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## Inner ear pressure during endolymphatic hydrops

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# Inner ear pressure during endolymphatic hydrops

T. J. Warmerdam

# **Inner ear pressure during endolymphatic hydrops**

**T.J. Warmerdam**

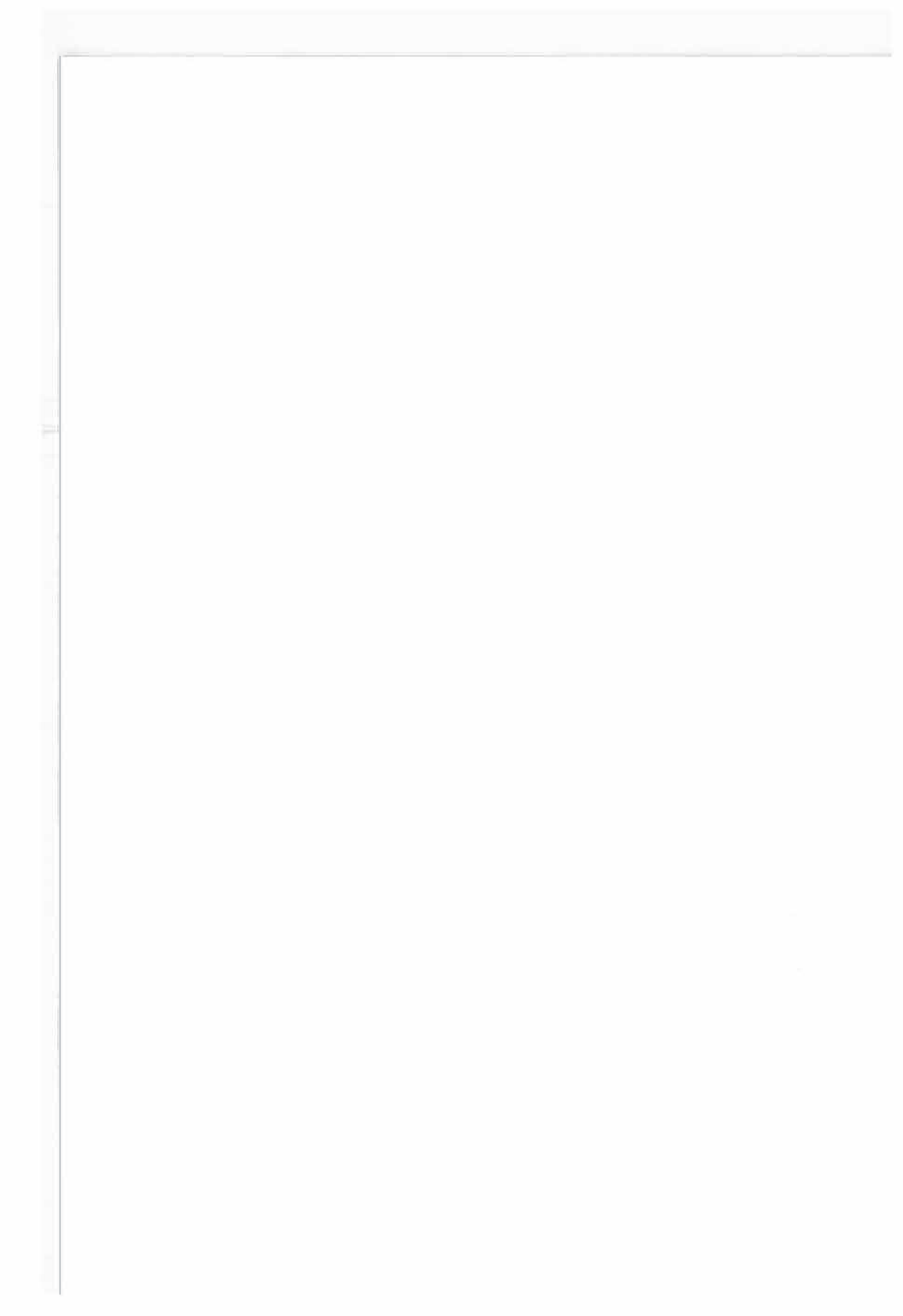


# Stellingen

behorende bij het proefschrift

## Inner ear pressure during endolymphatic hydrops

1. "Who knows if among this jumble there will one day be manifested two good lines to preserve". Prosper Menière over zijn verzameld werk (Pappas DG. *Otology through the Ages. Otolaryngology Head and Neck Surg* 1996;114:173-196).
2. Endolymfatische hypertensie lijkt geen belangrijke rol te spelen bij de ziekte van Menière, de volumetoename van het endolymfatische compartiment mogelijk wel (*dit proefschrift*).
3. De mechanische compliantie van de dunne membranen die de endolymfatische ruimte omgeven blijkt hoog te zijn (*dit proefschrift*).
4. Tijdens endolymfatische hydrops verandert de elektrofysiologie van het binnenoor (*dit proefschrift*).
5. De op dit moment grootste wetenschappelijke uitdaging voor de ziekte van Menière is het met beeldvormend onderzoek kunnen vaststellen van endolymfatische hydrops in patiënten met deze aandoening.
6. Als alles wat er is rook zou blijken te zijn, zouden neuzen daarin nog onderscheid kunnen maken (Heraclitus).
7. Een proefdruk is nog lang geen proefschrift.
8. Morele ondersteuning blijft een belangrijke pijler bij de behandeling van Menière-patiënten. (Proefschrift H.J. Rosingh, 1997).
9. Het is makkelijk te begrijpen waarom een koe in India een heilig dier is, wanneer men er eens bij stilstaat wat er tegenwoordig voor een biefstuk betaald moet worden.



Rijksuniversiteit Groningen

# **Inner ear pressure during endolymphatic hydrops**

## **Proefschrift**

ter verkrijging van het doctoraat in de  
Medische Wetenschappen  
aan de Rijksuniversiteit Groningen  
op gezag van de  
Rector Magnificus, dr. F. Zwarts,  
in het openbaar te verdedigen op  
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**Theodorus Jacobus Warmerdam**

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te Noordwijkerhout

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# Chapter 1

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## General introduction

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## Introduction

Menière's disease (MD) is a chronic illness that is characterised by disabling attacks of hearing loss, tinnitus and vertigo. In 1861 Prosper Menière (1) was the first to ascribe the inner ear as the anatomic location for this illness. Since then the aetiology and pathophysiology of this disease remain an enigma, despite a tremendous amount of research. In 1938 there seemed to have occurred a breakthrough in understanding this condition with the discovery of endolymphatic hydrops (EH) in temporal bones from patients with MD (2,3). EH is a post-mortem histopathological finding in the inner ear which can be best described as a situation wherein the endolymphatic spaces in the inner ear are dilated at the expense of the perilymphatic spaces. The endolymphatic spaces are bounded by membranes, filled with a fluid called endolymph and contain sensory cells for hearing and balance. EH is found in almost all patients with MD (4,5), but there are also reports of cases of EH who did not display the symptoms of MD (4). In that EH is not always present or solely limited to MD, and add to this that laboratory animals with EH do not show the symptoms of MD, some investigators question if it is an epiphenomena (6). However, it is the most striking and consistently found pathologic lesion in patients with MD. Therefore, and because there is still no alternative correlate, exploration of physiological mechanisms underlying this pathological substrate is inevitable.

The till now unknown aetiology of EH is not the subject of this thesis. We were interested in the pathophysiology of hydrops, i.e., the way it can be accounted for the clinical picture of MD.

Much has been speculated as to how EH leads to the symptoms hearing loss, tinnitus and vertigo. The most popular hypothesis has been that EH causes endolymphatic hypertension (2,7-9). The distended appearance of the membranous labyrinth has been considered to represent an elevated pressure in the endolymphatic system. Endolymphatic hypertension is a higher fluid pressure in the endolymphatic space as compared to the fluid pressure in the space that surrounds its membranes (i.e. perilymphatic space). Endolymphatic hypertension, which is thus dependent on the *mechanical compliance* of the boundary membranes of the endolymphatic compartments, could displace the basilar membrane from its resting position (10) or reduce the bloodflow to the organ of Corti (11), thereby impairing cochlear function (hearing). It may also produce vestibular dysfunction via displacement of the cupula (12). Hair cells may become partially decoupled from the tectorial membrane when the pressure in the endolymphatic space rises. There is a chance that this causes an elevation in the noise level at the hair cell input, the basis of cochlear tinnitus (7). All the above mentioned theories have however not been proven before now. Nonetheless the large impact of such hypotheses is made obvious by the fact that elimination of this hypertension is one of the therapeutic cornerstones of MD (8).

In humans, pressure measurements in the inner ear have for obvious reasons never been attempted. Pressure recordings are possible in the cochlea of animals but still impose important technical problems, the difficulties being the tiny dimensions of the inner ear fluid compartments. The first pressure measurements were performed in the cochlea of a dog in 1927(13). The study results then were not reliable because the experimental set up was to crude. Different experimental designs evolved, and in 1964 trustworthy pressure measurements in small volumes became possible with the introduction of the servocontrolled micropipette system (SCMS), which was developed for measuring bloodpressure in small mesenteric veins of the frog (14). It was not until 1975 that the SCMS was used for measuring the pressure in the inner ear (15).

With this new measuring device, there seems to be a general consensus that the pressure in the perilymphatic space equals the pressure in the endolymphatic space in the cochlea of normal guinea pigs (16-21).

In 1965 Kimura (22) developed the widely used animal model for endolymphatic hydrops in guinea pigs by surgical obliteration of the endolymphatic sac and duct. Damage to these structures causes a decrease in endolymph absorption and thus to an increase in endolymph volume, i.e., endolymphatic hydrops.

Since 1984 (23) different research groups have made attempts with the SCMS to analyse if endolymphatic hypertension occurs during EH. The outcome of these publications is variable, since some of them found endolymphatic hypertension (20) while others did not (18). These differences in data were thought to be due to the limitations of accuracy in the experimental set up. The inaccuracy being the use only one SCMS. This means that first the perilymphatic pressure was measured and subsequently, after perforating the basilar membrane with the micropipette, the endolymphatic pressure. During the time interval between measurements in both compartments, a pressure change could have taken place. Physiological pressure changes, which are known to occur in both compartments due to respiration and heartbeat (24), may have been responsible for such an alteration in pressure.

Later in 1990 Takeuchi et al. (25) were the first to solve the time interval problem by measuring the perilymphatic and endolymphatic pressure at the same time, thus not subsequently, using two SCMS. However, their experiments were not carried out in an endolymphatic hydrops model. They analysed both pressures during pressure manipulations in the perilymph and found that the pressure changes in the perilymph were directly transmitted to the endolymph and a pressure difference was not created between the two compartments.

The most sophisticated hydrops model up to now was introduced in 1991 (26). In this model EH was created via injection of artificial endolymph into the endolymphatic space. Two research groups have measured the pressure in both inner ear compartments simultaneously during this innovative creation of EH, and they found no endolymphatic hypertension (26,27).

Making use of this last model it is possible to calculate the mechanical compliance of the boundary membranes of the endolymphatic space in vivo, which to our knowledge has never been done before. Determination of the compliance is important to answer the question if development of endolymphatic hypertension is possible during EH. Because if the boundary membranes of the endolymphatic compartment can accommodate pressure increase in the endolymphatic space by stretching, then a volume increase need not be accompanied by a pressure increase. This would mean that EH is not synonymous with endolymphatic hypertension.

**Objectives of this study**

To measure the endolymphatic and perilymphatic pressure in the cochlea of normal guinea pigs, and to analyse if a quantitative difference exists between these two pressures

To analyse if endolymphatic hypertension occurs in a classic animal model where hydrops is created via a decrease in endolymph absorption, which is caused by a totally destructed endolymphatic sac

To evaluate if endolymphatic hypertension occurs in a recently developed animal model where hydrops is created via a decrease in endolymph absorption, which is caused by a partially functioning endolymphatic sac

To examine if endolymphatic hypertension occurs in an animal model where hydrops is created via injection of artificial endolymph into the endolymphatic system, and to calculate the mechanical compliance of the boundary membranes of the endolymphatic compartment



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## Chapter 2

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### History

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## History

In this thesis we try to find answers to some fundamental questions concerning the pathophysiology of Menière's disease (MD). Patients with MD suffer from a chronic illness that is characterised by disabling attacks of vertigo, hearing loss, tinnitus and a sensation of aural fullness.

In 1995 the American Academy of Otolaryngology-Head and Neck Surgery Committee on Hearing and Equilibrium (AAO-HNS CHE) published guidelines for the diagnosis and evaluation of therapy in MD. These are considered the golden standard in reporting results of therapy in MD world-wide and thus give us the best general impression of the clinical picture of MD to date. In 1972 the original AAO-HNS system for reporting results in the treatment of MD was set up. This system was later revised in 1985 and 1995 (1). The Committee defines MD as the presence of the following as further defined below: recurrent, spontaneous episodic vertigo; sensorineural hearing loss; aural fullness; and tinnitus. Either tinnitus or aural fullness (or both) must be present at the affected side. The spontaneous rotational vertigo lasts at least 20 minutes (commonly several hours), is often prostrating, and is accompanied by nausea and commonly by vomiting. At least two of these types of vertigo spells must occur to permit the diagnosis of definite Menière's disease. Diagnostic hearing loss may take any of the following forms: 1. The average of hearing thresholds at 0.25, 0.5 and 1 kHz is 15 dB or more



*Fig. 1. Prosper Menière, 1799 – 1862.*

higher than the average of 1, 2, and 3 kHz. 2. In unilateral cases, the average of threshold values at 0.5, 1, 2, and 3 kHz is 20 dB or more poorer in the ear in question than at the opposite side. 3. In bilateral cases, the average of threshold values at 0.5, 1, 2, and 3 kHz is greater than 25 dB in the studied ear. The Committee states that tinnitus and aural fullness are difficult to quantify. The Department of Otorhinolaryngology of the University Groningen has provided an alternative and more detailed definition of MD (2). Their criteria for a positive diagnosis are: a documented cochlear hearing loss (at least one threshold of 20 dB HL or worse in one of the six measured pure-tone audiogram frequencies) combined with tinnitus (being present now or in the past), vertigo attacks (at least two in the history), and other pathology excluded.

In 1861 Prosper Menière (figure 1) was the first who made the connection between the inner ear and an affliction characterised by sudden attacks of vertigo, tinnitus and hearing loss. He delivered a paper that summed up his work in a number of statements (3). A copy of part of that manuscript is shown in figure 2. A translation of it, is given below:

*II. About a type of profound hearing loss caused by a lesion of the inner ear.*

*By Mr. Menière (abstract by the author).*

*In: Bulletin de l'Academie Nationale de Médecine (Paris), January 21, 1861.*

*The author summarises his work in a number of statements:*

- 1. A hearing organ, perfectly sound until that moment, can become the source of functional troubles, consisting of continuous or intermittent sounds of variable nature, and these sounds will soon be accompanied by a more or less large diminution of hearing.*
- 2. These functional troubles, having their source in the internal hearing organ, can cause attacks thought to be of cerebral origin, like vertigo, dizziness, insecure walking, giddiness and falling, and on top of that they are accompanied by nausea, vomiting and a state of exhaustion.*
- 3. These accidents, that have an intermittent form, will surely be followed by deafness becoming more and more severe, and often the cochlea will be suddenly and completely destroyed.*
- 4. Everything supports to believe that the lesion that is the cause of these functional troubles resides in the semicircular canals.*

Before the latter half of the nineteenth century all forms of vertigo were believed to be due to a central neurologic problem.

Up to that time knowledge of the anatomy of the labyrinth was nearly complete. Yet, medical understanding of labyrinthine physiology was negligible. It was well known that the inner ear mediated sound perception, and there were a variety of competing theories of how we hear. The semicircular canals, however, were believed to be an extension of the auditory apparatus, also mediating the sensation of sound (4). The first clues that the semicircular canals were involved in the regulation of balance were provided by the experiments executed

II. *Sur une forme de surdité grave dépendant d'une lésion de l'oreille interne*, par M. MÉNIÈRE. (Commissaires : MM. Cruveilhier, Baillarger et Barth.)

(Extrait par l'auteur.)

L'auteur résume ce travail en un certain nombre de propositions :

1° Un appareil auditif, jusque-là parfaitement sain, peut devenir tout à coup le siège de troubles fonctionnels, consistant en bruits de nature variable, continus ou intermittents, et ces bruits s'accompagnent bientôt d'une diminution plus ou moins grande de l'audition.

2° Ces troubles fonctionnels ayant leur siège dans l'appareil auditif interne, peuvent donner lieu à des accidents réputés cérébraux, tels que vertiges, étourdissements, marche incertaine, tournoiements et chute, et de plus ils sont accompagnés de nausées, de vomissements et d'un état syncopal.

3° Ces accidents, qui ont la forme intermittente, ne tardent pas à être suivis de surdité de plus en plus grave, et souvent l'ouïe est subitement et complètement abolie.

4° Tout porte à croire que la lésion matérielle qui est cause de ces troubles fonctionnels réside dans les canaux demi-circulaires.

