, ROKUGO M, OHYAMA K, INAMURA N (1986), Saccular and cochlear endolymphatic potentials in experimentally induced endolymphatic hydrops of guinea pigs Acta Otolaryngol 101 27

LANG G (1863), Das Gehororgan der Cyprinoiden mit besonderer Berucksichtigung der Nervenendapparate Ztschr f wissensch Zool 13 303

LAWRENCE M, WOLSK D, LITTON WB (1961), Circulation of the inner ear fluids Ann Otol 70 753

LAWRENCE M (1966), Histological evidence for localised radial flow of endolymph Arch Otolaryngol 83 406

LIM DJ, LANE W (1969), Threedimensional observation of the inner ear with the scanning electron microscope Trans Am Acad Ophthalmol Otolaryngol 73 842

LIM DJ (1970), Morphology and function of the interdental cell An ultrastructural observation. J Laryngol Otol 84 1241

LIM DJ, SILVER P (1974), The endolymphatic duct system A light and electron microscopic observation Barany Soc Meet, Los Angeles

LIM DJ, ERWAY LC (1974), Influence of manganese on genetically defective otolith Ann Otol 83 565

LIM DJ (1980), Morphogenesis and malformation of otoconia A review In Gorlin RJ (ed) Morphogenesis and malformation of the ear Alan R Liss Inc, New York, p 111

LIM DJ, GLASSCOCK ME III (1981), Fine morphology of the endolymphatic sac in Ménière's disease In Vosteen KH et al (eds) Meniere's Disease Pathogenesis, Diagnosis and Treatment Thieme, New York, p 115

LINDEMAN H (1969), Regional differences in structure of the vestibular sensory regions J Laryngol Otol 83 1

LINDSAY JR (1942), Labyrinthine dropsy and Ménière's disease Arch Otolaryngol 39 853

LINDSAY JR (1947), Effect of obliteration of the endolymphatic sac and duct in the monkey Arch Otolaryngol 45 1

LINDSAY JR, SCHUKNECHT HF, NEFF WD, KIMURA RS (1952), Obliteration of the endolymphatic sac and the cochlear aqueduct Ann Otol 61 697

LISTON SL, PAPARELLA MM, MANCINI F, ANDERSON JH (1984), Otosclerosis and endolymphatic hydrops Laryngoscope 94 1003

LORENTO DE NO R (1933), Anatomy of the eight nerve The central projection of the nerve endings of the internal ear Laryngoscope 43 1

LUFT JH (1961), Improvements in epoxyresin embedding methods J Biophys Biochem Cyt 9 409

LUNDQUIST PG, KIMURA RS, WERSÄLL F (1964), Ultrastructural organisation of the epithelial lining in the endolymphatic duct and sac in the guinea pig Acta Otolaryngol 57 65

LUNDQUIST PG (1965), The endolymphatic duct and sac in the guinea pig An electron microscopic and experimental investigation Acta Otolaryngol Suppl 201 1

LUNDQUIST PG (1976), Aspects on the endolymphatic sac morphology and function Arch Otorhinolaryngol 212 231

MAGNUS R (1924), Körperstellung Springer, Berlin

MAKIMOTO K, SILVERSTEIN H (1974), Sodium and potassium concentrations in the endolymph and perilymph of the cat Ann Otol 83 174

MANNI JJ, KUIJPERS W, WICHEN VAN P (1986), Experimental endolymphatic hydrops in the rat Arch Otolaryngol 112 423

MANNI JJ, KUIJPERS W (1987), Longitudinal flow of macromolecules in the endolymphatic space of the rat An autoradiographic study Hearing Research 26 229

MARTIN GK, SHAW DWW, DOBIE RH, LONSBURY-MARTIN BL (1983), Endolymphatic hydrops in the rabbit Auditory brainstem responses and cochlear morphology Hearing Res 12 65

MATCHINSKY FM, THALMANN R (1967), Quantitative histochemistry of microscopic structures of the cochlea Ann Otol 76 638

MATSUNAGA T, NAKAJIMA R, UMEMOTO T, TOMIYAMA Y, NAIRO T (1972), Preliminary report on the vestibular function in cases of experimental labyrinthine hydrops Equilibrium Res Suppl 3 3

MITANI Y, KOSAKA N, KONISHI S, OGURA Y (1973), Observation of luminal surface of the endolymphatic sac in guinea pig Acta Otolaryngol 76 149

MIYAMOTO H, MORGENSTERN C (1981), Role of the electrolytes in the different parts of the endolymphatic space In Vosteen KH et al (eds) Meniere'sdisease Pathogenesis, Diagnosis and Treatment Thieme, New York, p 39

MORGENSTERN C, MIYAMOTO H (1979), DC-potential and $K^+$ activity in experimental endolymphatic hydrops Arch Otorhinolaryngol 222 273

MORGENSTERN C, LAMPRECHT J (1984), Morphological and functional findings in experimental endolymphatic hydrops Rev de Laryngol 105 223

MORIZONO T, COHEN J, SIKORA MA (1985), Measurement of action potential thresholds in experimental endolymphatic hydrops Ann Otol 94 191

MORRISON D, SCHINDLER RA, WERSALL J (1975), A quantitative analysis of the afferent innervation of the organ of Corti in guinea pig Acta Otolaryngol 79 11

MORSE DE, LOW FN (1972), The fine structure of subarachnoid macrophages in the rat Anat Rec 174 469

MYGIND SH (1952), The function and the diseases of the labyrinth Lectures on aural medicine Acta Otolaryngol 41 235

NADOL J, WEISS A, PARKER S (1975), Vertigo of delayed onset after sudden deafness Ann Otol 84 841

NAFTALIN L, HARRISON MS (1958), Circulation of labyrinthine fluids J Laryngol Otol 72 118

NAITO T (1950), Experimental studies on Meniere's disease J Otolaryngol Jap 53 19

NAKAI Y, HILDING D (1968), Vestibular endolymph producing epithelium Electron microscopic study of the development and histochemistry of the dark cells of the crista ampullaris Acta Otolaryngol 66 120

NAKAMURA T (1967), Experimental obliteration of the endolymphatic sac and the perilymphatic duct J Otolaryngol Jap 70 932

ODENIUS MV (1867), Über das Epithel der Maculae acusticae beim Menschen Arch Mikr Anat 3 115

OHASHI K, IGARASHI M (1985), Statoconia displacement in squirrel monkey ears ORL 47 242

OIGAARD A, THOMSON J, JENSEN J, DORPH S (1976), The vestibular aqueduct in Meniere's disease Acta Otolaryngol 82 279

PAPARELLA MM, KIM CH, SHEA D (1980), Sac decompression for refractory luetic vertigo Acta Otolaryngol 90 541

PAPARELLA MM, DE SOUSA LC, MANCINI F (1983), Meniere's syndrome and otitis media Laryngoscope 93 1408

PAPARELLA MM, MANCINI F (1983), Trauma and Ménière's disease Laryngoscope 93 1004

PAPARELLA MM, MANCINI F, LISTON S (1976), Otosclerosis and Meniere's syndrome Laryngoscope 76 58

PAPARELLA MM (1985), The cause (multifactorial inheritance) and pathogenesis (endolymphatic malabsorption) of Meniere's disease and its symptoms (mechanical and chemical) Acta Otolaryngol 99 445

PARTSCH CJ (1966), Zur Entwicklung des Ductus und Saccus endolymphaticus und ihr Verhalten bei pathologischen Zustanden Arch klin exp Ohr- Nas- Kehlk Heilk 187 581

PEARSE AGE (1972), *Histochemistry, theoretical and applied* 3d ed *Churchill Livingstone, London*

PLANTENGA KF, BROWNING GG (1979), The vestibular aqueduct and endolymphatic sac and duct in endolymphatic hydrops Arch Otolaryngol 105 546

POLITZER A (1956), Geschichte der Ohren Heilkunde I, II Georg Ohms Verlag, Hildesheim

RASK-ANDERSEN H, STAHLE J (1979), Lymphocyte-macrophage activity in the endolympatic sac An ultrastructural study of the rugose endolymphatic sac in the guinea pig ORL 41 177

RASK-ANDERSEN H, STAHLE J (1980), Immunodefence of the inner ear? Lymphocyte-macrophage interaction in the endolymphatic sac Acta Otolaryngol 89 283

RASK-ANDERSEN H, BREDBERG G, LYTTKENS L, LÖÖF G (1981[a]), The function of the endolymphatic duct An experimental study using ionic lanthanum as a tracer In Cohen B ed Vestibular and oculomotor physiology Ann NY Acad Sci 374 11