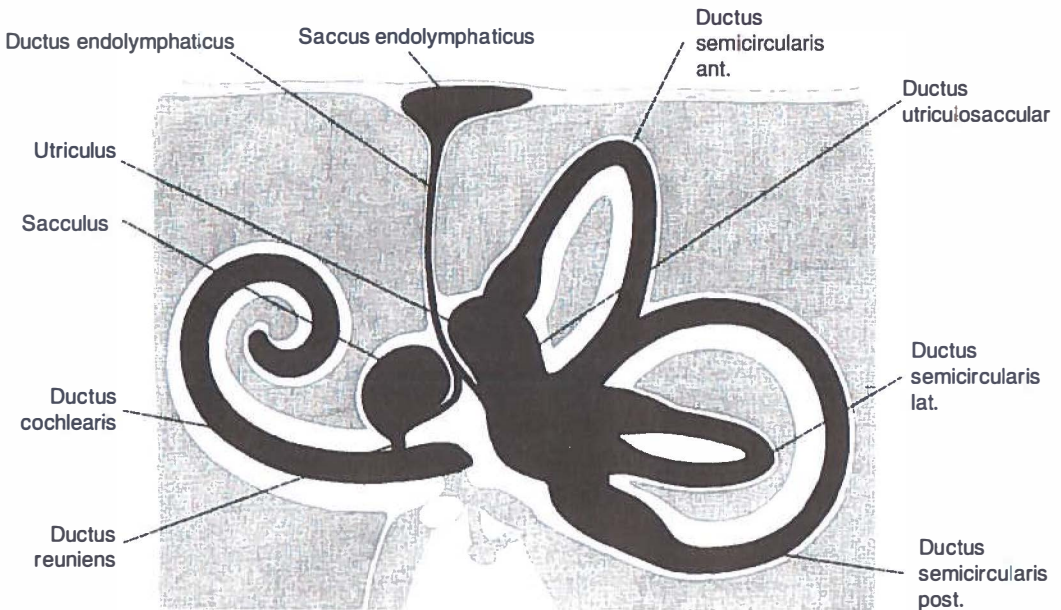
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Fig. 2. Copy of part of the original paper wherein Prosper Menière summarised his work (2).

by Flourens in 1824 (5). He demonstrated in pigeons that lesions in the horizontal semicircular canals caused the animal to turn around its vertical axis, while posterior canal lesions caused the birds to roll over backwards. Flourens concluded from his results that the semicircular canals influenced the directional movements of pigeons, rather than being the organ of equilibrium.

By the clinical practitioners in 1860, all conditions which included the symptoms of vertigo were lumped under the category of "apoplectiform cerebral congestion", a condition thought to result from the overfilling of blood vessels in the brain. Those unfortunate enough to have received this diagnosis were occasionally subjected to bloodletting and violent purgations, from which it took several months to recover (4). It was not until Prosper Menière's landmark studies in 1861, drawing on the work of Flourens and his own experience at the Institute for Deaf-Mutes in Paris where he was appointed chief physician in 1838, that patients with vertigo were recognised as having a distinct disorder whose underlying pathology resided in the labyrinth. He also mentioned that vertigo most often had a benign course and that the common drastic treatments at that time, such as bloodletting, often did more harm than good. It is important to note that he was not attempting to define a disease or syndrome but rather to



*Fig. 3. Diagram of the inner ear. The endolymphatic space is shown in black, the perilymphatic compartment is diagrammed as white, the bony structures are coloured grey.*

emphasise that vertigo could originate from damage to the inner ear. In 1862, Prosper Menière died from pneumonia.

In 1872 Duplay came up with the term 'maladie de Menière' for the illness characterised by auditory and vestibular symptoms (6).

Shambaugh stressed the clinical significance of aural fullness as an additional clinical component next to the triad of symptoms - vertigo, hearing loss, and tinnitus – in the second half of the 20th century (7).

The inner ear is a collective name for the sensory organs of hearing (cochlea) and equilibrium (sacculle, utricle, and semicircular canals) which are interconnected (figure 3). Both these sensory organs are composed of two hollow spaces. The first space, the endolymphatic compartment, is bounded by membranes and contains the sensory cells for hearing and equilibrium. This membranous endolymphatic system is a series of communicating structures which are called: the cochlear duct, ductus reuniens, sacculle, utricle, utriculo-saccular duct, semicircular canals, endolymphatic duct and the endolymphatic sac. The second space, the perilymphatic compartment, surrounds the endolymphatic space and is bordered by the bony otic capsule. The perilymphatic system comprises a series of continuous osseous cavities: the cochlea, the vestibule, and the semicircular canals.



The two spaces are filled with fluid. The endolymphatic compartment is filled with endolymph in contradistinction to the perilymph present in the perilymphatic space. Endolymph contains a very high concentration of potassium and a very low concentration of sodium, which is exactly opposite to the perilymph. An electrical potential of approximately + 80 millivolts exists all the time between the endolymph and perilymph, with positivity inside the endolymphatic space and negativity outside. This is called the endocochlear potential that sensitises the sensory cells of the inner ear.

The endolymphatic sac is assumed to perform *volume* regulation of the endolymphatic compartment with a greater capacity than other parts of the endolymphatic system. In 1927 Guild published his work on the longitudinal flow of endolymph towards the endolymphatic sac (8). Since then endolymph absorption was considered the only function of the endolymphatic sac for a long time. However today we know that its function is probably more complicated and that the sac may also be capable of producing endolymph next to absorbing endolymph (9).

In 1938 there seemed to have occurred a breakthrough in understanding MD with the discovery of *endolymphatic hydrops* (EH) in temporal bones from patients with MD. EH is a post-mortem histopathological finding in the inner ear which can be best described as a situation wherein the endolymphatic spaces in the inner ear are dilated at the expense of the perilymphatic spaces (figures 4A and 4B). A British research group (Hallpike and Cairns (10)) and a Japanese scientist (Yamakawa (11)) found this pathological condition in MD, and they

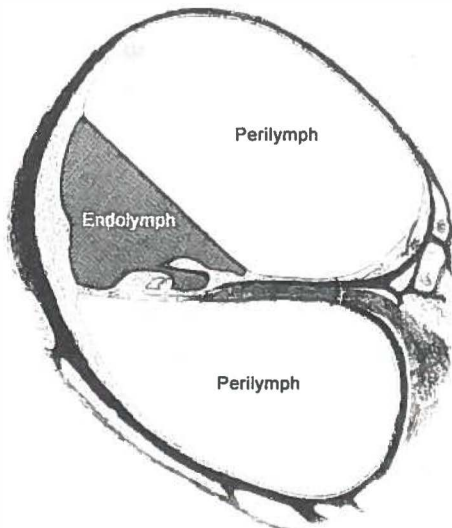


Fig. 4A. Schematic section through a normal cochlea.

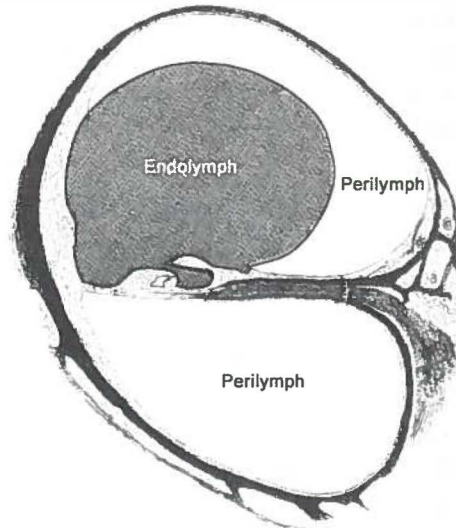
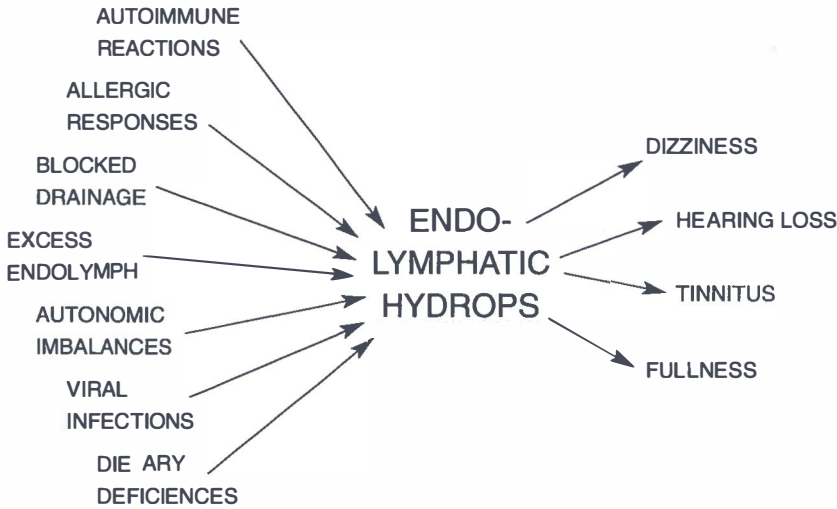


Fig. 4B. Schematic section through a cochlea with endolymphatic hydrops.



*Fig. 5. The central dogma for Menière’s disease. At the left is a partial list of factors that have, at one time or another, been suggested as leading to, or contributing to the development of endolymphatic hydrops. The dogma is that the symptoms arise from endolymphatic hydrops or its sequelae so that the common final path for the multiple etiologies is endolymphatic hydrops. On the right side is a list of the symptoms associated with Menière’s disease (14).*

published their findings at almost the same time. It appears that neither Yamakawa nor Hallpike and Cairns had any knowledge of each other’s work. They all had derived the term EH from Wittmaack’s description (1926) of a similar type of inner ear reaction ‘hydrops labyrinthi’ to toxins (12).

EH is found in almost all patients with MD (13, 14), but there are also reports of cases of EH who did not display the symptoms of MD (13). In that EH is not always present or solely limited to MD, and add to this that laboratory animals with EH do not show the symptoms of MD, some investigators question if it is an epiphenomenon (15). However, it is the most striking and consistently found pathologic lesion in patients with MD. Therefore, and because there is still no alternative correlate, exploration of physiological mechanisms underlying this pathological substrate is inevitable.

EH and its presumed sequelae are *thought of* as generating the symptoms of MD, but the hydrops itself could be produced in many ways and be influenced by a number of factors that, theoretically at least, affect fluid management in the inner ear (figure 5).

The origin of EH is commonly believed to be the result of an overaccumulation of endolymph produced in the labyrinth, or in a failure of the endolymphatic sac to absorb the excess volume.

The most popular hypothesis concerning the pathophysiology of MD until today is that the EH causes *endolymphatic hypertension* (10, 16-18), which is a higher hydrostatic pressure in the endolymphatic compartment as compared to the perilymphatic space. This seems a reasonable supposition since the balloonlike distension of the boundary membranes of the endolymphatic space creates an impression of a compartment suffering from increased pressure.

Earlier in 1871 Knapp already suggested that an increased inner ear pressure might be the basis for the pathophysiology of MD (19). In 1927, Portmann even tried to alleviate the inner ear pressure in patients with MD via opening of the endolymphatic sac in a transmastoid procedure (20). Remember that this was in a time where no pressure measurements in the inner ear had ever been done and where the pathological finding of endolymphatic hydrops in patients with MD was not made until more than 10 years later. Knapp and Portmann made no distinction between endolymphatic hypertension and increased inner ear pressure. Increased inner ear pressure means that the pressure in both compartments of the inner ear has equally increased as opposed to endolymphatic hypertension where the pressure in the endolymphatic compartment is higher as compared to the perilymphatic compartment.

Endolymphatic hypertension in the closed endolymphatic compartment is dependent on the *mechanical compliance* of the boundary membranes of the endolymphatic space and on the fluid volume. If the walls of the fluid compartment are rigid (low compliance) and because the fluid is incompressible, a small increase of volume results in a large increase in pressure. If the walls of the fluid compartment have a high compliance, an increase of the fluid volume distends them and then a volume increase need not be accompanied by a substantial pressure increase.

Endolymphatic hypertension could displace the basilar membrane from its resting position (21) or reduce the bloodflow to the organ of Corti (22), thereby impairing cochlear function (hearing). It is proposed to produce vestibular dysfunction via displacement of the cupula (23). Hair cells may become partially decoupled from the tectorial membrane when the pressure in the endolymphatic space rises. There is a chance that this causes an elevation in the noise level at the hair cell input, the basis of cochlear tinnitus (16). It seems natural that a sense of aural fullness might accompany endolymphatic hypertension during EH. Moreover there is evidence for the presence of neuroreceptors in membranes of the inner ear that may lead to that feeling (24).

All the above mentioned theories have not been proven until now. Nonetheless the large impact of such hypotheses is made obvious by the fact that elimination of this hypertension is one of the therapeutic cornerstones of MD (17).

All empirical research concerning pressure measurements during EH, in order to identify endolymphatic hypertension, is based on animal models. The inner ear in humans is for obvious reasons not available for such experiments.

In 1965 Kimura developed the almost exclusively used animal model for EH, in guinea pigs, by surgical obliteration of the endolymphatic sac and duct. Damage to these structures causes an increase in endolymph volume, i.e., endolymphatic hydrops (25).

Naito was the first to develop EH in guinea pigs, however his surgical technique had a much lower percentage of success for inducing hydrops than Kimura's model (26). Attempts to induce hydrops in other animals like monkeys, cats, and rats failed or had limited success (27-29).

Interestingly, as mentioned earlier, animals with endolymphatic hydrops have never been shown to have signs of vestibular disturbances. Thus, one may conclude that animal models are useful because they provide insight into the (patho)physiology of inner ear fluid balance and its relationship to endolymphatic hydrops but maybe not into MD (30).

Pressure recordings are possible in the cochlea of animals, but still impose important technical problems, the difficulties being the tiny dimensions of the inner ear fluid compartments.

Inner ear hydrostatic pressure measurements were first attempted by Szász in 1925, who advanced glass capillaries with internal diameters of 0.50 to 0.75 mm through the round window into the inner ear of dogs, and measured the displacement of the fluid column in the capillary, which reflected the relative, but not the absolute, pressure (31). Several decades later Weille et al., Martinez and Beentjes (32-34) used microcanulas with an internal tip diameter of about 20-30 micrometer connected to a capacitance electromanometer. These experiments using the electromanometer found high pressures and contradicting results concerning the pressure gradient between the two compartments of the inner ear. This was probably due to the still relatively large recording device, which caused displacement of inner ear fluid.

In 1964 reliable pressure measurements in small volumes became possible with the introduction of the servocontrolled micropipette system (SCMS), which was developed for measuring bloodpressure in small mesenteric veins of the frog (35). It was not until 1975 that the SCMS was used for measuring the pressure in the inner ear (36). This device measures the perilymphatic or endolymphatic pressure through a small micropipette tip with negligible volume displacement. Today this device is still the standard for measuring hydrostatic pressure in limited volume compartments.

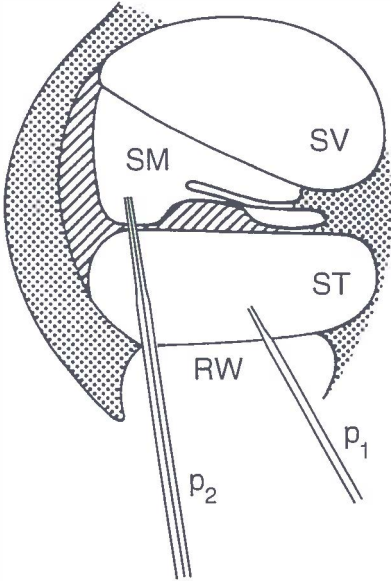
The SCMS works basically as follows. A micropipette links a small (inner ear) fluid compartment in which pressure has to be recorded with a large fluid compartment fitted with pressure transducer and a pump. The latter can rapidly change the pressure in the large compartment. A high molar electrolyte solution in the large compartment interfaces at the tip of the micropipette with the low molar electrolyte solution (endolymph or perilymph) in the small compartment. Small shifts of the high molar-low molar interface in the tip of the pipette allow for converting pressure differences between the two compartments in an electrical loop

between the two compartments in an electrical signal (change in resistance). The resistance at the electrical loop between the small compartment with low electrolyte concentration and the high molar solution in the micropipette depends on the position of the interface between high and low molar electrolyte solutions in the pipette tip. When the pressure in the small compartment increases, lower molar fluid penetrates into the micropipette and the resistance starts to increase. This signal is used as an error signal for the servo-controlled pump to increase pressure in the large compartment to the same level. Thus the pressure in the large compartment, easily recorded with a pressure transducer, represents the pressure in the small compartment. In addition, essential volume displacements in the small compartment are prevented. This system records only changes in hydrostatic pressure. A reference is needed to indicate absolute values of inner ear fluid pressure. The most easily available reference is the hydrostatic pressure in a fluid open to the atmosphere with the surface at the level of the inner ear, for instance, a drop of Ringer solution on the round window membrane (37).

In the beginning of the era of pressure measurements in the inner ear, perilymphatic and endolymphatic pressure were measured in succession using only one SCMS (38, 39). Through the round window membrane, the tip of the micropipette was inserted into the perilymphatic space, where pressure was measured. After that the basilar membrane was perforated, whereupon the pressure was measured in the endolymphatic space. This research method is vulnerable to inaccuracy for detecting endolymphatic hypertension, because during the time interval between measurements in both compartments, a pressure change could have taken place. Physiological pressure changes, which are known to occur in both compartments due to respiration and heartbeat (36), may have been responsible for such an inexact measurement.

Several years later this flaw in experimental set-up was solved by using two SCMSs. By that it is possible to insert a micropipette in both inner ear compartments, and then measure the perilymphatic and endolymphatic pressure at the same time (40).