RASK-ANDERSEN H, BREDBERG G, STAHLE J (1981[b]), Structure and function of the endolymphatic duct In Vosteen KH et al (eds) Meniere's disease Pathogenesis, Diagnosis and Treatment Thieme, New York, p 99

RASK-ANDERSEN H, BAGGER-SJÖBÄCK D, LUNDQUIST PG (1983), The fenestrated blood vessels of the endolymphatic sac A freeze-fracture and transmission electron microscopic study Am J Otol 4 214

RASK-ANDERSEN H, FRIBERG U, BAGGER-SJÖBÄCK D (1984), The ultrastructure of the human endolymphatic duct Acta Otolaryngol 406 61
RASMUSSEN A (1940), Studies of the VIIIth cranial nerve of man Laryngoscope 50 67
RAUCH S (1964), Biochemie des Hörorgans Thieme, Stuttgart
REISSNER M (1852), Zur Kenntnis der Schnecke im Gehörorgan d Menschen m d Säugetiere Arch J Anat u Phys
RETZIUS G (1884), Das Gehörorgan der Vogel und der Säugetiere Stockholm
REYNOLDS ES (1963), The use of lead citrate at high pH as an electron opaque stain in electron microscopy J Cell Biol 17 208
RODRIGUES-ECHANDIA EL, BURGOS MH (1965), The fine structure of the stria vascularis of the guinea pig inner ear Z Zellforsch 67 600
ROLLIN H (1940), Zur Kenntnis des Labyrinthhydrops und des durch in bedingten Meniere Hals-Nasen-Ohrenarzt 31 73
ROSS MD (1974), The tectorial membrane of the rat Am J Anat 139 449
ROSS MD (1979), Calcium ion uptake and exchange in otoconia Adv Oto Rhino Laryngol 25 26
RUDERT H (1969a), Experimentelle Untersuchungen zur Resorption der Endolymphe im Innenohr des Meerschweinchens I Lichtmikroskopische Untersuchungen nach Injektion von Trypanblau in den Ductus cochlearis Arch klin exp Ohr- Nas- Kehlk Heilk 193 138
RUDERT H (1969b), Experimentelle Untersuchungen zur Resorption der Endolymphe im Innenohr des Meerschweinchens II Versuche mit radioaktiv markierten Substanzen und ihre autoradiografische Auswertung Arch klin exp Ohr- Nas- Kehlk Heilk 193 156
RUSSELL IJ, SELLICK PM (1978), Intracellular studies of hair cells in the mammalian cochlea J Physiol 284 261
RUSSELL IJ (1983), Origin of the receptor potential in inner haircells of the mammalian cochlea – evidence for Davis' theory Nature 301 334

SAAD JJ (1984), Pars superioris in endolymphatic hydrops Arch Otolaryngol 110 679
SALAMAT MS, ROSS MD, PEACOR DR (1980), Otoconial formation in the fetal rat Ann Otol 89 229
SANDO I, IKEDA M (1984), The vestibular aqueduct in patients with Meniere's disease A temporal bone histopathological investigation Acta Otolaryngol 97 558
SAXN A (1951), Histological studies of endolymph secretion and resorption in the inner ear Acta Otolaryngol 40 23
SCARPA A (1789), Anatomicae disquisitiones de auditu et olfactu Ticini
SCHÄTZLE W, HAUBRICH J (1966), Über Verteilung von Glycosidasen, Esterasen und Eiweißbausteinen im Saccus endolymphaticus des Meerschweinchens Arch klin exp Ohr- Nas- Kehlk Heilk 186 373
SCHÄTZLE W (1971), Histochemie des Innenohres Urban u Schwarzenberg, München
SCHINDLER RA (1980), The ultrastructure of the endolymphatic sac in man Laryngoscope 90 Suppl 21 1
SCHMIDT RS, FERNÁNDEZ C (1963), Development of mammalian endocochlearpotential J Exp Zool 153 227
SCHUKNECHT HF, KIMURA RS (1953), Functional and histological findings after obliteration of the periotic duct and endolymphatic sac in sound conditioned cats Laryngoscope 63 1170
SCHUKNECHT HF, BENITEZ JT, BEEKHUIS J (1962), Further observations on the pathology of Meniere's disease Ann Otol 71 1039
SCHUKNECHT HF, EL SEIFI A (1963), Experimental observations on the fluid physiology of the inner ear Ann Otol 72 687
SCHUKNECHT HF, NORTHROP C, IGARASHI M (1968), Cochlear pathology after destruction of the endolymphatic sac in the cat Acta Otolaryngol 65 479
SCHUKNECHT HF (1974), Pathology of the ear Mass Harvard University Press, Cambridge
SCHUKNECHT HF, BELAL AA (1975), The utriculo-endolymphatic valve its functional significance J Laryngol Otol 89 985
SCHUKNECHT HF (1976), Pathophysiology of endolymphatic hydrops Arch Otorhinolaryngol 212 253
SCHULTZE M (1858), Über die Endigungsweise des Gehörnerven im Labyrinth Arch f Anat Physiol u wiss Med 343
SECRETAN JP (1944), De l'histologie normale du sac endolymphatique chez l'homme Acta Otolaryngol 32 119
SELLICK PM, JOHNSTONE JR, JOHNSTONE BM (1972), The electrophysiology of the utricle Pflugers Arch 336 21