The newest development in the search for endolymphatic hypertension during EH is the use of a double-barrelled micropipette in the endolymphatic space (41, 42) (figure 6). It replaces the single-barrelled pipette in the endolymphatic system that is mentioned above. One barrel of the pipette is used to measure pressure. Through the other barrel artificial endolymph can be injected into the endolymphatic compartment. Next to the double-barrelled pipette, a single-barrelled pipette is used to measure perilymphatic pressure. Both pipettes are connected to a SCMS. The double-barrelled pipette delivers two new parameters: volume increase of the endolymphatic space and the rate of volume increase. The volume increase can be obtained from the displacement of the fluid meniscus in the pipette, for which the inner diameter is precisely known. The rate of volume increase is calculated as the total injected volume divided by the total injection time. The greatest benefit of gaining these two



*Fig. 6. Position of the micropipette tips. The single barrel pipette measures perilymphatic pressure ( $p_1$ ) in scala tympani (ST). The double-barrelled pipette injects artificial endolymph into the endolymphatic space (scala media = SM), and measures endolymphatic pressure ( $p_2$ ). (RW = round window; SV = scala vestibuli).*

parameters next to the inner ear pressure, is that now the mechanical compliance of the boundary membranes of the endolymphatic compartment can be calculated in vivo for the first time. The compliance delivers crucial knowledge to the question if endolymphatic hypertension can occur during EH.

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## Chapter 3

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### Perilymphatic and endolymphatic pressure in the normal guinea pig

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Warmerdam TJ, FHHJ Schröder, Wit HP, Albers FWJ. Perilymphatic and endolymphatic pressure in the normal guinea pig. *ORL J Otorhinolaryngol Relat Spec* 1999;61(2):71-73



## **Introduction**

Elucidation of intralabyrinthine fluid regulation is necessary to understand the pathophysiology of signs and symptoms occurring after a change in hydrostatic inner ear pressure. Endolymphatic hydrops is a consistent finding in patients with Menière's disease. Endolymph homeostasis is postulated to be disturbed, causing a volume increase and an associated pressure rise in the endolymphatic compartment, thus leading to inner ear symptoms. Human studies have shown that a presumed increase (via raised intracranial pressure [1]) or decrease (e.g. after cerebrospinal fluid loss [2-4] or a perilymph fistula [5]) in inner ear pressure causes hearing thresholds to shift upward. An animal study by Yoshida et al. [6] revealed a small but reversible suppression of the cochlear microphonic potential during variation of endolymphatic and perilymphatic pressure.

The measurement of the hydrostatic fluid pressures of the cochlear compartments in the guinea pig has been subject of several investigations. Recent values for perilymphatic pressure range from 1.2 cm H<sub>2</sub>O to 4.7 cm H<sub>2</sub>O [7-12], while hydrostatic pressures in the scala media range from 1.2 cm H<sub>2</sub>O to 4.1 cm H<sub>2</sub>O [7-12]. Most studies recorded no significant pressure difference between the endolymphatic compartment and the perilymphatic space.

The present study was designed to measure perilymphatic and endolymphatic pressure in the inner ear of the guinea pig and to obtain normative values for further research on inner ear pressure regulation under variable conditions.

## **Material and methods**

Successful experiments were performed in 10 female albino guinea pigs (Harlan Laboratories U.K.; 250 g bodyweight), under general anesthesia induced by intramuscular administration of ketamine (Ketalar®, Parke-Davis; Hoofddorp, The Netherlands; 60 mg/kg) and xylazine (Rompun®, Bayer, Mijdrecht, The Netherlands; 3.5 mg/kg). During the experiments the animals were breathing spontaneously and situated in a prone position on a heating pad, which kept the body temperature at approximately 38°C. Animal care and use were approved by the Experimental Animal Committee of the Groningen University, protocol number 0940 - 1194 / 1295, in accordance with the principles of the Declaration of Helsinki.

The animal's head was secured in a stationary position with the contralateral ear down by means of a steel bolt fixed to the skull with dental cement. The bulla was opened by a retroauricular approach and the round window exposed. The middle ear was filled with saline until just above the round window membrane and a reference electrode of the pressure recording system was placed in the musculature of the neck. With the aid of a operating microscope and a micromanipulator a bevelled micropipette with a tip diameter of 10 μm was

advanced into the saline on the round window, where a reference pressure was measured. The pipette was then inserted through the round window into the scala tympani where the perilymphatic pressure was recorded, and subsequently through the basilar membrane into the scala media where the endolymphatic pressure was measured. Simultaneously the DC potential was recorded to verify the location of the pipette tip.

The pipette was connected to a WPI 900A micropressure system (World Precision Instruments, Inc., Sarasota, USA) which has been designed to measure hydrostatic pressure in limited volume compartments, with an accuracy of approximately 1 mm H<sub>2</sub>O. Pressure calibration was done by lowering the micropipette 1 cm in an electrically grounded beaker of normal saline.

Both hydrostatic pressure and electric potential were read from digital displays and were also recorded as analog signals on a polygraph.

Statistical calculations were applied to the data using the paired-samples t-test (SPSS for Windows 7.0).

## Results

In 10 guinea pigs the mean perilymphatic pressure was 2.26 cm H<sub>2</sub>O (SD = 1.25 cm H<sub>2</sub>O) and mean endolymphatic pressure was 2.31 cm H<sub>2</sub>O (SD = 1.25 cm H<sub>2</sub>O). The perilymphatic pressure was not statistically different from the endolymphatic pressure ( $P > 0.17$ ).

The largest difference in pressure between the scala tympani and scala media in the same guinea pig was 0.3 cm H<sub>2</sub>O. The difference in pressure between animals was much larger, with the perilymphatic pressure varying with as much as 3.5 cm H<sub>2</sub>O and the endolymphatic pressure with 3.7 cm H<sub>2</sub>O (figure 1).

## Discussion

Inner ear hydrostatic pressure measurements were first attempted by Szász in 1925 [13], who advanced glass capillaries with internal diameters of 0.50 to 0.75 mm through the round window into the inner ear of dogs, and measured the displacement of the fluid column in the capillary, which reflected the relative, but not the absolute, pressure. Several decades later Weille et al. [14] used microcanulas with an internal tip diameter of about 20-30 micrometer connected to a capacitance electromanometer. They found a normal perilymphatic pressure of 34.0 cm H<sub>2</sub>O and a normal endolymphatic pressure of 11.6 cm H<sub>2</sub>O, and concluded that the perilymphatic pressure was higher than the endolymphatic pressure. Martinez (1968) [15], in an extensive experiment on 1300 guinea pigs, also found a pressure difference between the

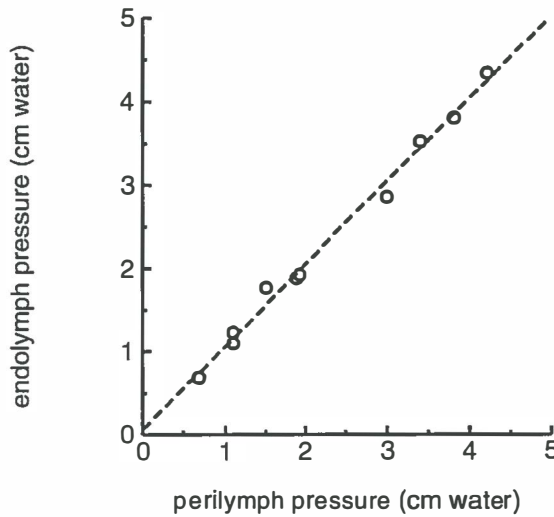


Figure 1. Endolymphatic pressure versus perilymphatic pressure for individual animals ( $n = 10$ ). Dashed line: least squares fit with  $y = ax + b$ , yielding  $a = 0.997$ ,  $b = 0.059$ .

perilymphatic and endolymphatic compartment, although his mean pressure values were lower than the values found by Weille et al.. Beentjes [16] in 1972, also using the same electromanometer with cats, was the first to measure no pressure gradient between scala media and scala tympani. His mean pressure value for both compartments was 14 cm H<sub>2</sub>O.

In summary, experiments using the electromanometer found high pressures and contradictory results concerning the pressure gradient between the two compartments of the inner ear. This was probably due to the relatively large recording device, which caused displacement of inner ear fluid.

Reliable measurements of inner ear pressure became possible with the introduction of the servocontrolled micropipette system (SCMPS). This system measures the perilymphatic or endolymphatic pressure through a small micropipette tip without volume displacement [17,18].

Inner ear pressures recorded in normal guinea pigs with the use of a SCMPS are summarised in table 1. As shown in this table, the perilymphatic and endolymphatic pressures measured in our laboratory are within the spectrum of values recorded in other studies.

Furthermore we found no pressure difference between perilymph and endolymph, also in accordance with the results found by others. That such a pressure difference does indeed not exist was convincingly demonstrated by Takeuchi et al. [19], who used two SCMPS simultaneously.

The consistent data from our study and reported experiments in literature form a solid

**Table 1.** Measured perilymphatic and endolymphatic pressure compared with values in literature. The fifth column shows if a pressure difference was found between the two compartments. The measurement systems are either home-made servo-controlled (“servo”) or commercially available (“WPI”) using the same principle.

PLP = perilymphatic pressure. ELP = endolymphatic pressure.

All numbers are given with the same accuracy to facilitate comparison.

author(s)	PLP (cmH <sub>2</sub> O)	ELP (cmH <sub>2</sub> O)	n PLP/ELP	difference PLP/ELP	method
<b>Feldman et al., 1979</b>	4.7 (± 0.4)	3.3 (± 0.6)	5/5	unknown	servo
<b>Yoshida and Lowry, 1984</b>	4.4 (± 1.2)	4.1 (± 0.8)	5/5	no	servo
<b>Long and Morizono, 1987</b>	2.6 (± 1.8)	2.7 (± 2.2)	25/25	no	WPI
<b>Nakashima et al., 1987</b>	2.5 (± 1.0)	2.5 (± 1.0)	9/9	no	WPI
<b>Warmerdam et al., 1998</b>	2.3 (± 1.3)	2.3 (± 1.3)	10/10	no	WPI
<b>Böhmer and Andrews, 1989</b>	2.0 (± 0.7)	2.0 (± 0.7)	10/10	no	servo
<b>Andrews et al., 1991</b>	1.2 (± 2.9)	1.2 (± 3.0)	10/10	no	servo

fundament for further experimental investigations of inner ear fluid regulation under pathological conditions.

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## **Chapter 4**

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### **Perilymphatic and endolymphatic pressure during endolymphatic hydrops**

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## **Introduction**

The purpose of measuring the fluid pressure in an endolymphatic hydrops animal model, is to gain more insight into the unknown pathophysiology of Menière's disease (MD), which is a chronic illness characterized by disabling attacks of hearing loss, tinnitus, vertigo and a sensation of aural fullness. The underlying mechanism by which this disease generates its symptoms appears complex, and is still not understood, despite the fact that it has been the subject of much investigation since the disease was first documented in 1861[15]. A major breakthrough in understanding seemed to have occurred in 1938 [8], when EH, which is a larger than normal volume of endolymph, was visualized for the first time in the inner ear of deceased patients but caution here should be noted [11]. Later temporal bone studies confirmed that this histopathological finding at death was apparent in 93 - 100% of the cases [16,23].

Since its discovery, EH has been the source of many hypotheses concerning the pathophysiology of MD, some of them having a biochemical basis [9], whereas others are grounded on mechanical principles [19,20]. We have concentrated on the latter group, and this is the subject of our study.

A popular mechanical supposition declares that the balloonlike distension of the membranous labyrinth in EH causes endolymphatic hypertension, which is a positive pressure difference between the endolymphatic and perilymphatic compartments. This pressure difference, which is dependent on the mechanical compliance of the membranous labyrinth, could displace the basilar membrane from its resting position, thereby impairing cochlear function [13,19], and may also produce vestibular dysfunction via displacement of the cupula [20]. The impact of such hypotheses is made obvious by the fact that elimination of this overpressure is one of the therapeutic cornerstones of MD [3]. However, the empirical evidence for endolymphatic hypertension during EH is weak because some investigators report finding hypertension [4] and others do not [14].

Therefore, the aim of our study was to measure if endolymphatic hypertension occurs during endolymphatic hydrops, which was surgically induced in the inner ear of the guinea pig, using the well known operation designed by Kimura [12] which leads to a decrease in endolymph absorption.

## **Materials and methods**

Successful experiments were performed on 8 out of 12 female albino guinea pigs (Harlan Laboratories U.K.; 250 g body weight). An experiment was considered to be successful when both perilymphatic and endolymphatic pressure could be measured in an ear that developed

EH. Animal care and use were approved by the Experimental Animal Committee of Groningen University, protocol number 1061, in accordance with the principles of the Declaration of Helsinki.

The animals were operated on twice and inner ear pressure was measured after the second operation.

The first operation was performed on the left ear under sterile conditions with the aid of a Zeiss operation microscope. Anaesthesia was used with a combination of halothane (Fluothane®, Zeneca; Ridderkerk, the Netherlands; 1%), oxygen (0.3 litre/min) and N<sub>2</sub>O (0.6 litre/min). During surgery, body temperature was stabilized using an electrically heated pad. Through an extradural posterior approach, the endolymphatic sac and distal part of the endolymphatic duct were removed and the created space was filled with bonewax.

The second operation, done 3 months later on both ears, was carried out directly before the pressure measurements, under general anesthesia induced by intramuscular administration of ketamine (Ketalar®, Parke-Davis; Hoofddorp, the Netherlands; 60 mg/kg) and xylazine (Rompun®, Bayer; Mijdrecht, the Netherlands; 3.5 mg/kg). The head of the animal was secured in a stationary position by means of a steel bolt fixed to the skull with dental cement, with the contralateral ear downwards. The bulla was opened by a retroauricular approach and the round window was exposed. The middle ear was filled with saline until just above the round window membrane, and a reference electrode of the pressure recording system was placed in the musculature of the neck. With the aid of an operating microscope and a micromanipulator, a bevelled micropipette with a tip diameter of 10 micrometer was advanced into the saline on top of the round window, where the reference pressure was measured. The pipette was then inserted through the round window into the scala tympani, where the perilymphatic pressure was recorded, and subsequently, through the basilar membrane into the scala media, where the endolymphatic pressure was measured. Simultaneously, the DC potential was recorded to verify the location of the pipette tip.

The pipette was connected to a WPI 900A micropressure system (World Precision Instruments Inc., Sarasota, Fla., USA), which is designed to measure pressure in limited volume compartments, with an accuracy of approximately 1 mm H<sub>2</sub>O. The system was calibrated by lowering the micropipette by 1 cm into normal saline.

Both pressure and electric potential were read from digital displays and were also recorded as analog signals on a polygraph. The right ears were controls.

During the experiments, the animals were breathing spontaneously and were placed in a prone position on a heated pad, which kept the body temperature at approximately 38 °C.

After the pressure measurements were concluded all the animals were killed with Pentobarbital (6mg/kg) via intraperitoneal injection. After decapitation, the bullae were removed and opened. The stapes was extracted and a hole was made in the apex of the cochlea near the helicotrema. The oval and round windows were perforated. Fixative was

perfused through the orifice in the apex. The entire procedure was completed within 4 minutes. The specimens were immersed in fixative: 2.5% glutaraldehyde, buffered in 0.1M cacodylate at pH 7.4. The specimens were decalcified in 10% EDTA, pH 7.4, and then postfixed in 1% OsO<sub>4</sub> with 1% K<sub>4</sub>Ru(CN)<sub>6</sub>. They were then rinsed in distilled water; dehydrated in a graded ethanol series followed by propylene oxide; infiltrated with a mixture of 1:1 propylene and Spurr's low viscosity resin for two hours; and finally with the pure resin overnight. Polymerization took place at 70 °C after exsiccation in a vacuum. The cochleas were cut in the midmodiolar plane and stained with toluidine blue. The hydrops was rated according to Sperling et al. [17] as: (1) slight hydrops: bulging of Reissner's membrane without contact with the bony wall of the scala vestibuli; (2) moderate hydrops: displacement of Reissner's membrane with contact with the bony wall but with an angle of less than 90° with the osseous spiral lamina; (3) severe hydrops: displacement of Reissner's membrane with bony contact that makes an angle of more than 90° with the spiral lamina.

Statistical calculations were applied to the pressure data using the paired samples t-test (SPSS for Windows 10.0).

## **Results**

In eight of the twelve hydropic ears, endolymphatic and perilymphatic pressure was measured. In the other four ears both types of pressures could not be measured due to complications such as broken micropipettes or blockage of the micropipette orifice with inner ear tissue.

In these eight ears the hydrops score was as follows: one slight, three moderate and four severe hydrops.

In the control ears, average endolymphatic pressure (ELP) was 2.3 cm H<sub>2</sub>O (range: 0.5 – 3.9 cm H<sub>2</sub>O; standard deviation (SD) = 1.0 cm H<sub>2</sub>O), and mean perilymphatic pressure (PLP) was 2.2 cm H<sub>2</sub>O (range: 0.5 – 3.9 cm H<sub>2</sub>O; SD = 1.1 cm H<sub>2</sub>O). The average ELP in these ears was statistically not different from the average PLP ( $p = 0.15$ ).

The results for the ears with EH are given in table 1. In these ears, the average ELP was 2.6 cm H<sub>2</sub>O (range: 1.9 – 3.3 cm H<sub>2</sub>O; SD = 0.5 cm H<sub>2</sub>O), the mean PLP was 2.6 cm H<sub>2</sub>O (range: 1.9 – 3.2 cm H<sub>2</sub>O; SD = 0.5 cm H<sub>2</sub>O). The average endolymphatic hypertension (ELP minus PLP) was 0.4 mm H<sub>2</sub>O (SD = 1.3 mm H<sub>2</sub>O). This value was statistically not different from zero ( $p = 0.48$ ).

The mean endocochlear potential (EP) in hydropic ears was 54 mV (SD = 25.6 mV) and 77 mV in control ears (SD = 14.3 mV). The value for EP in a hydropic ear was on average 73% (SD = 33%) of the value for EP in the control ear of the same animal (table 1). This value is statistically smaller than 100% ( $p > 0.99$ ).

Table 1. Results for hydroptic ears. The perilymphatic (PLP) and endolymphatic pressure (ELP) are given in centimetres water (cmH<sub>2</sub>O), the endocochlear potential (EP) in millivolts (mV) and the endolymphatic hydrops score as: sl = slight, mod = moderate, sev = severe.

Hydroptic ear #	PLP (cmH <sub>2</sub> O)	ELP (cmH <sub>2</sub> O)	EP (mV)	Hydrops score
1	3.0	3.3	66	mod
2	3.1	2.9	74	sl
3	2.5	2.6	67	mod
4	2.9	2.9	86	sev
5	2.1	2.1	38	mod
6	1.9	1.9	4	sev
7	2.1	2.2	44	sev
8	3.2	3.2	55	sev

## Discussion

Within the accuracy of our experimental setup we found no statistically significant endolymphatic hypertension during distinctly present EH. When reviewing the literature, eight papers from four research groups were found, giving results of inner ear fluid pressure measurements during EH [2,4-6,10,14,21,this study]. Five papers [2,4-6,10] reported endolymphatic hypertension and three [14,21,this study] did not, showing a remarkable discrepancy in the interpretation of the measurements in these studies. EH is a higher than normal endolymph volume. Up to now the exact mechanism for the creation and maintenance of EH is unknown. Possibly osmotic processes play a role. It is for instance possible to induce pressure differences in the cochlea by manipulating the osmolality of artificial perilymph [14]. Whether the increased endolymph volume leads to endolymphatic hypertension depends on the compliance of the membranes surrounding the endolymphatic compartment [23].

The average EH score in our experiments was “moderate”, which is in accordance with the creation of an acute pressure increase in the scala media [22] in the order of 10 Pa (= 1 mm H<sub>2</sub>O). This value is on the border of our measurement accuracy, which is approximately 1 mm H<sub>2</sub>O, and does not disagree with our average measured endolymphatic hypertension of 0.4 mm H<sub>2</sub>O. It is thus not unlikely that there was a slight overpressure during EH, which was however undetectable with our micropressure measurement system. On the other hand, the long duration of the EH, which was several months, may have caused the prolonged distended membranous labyrinth to have lost its elasticity, which would then be unable to sustain any pressure gradient across itself.

In this study we also compared the endocochlear potential between ears with EH and control ears. We found that the EP was statistically significantly decreased in ears with EH. There are many studies that confirm this finding [2,4-6,14,21]. It is generally accepted that the

marginal cells of the stria vascularis are responsible for the generation of the EP via an electrogenic transport of potassium into the scala media. In addition, histological studies have reported damage to the marginal cells during EH [1]. Therefore the EP decline could be explained on the basis of dysfunctional marginal cells.

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## Chapter 5

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### **Perilymphatic and endolymphatic pressure in the guinea pig after distal dissection of the endolymphatic sac**

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Warmerdam TJ, Schröder FHHJ, Wit HP, Albers FWJ. Perilymphatic and endolymphatic pressure after distal dissection of the endolymphatic sac. *Otol Neurotol* 2001;22(3):373-376



## **Introduction**

Endolymphatic hydrops (EH) is the most evident and recurrent histological finding in Menière's disease. This has led to the widespread hypothesis that hydrops generates the clinical symptoms of this illness. Many factors have been proposed as leading to EH. These have included decreased endolymph absorption (via diminished function of the endolymphatic duct (ED) and sac (ES)), overproduction of endolymph or a combination of both. Decreased endolymph absorption is the most investigated supposition and is also the main target of our study. Histological (1,2) and radiological studies (3), although not all (4), show that the vestibular aqueduct in Menière patients is thin or difficult to visualize. Narrowing of the bony canal, which houses the ED and partly the ES, could compromise the resorptive function of the ED and ES. Histological publications (2,4-7) on temporal bones from Menière patients describe pathologic changes, such as reduced vascularization and fibrosis in the perisaccular tissue, which may be responsible for reducing the resorptive capacity of the ES. In most cases, these studies report a normal or reduced lumen of the ED and ES, and sometimes an obstruction. Furthermore, EH develops in guinea pig models (8) with an obliterated ED and ES.

The difference between the clinical situation and the guinea pig model draws attention, because in the majority of Menière patients, the ED and ES are patent, while in the animal model they are not. This means that the widely used guinea pig model lacks the functional contribution of the endolymphatic sac.

In normal guinea pigs (12-18), perilymphatic pressure is identical to that of endolymph. The extension of the membranous labyrinth in endolymphatic hydrops is thought to be the result of endolymphatic hypertension (9-11). In five (16, 17, 19-21) of six articles (14, 16, 17, 19-21) that can be found regarding pressure changes in the cochlea during hydrops, a significantly higher endolymphatic than perilymphatic pressure is reported.

The present study was designed to evaluate if endolymphatic pressure exceeds perilymphatic pressure in EH, in a guinea pig model (described by Dunnebier et al (22)) that further approximates the morphopathology of Menière's disease as compared to the widely used animal model, by only partially damaging the endolymphatic sac and leaving the ED patent.

## **Material and methods**

Successful experiments were performed on 8 out of 19 female albino guinea pigs (Harlan Laboratories U.K.; 250 g body weight). An animal experiment was considered successful when perilymphatic and endolymphatic pressure could both be measured in an ear that developed

EH. Animal care and use were approved by the Experimental Animal Committee of the University of Groningen, protocol number 1061, in accordance with the principles of the Declaration of Helsinki.

The animals were operated on twice, inner ear pressure was measured after the second operation. The first operation was performed under sterile conditions with the aid of a Zeiss operation microscope. Anesthesia was used with a combination of halothane (Fluothane®, Zeneca; Ridderkerk, the Netherlands; 1%), oxygen (0.3 liter / min) and N<sub>2</sub>O (0.6 liter / min). During surgery, body temperature was stabilized, using an electric heating pad. Through an extradural posterior approach, the distal part of the endolymphatic sac of the left ear was dissected from the sigmoid sinus, and a small sheet of latex was inserted in the space created between the endolymphatic sac and the sigmoid sinus. The second operation, 3 (n = 5) and 6 months (n = 3) later, was carried out under general anesthesia induced by intramuscular administration of ketamine (Ketalar®, Parke-Davis; Hoofddorp, the Netherlands; 60 mg/kg) and xylazine (Rompun®, Bayer; Mijdrecht, the Netherlands; 3.5 mg/kg). During the experiments, the animals were breathing spontaneously and were placed in a prone position on a heating pad, which kept the body temperature at approximately 38°C.

The head of the animal was secured in a stationary position by means of a steel bolt fixed to the skull with dental cement, with the contralateral ear down. The bulla was opened by a retroauricular approach and the round window was exposed. The middle ear was filled with saline until just above the round window membrane, and a reference electrode of the pressure recording system was placed in the musculature of the neck. With the aid of an operating microscope and a micromanipulator, a bevelled micropipette with a tip diameter of 10 μm was advanced into the saline on the round window, where the reference pressure was measured. The pipette was then inserted through the round window into the scala tympani, where the perilymphatic pressure was recorded, and, subsequently, through the basilar membrane into the scala media, where the endolymphatic pressure was measured. Simultaneously, the DC potential was recorded to verify the location of the pipette tip.

The pipette was connected to a WPI 900A micropressure system (World Precision Instruments Inc., Sarasota, Fla., USA), which was designed to measure hydrostatic pressure in limited volume compartments, with an accuracy of approximately 1 mm H<sub>2</sub>O. The system was calibrated by lowering the micropipette 1 cm into an electrically grounded beaker of normal saline.

Both hydrostatic pressure and electric potential were read from digital displays and were also recorded as analog signals on a polygraph. The right ears were controls.

After the pressure measurements, all animals were killed with Pentobarbital (6 mg/kg) via intra-peritoneal injection. After decapitation, the bullae were removed and opened. The stapes was extracted and a hole was made in the apex of the cochlea near the helicotrema. The oval and round window were perforated. Fixative was perfused through the orifice in the apex. The

entire procedure was completed within 4 minutes. The specimens were immersed in fixative: 2.5% glutaraldehyde, buffered in 0.1 M cacodylate at pH 7.4. The specimens were decalcified for 5 days in 10% EDTA, pH 7.4, postfixed in 1% OsO<sub>4</sub> with 1% K<sub>4</sub>Ru(CN)<sub>6</sub> for 2-3 hours, rinsed in distilled water, dehydrated in a graded ethanol series followed by propylene oxide, infiltrated with a mixture of 1:1 propylene oxide and Spurr's low viscosity resin for two hours and finally with pure resin overnight. Polymerization took place at 70°C after exsiccation in a vacuum. The cochleas were cut in the midmodiolar plane and stained with toluidine blue. The hydrops was rated according to Sperling et al. as: (1) slight hydrops: bulging of Reissner's membrane without contact with the bony wall of the scala vestibuli; (2) moderate hydrops: displacement of Reissner's membrane with contact with the bony wall but with an angle of less than 90° with the osseous spiral lamina; (3) severe hydrops: displacement of Reissner's membrane with bony contact that makes an angle of greater than 90° with the spiral lamina.

Statistical calculations were applied to the data using the paired and independent samples t-test (SPSS for Windows 8.0).

## **Results**

Eleven of the 19 guinea pigs (58%) developed EH, of which two had moderate and nine had severe hydrops.

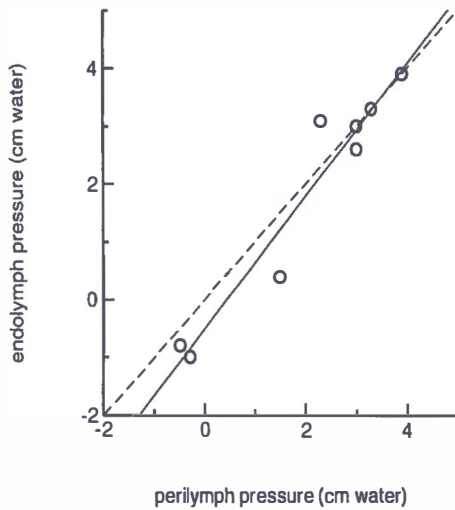
In 8 (one moderate and seven severe hydrops) of eleven hydroptic ears, endolymphatic and perilymphatic pressure were measured. In the other three ears both pressures could not be measured due to complications such as blockage of the micropipet orifice with inner ear tissue.

In all control ears, endolymphatic pressure equalled perilymphatic pressure (within the accuracy of the measuring apparatus). In these ears, the average pressure was 2.1 cm H<sub>2</sub>O (range: 0.0 - 4.9 cm H<sub>2</sub>O; SD = 1.6 cm H<sub>2</sub>O).

The results for dissected ears with EH are given in figure 1. In these ears, the average perilymphatic pressure (PLP) was 2.0 cm H<sub>2</sub>O (range: -0.5 - 3.9 cm H<sub>2</sub>O; SD = 1.7 cm H<sub>2</sub>O) and the average endolymphatic pressure (ELP) was 1.8 cm H<sub>2</sub>O (range: -0.8 - 3.9 cm H<sub>2</sub>O; SD = 2.0 cm H<sub>2</sub>O). The PLP in control ears was not significantly different from the PLP in hydroptic ears and the ELP in control ears was also not significantly different from its corresponding pressure in dissected ears with EH. There was no pressure difference between the endolymphatic and perilymphatic compartment in hydroptic ears ( $P > 0.33$ ).

Statistical comparison of pressures that were measured 3 or 6 months after distal dissection was not possible because the groups were too small.

In all successful pressure measurements ( $n = 5$ ) in operated ears that did not develop EH, PLP and ELP were even, and in the same range as in control ears and ears with EH.



*Figure 1. Measured perilymphatic and endolymphatic pressure in ears with endolymphatic hydrops. Solid line: best fit to data points with straight line. Dashed line: endolymphatic pressure equals perilymphatic pressure.*

The mean endocochlear potential (EP) in hydropic ears was 57 mV ( SE = 4.47 mV) and 81 mV ( SE = 2.24 mV) in control ears. The difference between the potentials was statistically significant (  $P < 0.002$  ). The mean EP in the 8 ears that were operated on, but did not develop EH, was 76 mV. This was statistically significantly higher ( $P < 0.18$ ) than the 57 mV in hydropic ears.

## Discussion

Endolymphatic hydrops animal models were developed to understand the pathophysiology of Menière's disease. Many insults to the inner ear have been used to induce hydrops in animals (26). Surgical blockage of the ED and ES in the guinea pig, because of its high success rate of inducing hydrops, has been the most widely used model. This model, probably contrary to the situation found in Menière patients, lacks the function of the ES. Therefore, more refined animal models were recently designed (24-26) by creating dysfunction, not inactivation, of the ES. In these models, endolymph resorption in the ES was reduced via immunological or pharmacological interventions and cauterization. In our model, mild perisacculary fibrosis was surgically induced, via dissection of the distal portion of the sac adjacent to the sigmoid sinus, resulting in a partially functioning ES, as described by Dunnebier et al (22). The fact that EH develops after dissecting the ES suggests that the resorptive action of the sac is defective.



To our knowledge, results of hydrostatic pressure measurements in the inner ear of a refined guinea pig hydrops model have never been published before. All six articles present in the literature (table 1), concerning pressure measurements in the perilymphatic and endolymphatic compartment, were based on the classic guinea pig model, in which ES function is totally lacking. The first two articles on hydrostatic pressure measurements in EH guinea pigs were published in 1987. Ito et al. (21) found a pressure difference in favour of endolymph, while Long III and Morizono (14) found no significant difference in pressure between operated ears and control ears. When only the hydropic ears were considered, there appeared to be a slight, relative pressure elevation in the scala media during the first 7 days after endolymphatic duct obstruction. Long III and Morizono assumed that with prolonged distension, Reissner's membrane would lose its elasticity and cease to sustain any pressure gradient across itself. The four other articles, all from one research group, concluded that pressure differences larger than 0.5 cm H<sub>2</sub>O occur only in the late stages (> 5 weeks) of EH and only in two-thirds of ears. They speculated that this pressure difference is due to loss of compliance of Reissner's membrane after long-standing distension. In summary, the presence of a pressure difference between the endolymphatic and perilymphatic space in EH is variable among different publications, although all these studies used the same hydrops model and measuring equipment. Only one study (20) compared the mean PLP and ELP in control ears and in hydropic ears, and found no difference. We recorded no hydrostatic pressure difference between the endolymphatic and perilymphatic compartment, but in a different, more realistic hydrops model. Eye-catching in figure 1 are three points that stand off from the dashed line, indicating a substantial intra individual pressure difference between scala media and scala tympani, which was also found by Long and Morizono (14). One plausible explanation for this variation in pressure difference could be an artifact of the experimental method used by dimpling membranes or by causing tiny leaks. Another interpretation is that these pressure differences are real and that they represent the oscillating behaviour of a homeostatic mechanism. In accordance with our results, Takeuchi et al. (27)

Table 1. Presence of pressure difference between the perilymphatic and endolymphatic space, and of decreased endocochlear potential (EP), in ears with hydrops, compared with literature.

author(s)	n	pressure difference	decreased EP
Long III and Morizono, 1987	21	no	yes
Ito et al., 1987	5	yes	no
Böhmer and Andrews, 1989	3	yes	yes
Böhmer et al., 1989	18	yes	yes
Böhmer and Dillier, 1990	11	yes	yes
Andrews et al., 1991	11	yes	yes
Present study	8	no	yes

and Demott and Salt (28) detected no pressure difference between the two compartments during a study of artificially created hydrops. In this dynamic hydrops model, hydrostatic pressure measurements were simultaneously performed in the endolymphatic and perilymphatic space, during infusion of artificial endolymph into the endolymphatic compartment. As reported by others (20), we found that the mean PLP and ELP in normal ears were in the same range as in hydropic ears.

The only difference that we found between normal ears and ears with hydrops was a decrease in EP in the latter category. A similar finding was reported by others (table 1). Interestingly, the EP in hydropic ears was also statistically significantly decreased compared to ears that were operated on, but did not develop EH. This means that the decreased EP is not caused by surgically induced damage, but is an effect of EH.

In the literature, much is speculated about the pathophysiology of the EP decrement, with emphasis on electrochemical shifts in the scala media (for a review see (29)). The consistent data from our study suggest that electrophysiological changes, more than endolymphatic hypertension, play a role in the pathophysiology of EH.