SELLICK PM, JOHNSTONE BM (1972), The physiology of the saccule Pflugers Arch 336 28
SELLICK PM, JOHNSTONE BM (1975), Production and role of inner ear fluid Progr Neurobiol 5 337
SEYMOUR JC (1954), Observations on the circulation in the cochlea J Laryngol Otol 68 689
SHAMBAUGH GE Jr, CLEMIS JD, ARENBERG IK (1969), The endolymphatic sac and duct in Meniere's disease I Surgical and histopathologic observations Arch Otolaryngol 89 816
SHINOSAKI N, KIMURA RS (1980), Scanning electron microscopic observations on the distended Reissner's and saccular membrane in the guinea pig Acta Otolaryngol 90 370
SIEBENMANN F (1919), Anatomische Untersuchungen uber den Saccus und Ductus endolymphaticus beim Menschen Passow u Schaefer Beitr Anat Ohres 13 59
SIIRALA U (1942), Über den Bau und die Funktion des Ductus und Saccus endolymphaticus bei alten Menschen Anat Ent 3 246
SILVERSTEIN H (1966[a]), Biochemical studies of the inner ear fluids in the cat Ann Otol 75 48
SILVERSTEIN H (1966[b]), Biochemical and physiologic studies of the endolymphatic sac in the cat Laryngoscope 76 498
SMITH CA, LOWRY OH, WU ML (1954), The electrolytes of the labyrinthine fluids Laryngoscope 64 141
SMITH CA (1955), Electron microscopic studies of the organ of Corti Anat Rec 121 451
SMITH CA, DAVIS H, DEATHERAGE BH, GESSERT CF (1958), DC potentials of the membranous labyrinth Amer J Physiol 193 203
SMITH C, SJOSTRAND F (1961), Structure of the nerve endings on the external hair cells of the Guinea pig cochlea as studied by serial sections J Ultrastruct Res 5 523
SORNAS R (1971), Transformation of mononuclear cells in cerebrospinal fluid Acta Cytol 15 545
SOUDIJN ER (1976), Scanning electron microscopic study of the organ of Corti in normal and sound-damaged guinea pigs Ann Otol Suppl 29 1
SPOENDLIN H (1956), Elektronenmikroskopische Untersuchungen am Cortischen Organ Pract Otorhinolaryngol 18 246
SPOENDLIN H (1957), Elektronenmikroskopische Untersuchungen am Cortischen Organ des Meerschweinchens Pract Otorhinolaryngol 19 192
SPOENDLIN H (1969), Innervation patterns in the organ of Corti of the cat Acta Otolaryngol 67 239
SPRING KR (1982), Salt and water transport by epithelial cells Am J Otolaryngol 3 361
STEEL KP (1985), Composition and properties of the mammalian tectorial membrane In Drescher (ed) Auditory biochemistry Thomas, Springfield, p 351
STEIFENSAND K (1835), Untersuchungen über die Ampullen des Gehörorgans Arch f Anat u Physiol 2 175
STERKERS O, SAUMON G, TRAN BA HUY P, AMIEL C (1982), Evidence for a perilymphatic origin of the endolymph Application to the pathophysiology of Meniere's disease Am J Otolaryngol 3 367
STERZI G (1909), Il sacco endolinfatico Richerche anatomiche ed embiologiche Gegenbauers Morphol Jahrb 39 446
SUH KW, CODY DTR (1974), Obliteration of vestibular and cochlear aqueducts in the guinea pig Laryngoscope 84 1352
SUH KW, CODY DTR (1977), Obliteration of vestibular and cochlear aqueducts in animals Trans Am Acad Ophthalmol Otol 84 359