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## Chapter 6

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### **Artificial hydrops in the guinea pig by direct fluid injection: a way to measure endolymphatic compartments**

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## **Introduction**

The aetiology and pathophysiology of Menière's disease (MD) are still unclear. The association of this disease with endolymphatic hydrops (EH) (1), which is a volume increase of the endolymphatic spaces in the inner ear, led to the formation of a central theory that is still to be conclusively tested. The basic idea is that EH and its presumed sequelae are thought of as generating the symptoms of MD but the hydrops itself could be produced in many ways and be influenced by a number of factors, that theoretically at least, affect fluid management in the inner ear (2). One theorized outcome of EH is endolymphatic hypertension (1,3-5) that is a positive pressure difference between the endolymphatic and perilymphatic compartment in the inner ear. This endolymph-perilymph fluid pressure imbalance may be responsible for perceptive hearing loss in MD, via reduced blood flow to the organ of Corti (6) or displacement of the basilar membrane toward the scala tympani (7). Vertigo and tinnitus, other symptoms of MD, have also been associated with endolymphatic hypertension (3,5,8). Furthermore some therapies for MD, medical and surgical (4), are based on the concept of endolymphatic hypertension.

However if membranes such as Reissner's membrane can accommodate pressure increase in the endolymphatic space by stretching, then a volume increase need not be accompanied by a pressure increase. Which means that the elastic property (mechanical compliance) of the membranous labyrinth plays a crucial role in whether or not EH actually leads to endolymphatic hypertension.

Early studies verifying the presence of endolymphatic hypertension during EH in the cochlea of guinea pigs used a somewhat coarse technique, where the perilymphatic and endolymphatic pressure were measured consecutively using only one micropressure detection system, and they had variable results. For instance Long and Morizono (9) found no significantly higher endolymphatic than perilymphatic pressure but Böhmer and Andrews (10) did. More elegant experiments done later, where perilymphatic and endolymphatic pressure were measured simultaneously using two micropressure detection systems while artificial endolymph was injected in scala media (11,12), registered no endolymphatic hypertension.

The aim of the present study was to analyse if endolymphatic hypertension occurs during creation of endolymphatic hydrops in the guinea pig via injection of artificial endolymph into scala media, using the refined technique by which the pressure in both compartments of the cochlea are measured at the same time. Another goal was to calculate the compliance of the membranous labyrinth from the measured results.

## Material and methods

Successful experiments were performed in 10 female-pigmented guinea pigs (Harlan laboratories UK; 500-800 g body weight). An animal experiment was considered successful when perilymphatic and endolymphatic pressure could simultaneously be measured during infusion of artificial endolymph in scala media. For the duration of the experiment the animals were under general anaesthesia induced by intramuscularly administration of ketamine (Ketalar®, Parke-Davis; Hoofddorp, the Netherlands; 60 mg/kg) and xylazine (Rompun®, Bayer; Mijdrecht, the Netherlands; 3.5 mg/kg) and suxamethonii chloridum (Centrafarm; Eetten-leur, the Netherlands; 2.5 mg/kg). The animals were artificially ventilated through a tracheostoma (Columbus instruments model 7950, Columbus, Ohio, USA) and the body temperature was maintained at 38 °C. The animal's head was kept in a stationary position by means of a steel bolt fixed to the skull with dental cement. The bulla was opened by a retroauricular approach and the round window exposed. Through the round window membrane, the tip of a double-barrelled micropipette was inserted into scala tympani, and after subsequent perforation of the basilar membrane into scala media. DC potential at the pipette tip was measured to verify its position. Double barrelled micropipettes were drawn from borosilicate glass (1.5/0.84 mm per barrel) and the tips were bevelled (Narishige EG-40, Tokyo, Japan). Tip diameters were around 30 µm per barrel, which is a compromise between low enough flow resistance for fluid injection and tip smallness. One barrel of the pipette was used to measure inner ear pressure and DC potential (World Precision Instruments inc. (WPI), 900A micropressure system, Sarasota, Fla., USA). Through the other barrel, artificial endolymph (140 mM KCL + 25 mM KHCO<sub>3</sub>; (12)) could be injected with a constant flow rate into the inner ear by applying a controllable pneumatic pressure (WPI PV830 Picopump) to the barrel end. The fluid injection rate was calculated as the total injected volume divided by the total injection time. The injected volume was measured as displacement of the fluid meniscus in the pipette, for which the inner diameter is precisely known (0.84 mm).

Apart from the double-barrelled pipette, a single-barrelled pipette (diameter of bevelled tip 10 µm) was also introduced through the round window rim into scala tympani, to measure the perilymphatic pressure with a second WPI 900A system. During an experiment, endolymphatic and perilymphatic pressure values were stored digitally with a rate of 10 Hz, after low pass filtering (cut-off frequency 5 Hz). The response time of the micropressure system was calibrated to be better than 0.1 s. Recordings were processed off-line with an appropriate software package.

A measurement started with injection of a small amount of fluid, while both pipette tips were in scala tympani. This was done to detect a possible difference in calibration between the two WPI systems, for which then could be corrected off line. After this, the double barrelled pipette was advanced into scala media (fig 1).



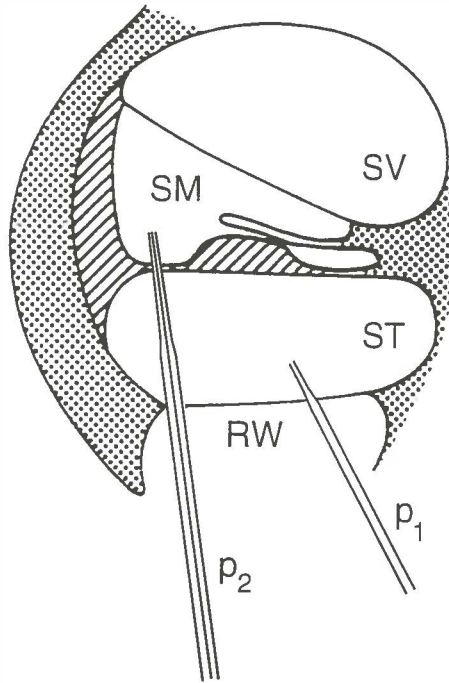


Figure 1. Position of micropipette tips. The single barrel pipette measures perilymphatic pressure ( $p_1$ ) in scala tympani (ST). The double-barrelled pipette measures endolymphatic pressure ( $p_2$ ) and potential in scala media (SM). (RW = round window; SV = scala vestibuli).

Then a small hole was made in the rim of the round window with a sharp metal tip that was attached to a micromanipulator. This opening reduces disturbing physiological pressure fluctuations (mainly caused by breathing and heartbeat) to a large extent. A measurement series consisted typically of 20 injections of fluid into scala media. As injection time (10 s) and injection repetition rate (0.02 per s) were controlled with a precision electronic timer (Stanford DG535, USA), subsequent pressure profiles or measurement series from different ears could be averaged if necessary.

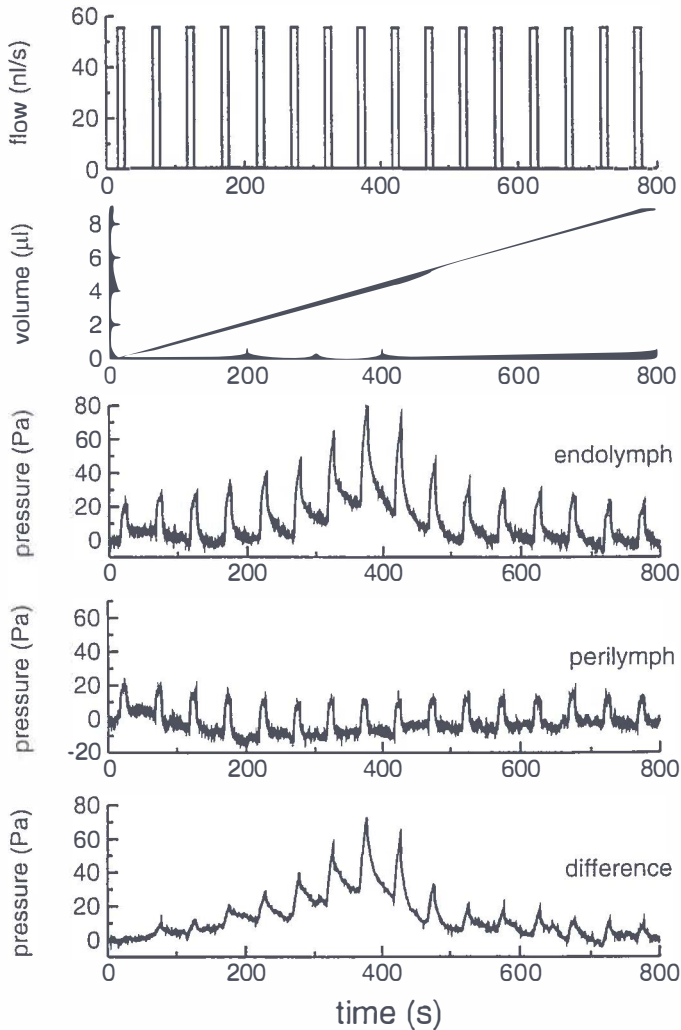
The Groningen University Animal Care and Use Committee under number DEC 2321 approved the care and use of the guinea pigs reported on in this study.

## Results

The essence of the experiments is captured in figure 2, which shows an example of registrations done in one guinea pig. The importance of simultaneous measurement of

perilymphatic and endolymphatic pressure is directly visible in panel 4 of this figure, because pressure increases are still measurable in the perilymph, during fluid injection in scala media, despite the fact that an opening was made in the round window.

Endolymphatic hypertension was measured in the example experiment as shown in panel



*Figure 2. Results from fluid pressure measurements in a guinea pig inner ear. Panel 1 (upper panel): flow profile; every 50 s, artificial endolymph is injected during 10 s with a constant rate. Panel 2: volume of injected fluid. Panel 3: pressure profile in endolymph. Panel 4: pressure profile in perilymph. Panel 5 (lower panel): endolymphatic pressure minus simultaneously measured perilymphatic pressure.*

5 of figure 2. Moreover, in all 10 ears a positive pressure difference between the endolymphatic and perilymphatic compartment was registered. The variation between animals concerning the magnitude of endolymphatic hypertension was small and is, for easier comparison, partially reflected in figure 3 which gives the difference between endolymphatic and perilymphatic pressure for four ears. Fluid flow during injection was approximately the same for these four recordings (50 nl/s), as apposed to the other six that had different flow rates.

In all experiments a striking occurrence was observed during the registration of endolymphatic pressure. This event is visible in the middle panel of figure 2, it is a maximal value of endolymphatic pressure appearing around  $t = 400$  s, after which the pressure decreases while artificial endolymph is still injected. It is evident in panel 4 of the same figure that it does not occur in the perilymphatic space. We interpreted this phenomenon as the development of permanent damage to the endolymphatic system. Figure 4 shows that this "catastrophe" occurs after the injection of approximately  $4 \mu\text{l}$  of fluid, independent of the flow rate with which artificial endolymph was injected into scala media.

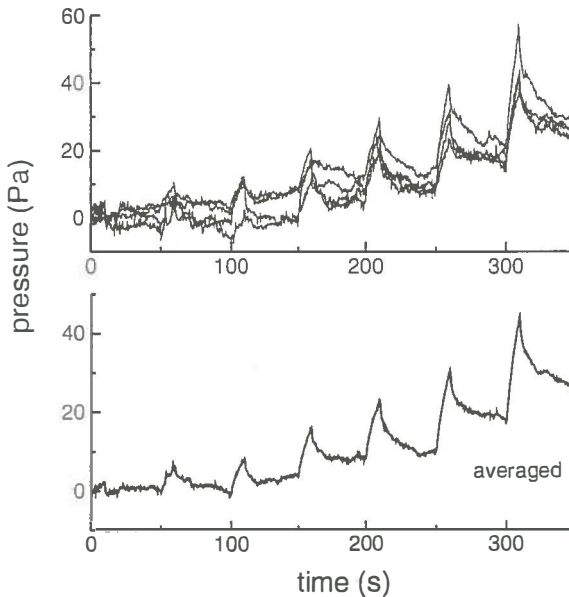


Figure 3. Upper panel: endolymphatic minus perilymphatic pressure in four different ears. One of the curves is equal to that shown in the lower panel of Fig. 4. Lower panel: average of the curves shown in the upper panel. Note that the first injection of artificial endolymph starts at  $t = 0$ ; this causes no measurable pressure difference.

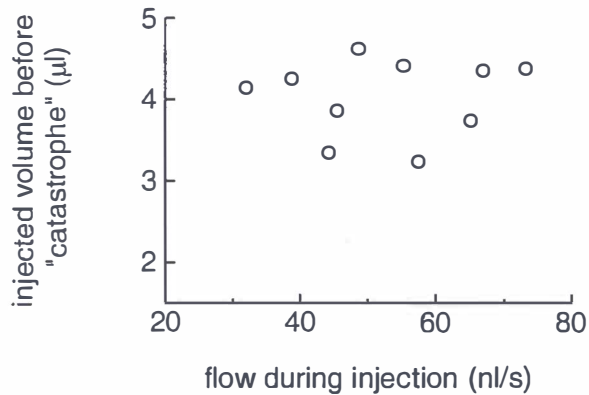


Figure 4. Relation between rate of fluid injection into scala media and the injected volume at which (most probably) a tear forms in one of the membranes bordering the endolymphatic compartment.

## Discussion

The typical shape of the pressure difference curve (figures 2 and 3, lower panels) could be explained with a two-compartment model for the endolymphatic system (14). It begins with a rapid rise in endolymphatic pressure during injection of fluid into scala media that is the first compartment. The slope of the pressure versus time curve during this phase is equal to  $f/C_1$ , in which  $f$  is flow rate and  $C_1$  is the compliance of scala media. (The larger  $f$ , the faster pressure increases. The larger  $C_1$ , the slower pressure increases during injection).

After that, pressure equilibrium is created during the 40 seconds intervals between injections, because fluid flows from scala media through the narrow ductus reuniens (Hensen's duct) into the vestibular part of the endolymphatic system that is the second compartment. This equilibrium pressure following each injection, rises to a higher value until the "catastrophe" occurs.

Figure 5 gives a scatter plot of these equilibrium pressure values as a function of the volume of injected fluid for all 10 animals. From the curve fitted to the data points in this figure the total compliance  $C$  of the endolymphatic system can be derived. This compliance can be approximated by  $C = 0.17 \mu\text{l}/\text{Pa}$ . ( $C = C_1 + C_2$ , with  $C_1 =$  compliance of scala media and  $C_2 =$  compliance of vestibular part of the endolymphatic system). The compliance decreases with increasing endolymph volume, because if the compliance had been independent of volume the solid line in figure 5 would have been straight.

In addition to the characteristic form of the pressure difference curve, another observation can be made: relatively large volumes (minimal  $2 \mu\text{l}$ ) of rapidly injected ( $50 \text{ nl/S}$ ) fluid were needed to create endolymphatic hypertension that was barely measurable ( $10 \text{ Pa} = 1 \text{ mm H}_2\text{O}$ ). It is than not remarkable that other studies with the same experimental set-up (11,12) were unable to measure endolymphatic hypertension, since they injected a maximum of  $2 \mu\text{l}$  artificial endolymph and because the resolving power of the WPI 900A micropressure system is almost of the same magnitude as the measured hypertension ( $1 \text{ mm H}_2\text{O}$ ).

It's possible to give an impression of the amount of hydrops formation needed for measurable endolymphatic hypertension to occur. The volume increase of scala media can be calculated after injecting a known fluid volume, because  $C_1$  and  $C$  can be assessed. For instance: after  $2 \mu\text{l}$  fluids are injected, the compliance  $C_1$  is five times smaller than the total compliance  $C$  ((14), fig.11 and fig.12). So in the resting phase, after fluid injection, 20% ( $0.4 \mu\text{l}$ ) of the fluid is in the cochlear duct and 80% in the vestibular part of the endolymphatic system. The compliance of Reissner's membrane is much larger than that of the basilar membrane. Therefore the volume increase will mainly lead to expansion of Reissner's membrane. This dislocation can be estimated because the dimensions of Reissner's membrane are known (width =  $0.4 \text{ mm}$ , length =  $18 \text{ mm}$ , (15)). A calculation then delivers, considering the cochlear duct to be cylindrical in shape with its volume being approximated by

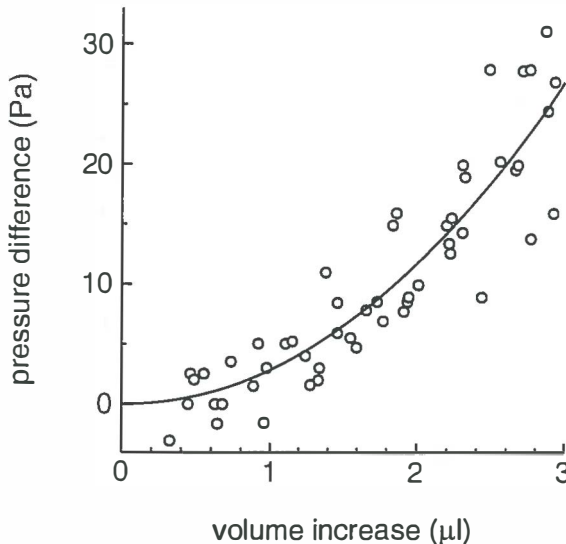


Figure 5. Open circles: compilation of results from measurements in 10 different ears. Increase in pressure difference between endolymph and perilymph versus volume of fluid injected into scala media. The solid line is a fit with  $p = aV^b$  ( $p$  = pressure,  $V$  = volume), yielding  $a = 2.86$  and  $b = 2.04$ .

$V = \pi R^2 h$ , that Reissner's membrane is displaced with 100  $\mu\text{m}$ . This is substantial hydrops formation. However, as can be seen in figure 5, it is accompanied by only very slight endolymphatic hypertension (10 Pa = 1mm H<sub>2</sub>O).

The expansion of Reissner's membrane during endolymphatic hydrops has been elegantly measured and visualized in a recent publication by Flock & Flock (16). According to figure 4 in their article, a pressure between 5 and 10 cm H<sub>2</sub>O is needed to displace Reissner's membrane with 100  $\mu\text{m}$ . To generate hydrops they used an artificially induced perilymph current, running from the basal to the apical part of scala vestibuli. The bottom part of Reissner's membrane is then pressed towards the basilar membrane, while the top part is distended. The pressure mentioned by Flock & Flock to create this local hydrops in the apical part of the cochlea was the one needed to realize the current in the perilymphatic space. It is important to note that this is not the same pressure that displaced Reissner's membrane. However estimation can be made of this last pressure: the length of scala vestibuli through which the fluid streams is about 1 cm and the mean diameter 0.6 mm ((16), fig.1). When we use Poiseuille's formula (14) we can calculate for scala vestibuli a flow resistance of 2.2 Pa.s/ $\mu\text{l}$ . The flow rate through scala vestibuli in the experiments of Flock & Flock is almost 1  $\mu\text{l/s}$ . This means that the pressure difference between the basal and apical part of scala vestibuli that is the pressure that displaces Reissner's membrane, is in the order of Pascal's. Therefore the results of Flock & Flock do not contradict ours.

In conclusion, a substantial volume increase of the membranous labyrinth is needed to be accompanied by a small positive pressure difference between scala media and the perilymphatic compartment due to the large elastic properties of the membranes surrounding the endolymphatic compartment. This means that endolymphatic hydrops is not always followed by endolymphatic hypertension, unless we consider 1mm H<sub>2</sub>O or less hypertension.

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## Chapter 7

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### Measurement of the mechanical compliance of the endolymphatic compartments in the guinea pig

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Wit HP, Warmerdam TJ, Albers FWJ. Measurement of the mechanical compliance of the endolymphatic compartments in the guinea pig. *Hear Res* 2000;145:82-90



## **Introduction**

Permanent displacement of the basilar membrane from its equilibrium position by endolymphatic hypertension has been put forward as an explanation for hearing loss associated with Menière's disease (Tonndorf, 1957; Klis and Smoorenburg, 1985). Endolymphatic hypertension is a permanent positive pressure difference between endolymphatic and perilymphatic compartments in the inner ear, and is thought to be a consequence of endolymphatic hydrops, which is a higher than normal endolymph volume.

However, attempts to create endolymphatic hypertension by injection of artificial endolymph into scala media of guinea pigs failed: Takeuchi et al. (1991) injected 2  $\mu\text{l}$  of artificial endolymph and simultaneously measured endolymphatic and perilymphatic pressure with micropipettes. No pressure difference was found within the accuracy of these measurements (0.1 mm Hg). These results were a few years later confirmed by DeMott and Salt (1997b), who also found no difference in simultaneously measured peri- and endolymphatic pressure, when injecting artificial endolymph, up to a volume of approximately 1  $\mu\text{l}$ .

In a previous paper (Wit et al., 1999) we reported about the dynamics of inner ear pressure release after injection of artificial endolymph into scala media of the guinea pig through one barrel of a double-barreled micropipette. The other barrel was used for simultaneous measurement of endolymphatic pressure and potential. As pressure differences between endo- and perilymph are non-existing (or at least very small), the pressures measured in this series of experiments with only one double-barreled pipette are total inner ear pressure (perilymphatic pressure = endolymphatic pressure). And the dynamics of pressure release could be explained with a simple model, incorporating a compliance (mainly the round window membrane) and a flow resistance (mainly the cochlear aqueduct).

In the present paper results are given from simultaneous measurements of endolymphatic and perilymphatic pressure, during and after injection of artificial endolymph. Use was made of two micropipettes: a single barrel pipette measured perilymphatic pressure and another double-barreled pipette measured endolymphatic pressure and was used for fluid injection.

In contrast with the earlier reports, a small but reproducible pressure difference could be measured between endolymph and perilymph, most probably because higher fluid injection rates were used and more fluid was injected.

## **Theoretical model**

Consider the situation of figure 1, in which two compartments with compliant walls (compliances  $C_1$  and  $C_2$ ) are connected by a flow resistance  $R_1$ . Fluid is injected into the first

compartment with a rate  $f$ . The pressure difference across the walls of the compartments is given by  $p_1$  and  $p_2$ .  $C_1$ ,  $C_2$ ,  $R_1$ , and  $f$  are supposed to be constant.

Fluid volume change per unit time in a compartment is equal to the net flow of fluid into it.

In formula for compartment 1:  $\frac{dV_1}{dt} = f - \frac{1}{R_1}(p_1 - p_2)$ ;

and for compartment 2:  $\frac{dV_2}{dt} = \frac{1}{R_1}(p_1 - p_2)$ .

Combining this with the definition for compliance  $C = \frac{dV}{dp}$ , and introducing the abbreviations

$\alpha = \frac{f}{C_1}$ ,  $\tau_1 = R_1 C_1$  and  $\tau_2 = R_1 C_2$  gives the set of coupled differential equations:

$\tau_1 \frac{dp_1}{dt} = \tau_1 \alpha - (p_1 - p_2)$  and  $\tau_2 \frac{dp_2}{dt} = p_1 - p_2$

with solutions:  $p_1(t) = \alpha \tau \left[ \frac{\tau}{\tau_1} (1 - e^{-\frac{t}{\tau}}) + \frac{t}{\tau_2} \right]$  and  $p_2(t) = \alpha \tau \left[ -\frac{\tau}{\tau_2} (1 - e^{-\frac{t}{\tau}}) + \frac{t}{\tau_2} \right]$ ;

If  $p_1(0) = p_2(0) = 0$  and with  $\frac{1}{\tau} = \frac{1}{\tau_1} + \frac{1}{\tau_2}$ .

If fluid is injected during time  $T$ , the pressures in compartments 1 and 2 will have reached the values  $p_1(T)$  and  $p_2(T)$  respectively. If at this time injection is ceased, fluid continues to flow from compartment 1 into compartment 2 (because  $p_1(T) \geq p_2(T)$  in the general case), until  $p_2$  equals  $p_1$ .

As  $\alpha = 0$  when fluid is no longer injected, the differential equations become:

$\tau_1 \frac{dp_1}{dt} = -(p_1 - p_2)$  and  $\tau_2 \frac{dp_2}{dt} = p_1 - p_2$ ,

with solutions (for  $t \geq T$ ):

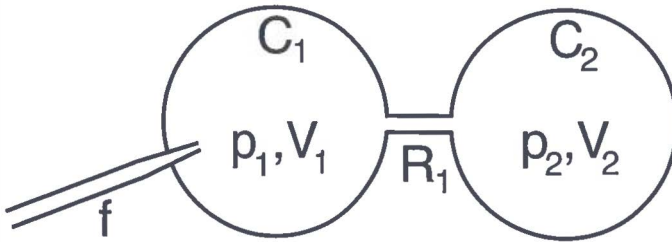


Figure 1. Two fluid filled compartments with compliant walls (compliances  $C_1$  and  $C_2$ ), connected by a flow resistance  $R_1$ . The pressure difference across the compartment walls is given by  $p_1$  and  $p_2$ , while the fluid volumes inside the compartments are  $V_1$  and  $V_2$ . During some time extra fluid is injected into the first compartment with a rate  $f$ .

$$p_1(t) = \tau \left[ \frac{p_1(T) - p_2(T)}{\tau_1} e^{-\frac{t-T}{\tau}} + \frac{p_2(T)}{\tau_1} + \frac{p_1(T)}{\tau_2} \right] \text{ and}$$

$$p_2(t) = \tau \left[ \frac{p_2(T) - p_1(T)}{\tau_2} e^{-\frac{t-T}{\tau}} + \frac{p_2(T)}{\tau_1} + \frac{p_1(T)}{\tau_2} \right]$$

With  $\varepsilon = 1 - e^{-\frac{T}{\tau}}$  these solutions can be written as:

$$p_1(t) = \alpha \tau \left[ \varepsilon \frac{\tau}{\tau_1} e^{-\frac{t-T}{\tau}} + \frac{T}{\tau_2} \right] \text{ and}$$

$$p_2(t) = \alpha \tau \left[ -\varepsilon \frac{\tau}{\tau_2} e^{-\frac{t-T}{\tau}} + \frac{T}{\tau_2} \right]$$

The limit for  $t \rightarrow \infty$  for these solutions is  $p_1(\infty) = p_2(\infty) = \frac{\alpha \tau T}{\tau_2}$ . This can be rewritten as

$$p(\infty) = \frac{fT}{C_1 + C_2} \text{ or } C_1 + C_2 = \frac{fT}{p(\infty)} = \frac{\Delta V}{\Delta p}, \text{ in which } \Delta V \text{ is the volume increase caused by fluid}$$

injection with a rate  $f$  during time  $T$ , and  $\Delta p$  is the accompanying pressure increase.

It can easily be verified that  $\frac{dp_1}{dt} = \alpha = \frac{f}{C_1}$  at  $t = 0$ .

As an illustration of the above theory, figure 2 gives  $p_1(t)$  and  $p_2(t)$  for  $f = 50$  nl/s,  $T = 10$  s,  $C_1 = 20$  nl/Pa,  $C_2 = 80$  nl/Pa and  $R_1 = 0.3$  Pa.s/nl.

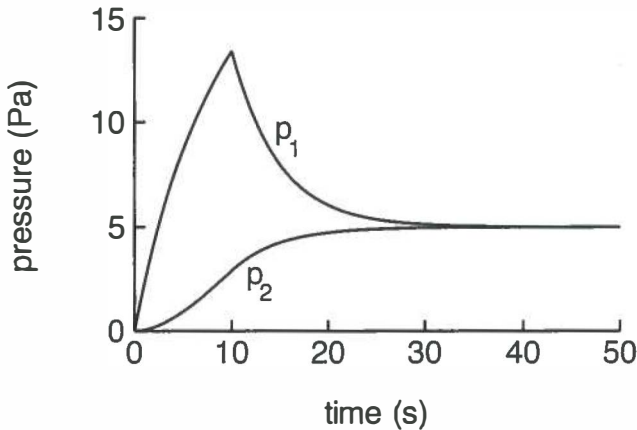


Figure 2. Calculated pressure profiles inside the compartments of figure 1, if fluid is injected with a rate of 50 nl/s during 10 seconds (starting at  $t = 0$ ), for  $C_1 = 20$  nl/Pa,  $C_2 = 80$  nl/Pa and  $R_1 = 0.3$  Pa.s/nl.

## Materials and methods

Successful experiments were performed in 10 female pigmented guinea pigs (Harlan Laboratories U.K.; 500-800 g bodyweight), under general anesthesia induced by intramuscular administration of ketamine/xylazine (60/3.5 mg/kg). Muscle relaxation was obtained with succinylcholine (2.5 mg/kg). The animals were artificially ventilated through a tracheostoma (Columbus Instruments, model 7950) and body temperature was maintained at 38 °C. The animal's head was kept in a stationary position by means of a steel bolt fixed to the skull with dental cement. The bulla was opened by a retroauricular approach and the round window exposed. Through the round window membrane the tip of a double-barreled micropipette was inserted into scala tympani, and after subsequent perforation of the basilar membrane into scala media. DC potential at the pipette tip was measured to verify its position. Double-barreled micropipettes were drawn from borosilicate glass (1.5/0.84 mm diameter per barrel) and the tips were bevelled (Narishige EG-40). Tip diameters were around 30 micrometer per barrel, which is a compromise between low enough flow resistance for fluid injection and tip smallness. One barrel of the pipette was used to measure inner ear pressure and DC potential (WPI 900A micropressure system). Through the other barrel artificial endolymph (140 mM KCl + 25 mM KHCO<sub>3</sub>; Salt and DeMott, 1997) could be injected with a constant flow rate into the inner ear by applying a controllable pneumatic pressure (WPI PV830 Picopump) to the barrel end. Fluid injection rate was calculated as the total injected volume divided by total injection time. Injected volume was measured as displacement of the fluid meniscus in the pipette, for which the inner diameter is precisely known (0.84 mm).

Apart from the double-barreled pipette a single-barreled pipette (diameter of beveled tip 10 micrometers) was also introduced through the round window rim into scala tympani, to measure the perilymphatic pressure with a second WPI 900A system. During an experiment endolymphatic and perilymphatic pressure values were stored digitally with a rate of 10 Hz, after low pass filtering (cut-off frequency 5 Hz). The response time of the micropressure system was calibrated to be better than 0.1 s. Recordings were processed off-line with an appropriate software package. A measurement started with injection of a small amount of fluid, while both pipette tips were in scala tympani. This was done to detect a possible difference in calibration between the two WPI systems, for which then could be corrected off-line. After this the double-barreled pipette was advanced into scala media (figure 3). Then a small hole was made in the rim of the round window with a sharp metal tip attached to a third micromanipulator.

A measurement series consisted typically of 20 injections of fluid into scala media. As injection time (10 seconds) and injection repetition rate (0.02 per second) were controlled with a precision electronic timer (Stanford DG535) subsequent pressure profiles or measurement series from different ears could be averaged if necessary.

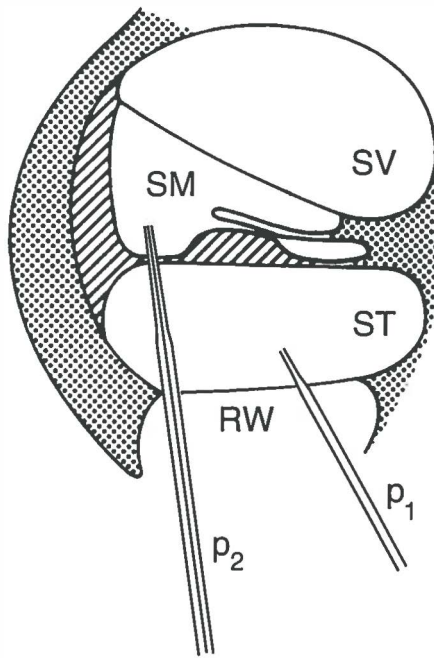


Figure 3. Position of micropipette tips. The single barrel pipette measures perilymphatic pressure ( $p_1$ ) in scala tympani (ST). The double-barreled pipette measures endolymphatic pressure ( $p_2$ ) and potential in scala media (SM). (RW = round window; SV = scala vestibuli.)

The care and use of the guinea pigs reported on in this study were approved by the Groningen University Animal Care and Use Committee under number DEC 2321.

## Results

An illustrative result of an inner ear pressure measurement during fluid injection is given in figure 4. Despite the fact that a hole was made in the round window, a pressure increase is measurable in scala tympani during fluid injection, as can be seen in panel 4 of figure 4. This shows that simultaneous measurement of pressures in perilymph and endolymph is crucial to obtain reliable results. The endolymphatic pressure profile (middle panel) shows a typical maximum around  $t = 400$  seconds. Such a maximum is not seen in the perilymphatic pressure. The occurrence of this maximum was used as a criterion for a measurement to be successful. It was interpreted as follows: each time fluid is injected into scala media (during 10 seconds) pressure difference between endolymph and perilymph increases until about  $4 \mu\text{l}$  of artificial endolymph is injected. If then still more fluid enters scala media, this causes a

pressure rise during injection, but in the intervals between injections pressure difference returns to zero, most probably because a permanent leak was created somewhere in the walls of the endolymphatic system. The total injected volume before this “catastrophe” occurs is displayed as a function of fluid injection rate in figure 5.

To show the variation between results obtained from different ears, figure 6 gives the difference between endolymphatic and perilymphatic pressure for 4 ears. One of these results is the same as that shown in the lower panel of figure 4. Fluid flow during injection was approximately the same for all 4 recordings (average 47 nl/s).

The shape of the individual peaks in figure 6, especially in the averaged curve (lower

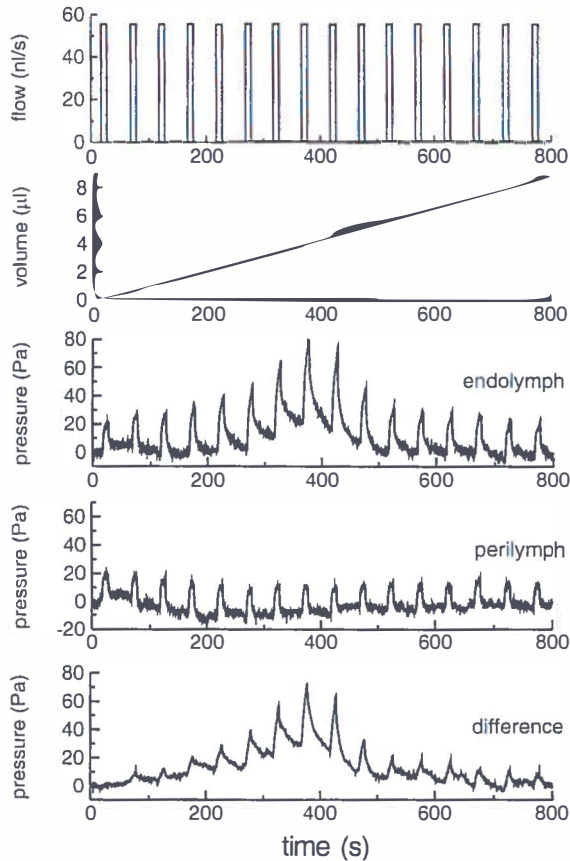


Figure 4. Results from fluid pressure measurements in a guinea pig inner ear. Panel 1 (upper panel): flow profile; every 50 seconds artificial endolymph is injected during 10 seconds with a constant rate. Panel 2: volume of injected fluid. Panel 3: pressure profile in endolymph. Panel 4: pressure profile in perilymph. Panel 5 (lower panel): endolymphatic pressure minus simultaneously measured perilymphatic pressure.



panel), shows a strong resemblance with the curve predicted for  $p_1$  in the Theory Section (see figure 2). This is even better illustrated in figure 7, where the peak starting at  $t = 150$  s in the lower panel of figure 6 is fitted with a  $p_1$ -curve, for which the formulas are given in the Theory Section. This least squares fit yielded:  $C_1 = 21$  nl/Pa,  $C_2 = 98$  nl/Pa and  $R_1 = 0.28$  Pa.s/nl.