TACHIBANA M, SAITO H, MACHINO M (1973), Sulfated acid mucopolysaccharides in the tectorial membrane Acta Otolaryngol 76 37
TAKAHASHI K (1961), Study on the distribution of several chemical substances in membranous labyrinth of normal guinea pig J Otolaryngol Jap 64 266
TASAKI I, DAVIS H, ELDREDGE DH (1954), Exploration of cochlear potentials in guinea pig with microelectrode J Acoust Soc Am 26 765
TOMIYAMA S, HARRIS JP (1986), The endolymphatic sac its importance in inner ear immune responses Laryngoscope 96 685

UZIEL A, ROMAND R, MAROT M (1981), Development of cochlear potentials in rats Audiology 20 89

VEENHOF VB (1969), The development of statoconia in mice North-Holland, Amsterdam
VOLDRICH L (1967), Morphology and function of the epithelium of the limbus spiralis cochleae Acta Otolaryngol 63 503
VOSTEEN KH, MORGENSTERN C (1986), Biochemical aspects of inner ear pathophysiology In CR Pfaltz (ed) Controversial aspects of Meniere's disease Thieme, New York, p 16

WACKYM PA, FRIBERG U, BAGGER-SJOBACK D, RASK-ANDERSEN H (1986), Human endolymphatic duct, possible mechanisms of endolymph outflow Ann Otol 95 409

WATSON ML (1958), Staining of tissue sections for electron microscopy with heavy metals J Biophys Biochem Cytol 4 475

WERSALL J (1956), Studies on the structure and innervation of the sensory epithelium of the cristae ampullares in the guinea pig A light and electron microscopic investigation Acta Otolaryngol Suppl 126 1

WEVER EG, BRAY C (1930), Action currents in the auditory nerve in response to acoustic stimuli Proc Natl Acad Sci USA 16 344

WILBRAND HF, STAHLE J (1981), Temporal bone in patients with Meniere's disease In Vosteen KH et al (eds) Meniere's Disease Pathogenesis,Diagnosis and Treatment Thieme, New York, p 141

WISLOCKI GB, LADMAN AJ (1955), Selective and histochemical staining of the otolithic membranes, cupula and tectorial membrane of the inner ear Amer J Anat 89 3

YAMAKAWA K (1929), Die Wirkung der arsenigen Säure auf das Ohr Arch klin exp Ohr- Nas- Kehlk Heilk 123 238

YAMANE H, IMOTO T, NAKAI Y, IGARASHI M, RASK-ANDERSEN H (1984), Otoconia degradation Acta Otolaryngol suppl 406 263

YANAGI Y (1973), Patterns of hair cell damage of the cochlea duct due to intense sound stimulation and cochlear potentials in the guinea pig with experimentally induced endolymphatic hydrops Jap J Otol 76 61

ZECHNER G, ALTMANN F (1969), Histological studies on the human endolymphatic duct and sac Pract Otorhinolaryngol 31 65

I am greatly indebted to all who have contributed to the realization of this thesis.
I would like to express my sincere appreciation to mrs. E.L.G.M. Tonnaer B.Sc. for her tireless efforts to document the electron microscopic events of this study.
Mr. H. Huibers, I thank you for your dedication and humour while solving technical disasters during evoked response audiometry.
I am most grateful to mrs. E.W.M. Camps, mr. P. van Wichen, mr. M.G.M. Nicolasen and mr. T. Hafmans for their skilful technical assistance.
Mr. P. Spaan and his co-workers of the Central Animal Laboratory (Head Dr. W.J.S. van de Gulden) are credited for taking good care of the animals used for these experiments.
I am much indebted to Prof.Dr. J.J. Eggermont, Dr. P.H.K. Jap, Ir. G.C.J.H. Hombergen for their interest and many valuable discussions.
The drawings were prepared by mrs. M. Ackermans-de Leeuw.
I am extremely grateful to mrs. M.W. Helsper-Peters and mrs. H.C.M. Beukering for typing the many versions of this thesis and to mr. J.D.K. Dawes B.Sc., M.D., F.R.C.S. of Newcastle upon Tyne, U.K., for correcting the manuscript.
I thank my parents who supported my academic study and my wife and children for being a constant source of joy and inspiration.

Financial support was kindly provided by Entermed B.V., Linschoten.

## CURRICULUM VITAE

Johannes Jan Manni was born in 1942 in Apeldoorn, The Netherlands. He enrolled to study medicine at the University of Utrecht in 1960, where he graduated in 1966 and at the Erasmus University, Rotterdam, where he gained his licence to practice medicine in 1968. Thereafter he was appointed as house officer in surgery at the Harbour Hospital, Rotterdam and surgery and obstetrics at Juliana Hospital, Ede respectively. From 1969 he was working as medical officer in charge at the Holy Family Hospital, Techiman, Ghana. From 1974 till 1978 he specialized in Otorhinolaryngology at the Departments of Otorhinolaryngology at the University Hospital St. Radboud, Nijmegen (Head Prof.Dr. W.F.B. Brinkman) and the Georg August University, Göttingen, B.R.D. (Head Prof.Dr. A. Miehlke, Februari-September 1977). He was registered as a specialist in Otorhinolaryngology in april 1978. Thereafter he joined the Directorate General for International Cooperation, Ministry for Foreign Affairs of the Government of the Netherlands. From May 1978 until June 1980 he was seconded to the Ministry of Health of Tanzania as lecturer in Otorhinolaryngology at the Medical Faculty of the University of Dar es Salaam and was Head of the Department of Otorhinolaryngology at the University Hospital Muhimbili Medical Centre. He spent part of his sabbatical months following this period as research fellow at the Research Department (Head Dr. R.S. Kimura) of Massachusetts Eye and Ear Infirmary, Harvard University, Boston, U.S.A. (Head Prof.Dr. H.F. Schuknecht).
Since September 1980 he has been employed as lecturer and consultant specialist at the Department of Otorhinolaryngology, St. Radboud Hospital, University of Nijmegen (Head Prof.Dr. P. van den Broek).
He is married to Pieternella van den Heuvel, and has three daughters Mirjam C., Marlies P. and Marije J.

## STELLINGEN

I

De saccus endolymphaticus heeft een resorberende functie *(dit proefschrift).*

II

Het nut van de shunt operatie van de saccus endolymphaticus als therapie voor de ziekte van Ménière dient op grond van in dit proefschrift beschreven onderzoek betwijfeld te worden.

III

De door Tos waargenomen toename van het aantal secretoire cellen in het middenoorslijmvlies na tuba-obstructie is het gevolg van een infectieus proces *(Acta Otolaryngol. 1981; 92 : 51).*

IV

Het bepalen van de groeisnelheid van subcutaan in de naakte muis getransplanteerde tumoren via uitwendige volumemetingen is discutabel *(Elprana et al. Eur. J. Cancer Clin. Oncol. 1986; 22 : 1211).*

V

De incisie volgens MacFee verdient de voorkeur boven andere incisies beschreven voor halsklierdissecties.

VI

Indien bij oncologische patiënten met ernstige pijn opiaten in de orale toedieningsvorm niet toereikend zijn en zenuwblokkering niet mogelijk is, dient epidurale of intrathecale toediening van opiaten te worden overwogen.

VII

Hyperostosis cranialis interna is een nieuw syndroom met autosomale dominante overerving *(eigen waarneming).*

VIII

De ontwikkeling in de leeftijdsopbouw van de Nederlandse bevolking maakt het waarschijnlijk dat de geriatrische keel- neus- en oorheelkunde zeker zoveel toekomst heeft als haar paediatrische pendant.

IX

Het door de wereldgezondheidsorganisatie geïntroduceerde universele systeem van standdaardsymbolen voor het gebruik van geneesmiddelen en gevaarlijke stoffen heeft te weinig oog voor visueel analphabetisme in ontwikkelingslanden.

X

De Groninger spreekprothese blijkt nationaal en internationaal de communicatie te verbeteren.

XI

De sportverenigingen in Nederland dienen in te spelen op de groep 55-plussers waarvan het aantal toeneemt.

XII

Het voorzien van verkeersdrempels met reflectoren vergroot de verkeersveiligheid.

J.J. Manni

Nijmegen, 15 juni 1987.