In figure 8 part of the lower panel of figure 4 is again shown, with straight horizontal line segments fitted to the last 10 seconds of all 40 second periods of pressure recovery after injection of artificial endolymph. The position of these segments along the vertical axis gives the increase in static pressure difference between endolymph and perilymph caused by (cumulative) fluid injection. The relation between this pressure difference increase and the volume of injected fluid is given in the upper panel of figure 9. The sloping line segments in figure 8 are fits with second order curves (parabola segments) to the first 5 seconds of pressure increase during fluid injection. From these fits the slope of the curve at the start of injection was derived. This slope is equal to  $f/C_1$ , in which  $f$  is fluid injection rate and  $C_1$  is the compliance of the wall of the compartment into which fluid is injected, as was derived in the Theory Section. With the known (average) value for  $f$  the compliance  $C_1$  was calculated and shown in the lower panel of figure 9, again as a function of the volume of injected fluid.

The procedure to construct figure 9 was applied to the data from all 10 successful measurements. This resulted in the compilations shown in figure 10 for pressure difference increase and in figure 11 for scala media compliance decrease.

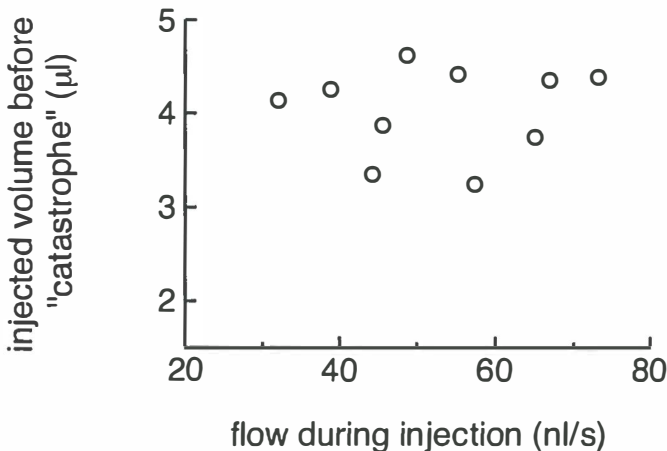


Figure 5. Relation between rate of fluid injection into scala media and the injected volume at which (most probably) a tear forms in one of the membranes bordering the endolymphatic compartment.

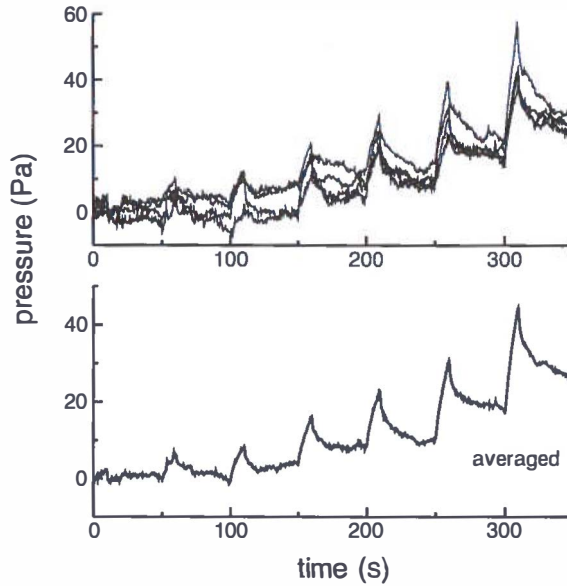


Figure 6. Upper panel: endolymphatic minus perilymphatic pressure in 4 different ears. One of the curves is equal to that shown in the lower panel of figure 4. Lower panel: average of the curves shown in the upper panel. Note that the first injection of artificial endolymph starts at  $t = 0$ ; it causes no measurable pressure difference.

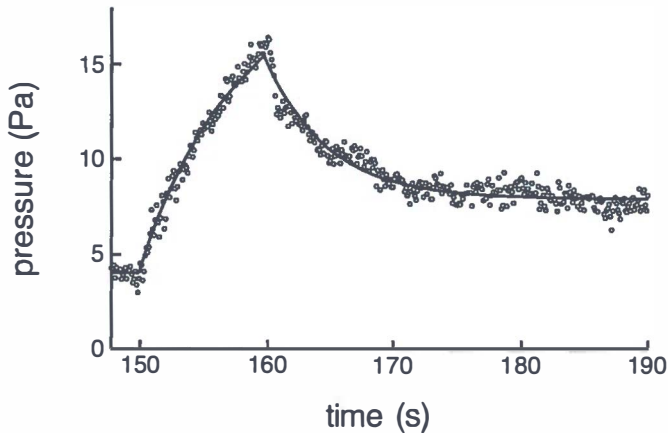


Figure 7. Open circles: part of figure 6, lower panel. Solid line: least squares fit with theoretical curve as derived in the Theory Section, which is also shown in figure 2 ( $p_1$ ).

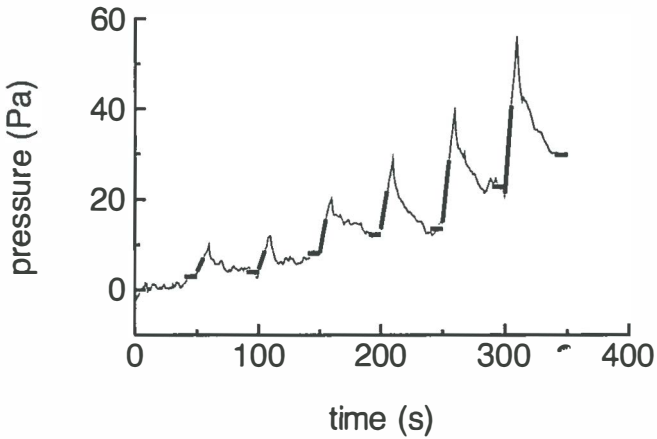


Figure 8. Thin line: slightly smoothed first part of the curve in the lower panel of figure 4. Thick lines: least squares fits to parts of the thin line, to derive static pressure increase (horizontal segments) and compartment compliance (sloping segments) from it.

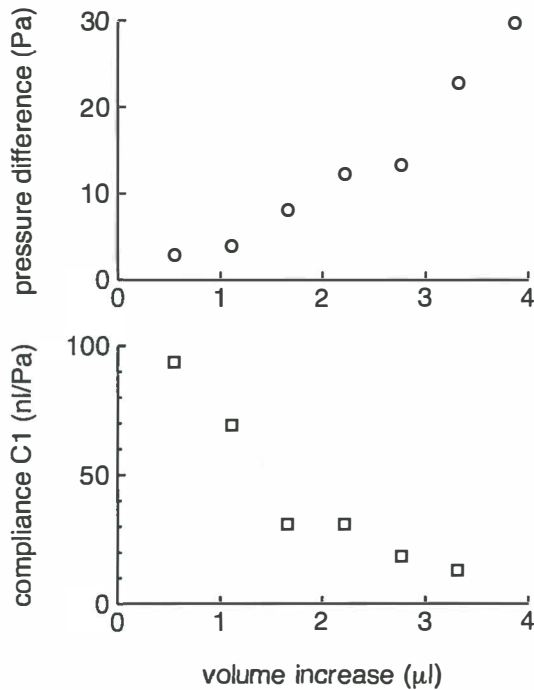


Figure 9. Results derived from the data shown in figure 8. Pressure difference between endolymph and perilymph (upper panel) and compliance of the membranes bordering scala media (lower panel) as a function of injected fluid volume.

## Discussion

In a previous preliminary study (Wit et al., 2000) both peri- and endolymphatic pressures were measured in the guinea pig during fluid injection into scala media (like in the present study), but no hole was made in the round window membrane. In that case inner ear fluid pressure increased by about 6 cm water (600 Pa) during injections with rates comparable to those in the present study, and its dynamic behaviour was dominated by the bony surroundings of the inner ear, combined with the round window compliance and the cochlear aqueduct flow resistance (Wit et al., 1999). After making a hole in the round window, pressure difference between endo- and perilymph during fluid injection into scala media is of the same order of magnitude as the increase in perilymphatic pressure (figure 4, panels 4 and 5), and therefore more directly observable. Furthermore cochlear perforation reduces disturbing physiological pressure fluctuations (mainly caused by breathing and heartbeat) to a large extent.

Takeuchi et al. (1991) injected 2  $\mu$ l of artificial endolymph in the guinea pig ear and were not able to record an increase in the pressure difference between endolymph and perilymph during infusion. They state that if a pressure difference had been created, it would have been below the resolving power of their method ( $\pm 0.1$  mm Hg). According to our figure 10, injection of 2  $\mu$ l of fluid creates on average a pressure difference of 12 Pa (= 0.09 mm Hg). This is indeed just below the resolving power of Takeuchi et al.'s method. As DeMott and Salt (1997b) injected even smaller volumes of artificial endolymph, it is not remarkable that they also did not observe a difference between endo- and perilymphatic pressure.

The endolymph filled space in the guinea pig inner ear actually consists of more than one compartment (e.g. Konishi, 1977): scala media is connected with the saccule through the narrow ductus reuniens (or Hensen's duct). The saccule in turn is connected with the endolymphatic sac through the saccular and the endolymphatic duct. Close to the saccule the utricular duct connects the utricule (and the membranous semicircular canals) with the saccular duct.

This system was modeled by two compliant compartments, connected through a narrow tube, as shown in figure 1. The first compartment in this model is scala media, while the second compartment represents the remaining part of the endolymphatic system. The narrow tube between the two compartments then is the ductus reuniens. Although this model is a simplified representation of the real endolymphatic system, it can very well describe the shape of the curves for the difference in pressure between endolymph and perilymph during and after injection of artificial endolymph into scala media.

For instance the fit as shown in figure 7 is good. The values derived from this fit ( $C_1 = 21$  nl/Pa,  $C_2 = 98$  nl/Pa,  $R_1 = 0.28$  Pa.s/nl) are thus a good approximation for the compliance of scala media, for the compliance of the saccule etc., and for the flow resistance of the ductus reuniens respectively.

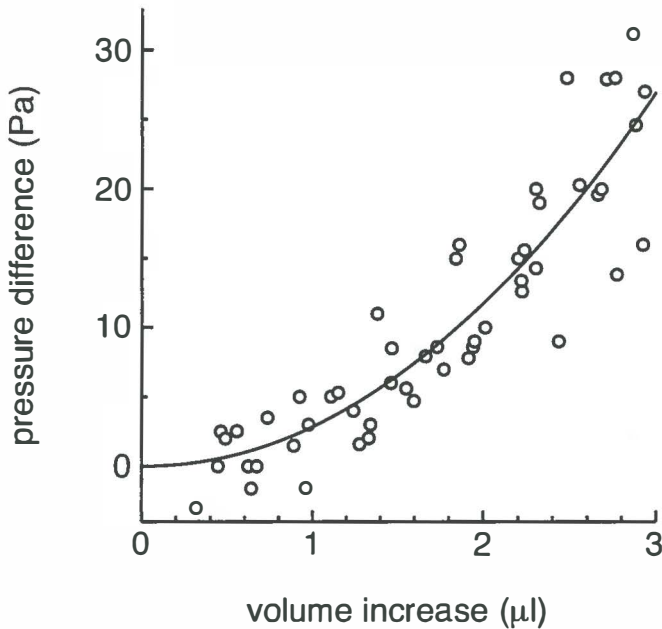


Figure 10. Open circles: compilation of results from measurements in 10 different ears. Increase in pressure difference between endolymph and perilymph versus volume of fluid injected into scala media. The solid line is a fit with  $p = \alpha V^b$  ( $p$  = pressure,  $V$  = volume), yielding  $a = 2.86$  and  $b = 2.04$ .

For the derivation of the shape of the  $p_1$ -curve and the  $p_2$ -curve (figure 2) it was assumed that  $C_1$ ,  $C_2$  and  $R_1$  are constants. If this were the case then the relation between pressure increase and injected fluid volume, as shown in figure 10, would have been linear. And figure 11 would have shown a constant value for  $C_1$ , independent of volume increase, which is clearly not the case.

The shape of the curve made up by the compilation of data in figure 10 can well be described with  $p = \alpha V^b$ . Combining this expression with the definition for compliance  $C = \frac{dV}{dp}$  gives  $C = \frac{1}{ab} V^{1-b}$ . The curve for  $C$ , which is the total compliance of the endolymphatic system

( $C_1 + C_2$  in the two-compartment model), is shown in figure 12. Because  $b > 1$ , this compliance is very large for small increases of endolymph volume. (Whether or not  $C$  actually goes to infinity for  $\Delta V \rightarrow 0$  cannot be decided; for that the spread in the data points in figure 10 is too large.)

The model, as described in the theory section, predicts endolymphatic pressure during and after fluid injection with respect to the pressure outside the compartments shown in figure

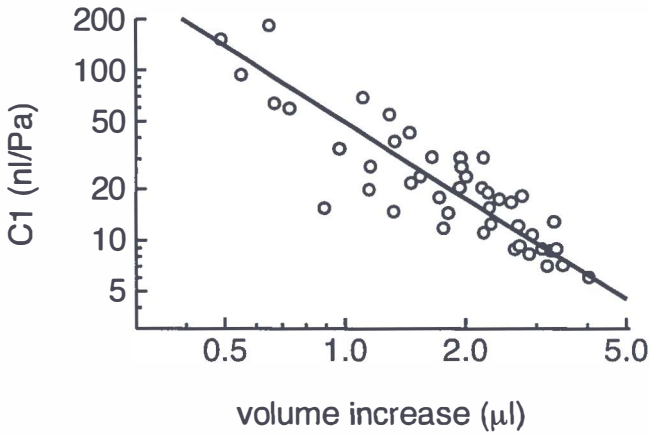


Figure 11. Open circles: compilation of results from measurements in 10 different ears. Compliance of membranes bordering scala media versus volume of fluid injected into scala media. The solid line is a fit with  $C = \alpha V^{-\beta}$  ( $C$  = compliance,  $V$  = volume), yielding  $\alpha = 49.6$  and  $\beta = 1.49$ .

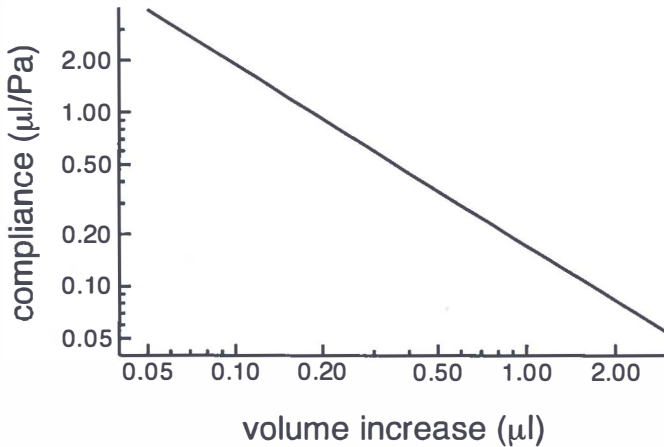


Figure 12. Total compliance of the guinea pig endolymphatic system as a function of endolymph volume increase, derived from the solid line fit in figure 10. (Total compliance = sum of compliances of the different endolymphatic compartments.)

1. This outside pressure, which is perilymphatic pressure, is assumed to be zero. In reality this is not the case, even not although a hole was made in the round window. But because it is the pressure difference between endolymph and perilymph (figure 4, lower panel) that was further analyzed, the model does not lose its predictive value, unless perilymphatic pressure has a value outside compartment 1 different from the value outside compartment 2. This is,

however, unlikely because there are no structures with large flow resistances between the different parts of the perilymphatic space (Salt and Konishi, 1986).

As a consequence of the fact that endolymph pressure increase (with respect to perilymph) is very small for small volume increases it is questionable if endolymph volume is regulated by detection of pressure change. It could be that such small (static) pressure changes are detectable by hair cells: Zucca et al. (1991) found changes in spontaneous and evoked nerve activity in a preparation of the vestibular system of the frog at pressure differences between endo- and perilymph as low as 3 Pa. But if hair cells would really be involved in volume regulation, damaging of hair cells in cases of hearing loss or loss of vestibular function would lead to a disturbed volume regulation mechanism as well.

It has been speculated that stretch activated channels, as found in Reissner's membrane (Yeh et al., 1997), might play a role in volume regulation. However these channels do not open before pressure has increased by about 15 mm Hg, which is a similar pressure as found to open stretch activated channels in other preparations (Chamberlin and Strange, 1989), but which is two orders of magnitude larger than the pressure increase caused by an endolymph volume increase of 2.5  $\mu\text{l}$  as shown in figure 10. This volume increase should be compared with the total inner ear endolymph volume, which is around 5  $\mu\text{l}$  (Rauch, 1964).

Comparison of figures 11 and 12 gives that  $C_2$  in the two-compartment model is 3 to 5 times larger than  $C_1$ . This is in accordance with results obtained by Salt and DeMott (1997). Based on endolymphatic flow measurements induced by microinjections of artificial endolymph at low rates these authors concluded that (highly compliant) structures outside the cochlea play a role in scala media endolymph volume disturbances. This highly compliant structure outside the cochlea could well be the saccule, because the saccule is observed to be one of the first labyrinthine structures to distend if an endolymphatic hydrops is induced in a guinea pig ear by destruction of the endolymphatic sac (Konishi, 1977).

The flow resistance  $R_1$  in the two-compartment model is made up by the ductus reuniens (Hensen's duct) between the basal turn of scala media and the saccule. The value for this flow resistance (0.28 Pa.s/nl), obtained from the fit to the data in figure 7 is a reasonable one, considering the dimensions of the ductus reuniens: We assume Poiseuille's formula

$f = \frac{\pi p r^4}{8 \eta l}$  to be valid for flow through a small cylindrical duct (Allen, 1987). ( $f$  = volume flow,

$p$  = pressure,  $r$  = duct radius,  $\eta$  = fluid viscosity and  $l$  = duct length). With  $R = \frac{dp}{df}$  this gives:

$R = \frac{8 \eta l}{\pi r^4}$ . Substituting  $R = 0.28$  Pa.s/nl and  $\eta = 6.9 \times 10^{-4}$  Pa.s (for saline) fixes the relation

between  $l$  and  $d (= 2r)$ . This relation is shown in figure 13. The length of the ductus reuniens is at least 0.6 mm (Konishi, 1977), so its diameter is around 0.1 mm, according to figure 13.

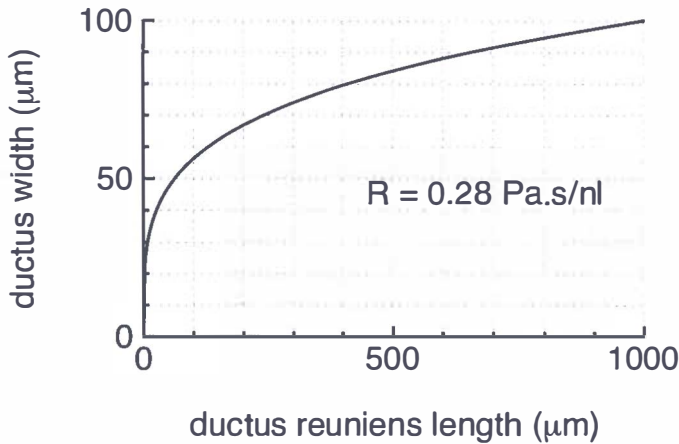


Figure 13. Relation between ductus reuniens length and diameter for the flow resistance as measured in one guinea pig ear.

Values for this diameter are given by Konishi (1977): 0.11 mm in hydroptic ears and not more than 0.03 mm in the unoperated control ear. So our estimated value of 0.1 mm is larger than that in an undisturbed ear, but it might well be that injection of artificial endolymph dilates the ductus reuniens, making its diameter comparable to that in a hydroptic ear.

Endolymphatic pressure in guinea pigs changes on a time scale of minutes after intravenous (Yoshida et al., 1985) or intraesophageal (Takeda et al., 1990) injection of glycerol. So it is imaginable that a pressure difference between endo- and perilymph can be created by injecting fluid with the wrong osmolarity. Therefore the artificial endolymph that was injected was carefully chosen to have the same composition as natural endolymph (Salt and DeMott, 1997). Furthermore, it was shown by Zucca et al. (1995), in whole labyrinth preparations of the frog, that the effects of change of (perilymph) osmolarity reach a steady state only after 20-25 minutes. We would therefore expect a more or less monotonic and slow change of pressure, if fluid with the wrong osmolarity should have been injected, instead of the typical pressure profiles as seen in figure 4.

As permanent displacement by endolymphatic hypertension has been put forward as an explanation for hearing loss in Menière's disease (Tonndorf, 1957; Klis and Smoorenburg, 1985), it is worthwhile to estimate this displacement for pressure differences in the order of 10 Pa. Such differences in pressure between endolymph and perilymph are created by injection of a few microliters of artificial endolymph into a healthy guinea pig inner ear (see figure 10). Direct measurement of basilar membrane stiffness has only been performed at basal locations in different animals. Ruggero et al. (1990; Table IV) give an average compliance of  $6.6 \times 10^{-9} \text{ cm}^3/\text{dyn}$  ( $= 0.66 \text{ nm}/\text{Pa}$ ) for the basilar membrane in the basal turn of the guinea pig. This means that a pressure of 10 Pa will give a basilar membrane displacement at this



location of 6.6 nm, which is negligible with respect to the dimensions of the organ of Corti. But the most compliant part of the basilar membrane is in the apical turn. To estimate the displacement at this location, a value for the (volume) compliance of the total basilar membrane, given by Décory et al. (1990), can be used. This value is given as  $0.9 \times 10^{-13} \text{ m}^5/\text{N}$ , which is equal to 0.09 nl/Pa (a value that should be compared with the compliance of the walls of scala media as given in figure 11). This means that a pressure of 10 Pa will give a volume displacement of the basilar membrane of 0.9 nl. If we assume that this volume displacement completely takes place at the upper 3 mm of the basilar membrane (corresponding to the frequency range 50-500 Hz (Greenwood, 1990), the average for the maximum displacement of the basilar membrane is 2  $\mu\text{m}$  (taking a width of 0.24 mm for the membrane (Lewis et al., 1985) and a parabolic shape for its cross section). Such a displacement is about two times the thickness of the membrane (Fernandez, 1952), but still small in comparison with the dimensions of the organ of Corti. So it remains a question how critically hearing threshold depends on a small permanent displacement of the basilar membrane. This dependence can not reliably be derived from measurements in which the position of the basilar membrane is cyclically changed by low frequency sound stimulation (Zwicker, 1977; Klis and Smoorenburg, 1985; Mrowinski et al., 1996), because the latter is a dynamical proces with a period of about 30 ms , and because it is imaginable that the organ of Corti can adapt to small permanent displacements.

This problem was addressed by Klis and Smoorenburg (1994), who investigated the effects of static displacement of the basilar membrane by perfusing the perilymphatic spaces in the guinea pig with hypo- and hypertonic solutions. They found effects on the summing potential and the compound action potential, comparable to the effects found during low-frequency biasing experiments, and conclude that basilar membrane displacement towards scala tympani is an important contributing factor to the electrophysiological changes in endolymphatic hydrops. But up to now the results of such osmotic pressure difference experiments can only be interpreted in a qualitative way, because, as far as we know, the actual pressure difference across the basilar membrane has not been measured and estimations for the displacement of the basilar membrane have not been given.

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## **Chapter 8**

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**Summary and conclusions**

**Samenvatting en conclusies**

**Dankwoord**

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## **Summary and conclusions**

With the equipment now available for doing reliable fluid pressure measurements in small volumes we evaluated the endolymphatic and perilymphatic pressures in the inner ear of normal guinea pigs and in three different in time evolved endolymphatic hydrops animal models. Our main interests were the possible development of endolymphatic hypertension in EH, and the *in vivo* determination of the mechanical compliance of the boundary membranes of the endolymphatic system.

In chapter 3 we measured the perilymphatic and endolymphatic pressure consecutively with one micropipette in the cochlea of normal guinea pigs. We found that the perilymphatic and endolymphatic pressure were equal in these ears.

In chapter 4 the perilymphatic and endolymphatic pressure were analysed subsequently, with one micropipette, in the classic endolymphatic hydrops animal model which was designed by Kimura. In this model the endolymphatic sac is totally destructed, leading to a decrease in endolymph resorption and thus to EH.

We found no endolymphatic hypertension, both in ears with hydrops and in control ears. Remarkable was the significantly decreased EP in hydropic ears when compared to control ears. Studies that confirm this finding were brought forward. The EP was measured to verify the location of the pipette tip in the inner ear. We suggested that dysfunctional marginal cells could be responsible for this decline in EP.

In chapter 5 the perilymphatic and endolymphatic pressure were also determined consecutively with one micropipette. However, this time in an endolymphatic hydrops animal model which further approximated the histopathological situation in MD. This recently designed model is distinct from the classic hydrops model, because it has a partially functioning endolymphatic sac as opposed to an endolymphatic sac that has lost all function. We presented histological confirmation that EH was achieved with a partially functioning endolymphatic sac.

We detected no endolymphatic hypertension in this hydrops model and in control ears. s well as in the Kimura hydrops model we found a significantly decreased endocochlear potential during hydrops. Interestingly, the endocochlear potential in hydropic ears was also statistical significantly decreased compared to ears that were operated, but did not develop EH. This means that the decreased endocochlear potential was not caused by surgically induced damage, but was an effect of EH. We proposed that electrophysiological changes, more than endolymphatic hypertension, may play a role in the pathophysiology of EH.

In chapter 6 and 7 we analysed the perilymphatic and endolymphatic pressure simultaneously in the most sophisticated hydrops model extant to this day. In this hydrops model an increase in volume of the endolymphatic system is created via injection of fluid into scala media, thus not by impairing endolymph resorption. Use was made of two micropipettes: a single barrelled pipette measured perilymphatic pressure and another double-barrelled pipette measured endolymphatic pressure and was used for fluid injection.

We detected a small amount of endolymphatic hypertension in all ears, but only after substantially expanding the endolymphatic compartment in a relatively short time with 2–4  $\mu\text{l}$  artificial endolymph. We compared our data with the only two other comparable experiments in literature. These publications found no endolymphatic hypertension. Our measurement outcome probably differed from the two studies because we injected more artificial endolymph into scala media and used a higher injection rate.

The endolymphatic system was damaged after injection of more than 4  $\mu\text{l}$  fluid.

The shape of the pressure-time curve during and between repetitive injections of fluid could well be described with a two-component model for the endolymphatic system, consisting of two compartments with compliant walls, connected through a flow resistance. The first compartment in this model is scala media, while the second compartment represents the remaining part of the endolymphatic system. The narrow tube between the two compartments then is the ductus reunions (Hensen's duct). With this model, a larger mechanical compliance was found for the boundary membranes of the second compartment (vestibular part of the endolymphatic system) than the surrounding membranes of the first compartment, into which fluid was injected (scala media).

We concluded that a substantial volume increase of the membranous labyrinth is needed to be accompanied by a small positive pressure difference between scala media and the perilymphatic compartment due to the large elastic properties of the boundary membranes of the endolymphatic system. This means that endolymphatic hydrops is not always followed by endolymphatic hypertension, unless we consider 1 mm H<sub>2</sub>O or less hypertension.

Translated to the clinical situation the meaning of the above mentioned conclusion implies that we do not share in the enthusiasm for the development of medical and surgical therapies which are aimed at reducing endolymphatic hypertension in Menière's disease.

Our advice not to strive for a reduction in endolymphatic hypertension does not mean that one should not try to reduce the endolymphatic volume in endolymphatic hydrops. It is important that we add this last remark because there is no prove that the endolymphatic volume is not disturbed in Menière's disease.



## **Samenvatting en conclusies**

Met de nu beschikbare instrumenten voor het verrichten van betrouwbare drukmetingen in kleine ruimten evalueerden wij de endolymfatische- en perilymfatische druk in het binnenoor van cavia's. Er werd gemeten in de cochlea van normale cavia's en in de cochlea van drie in de tijd geëvolueerde diermodellen voor het creëren van endolymfatische hydrops. We waren met name geïnteresseerd in de mogelijke aanwezigheid van endolymfatische hypertensie tijdens endolymfatische hydrops, en in het bepalen van de mechanische compliantie van de membranen die de endolymfatische ruimte begrenzen.

In hoofdstuk 2 werden achtereenvolgens de perilymfatische- en endolymfatische druk gemeten met één micropipet, in cavia's met normale cochlea's. De perilymfatische- en endolymfatische druk bleken gelijk in deze oren.

In hoofdstuk 3 werden de perilymfatische- en endolymfatische druk ook successievelijk gemeten met één micropipet, maar dan in het klassieke endolymfatische hydropsmodel, dat ontworpen werd door Kimura. In dit diermodel wordt de functie van saccus endolymfaticus geheel uitgeschakeld, wat leidt tot een afname in endolymferesorptie, en daardoor tot endolymfatische hydrops.

Zowel in de controle-oren als in de oren met hydrops werd geen endolymfatische hypertensie gemeten.

Een interessante vondst was de significant afgenomen EP in oren met hydrops vergeleken met de controle-oren. Gerefereerd wordt aan andere studies met dezelfde ontdekking. De EP werd gemeten om de locatie van de micropipet in het binnenoor te bepalen. We suggereren dat disfunctionele marginale cellen verantwoordelijk kunnen zijn voor de daling van de EP.

In hoofdstuk 4 werden de perilymfatische- en endolymfatische druk wederom opeenvolgend bepaald met één micropipet. De metingen vonden hier echter plaats in een hydropsdiermodel dat de histopathologie van de ziekte van Menière beter benadert. Dit recent ontworpen model is verschillend van het klassieke hydrops model, omdat het een partieel functionerende saccus endolymfaticus heeft in plaats van een saccus endolymfaticus zonder enige functie. We lieten aan de hand van histologische coupes zien dat EH kan ontstaan bij een partieel functionerende saccus endolymfaticus.

We detecteerden geen endolymfatische hypertensie in dit hydropsmodel en in de controle-oren.

Evenals in het hydropsmodel van Kimura vonden we een significant afgenomen EP bij hydrops. Interessant in dit experiment was dat de EP in oren met hydrops ook significant

verlaagd was vergeleken met geopereerde oren die geen hydrops ontwikkelden. Dit betekent dat de afgenomen EP dus veroorzaakt werd door EH en niet door chirurgische schade. Het lijkt er dus op dat elektrofysiologische veranderingen, meer dan endolymfatische hypertensie, een rol spelen in de pathofysiologie van EH.

In hoofdstuk 5 en 6 analyseerden we de perilymfatische- en endolymfatische druk tegelijkertijd in het tot op heden meest geavanceerde hydropsproefdiermodel. In dit hydropsmodel wordt een toename van het volume van de endolymfatische ruimte gecreëerd door kunstmatige endolymfe in de scala media te injecteren. De hydrops wordt in dit model dus niet veroorzaakt door een afname in endolymferesorptie. De drukmetingen werden verricht met twee soorten micropipetten. Een enkelloospipet analyseerde de perilymfatische druk. Een dubbelloospipet bepaalde de endolymfatische druk met de ene loop en met de andere werd vloeistof in de endolymfatische ruimte gebracht.

In alle oren vonden we een heel lage endolymfatische hypertensie, en dat alleen nadat het volume van de endolymfatische ruimte in korte tijd substantieel werd vergroot met 2-4  $\mu\text{l}$  kunstmatige endolymfe. We legden onze data naast de enige twee andere vergelijkbare onderzoeken in de literatuur. Die twee studies vonden geen endolymfatische hypertensie. Onze meetuitkomsten verschilden waarschijnlijk van deze publicaties omdat wij meer vloeistof injecteerden in de scala media en met een hogere injectiesnelheid.

Het endolymfatische systeem leek beschadigd nadat er meer dan 4  $\mu\text{l}$  vloeistof in werd geïnjecteerd.

De vorm van de grafiek van druk versus de tijd gedurende en tussen opeenvolgende vloeistofinjecties, kon verklaard worden met een twee-compartimenten model voor het endolymfatische systeem. Het gaat dan om twee door membranen begrensde ruimten die verbonden zijn door een nauwe buis met een weerstand. Het eerste compartiment in dit model representeert de scala media, de tweede stelt de rest van het endolymfatische systeem voor. De nauwe buis tussen deze ruimten geeft de ductus reuniens (ductus van Hensen) weer. Met dit model werd bepaald dat de compliantie van de omgevende membranen van het tweede compartiment (het vestibulaire deel van het endolymfatische systeem) hoger was dan die van de membranen die het eerste compartiment omsluiten (scala media).

We concluderen dat als gevolg van de hoge elastische eigenschappen van de begrenzende membranen van het endolymfatische systeem, een forse volumetoename van het membraneuze labyrint slechts leidt tot een laag positief drukverschil tussen de scala media en het perilymfatische compartiment. Dit betekent dat endolymfatische hydrops lang niet altijd gepaard gaat met endolymfatische hypertensie, tenzij we een druk van 1 mm H<sub>2</sub>O of minder als hypertensie beschouwen.

Vertaald naar de klinische situatie impliceert de betekenis van bovengenoemde conclusie dus dat wij niet delen in het enthousiasme voor het ontwikkelen van medicamenteuze en

chirurgische therapieën die gericht zijn tegen endolymfatische hypertensie bij de ziekte van Menière.

Ons advies om niet te streven naar een reductie van endolymfatische hypertensie houdt niet in dat niet gepoogd moet worden het endolymfatische volume te verkleinen bij hydrops. Deze toevoeging noemen wij met name omdat er nog geen bewijs is dat bij de ziekte van Menière het volume niet verstoord is.

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